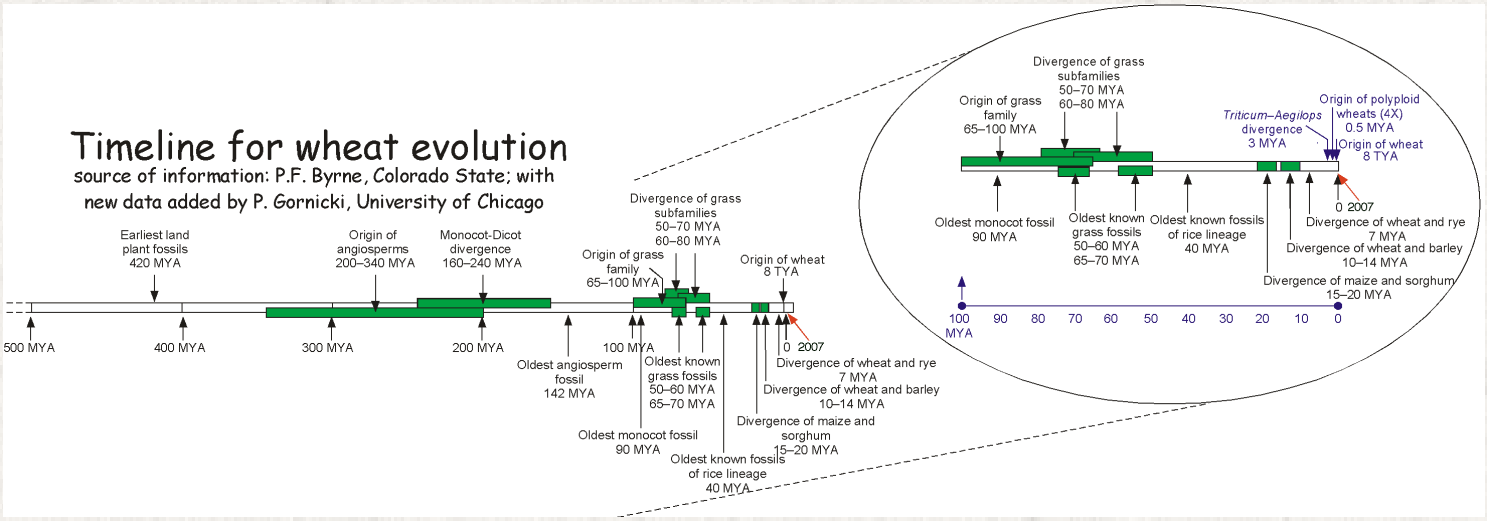
ANNUAL WHEAT NEWSLETTER

Volume 58



Contribution no. 13-004-D from the Kansas Agricultural Experiment Station,
Kansas State University, Manhattan.

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IN DEDICATION TO DR. ARNULF MERKER

Arnulf Merker died in September, 2010, at the age of 65. He was appointed to the Swedish University of Agricultural Sciences (SLU) at Alnarp and specialized in plant breeding. In 1990, Arnulf became professor at the Swedish University of Agricultural Sciences in Uppsala; in 1997 it tied him to the SLU unit at Alnarp, i.e., moving him back to Skåne.

A. Merker (right) on a field excursion during the International Rye Conference, Rostock, Germany, 2007.

Arnulf was born in Czechoslovakia during World War II. His father was a Sudeten German and his mother Swedish. The family fled to Sweden and lived for the first few years in northern Sweden. In 1950, the family moved to Helsingborg, where his father was working as an agronomist. He received his particular interest in biology and nature from his father Helmut Merker, the first employee at the Botanical Garden of Lund and later head of the Frederick Valley Gardens in Helsingborg.

After basic studies in biology, chemistry, and geology at Lund University, Arnulf's interest was captured by genetics. Consequently, he received his Ph.D. degree in genetics from Lund University in 1973. His thesis focused on chromosome studies in triticale under the guidance of the famous Prof. A. Müntzing. From 1974 he was employed by the Swedish Seed Association, followed by a successful postdoctoral period at the International Maize and Wheat Research Center (CIMMYT) in Mexico. He published many papers on the cytogenetic instability of triticale, resulting in the discovery of spontaneous wheat–rye chromosome substitutions in Mexican spring triticales. His first Swedish triticale variety, Uno, was released in 1990. For this, he received the Scanian Lantmännen award for having established triticale as a crop in Sweden. He continued as plant breeder and researcher at Svalöf AB until 1990.

Arnulf's main research was related to triticale and rye and utilization of wild species in breeding of wheat, rye, and barley. Dr. Merker was linked to the Swedish University of Agricultural Sciences as an assistant professor in the Department of Plant Breeding in 1986 and was appointed in 1990 as professor of plant breeding with placement at the Swedish Agricultural University in Ultuna (Uppsala), and later in Svalöv and Alnarp. His contribution to rye and wheat research continued throughout his career. Induced alien introgressions and domestication of crop plants has been widely reported. He had great interest in wheat hybrids with wild perennial wheat relatives, *Leymus arenarius*, *L. mollis*, *L. racemosus*, and *Thinopyrum junceiforme*, which was part of an inspiring collaboration with Kesara Anamthawat-Jónsson. By selection in wild populations and interspecific hybrids, he produced several prebreeding lines of great agronomical potential.

During recent years, he was heavily involved in international projects with graduate students from Africa, Central America, and Asia with a focus was on local crops, such as cereal tef, wheat, cabbage, and oilseed guizotia from Ethiopia; sesame in Southeast Asia; and indigenous forms of maize in Nicaragua. During the last years, he was able to lead a very large project in Central Asia to building modern seed and plant breeding activities.

An important part of his professional achievement was teaching at different levels. He became strongly involved in basic education of genetics and plant breeding at University of Agricultural Sciences, where he was a very popular teacher. Arnulf was elected to the Royal Physiographic Society of Sweden, Lund, and, in 2002, he received the National Agriculture and Forestry Academy Award for exemplary contributions to research information. For several years he was a member of the Swedish Gene Technology Advisory Board.

Arnulf Merker also was one of the most politically active students in Lund during the 1960s. He started as a radical pacifist and was sentenced to prison for draft resistance. He was then involved in FNL groups and was against the U.S. war in Vietnam. In the 1980s, he sat for a few years on the Höganäs City Council as a representative of the 'Left'. In recent years, and as a professor of plant breeding, Arnulf was challenged by radical forces, such as Greenpeace and organic farmers, who wanted to stop the development of genetically modified crops. On the contrary, he argued that genetic engineering can make farming more environmentally friendly and that resistance against it is due to ignorance.

Since 1997, Arnulf was living in Malmö. He is survived by his wife Ann-Charlotte Alverfors, sons Emil and Pål, stepdaughter Rebecca, and their families. Arnulf Merker's legacy will remain in memory of all his students, colleagues and friends.

Rolf Schlegel, Gatersleben, Germany & Kesara Anamthawat-Jónsson, Reykjavik, Iceland.

WHEAT WORKER'S CODE OF ETHICS

This seed is being distributed in accordance with the 'Wheat Workers' Code of Ethics for Distribution of Germ Plasm', developed and adopted by the National Wheat Improvement Committee on 5 November, 1994. Acceptance of this seed constitutes agreement.

1. The originating breeder, institution, or company has certain rights to the material. These rights are not waived with the distribution of seeds or plant material but remain with the originator.

2. The recipient of unreleased seeds or plant material shall make no secondary distributions of the germ plasm without the permission of the owner/breeder.

3. The owner/breeder in distributing seeds or other propagating material grants permission for its use in tests under the recipient's control or as a parent for making crosses from which selections will be made. Uses for which written approval of the owner/breeder is required include:

   (a) Testing in regional or international nurseries;
   (b) Increase and release as a cultivar;
   (c) Reselection from within the stock;
   (d) Use as a parent of a commercial F_1 hybrid, synthetic, or multiline cultivar;
   (e) Use as a recurrent parent in backcrossing;
   (f) Mutation breeding;
   (g) Selection of somaclonal variants; or
   (h) Use as a recipient parent for asexual gene transfer, including gene transfer using molecular genetic techniques.

4. Plant materials of this nature entered in crop cultivar trials shall not be used for seed increase. Reasonable precautions to ensure retention or recovery of plant materials at harvest shall be taken.

ITMI and NWGC Workshop Speaker Abstracts

25–29 June, 2012
Ramada Plaza and Suites Hotel–Fargo, ND, USA

SESSION I: GENOME SEQUENCING AND UTILIZATION

(Speakers denoted in **bold** lettering).

Sequences of 14,600 'gene-bearing' MTP BACs of Morex barley.

Timothy J Close. Department of Botany & Plant Sciences, University of California, Riverside, California, USA.

In a USDA–AFRI NIFA project entitled "Advancing the Barley Genome", we applied combinatorial pooling and Illumina sequencing to ~14,600 BACs that constitute a minimal tiling path (MTP) from 83,831 gene-bearing BACs of Morex barley. The BAC library was developed by Yu et al. (2000. Theor Appl Genet 101:1093-99) and these BACs account for roughly 70% of all expressed genes but only about 30% of the entire genome. Identification of gene-bearing BACs, fingerprinting, BAC contig assembly, MTP definition and assignment of an initial subset of about 2,000 MTP BACs to the genetic map were the results of prior projects supported by NSF and USDA, and from contributions of many individuals. These individuals, and others involved in the BAC sequencing, will be cited during the presentation. Most of these ~14,600 newly generated BAC sequences have been anchored to the genetic map. Anchoring methods include Illumina GoldenGate assays to relate mapped SNP loci to BACs, BLAST of BAC sequences to flow-sorted chromosome 1H and the short and long arms of 2H-6H, and BLAST of BAC sequences to other grass genomes to enhance physical map resolution based on synteny. A consequence of the combinatorial sequencing method is that each BAC assembly is composed of about 100 unordered contigs with a N50 in the range of 6 kb; the result is not end-to-end finished BAC sequences. In addition, the BAC assemblies in general do not include highly repetitive sequences. The available BAC sequences are searchable by BLAST via www.harvest-blast.org and can be retrieved from www.harvest-web.org as a single concatenated FASTA file for each BAC, with headers providing additional information. HarvEST:barley (http://harvest.ucr.edu) provides a Windows interface to facilitate retrieval of BAC sequences starting either with genetic map position or names of MTP BACs or other BACs contained in the same contigs from which gene-bearing MTP clones were selected for sequencing. Some example queries will be shown. Improvements of user interfaces and integration of these new BAC sequences with other International Barley Sequencing Consortium (IBSC) resources are in progress, with updates posted on the IBSC website (www.barley-genome.org).

Same, same but different: complementary analytical approaches highlight the different shades of polyploidy in rye and wheat.

Rachel Brenchley [1], Manuel Spannagl [2], Matthias Pfeifer [2], Gary L.A. Barker [3], Rosalinda D'Amore [1], Alexandra M. Allen [3], Neil McKenzie [4], Melissa Kramer [5], Dan Bolser [6], Suzanne Kay [1], Darren Waite [4], Yong Gu [7], Naxin Huo [7], Ming-Cheng Luo [8], Sunish Sehgal [9], Sharyar Kianian [10], Martin Trick [4], Ian Bancroft [4], Bikram S. Gill [9], Olin Anderson [7], Jan Dvorak [8], Paul Kersey [6], Richard McCombie [5], Anthony Hall [1], **Klaus F.X. Mayer** [2], Keith J. Edwards [3], Michael W. Bevan [4], Neil Hall [1], Mihaela Martis [2], Hana Simkova [11], Jan Vrana [11], Jaroslav Dolezel [11], Susanne König [12], Ruonan Zhou [12], Thomas Schmutzer [12], Uwe Scholz [12], Viktor Korzun [13], Nils Stein [12], Chris-Carolin Schön [14], Eva Bauer [14], and Grit Haseneyer [14].

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The large 8-Gb diploid genome of diploid rye and the 17-Gb genome of allohexaploid bread wheat are major challenges for genome analysis due to their size and complexity. While the wheat genome is composed of three closely related and independently maintained homoeologous genomes, the rye genome is diploid and has undergone a series of rearrangements in comparison to the wheat and the barley genome. We followed different but complementary strategies to work towards highly enriched molecular knowledgebases for both species.

For wheat we used a novel comparative genomics strategy combined with a whole-genome shotgun approach to identify 90 k of wheat genes and assigned a significant proportion to the component A, B, and D genomes. Our analysis reveals a highly dynamic genome, with rapid and extensive loss of gene family members upon polyploidization and an abundance of gene fragments.

For rye, a strategy that involves genome fractionation, development of a dense marker map and syntenic integration (aka GenomeZipper) was employed. The dense marker scaffold allowed to delineate syntenic segments and chromosomal breaks with high resolution and allowed to position 22 k rye genes on the genome. The integration allows to analyse and compare the rye genome with unprecedented depth and allows to generate fundamental insights into the evolution of the rye genome and the genomic consequences of an outbreeding lifestyle.

Grass microRNA gene paleohistory unveils new insights into gene dosage balance in subgenome partitioning after whole genome duplication.

Michael Abrouk [1], Rongzhi Zhang [2], Florent Murat [1], Aili Li [2], Caroline Pont [1], Long Mao [2], and Jérôme Salse [2].

[1] INRA/UBP UMR 1095 Génétique, Diversité et Ecophysiologie des Céréales (GDEC), Laboratory Paléogénomique des Plantes pour l'Amélioration Variétale (PPAV), 234 avenue du Brézet, 63100 Clermont Ferrand, France, and [2] Institute of Crop Science, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Road, Haidian District, Beijing 100081, China.

The recent availability of plant genome sequences, as well as high resolution gene-based genetic maps, have recently offered the opportunity to compare modern genomes and model their evolutionary history from reconstructed founder ancestors on a very precise scale. *In silico* paleogenomic analyses have revealed the evolutionary forces that have shaped present-day plant genomes. They showed that polyploidisation followed by genomic rearrangements such as nested chromosomes fusions (NCFs) that lead to chromosome number reduction, and the return at a diploid state have been driving forces of plant genome evolution.

Comparative genomic analyses combined with a robust scenario of the evolution of modern monocot and eudicot karyotypes from their diploid ancestors, offer an opportunity to gain insights into the evolution of specific sequences. In this work, we studied more particularly the paleohistory of microRNA (miRNA) in plants. The characterization and comparison of miRNAs sequences and associated protein-coding targets in plants allowed us to unravel (i) contrasted genome conservation patterns of miRNAs in monocots and eudicots after whole genome duplication (WGD), (ii) an ancestral miRNA founder pool in the monocot genomes dating back to 100 million years ago, (iii) miRNA subgenome dominance during post-WGD diploidization process with selective miRNA deletion complemented with possible transposable element (TE)-mediated return flows, and (iv) the miRNA/target interaction-directed differential loss/retention of miRNAs following the gene dosage balance rule. Finally, our data suggest that in grass genomes under-retained miRNAs are mainly involved in the basic regulation of plant development while over-retained miRNAs are likely involved in the regulation of genes associated with stress responses and plant adaptation.

Comparative genomic hybridization reveals structural diversity in barley.

Gary J. Muehlbauer [1,2], M. Munoz-Amatriain [1], T. Richmond [3], J. Jeddeloh [3], A. Landreman [3], B. Steuernagel [4], S. Taudien [4], M. Platzer [4], U. Scholz [4], M. Mascher [4], R. Ariyadasa [4], T. Nussbaumer [5], K. Mayer [5], S.R. Eichten [2], N.M. Springer [2] and N. Stein [5].

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Structural variation is characterized by chromosomal rearrangements (inversions, translocation), copy number variation (CNV) and presence/absence variation (PAV). CNV and PAV have been identified in numerous animal and plant species and there is a growing awareness of the potential of these variants to impact phenotypes. Comparative genomic hybridization (CGH) is a powerful approach to assess CNV and PAV. We developed a CGH array for barley composed of 2.1M probes from 211,669 target fragments (10 probes per fragment) capturing ~50 Mbp of gene space. Initially, we validated the array on a wheat–barley addition line carrying the long arm of barley chromosome 3H and showed that the technology was highly robust. Subsequently, we used the array to examine structural variation in eight cultivated barleys, six wild barley accessions, and the Oregon Wolfe barley mapping population parents. Our results demonstrated that between 2.4–3.5% and 4.4–4.5% of the fragments exhibited structural variation in cultivated and wild barley, respectively. These results demonstrate, as expected, that wild barley germplasm carries a higher level of structural variation than cultivated barley. In total, 15.6% of the fragments exhibited structural variation, indicating that a substantial proportion of the barley gene space has the potential to exhibit structural variation. Over 35% of the events were detected in only a single genotype, suggesting that many of these events may be rare. The distribution of structural variants was predominately located near the telomeres. Noteworthy, chromosome 4H exhibited a reduced amount of structural variation indicating that recombination is restricted on this chromosome. Our results provide the first genome-wide view of the structural variation in the barley genome across a range of genotypes. Annotation and further characterization of the structural variants is ongoing and will be presented.

kmer-based contamination screening in the wheat chromosome survey sequencing project.

Jonathan Wright and Ricardo Ramirez-Gonzalez on behalf of the IWGSC. The Genome Analysis Centre, Norwich Research Park, Norwich, UK.

The Genome Analysis Centre (TGAC) is coordinating the hexaploid wheat chromosome survey sequencing project on behalf of the International Wheat Genome Sequencing Consortium (IWGSC). This project aims to generate sequence reads from each of the 42 chromosome arms of hexaploid wheat and assemble these reads using the ABySS assembler. Advances in chromosome sorting in wheat have enabled this approach as a viable method to reduce the complexity of this large, highly repetitive genome. Chromosome sorting uses the size of each chromosome arm to separate it from the other arms and purities of around 90% can be achieved.

After assembling the reads from each arm, we aligned bin-mapped wheat ESTs to the assemblies to check for gross contamination and observed a general trend of background contamination from other chromosome arms. In some cases, this background contamination was higher than expected. In order to investigate this further, we developed a novel kmer-based analysis whereby each assembly was reduced to kmers (overlapping words of length k), then compared with all kmers from each of the other assemblies. We found that when a large kmer length was used, each chromosome arm could easily be distinguished from the other arms using this method. In order to reduce high background contamination, we repeated flow-sorting and sequencing for chromosome arms that were heavily contaminated and observed either the same pattern of contamination (indicating the contamination was due to an artifact of the flow-sorting process), or a different pattern of contamination (indicating random contamination specific to each sequencing run), or a combination of both. To develop a bioinformatics cleaning strategy for the contaminated assemblies, we extended the kmer analysis to the sequence reads in order to identify reads from different runs that contain shared kmers and, thus, reject reads that appear to be random contamination. These reads were used to generate 'clean' assemblies. We found this kmer-based analysis enabled us to improve the quality of the problematic assemblies.

Current status of physical mapping on wheat chromosome 6B.

F. Kobayashi [1], S. Katagiri [1], W. Karasawa [1], Y. Hanawa [1], S. Kaneko [2], S. Nasuda [2], K. Hayakawa [3], H. Fujisawa1, Y. Ito1, Y. Mukai [1], J. Dolezel [4], T. Matsumoto [1], J. Wu [1], and **Hirokazu Handa** [1].

[1] National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; [2] Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; [3] Nisshin Flour Milling Inc., Tsukuba, Ibaraki, Japan; and [4] Institute of Experimental Botany, Olomouc, Czech Republic.

For the purpose of better understanding the genome structure in wheat and accelerating the development of DNA markers for gene isolations and future breeding, the Japanese research consortium, as a member of IWGSC, is now conducting a project for the physical mapping and genomic sequencing of Chinese Spring chromosome 6B (914Mb). With a sequence-based finger printing method (Whole Genome Profiling; Amplicon Express, KeyGene Inc.), physical maps for the short and long arms of chromosome 6B have been successfully established using the 6BS- and 6BL-specific BAC libraries, respectively, which consist of 2,667 and 1,842 BAC contigs. And the estimated chromosomal coverage is more than 80% of both arms. Overlap analysis between the neighboring clones within the BAC contigs resulted in a total number of 5,079 and 4,889 MTP (Minimal Tiling Path) BACs on 6BS and 6BL, respectively. For the confirmation and the chromosomal assignment of the BAC contigs onto their corresponding genomic regions, we currently develop a large number of 6B-specific DNA markers using the public marker resources, available EST databases, and the 6B survey sequence. To date, among 3,743 markers tested for their availability, 2,480 markers were found to be useful for the PCR screening of BACs, and 451 BAC contigs has been anchored by 627 markers to the specific genomic regions on chromosome 6B presumed on the basis of genetic maps, deletion maps and/or Genome Zipper. In parallel, a high-resolution radiation hybrid map and a genetic map using recombinant inbred lines are under development for further improvement of physical maps. This work was supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics Agricultural Innovation; KGS-1001 and KGS-1003) and funding from Nisshin Flour Milling Inc.

SESSION II: HISTORICAL PERSPECTIVES

ITMI: The early years and impacts on Triticeae research.

C.O. Qualset and P.E. McGuire. Department of Plant Sciences, University of California, Davis, CA 95616, USA.

The International Triticeae Mapping Initiative was launched at a meeting in Davis, California, in 1989. Motivation for a collaborative effort was twofold: find research funds and foster progress through collaboration. The informal nature of ITMI was meant to encourage independent research among the primary participating laboratories and at the same time encourage collaboration so that good maps would be obtained rapidly and materials and resources could be shared. The bigger challenge was funds for research. Several companies provided some support funds, but really only to conduct workshops and coordination meetings. CIMMYT provided support for producing the first public RFLP map in wheat and both CIMMYT and ICARDA provided funds in support of ITMI management. Barley workers in the U.S. were able to secure Cooperative Agreement funds from the USDA. A DOE/NSF/USDA Joint Program provided a grant that greatly assisted in conducting workshops and short courses and preparing research grant proposals. Several national ITMI groups were formed and a roster of more than 130 Affiliate Scientists was amassed. The ITMI was coordinated in the office of the California Genetic Resources Conservation Program at UC Davis for 12 years, 1989-2000; thereafter the coordination has been rotated to other countries. Coordinators of chromosome groups or species were appointed to collect data and monitor progress. The first successful grant for the U.S. ITMI scientists was obtained from the USDA for detailed RFLP mapping of four chromosomes of wheat. Oddly, when this project was successfully completed, the grant application for mapping the three remaining chromosomes was not funded. Later, NSF supported a large grant for producing and mapping ESTs. This was a very successful activity with about 100,000 ESTs produced and about 8,000 mapped, producing about 18,000 loci mapped to deletion bins in hexaploid wheat. Next efforts have emphasized sequencing and ITMI has retained its momentum in coordinating workshops almost every year till now with this 22nd workshop. Maps were produced, genetic stocks developed, and dozens of scientific papers have been produced by ITMI collaborating scientists. ITMI still remains a legacy of Ernie Sears, whose nulli-tetra and other aneuploid stocks facilitated much of the mapping research.

An Australian perspective on the early days of ITMI.

Rudi Appels. Centre for Comparative Genomics, Murdoch University, Perth WA 6150, Auatralia.

In the late 1970s and 1980s the cloning and sequencing of DNA segments of the wheat and rye genomes was initiated in several countries around the world. This activity focused on repetitive DNA sequences because they were more accessible for establishing the technologies required for using these sequences as molecular markers in genetic mapping and characterizing breeding lines. Early studies in Australia showed the value of the repetitive DNA sequences for characterizing chromosome arms through visualizing their distribution by *in situ* hybridization to root-tip squashes (Appels et al 1978). In view of the geographic location of Australia and the fact that it was only in the mid 1980s that FAX and email facilities became available, the discussions to start a loosely structured group of collaborators under the International Triticeae Mapping Initiative was a most welcome development. The Grains Research Development Corporation (GRDC) funded the formation of the Australian Triticeae Mapping Initiative in 1990 to complement ITMI and provide a focus for understanding the use and value of molecular markers in breeding and prebreeding. The ITMI network was the source of molecular markers to argue the case for major national molecular marker programs in wheat and barley that ran for ten years and formed the foundation (Marshall et al. 2001; Langridge and Barr 2003) of the prebreeding laboratories around Australia that are still active to this day. The genome sequencing and sequence-based genetic maps activities in wheat and barley trace their inspiration to the ITMI cooperative interactions.

Appels R, Driscoll C, and Peacock WJ. 1978. Heterochromatin and highly repeated DNA sequences in rye (*Secale cereale*). Chromosoma (Berl) 70:67-89.

Langridge P and Barr AR. 2003. Better barley faster: the role of marker assisted selection. Austr J Agric Res 54:1065-1408 (31 papers).

Marshall DR, Langridge P, and Appels R. 2001. Wheat breeding in the new century. Austr J Agric Res 52:1043-1423 (33 papers).

SESSION III: UTILIZING TRITICEAE RESOURCES AND DIVERSITY

DNA marker-assisted chromosome engineering for efficient alien gene introgression of stem rust resistance in wheat.

Steven S. Xu [1], Zhixia Niu [1], Daryl L. Klindworth [1], Qijun Zhang [2], Shiaoman Chao [1], Timothy L. Friesen [1], Justin D. Faris [1], Yue Jin [3], and Xiwen Cai [2].

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Cultivated wheat has a large number of related species that represent a valuable gene pool for wheat improvement. In the past 50 years, a number of effective stem rust resistance (*Sr*) genes identified from wild relatives of wheat have been transferred into wheat through genetic manipulation in the form of chromosome translocations and additions. In an effort to enhance the utility of the alien-derived *Sr* genes that are effective against Ug99 stem rust races, we developed an optimal scheme of chromosome engineering for efficient elimination of large amounts of alien chromatin surrounding the *Sr* genes. In this procedure, we first developed a large backcross population of homoeologous recombinants using the *ph1b* mutant in hexaploid wheat or *Ph1*-deficient aneuploid in tetraploid wheat. We then identified the recombinants carrying the gene of interest on small interstitial segments using robust DNA markers and high-throughput phenotyping and genotyping. By using this procedure, we developed wheat germplasm carrying four Ug99-resistant genes (*Sr37*, *Sr39*, *Sr43*, and *Sr47*) on minimal alien chromatin in a short period of time. We are currently applying this procedure to transfer more genes for resistance to stem rust and leaf rust from wild relative species into the wheat genome. This study demonstrated that integration of modern genomic and marker technology with classical chromosome engineering and breeding greatly improved the efficiency of transferring resistance genes from wild relatives into modern crops.

Barley germplasm and malting barley breeding.

Takashi Iimure [1], Takehiro Hoki [1] , Naohiko Hirota [1], Wataru Saito [1], Makoto Kihara [1], Kiyoshi Takoi [2], Tomokazu Takaoka [3], Shigeki Araki [4], Masahide Sato [4], Shinji Yamada [1], Brian G Rossnagel [5], and Kazuhiro Sato [6].

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In malting barley breeding, overall improvement of agronomic, malting and brewing traits is necessary and effective use of germplasm can greatly accelerate progress. We present two recent research works on beer foam and flavor stability using barley germplasm of Okayama University. Several proteins and enzymes have been identified as factors controlling foam and flavor stability. Although barley protein Z4 (Z4) and protein Z7 (Z7) have been suggested as factors responsible for foam stability, their effect is still under debate. To evaluate the effects of Z4 and Z7 on foam stability, we brewed beer samples from deficient mutants on each of these proteins. The cultivar Pirrka was used as a source of Z4 deficient, while a Z7 deficient mutant was unknown and screened from the Okayama University barley germplasm collection. The results of brewing trial suggested that the contribution of Z4 and Z7 on foam stability was not greater than that of other beer proteins. Secondly, we have developed lipoxigenase-1 (LOX-1) null barley to improve beer flavor stability. LOX-1 is a key enzyme for flavor stability. We screened LOX-1 deficiency in the Okayama University barley germplasm collection and found six LOX-1 null mutants. One of these was used in breeding by marker assisted backcrossing a recurrent parent CDC Kendall. We developed LOX-1 null variety, CDC PolarStar and brewing trial using CDC PolarStar and CDC Kendall clearly showed that the flavor stability of CDC PolarStar beer was superior.

Comparative sequence analysis of chromosomes isolated from two wild relatives of wheat (Aegilops umbellulata and Ae. biuncialis).

Mihaela Martis [1], István Molnár [2], Jan Vrána [3], Marie Kubaláková [3], **Hana Šimková** [3], Federica Cattonaro [4], Márta Molnár-Láng [2], Klaus Mayer [1], and Jaroslav Doležel [3].

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Wild species of Triticeae were not exposed to domestication, exhibit large genetic and phenotypic diversity and provide a rich source of novel genes and alleles for breeding improved varieties of wheat. However, effective transfer of alien genes and gene variants either via interspecific hybridization or transgene technology from wild species requires solid knowledge of their genomes. Chromosome genomics proved to be elegant method to analyze the complex genomes of Triticeae. This approach simplifies the analysis by dissecting genomes to small parts by flow cytometric sorting of mitotic chromosomes. In wheat, chromosome genomics has been used to develop markers from sub-genomic regions, identify genes and establish virtual gene order for the complete set of chromosome arms, as well as to construct physical maps of chromosome arms to facilitate map-based cloning and production of a reference genome sequence. Here we report on developing chromosome genomics in *Aegilops* spp. We have isolated by flow cytometric sorting chromosomes 1U, 3U, and 6U from diploid *Ae. umbellulata* and homologous chromosome $1U^b$ from allotetraploid *Ae. biuncialis*. Chromosomal DNA was amplified and shotgun sequenced to a high coverage by Illumina technology; low-copy, and genic regions were assembled and used to generate annotated syntenic builds. This advance provided first large-scale insights into the sequence composition of the *Aegilops* genomes. A virtual gene order was constructed for the four chromosomes using the GenomeZipper approach. In total, 11,513 non-redundant gene loci were ordered: 2,666 loci on 1U, 3,155 loci on 3U, 3,212 loci on 6U, and 2,480 loci on $1U^b$. Moreover, syntenic relationship established between the four *Aegilops* chromosomes and genomes of *Brachypodium*, rice, sorghum, barley, and wheat, enabled discovery of multiple chromosomal rearrangements in *Ae. umbellulata* and *Ae. biuncialis*, providing a valuable reference to study genome evolution in *Aegilops*.

Nonparametric tests reveal multiple selection events in the wheat genome.

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Wheat has been subjected to strong human-driven selection aimed at the development of cultivars adapted to local environments. Preferential selection of advantageous alleles changes the patterns of genetic variation and linkage disequilibria around selected loci. To detect such signatures of selection in the wheat genome, we used two nonparametric tests: 1) based on the patterns of genetic differentiation among wheat populations of different geographic origin and 2) based on the extent of haplotype sharing among accessions in wheat populations. A worldwide sample of wheat cultivars and landraces including 2,924 accessions was genotyped using the Illumina 9K iSelect assay. A total of 6,305 high-quality SNP calls were included into the analysis. An integrated genetic map was built using a combination of six bi-parental mapping populations and one MAGIC population. Using the distribution of F(st) statistics across a sliding window throughout the wheat genome we detected in total 40 selective sweeps events in the populations of spring and winter wheat. Haplotype sharing statistics identified more than 100 genomic regions that showed unusually long identical haplotypes. Some of these genomic regions showed strong differentiation in haplotype frequency among the populations of different geographic origin. An association of selected regions with genes, which are targeted in breeding programs, was used to validate our results. Here we demonstrate that by using the methods of population genetics it is possible to identify genomic regions that have been subjected to selection, providing valuable information for detailed analysis of marker-phenotype associations.

A genome-wide survey of leaf stripe resistance in a low-structured barley association panel.

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In barley (*Hordeum vulgare*), the application of marker platforms that provide dense genome-wide coverage of molecular polymorphism allows to elucidate the evolutionary history of natural populations and use their biodiversity to dissect traits of agronomic interest, through genome-wide association scan (GWAS) approaches. Here we describe the evaluation of a low-structured collection of ~210 spring, 2-rowed, European barley cultivars for their resistance to leaf stripe, a seed-borne disease caused by the fungal pathogen *Pyrenophora graminea*. For each line, 60 seeds were surface-sterilized and incubated in Petri dishes between two Potato Dextrose Agar layers colonized by an actively growing mycelium of the *P. graminea* isolate Dg5. After 20 days of incubation in the dark at 6°C, the emerged seedlings were transplanted to pots and grown in the greenhouse (20°C, 14 h light and 12°C, 10 h night). Resistance has been assessed as the percentage of plants showing leaf stripes symptoms, and the whole experiment has been repeated during three consecutive years. The same barley collection has been genotyped with a novel set of 7,864 gene-based SNPs incorporated into a single Illumina Infinium™ iSelect assay, in order to investigate: i) trends in the patterning of genetic diversity in European spring

barley cultivars in time and space; and ii) the utility of a low-structured population for discovering significant associations between genetically mapped markers and important agronomic traits. SNP markers mapping on the short arm of barley chromosome 6H (8.6–13.6 cM) showed a significant association with leaf stripe resistance. This genomic region is syntenic with a ~240 Kb of rice chromosome 2, where 42 genes were annotated, that could serve for the identification of candidate genes involved in barley resistance to pathogens or for the development of new SNP markers, in order to increase the resolution of the GWAS.

Haplotype diversity and evolutionary history of the Lr34 locus of a world wheat germplasm collection.

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The resistance gene *Lr34* has been a key gene in the genetic management of wheat leaf rust worldwide. However a little is known about the geo-genetic diversity, the history, and the origin of this unique gene. This study was conducted to provide a comprehensive analysis of the genetic diversity at the *Lr34* locus of a world wheat germplasm collection employing ten molecular markers located within the coding sequence of *Lr34* or closely linked to it. A total of 52 alleles were detected for the ten markers. Marker gwm1220 was the most polymorphic with 21 alleles, and the highest PIC value of 0.91. Marker caSNP12 was under positive selection while markers gwm1220 and cam11 were under balancing selection. On the basis of the *Lr34*-specific markers, the world collection was divided into five major haplotypes (H) of which H1 was consistently associated with the resistance phenotype (*Lr34*+). Combined analysis of the ten molecular markers resulted in dividing the major haplotypes into 118 different sub-haplotypes. Structure and clustering analyses grouped these sub-haplotypes into two main clusters and seven sub-clusters. Variance among main clusters represented the largest proportion of the total variation. H4, an *Lr34* haplotype, was hypothesized to be the most ancient haplotype and H1 the most recent, as it likely arose after the advent of hexaploid wheat. Analysis of geographical distribution showed that H1 was more frequent in Asian germplasm, although H4 predominated in European germplasm. *Lr34*, a gain of function mutation, is hypothesized to have originated in Asia.

SESSION IV: GENETICS AND GENOMICS OF IMPORTANT TRAITS

An analysis of variation in the saccharification potential of barley straw.

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Many people are concerned that using crops as renewable sources of energy/fuel could compete with food production, reducing supply and increasing prices. We have been exploring the potential of using agricultural wastes (straw, grain husks) as feedstocks for energy, fuel, and chemical generation. We are particularly interested in evaluating whether the feedstock can be modified to improve the ease with which fermentable sugars can be extracted, both by breeding through exploiting available natural genetic diversity and/or by transgenesis. Using all parts of a crop will improve the economics and incentive for food production, as well as reducing the overall carbon footprint of the industry. For example, in the United Kingdom, almost one-fifth of the greenhouse gases come from the food chain, with farming accounting for the largest share. Producing more food chain co-products, such as biofuels, has the potential to significantly reduce the carbon balance for each product.

The overall aim of the work I will present is primarily to evaluate the possibility of improving barley straw for next generation biofuel production, thereby producing an optimized raw material for industrial biotechnology while securing sustained grain production. Along with colleagues in the Universities of Dundee (Claire Halpin) and York (Simon McQueen-Mason), we have explored the power of combining GWAS with high-throughput biochemical (functional) phenotyping to identify genes involved in determining straw saccharification potential. We have identified and characterized barley lignin pathway genes and pathway regulators, and used transgenic biology both as a validation tool for the outcomes of GWAS and directly to test of the effect of drastically altering key lignin genes on plant growth and development, and interactions with the environment.

Mother of FT and TFL1 represses wheat germination and has potential breeding applications to improve seed dormancy.

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Pre-harvest sprouting is a major problem for stable production of wheat in Japan, where the rainy season coincides with the harvest season. To solve this problem, we need to develop wheat cultivars with strong seed dormancy; identifying genes that regulate the level of dormancy will inform breeding strategies for this purpose.

To identify regulators of dormancy, we focused on the wheat seed dormancy response to temperature during seed development, in which cooler temperatures increase seed dormancy. Since temperature-dependent transcriptional regulation may control this response, we analyzed this phenomenon using a transcriptomic approach. We found that *Mother of FT and TFL1* (*MFT*) was expressed at much higher levels in embryos of dormant seeds grown at a cooler temperature. *MFT* belongs to the phosphatidyl ethanolamine-binding protein family, which also includes the flowering inducer *Flowering locus T* (*FT*) and the flowering repressor *Terminal Flower 1* (*TFL1*). In this presentation, we report our analysis of *MFT* expression, mapping of the *MFT* locus, and transformation and transient assay analysis of *MFT* function. Our results suggest that *MFT* represses germination. Moreover, *MFT* on chromosome 3A is a promising candidate for a seed dormancy QTL, and a single nucleotide polymorphism in the *MFT* promoter seems to be the causal polymorphism for the QTL. We have developed a DNA marker for this SNP to select the allele that produces strong seed dormancy. Genotyping of various wheat cultivars suggests that breeders can use this marker to improve pre-harvest sprouting tolerance in wheat.

Barley's triple spikelet meristem is controlled by Vrs4 (six-rowed spike 4).

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The appearance of the triple spikelet meristem within the genus *Hordeum* is one of the important developmental and genus-specific features. Classically, barley (*Hordeum vulgare* L.) is subdivided into two- and six-rowed barleys depending upon lateral spikelet fertility. Until today, five different loci have been identified, which can convert two-rowed barley to six-rowed barley; they include *vrs1* (2HL), *vrs2* (5HL), *vrs3* (1HL), *vrs4* (3HS), and *int-c* (4HS). Komatsuda et al. identified the *Vrs1* gene as being an HD ZIP transcription factor and, recently, Ramsay et al. found *int-c* as the barley orthologue of maize *Teosinte Branched*. Among other vrs loci (*vrs2*, *vrs3*, and *vrs4*), *vrs4* is known to produce a prominent six-rowed phenotype with many fully fertile, long awned lateral spikelets. In the present study, we mapped the *vrs4* locus in two bi-parental mapping populations, (Barke × *vrs4.k* and *vrs4.k* × Golden Promise-96 individuals in each) using SNP-based CAPS markers and VeraCode technology. *vrs4* showed linkage to markers derived from chromosome 3HS. The corresponding marker-phenotype interval comprised 27 genes in *Brachypodium*, annotations of which revealed an important transcription factor involved in inflorescence development. Resequencing of the transcription factor in *vrs4.k* and its wild type MFB 104 showed a unique deletion in the *vrs4.k* mutant, resulting in a truncated protein product. Hence, we resequenced the gene in 18 *vrs4* mutant alleles available from NordGen, Sweden and USDA, USA; most of them showed nucleotide changes in the coding region, but also in upstream or downstream regions of the gene. High-resolution mapping in around 2,000 gametes and BAC library screening have established a 274-Kb physical contig containing a single gene (*vrs4* locus). Tissue localization of the vrs4 gene expression through *in situ* hybridizations, its genetic networks by microarray analysis, and a working model for the six-rowed spike pathway, involving *vrs4*, shall be presented at the meeting.

Komatsuda T, Pourkheirandish M, He C, Azhaguvel P, Kanamori H, et al. 2007. Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc Natl Acad Sci USA 104:1424-1429.

Ramsay L, Comadran J, Druka A, Marshall DF, Thomas WTB, et al. 2011. *INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1*. Nature Genet 43:169-172.

Towards cloning the powdery mildew resistance gene QPm.tut-4A introgressed to bread wheat from T. militinae.

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Introgression of agronomically important genes from wild relatives is one of the most effective means to improve wheat gene pool. However, introgressions often introduce collinearity and recombination aberrations. Recently, resistance to powdery mildew from the tetraploid wheat *Triticum militinae* was introgressed to hexaploid wheat cultivar Tähti. The locus with the major contribution in both seedling and adult stage was mapped on distal end of 4AL chromosome arm to the region delimited by wmc232 and wmc313 markers (~10 cM) and denominated as *QPm-tut-4A*. In the original mapping population from a cross of Tähti by resistant introgressive line 8.1, the region includes 12 markers. However, their order could not be resolved using 1,200 haplotypes. To resolve this obstruction, a combination of traditional approaches and recent advances in wheat genomics were used. For marker ordering, a 4AL-specific radiation hybrid panel and three additional recombination based mapping populations were employed. For efficient marker development, 4AL chromosome-specific BAC library was constructed, fingerprinted, and ordered into contigs. The region was anchored to the rice, *Brachypodium*, and *Sorghum* chromosome 6, 1, and 10, respectively. All genes from the collinear regions were mapped to our mapping populations. Marker development was enhanced using MDA amplified DNA from the 4AL chromosome arm of Chinese Spring, and the same arm carrying the translocation in combination with survey sequences of chromosomes 7A and 7D. Using these resources, the *QPm-tut-4A* gene was delimited in 0.2 cM region flanked with the gpw356 and gpw3079 markers. The remaining three SSR markers (barc70, gwm832, and gpw3556) completely linked to the *QPm-tut-4A* locus were used to identify three BAC contigs that comprise 158 BAC clones and cover about a 2 Mb region. From the contigs, 114 BAC ends were sequenced and used for marker development. The physical map, radiation hybrid lines, markers, and the remaining BAC-end sequences will be used to saturate the *QPm-tut-4A* map and, finally, clone the gene. This work has been supported by the MEYS of Czech Republic (Operational Programme Research and Development for Innovations No. ED0007/01/01), Internal Grant Agency PrF-2012-001, and by Estonian Ministry of Agriculture.

Cleistogamy in the Triticeae: genetic variation and its regulation.

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Cereals are requested for human energy and protein needs. Efforts in particular brought attention to concerns of seed-borne disease contamination as well as enhance seed production. In the typical grass flower, the florets gape as a result of the swelling of the lodicules (non-cleistogamous), but non-gaping (cleistogamous) variants are known in many species. The possibility of manipulating flower type is of particular relevance for the prevention of pollen flow from transgenic types and for the control of Fusarium head blight, a disease to which non-cleistogamous barley and wheat cultivars are especially vulnerable. In barley, the development of lodicule is genetically determined by allelic variation at the *cly1* (syn. *HvAP2*) locus. In the non-cleistogamous type flower (*Cly1.a*), *HvAP2* activity is suppressed by miR172-directed cleavage. A single nucleotide substitution at the microRNA target site generates the recessive allele *cly1.b*, in which no cleavage occurs, converting the flower into a cleistogamous type. *HvAP2* homologues among various diploid and polyploid wheats are regulated in the same fashion as in barley; in non-cleistogamous wheats, miR172-directed cleavage can be detected at each AP2 homoeologue. As the cleistogamous type is produced by a recessive *AP2* allele, to convert hexaploid wheat into a cleistogamous type, we would need to identify the recessive allele at each of the A, B, and D homoeoloci and then combine them into a single individual.

Variations in patterns of gene expression are central to evolution. A large-scale screen of barley germplasm has identified an alternative de-repressor of lodicule development SV235. The relevant *HvAP2* coding sequence is identical to that of *cly1.b*, so that *HvAP2* down-regulation is not associated with miR172-directed degradation; instead, transcriptional repression appears to be induced by the maintenance of the *HvAP2* promoter in a hyper-methylated state during the period when the vascular tissue in the lodicule would normally develop. The cleistogamous type can also arise in the presence of non-cleistogamous type lodicule development. In certain *Cly1.a* x *cly1.b* hybrids, the spikes remain within the boot during anthesis, and the lodicules remain shrunken even after the emergence of the spike from the boot.

Genetics and molecular evolution of a 3.1-Mb genomic region harboring both wheat prolamin and disease resistance gene families.

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Several important wheat prolamin and disease resistance gene loci have been mapped at similar locations in the short arm of the group-1 chromosomes in wheat. However, genetics and structural organization of genomic regions harboring these important traits have not been well characterized. We therefore sequenced a 3.1-Mb region harboring prolamin multigene families in the genome of *Ae. tauschii*, the D-genome donor of hexaploid bread wheat. The sequence revealed a much higher gene density (98 genes; one gene/31 kb) in the sequenced region than the average value (ca. one gene/112 kb) expected for the wheat D genome. The high gene density is primarily due to the large number of duplicated genes, present either in tandem or interspersed with other genes. In addition to different types of prolamin gene families (γ-gliadin, ω-gliadin, and LMW-glutenin), multiple NBS-LRR disease resistance gene homologues of *Lr21* (resistance to leaf rust) and *Pm3* (resistance to powdery mildew) were identified. Furthermore, leucine-rich receptor protein kinase (LRK) genes, representing a different class of resistance genes, also were highly duplicated in this region. Comparative analyses indicated that the orthologous regions in the rice, *Brachypodium*, and sorghum genomes are highly conserved, whereas in *Ae. tauschii*, only 16 out of 98 genes are syntenic. Most of these highly duplicated genes are unique in the *Ae. tauschii* genome, suggesting rapid evolution in this region. Genetic analysis revealed the sequenced region spanned over 10-cM genetic distance with 13 markers mapped to this region. Recombination rates ranged from ca. 200 to 1,000 kb/cM between two marker intervals. Both co-evolution and independent evolution in different gene families were observed. Mechanisms underlying the molecular evolution of prolamin and resistance gene families in this complex region will be presented.

SESSION V: YOUNG TRITICEAE RESEARCHERS

Genetic provenance and genetic providence in a diverse crop.

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Multiple factors converge to contribute to high levels of genetic and phenotypic diversity in barley. The progenitor species is ecotopically and genetically diverse, with a large geographic range and large effective population size. Cultivated barley arose from multiple source populations and became adapted to diverse agronomic conditions across much of Eurasia and North Africa over several millennia of human prehistory. Exploiting this diverse adaptive history for barley improvement is challenging if methods are limited to top-down approaches based on genotype-phenotype associations. A new generation of bottom-up approaches provides the opportunity to investigate the genetic differentiation and genetic provenance of adaptive allelic variation and associations between SNPs and ecogeographic variables. Both sets of approaches provide new opportunities for associating genetic variation with phenotypic traits of agronomic importance, including disease resistance and climate adaptation. We make use of genotyping data from 2,417 worldwide barley accessions from the USDA National Small Grains Collection (landraces and cultivars) genotyped with 7,800 SNPs.

We seek to identify the geographic regions that best explain the diversity in domesticated barley and associate genes or genomic regions with diverse environmental conditions.

Molecular tools and genetic resources for eyespot resistance in wheat.

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Eyespot is an important stem base disease of wheat caused by two species of fungi, *Oculimacula yallundae* and *Oculimacula acuformis*, both of which are widespread in northern Europe and the Pacific northwest USA. There are two main sources of resistance available; *Pch1*, a potent resistance gene introduced from *Aegilops ventricosa*, and the moderate resistance of the variety Cappelle Desprez.

Pch1 has not been used widely in European wheat varieties due to linkage between *Pch1* and yield-limiting genes. Breaking this linkage has proven difficult because of a low recombination frequency and a lack of molecular markers that function in both wheat and *Ae. ventricosa*. Utilising the *Brachypodium* genome sequence, we developed co-dominant Conserved Orthologous Sequence (COS) markers targeted to the *Pch1* region. These markers were used to identify recombinants and to locate *Pch1* on chromosome 7DL. By exploiting co-linearity between *Brachypodium*, rice and sorghum, we identified a candidate gene region as a prelude to the map-based cloning of the gene.

Resistance in Cappelle Desprez has been attributed to the partial resistance gene *Pch2*. We mapped *Pch2* resistance to *O. acuformis* as a QTL on the distal end of chromosome 7AL. However, it was not possible identify any resistance to *O. yallundae*. This indicates that *Pch2* operates specifically against *O. acuformis*, and that *Pch2* should not be relied upon as a stand-alone resistance in wheat varieties.

There is previous evidence of an adult-plant resistance on chromosome 5A of Cappelle Desprez. We showed this resistance to also be effective in seedlings and to provide protection against both pathogen species, indicating that it will be useful in commercial varieties. Two chromosome 5A recombinant populations were tested in seedling bioassays and field trials to locate a stable resistance QTL on 5AL and identify linked SSR markers for selection of the resistance.

In addition to characterising existing eyespot resistances, we screened 1,036 hexaploid genotypes from the Watkin's Worldwide Collection for resistance to *O. yallundae*. Some accessions exhibited a high level of resistance. Selected lines will be screened for resistance to *O. acuformis* and the genetic basis of any novel resistances will be determined.

Wheat Zapper, Illumina BSA, and radiation hybrids for synteny analysis of the scs region.

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Species cytoplasm specific (*scs*) genes are of great evolutionary importance because they guide the precise interaction between nucleus and cytoplasm. Recently, an *scs* gene of wheat (*Triticum* species) was localized to the pericentromere of chromosome 1DL. In an attempt to clone this gene, we generated a radiation hybrid (RH) *in vivo* population of ~1,500 individuals. EST-based markers were developed and used for genotyping the whole 1D chromosome. An online tool was developed to easily and rapidly study the colinearity between wheat and three sequenced plant species (*Sorghum bicolor*, *Brachypodium distachyon*, and *Oryza sativa*), namely the Wheat Zapper (http://wge.ndsu.nodak.edu/wheatzapper/). Employing this tool, together with functional analysis of the RH lines, we determined that the *scs* region corresponds to a region of paleofusion between two ancestral chromosomes corresponding to the modern Sb9-1, Bd3-2, or Os10-5. To

further investigate the events that lead to this fusion, we coupled a protocol for genome complexity reduction with the Illumina platform, to sequence at 40X coverage a bulk of seven RH lines carrying or missing the *scs* gene. The sequences identified in the first (positive) bulk but not in the second (negative), were considered to represent the *scs* region. These 3.2 K differential pair-ends sequences were then extended to 1–5 Kb in size, employing the publicly available 50X survey sequences of *Aegilops tauschii*. Furthermotr, these extended sequences were assembled with DNAStar into 556 contigs spanning 1.2 Mb with an N50 of 3Kb. This gapped sequence was then anchored to the scaffold RH map, pin-pointing the *scs* locus to an interval of just eight genes. The result of coupling Illumina sequencing, Wheat Zapper synteny analysis, and RH populations is discussed here in relation to the evolutionary importance of the *scs* region and its map based cloning.

Identification of a candidate barley stem rust susceptibility gene determining the recessive nature of Rpg4-mediated Ug99 resistance in barley.

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The *rpg4*/*Rpg5* locus in barley (*Hordeum vulgare*) provides recessive resistance against several wheat stem rust races (*Puccinia graminis* f. sp. *tritici*) including QCCJ and the highly virulent race TTKSK (aka Ug99). Three genes required for wheat stem rust resistance (*HvADF3*, *Rpg5*, and *HvRGA1*) were identified in the ~70kbp *rpg4*/*Rpg5* stem rust resistance locus using high-resolution mapping and virus induced gene silencing. The dominant rye stem rust resistance gene *Rpg5* is predicted to have the typical R-gene domains including the nucleotide-binding site (NBS), leucine rich repeat (LRR), and serine/ threonine protein kinase (STPK) domains. The *Rpg5* gene appears to condition compatible or incompatible interactions with the wheat stem rust races QCCJ and Ug99, because it is the only polymorphic gene correlating with resistance and susceptibility in the delimited *rpg4*/*Rpg5* region. Sequence analysis of *rpg5* susceptible alleles showed that they make up two groups. The group-1 susceptible lines contain an insertion/deletion region having a predicted functional protein phosphatase 2C gene (*HvPP2C*) in place of the *Rpg5* STPK domain (Harrington, Steptoe, and Sm89010). The group-2 susceptible lines have an intact STPK domain but a predicted nonfunctional *rpg5* allele due to a single cytosine insertion causing a frame shift mutation resulting in a premature stop codon (Golden Promise, OSU6, and MD2). Analysis of F_2 progeny from crosses between Q21861 and group-1 and -2 susceptible lines segregated in 1:3 ratio (resistant:susceptible) when the *HvPP2C* gene is present but in a 3:1 ratio (resistant:susceptible) when the *HvPP2C* gene is absent. Thus, it appears that the previously identified *Rpg5* dominant rye stem rust resistance gene also imparts *rpg4*-mediated wheat stem rust resistance and behaves as a dominant gene in the absence of the *HvPP2C* gene but as a recessive resistance gene in the presence of *HvPP2C*. The data suggests that components of *rpg4* and *Rpg5* resistance are not distinct and the difference in the dominant or recessive nature of resistance is due to the *HvPP2C* gene acting as a dominant susceptibility factor that suppresses *rpg4*/*Rpg5*-mediated resistance against the wheat stem rust races including Ug99.

Targeted re-sequencing of the wheat exome and the generation of public co-dominant single nucleotide polymorphism markers.

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The complex nature of the wheat genome has, until recently, resulted in a lack of single nucleotide polymorphism (SNP)-based molecular markers of practical use to wheat breeders. Recently, large numbers of SNP-based wheat markers have been made available via the use of next generation sequencing combined with a variety of genotyping platforms. However, many of these markers and platforms have difficulty distinguishing between heterozygote and homozygote indi-

viduals and are therefore of limited use to wheat breeders carrying our commercial scale breeding programs. To identify co-dominant SNP-based markers, which are capable of distinguishing between heterozygotes and homozygotes, we have used targeted re-sequencing of the wheat exome to generate large amounts of genic sequences from eight varieties. Using a bioinformatics approach, these sequences have been used to identify 95,266 putative, gene-based, single nucleotide polymorphisms, of which 10,251 were classified as being suitable markers for the discrimination of homozygote and heterozygote individuals. Validation of a sample of these markers confirmed that 81% could easily discriminate between heterozygous and homozygous individuals. Comparison of these co-dominant markers with dominant markers indicated that both marker types were distributed similarly across genetic maps. In addition, the use of both marker types across two U.K. mapping populations revealed that the two populations had different levels of polymorphism across the A, B, and D genomes. The new co-dominant markers described here are capable of complete genotypic classification of a segregating locus in polyploid wheat and can be used on a variety of genotyping platforms; as such they represent a powerful tool for wheat breeders. The markers and related information described here have been made publically available on an interactive web-based database in order to facilitate their use in genotyping programs worldwide.

Molecular adaptation to cooler climates and ecological diversification of Pooideae.

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Adaptation to temperate environments is a common feature in the grass subfamily Pooideae, suggesting an ancestral origin of low temperature stress tolerance dating back to the beginning of Pooideae taxonomic divergence. It also has been suggested that climate cooling during the Eocene-Oligocene transition (~34MYA) was important for cold climate adaptation in the core Pooideae clade, in which the Triticeae species is contained. Here we analyze the molecular evolution of genes involved in low-temperature stress response and present evidence for the importance these genes in the evolution of the Pooideae lineage.

Maximum likelihood-based phylogenetic methods were used to estimate substitution rates in Pooideae species relative to rice and test the hypothesis that cold induced loci were under positive selection during radiation of the Pooideae lineage. In addition we carried out in depth studies of the evolution of three Pooideae-specific gene families, *CBF*, *FST*, and *IRIP* genes, known to be central in core Pooideae low temperature stress responses.

Phylogenies of 4330 orthologous loci were produced, of which 388 loci were defined as low-temperature induced in Pooideae. A general increase in substitution rates was observed for all genes in the Pooideae lineage relative to rice, and this rate increase was higher for nonsynonymous substitutions (+7–20%) compared to synonymous substitutions (+0–7%). However, the nonsynonymous substitution rate increase in Pooideae was significantly higher in those loci defined as low-temperature induced. Tests for positive selection on the basal Pooideae branch showed a 3.3-fold increase in significant tests for low temperature induced loci compared to all loci. Analyses of Pooideae-specific gene families involved in low temperature stress responses identified both ancient evolutionary innovations (basal Pooideae), as well as more recent innovations in carbohydrate metabolism specific for core Pooideae.

The Pooideae lineage evolved from a tropical/subtropical ancestor to become a taxonomic group with ecological dominance in temperate ecosystems. Our results suggest that adaptive evolution of low temperature responses was of importance in the basal Pooideae, possibly enabling the ecological radiation into cooler ecosystems.

SESSION VI: PHYSICAL MAPPING AND MAP-BASED CLONING — YOUNG RESEARCHERS

Physical mapping of the wheat and Triticeae genomes using single gene FISH.

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The cytogenetic structure of wheat, *Triticum aestivum* (2n=6x=42, AABBDD), was analyzed intensively during the last century. Chromosomes were identified based on meiotic pairing affinities during the 1920s, by aneuploidy during the 1950s, banding techniques in the 1970s, and *in situ* hybridization with repeated DNA probes during the 1980s, which also allowed the mapping of genes to chromosomes and chromosomal arms. The chromosome bin physical maps of expressed sequence tags (ESTs) were developed in the 2000s using deletion stocks. Because of the large sizes of deletion bins, loci within the bins cannot be ordered. Moreover, most of these resources do not exist in wild species, which hinders their exploitation in crop improvement. Fluorescent *in situ* hybridization (FISH) allows mapping of particular sequences to specific chromosomal regions including those with low recombination rates as well as studying chromosomal rearrangements. FISH with wheat tandem repeats can be used for chromosome identification, but the distribution of repetitive elements varies among homoeologous chromosomes within a species or between species. Single-gene FISH can be a useful and tool for genome physical mapping and studying chromosome rearrangements in wheat and its relatives. The genic regions are highly conserved and homoeologous genomes of Triticeae are largely collinear. In our study, to develop a single gene FISH probe, the cytosolic acetyl-CoA carboxylase gene (*Acc-2*) was selected, and the probe was hybridized to chromosomes of bread wheat, *T. urartu*, *T. monoccocum*, *Aegilops speltoides*, *Ae. tauschii*, *T. turgidum*, and *T. timopheevii*. Additional cDNA FISH probes were developed and used for chromosome identification. The *Acc-2* probe was detected on the long arms of each of the group-3 homoeologous chromosomes, on 5DL and 4AL of bread wheat, and on homoeologous and nonhomoeologous chromosomes of the diploid and tetraploid species. In all the species tested, FISH detected more *Acc-2* gene sites or pseudogenes than those detected by PCR or Southern analysis. The *Acc-2* FISH mapping detected chromosome translocations in some of the wild species. The present study demonstrates the usefulness of the FISH technique for physical mapping of genic sequences in wheat and will have broad applications in genome analysis of the Triticeae.

Assembly of chromosome 1BS physical map and its utilization for positional cloning of disease resistance genes in wheat.

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A physical map of chromosome 1BS was constructed within the framework of the European consortium Triticeae Genome designed to develop physical maps of wheat and barley group 1 and 3 chromosomes. Fingerprinted BAC clones from a 1BS-specific library were assembled by LTC software into 385 contigs covering 274 Mb (87%) of 1BS (Sulston 10-25). These contigs were then re-organized (Sulston 10-15) into 52 long supercontigs (1–22 Mb, each), covering ~259 Mb (~82%) of 1BS. Verification steps were conducted using BAC-end sequences. Hybridization of a Nimblegen 40K wheat expression array with 57 MTP pools resulted in the assignment of wheat unigenes to 1BS physical supercontigs. The orientation and order of the supercontigs was determined based on parallel synteny between *Brachypodium* Bd2 and wheat 1BS unigenes. About 600 markers representing 400 different genes were assigned to individual BACs composing the 1BS physical map. Around 300 of these genes were *in silico* anchored to Group 1S Genome Zipper and their deletion bin position was estimated. The assembled 1BS physical map is now being utilized for positional cloning of disease resistance genes derived from wild emmer wheat, *Triticum turgidum* subsp. *dicoccoides*. *YrH52* is conferring broad spectrum resistance to stripe rust, one of the most destructive diseases of wheat. A large mapping population segregating for *YrH52* was developed. Comparative genomic analysis was used to anchor the *YrH52* interval to the colinear region on the *Brachypodium* Bd2, rice Os5, and sorghum Sb9 chromosomes. Screening of the 1BS MTP pools with *YrH52*

flanking markers resulted in the identification of BAC supercontigs that cover 19.2 Mb of the *YrH52* gene region. Further work is underway to refine the physical map and identify candidate gene(s) for *YrH52*. A similar strategy is employed for positional cloning of *Yr15* and *YrG303*, also derived from *T. turgidum* subsp. *dicoccoides*. Collaboration with other groups was established to promote the positional cloning of other wheat genes that reside on 1BS. These results demonstrate the importance of the genomic resources developed by TriticeaeGenome consortium for accelerating positional cloning of target genes in the complex genome of wheat.

High-resolution mapping of areas of low recombination and polymorphism containing the hexaploid wheat loci Pis1 and C.

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Gene mapping and map-based cloning studies in wheat are often complicated by lack of marker polymorphism and/or recombination. Radiation hybrid mapping can address both problems as it relies on radiation-induced breaks to order markers. Due to the nature of RH panels markers are scored as presence/absence, eliminating the need for polymorphism. We utilized this approach to physically map genes based on multiple overlapping deletions induced by γ irradiation. *Pis1* is a wheat floral mutant producing three fully functional pistils per floret instead of the usual single pistil. Compactum (*C*) is responsible for club head phenotype and maps to the low-recombination, pericentromeric region of 2D. The location of *C* with respect to the centromere is not well established. *Pis1* has proven difficult to map in an F_2 population due to lack of marker polymorphism. In this study, independent RH mapping panels were assembled for both genes (282 RH lines for each of *Pist1* and *Compactum*). For each gene, a set of 94 lines were selected and characterized with ESTs and SSRs, specific to the targeted regions. We mapped *Pist1* gene on chromosome 2D (deletion bin 2DL-9) using 14 SSRs and 27 ESTs. Total map distance was 145.0 cR1500 covering ~98 Mb. Even in this region of a recombination hot spot, a cM/cR1500 ratio was found as 1:8; with a mapping resolution of ~750 kb. The closest ESTs flanking *Pist1* are co-segregating and spanned only six rice genes. *Pis1*-linked ESTs were then mapped on a genetic mapping F_2 (Multiovary/Winsome) population. For the *Compactum* locus, 25 ESTs and 16 SSRs were used in mapping. A total map length created was 158.8 cR1500 with average marker retention of 80% and map resolution of ~710 kb. The cM/cR1500 ratio in this region of chromosome was found to be 1:60. Two ESTs flanking *C* locus on this RH map span 15 rice genes. ESTs flanking the *C* locus were mapped on two different bi-parental genetic populations to confirm the outcomes of RH mapping. Putative candidates are being used for developing gene specific markers and work on fine mapping and their eventual cloning is underway.

The rye gene Pm8 conferring resistance to wheat powdery mildew is a homologue of the wheat powdery mildew resistance gene Pm3.

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During wheat breeding, chromosomes of the rye (*Secale cereale* L.) genome have been introgressed into the wheat genome to enhance tolerance to biotic and abiotic stresses. The most widely used wheat–rye translocation nowadays is the 1RS chromosome arm derived from the rye cultivar Petkus carrying three rust resistance genes and the powdery mildew resistance gene *Pm8*. In wheat, *Pm* genes mediate resistance against powdery mildew, a major fungal pathogen. *Pm8* was mapped at the same gene-rich region on the short arm of wheat homoeologous group-1 chromosome as the powdery mildew resistance gene *Pm3* of hexaploid wheat and was, therefore, proposed to be an ortholog of *Pm3*. Southern hybridization analysis showed that there is a sequence in rye and in *Pm8* wheat lines with homology to the *Pm3* promoter region. In a homology-based cloning approach based on primers derived from the cloned wheat powdery mildew resist-

ance gene *Pm3*, we were able to amplify a *Pm3*-homologous gene from the chromosome arm 1RS. By means of transient expression of the *Pm8*-candidate gene in susceptible wheat lines and the generation of stable transgenic lines, we could show that the *Pm8*-candidate gene mediates *Pm8*-specific powdery mildew resistance to *Pm8* avirulent powdery mildew isolates. In two independent mapping populations, we could also confirm that the cloned resistance gene indeed maps to the *Pm8* locus. Furthermore, sequence comparison of *Pm8* with *Pm3* and *Pm3*-like genes revealed a complex mosaic of ancient haplotypes in these resistance genes. Since the *Pm8*-candidate gene is functional and localizes to the previously assigned *Pm8* locus, it is indeed *Pm8* and its high sequence similarity to *Pm3* shows that it is a homologous gene of *Pm3*.

SESSION VII: GENOMICS-ASSISTED BREEDING

Use of genomic selection in 21st century wheat breeding.

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The overall goal of wheat breeding efforts over the past 120 years has limited variability; combine the most positive alleles into one individual plant to maintain the economics and sustainability of wheat production locally and globally. One facet that has changed significantly is the tools at the disposal of current day wheat breeders to implement their breeding goals. The use of molecular markers over the past 25 years has opened new breeding approaches as we are now able to locate QTL and genes of interest, efficiently move them into adapted germplasm, and pyramid them effectively. More recently, the ability to saturate the genome of wheat with single nucleotide polymorphism (SNP) markers and genotyping by sequencing (GBS) has provided the tool of genomic selection. Genomic selection, which is used heavily in animal breeding, is a new tool in wheat breeding for improving quantitative traits in large breeding populations in an attempt to increase the accuracy of the prediction of breeding and genotypic values. By predicting the breeding values of lines in a population through analysis of phenotypes and high-density marker scores, the breeding cycle can accelerate, enhancing gains per unit time. Although most genomic selection models have been through computer simulations, the correlation between true breeding values and the genomic estimated breeding value has been reported to be as high as 0.85. Recently, the wheat breeding programs at Washington State University have begun to evaluate the usefulness of genomic selection in the wheat development effort. Training panels have been established and genotyped, and are in the process of being phenotyped. Perspectives on how this new tool will be used as part of a toolbox will be discussed as breeding programs are developed that more efficiently and effectively release wheat cultivars.

DArT and DArTseq genome profiling with relevant IT support.

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Diversity Arrays Technology (DArT) was developed over a decade ago to enable crop breeding with utilization of the whole-genome profile information. The technology has found numerous genetic and breeding applications in a variety of crops. At the moment, DArT has been developed in over 65 organisms, including all significant ITMI crops and their relatives. In the last two years, we have developed and launched commercially a new service using DArT complexity reduction methods combined with Next Generation Sequencing platforms. This new (DArTseq) platform has been applied to tens of thousands of wheat samples and tested successfully in practically all cultivated Triticeae crops. The technology scans over 100,000 loci in the genome for DNA variation targeting primarily genic regions of the genome. DArTseq integrates DArT markers (presence/absence of restriction fragment in genomic representation) based on SNP and methylation variation with 'traditional' SNP markers on the fragments detected in genomic representations. We will present a number of examples of application of DArT and DArTseq to crop breeding and genetics as well as in product purity and genetic ID testing. Our analytical pipeline for genome profile production and new information technologies for data storage processing will be also presented.

The usefulness of genomic selection in wheat breeding programs.

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With the expected development of thousands of molecular markers in most crops, the marker-assisted selection theory has recently shifted from the use of a few markers targeted in QTL regions (or derived from candidate genes) to the use of many more markers covering the whole genome. Provided that a sufficient level of linkage disequilibrium exists between the markers used for genotyping and the true genes underlying QTL for complex traits, these genome-wide markers can be used to predict the true breeding value. To be useful for breeding purposes, the accuracy of this Genome Estimate of Breeding Value (GEBV) should not be worse than the estimation based on phenotype, which is not always the best predictor of breeding value, particularly in the presence of 'G x E' interactions. Moreover, because GEBV allows shorter selection cycles, an overall improvement of genetic progress per time unit is expected. We present a case study using DArT markers on the INRA wheat breeding program, in an attempt to implement whole-genome selection as an alternative to phenotypic selection. This investigation assesses different models – Pedigree BLUP, Ridge Regression BLUP, Bayesian Ridge Regression, and Bayesian Lasso – ability to predict either simulated or real data (yield in a multisite, multi-annual network). The prediction coefficients obtained when the target population is a random sample (i.e., cross-validation) of the data are of the same magnitude of those reported in the literature. However, when the target population is a subset of genotypes studied in a given year (out of 10), the prediction quality is much lower. However, from a practical point of view, the ranking of (best) genotypes is more important than the prediction accuracy. Again the goodness of fit of best genotypes ranking is highly variable from year to year. Implication of results for practical implementation of genomic prediction in breeding programmes is discussed.

Development of gene specific KASP markers: a toolbox for marker-assisted selection in wheat.

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Whole-genome, SNP detection technologies now available in wheat provide fast and cost-effective approaches for construction of linkage maps, genome-wide association mapping, and development of genomic selection models. However, these technologies are not suited for marker-assisted breeding applications where labs need to cost-effectively assay the same polymorphism on tens of thousands of plants in the segregating generations of a breeding program. Predictive markers amenable to high-throughput genotyping are needed for use at low-plex levels in small-scale programs and in combination with other marker-assisted breeding approaches in larger programs. We report the initial results of an international collaboration to develop and make publicly available allele-specific assays for important genes in wheat. Stream-lined homogeneous assays were developed using the KASPar technology. KASP provides flexibility in assay design, making it well-suited for use in polyploid wheat, and is low cost relative to other closed-tube genotyping methods. Reported sequence variation (SNPs and indels) were targeted for development of assays specific for alleles of the reduced height, vernalization, and photoperiod-response genes. In addition, allele-specific assays were developed for cloned disease resistance and end-use quality genes. In some cases, it has been difficult to develop assays based on the causal gene sequence. Also, a number of genes in wheat are well mapped but not yet cloned. To develop KASPar assays for these, associated SSR and STS markers were evaluated on a panel of lines genotyped with the 9,000 SNP Wheat Infinium Assay. SNP in linkage disequilibrium (r^2 >0.9) with the SSR and STS markers were identified. Using this approach, markers were developed that are highly predictive for the presence of the T1RS·1BL translocation, *Vrn-A1a*, the *Sr36* and *Sbm1* resistance genes, and alleles at the *Glu-D1* locus. Results from the plethora of on-going linkage and association mapping experiments, coupled with SNP genotyping and current sequencing projects in wheat, will result in identification of numerous sequence targets for development of new predictive, homogeneous assays for important genes. We encourage researchers to contribute sequences for development of additional publicly available KASPar assays for use in wheat improvement programs world-wide.

An integrated SNP, SSR, DArT map in the wheat population 'Kenyon/86ISMN 2137'.

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Single nucleotide polymorphism (SNP) markers are a powerful new tool for wheat genetics. Most current SNP markers are gene-based markers, which facilitates comparative mapping with *Brachypodium* and rice. This feature enables development of additional markers from the publicly available genome sequences of these related species for map-based cloning projects. A recombinant inbred line (RIL) population developed from the cross 'Kenyon/86ISMN 2137' was tested with the 9,000 SNP Infinium iSelect BeadChip panel developed by researchers in the U.S. and Australia. An integrated map of SNP, SSR, and DArT markers has been developed. The 'Kenyon/86ISMN 2137' RIL population segregates for reaction to multiple wheat pathogens, including *Puccinia triticina*, *Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, *Tilletia* spp., and *Fusarium graminearum*. Efforts to map resistance to these pathogens are in various stages of completion.

SESSION VIII: HIGH-THROUGHPUT PHENOTYPING

State of the art and future developments in phenotyping: half a century of expertise in ARVALIS Institut du végétal.

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Arvalis is a technical institute created by the will of cereal-growing French farmers. For more than 50 years, Arvalis employees have conducted research experiments with the primary goal of increasing the competitiveness of farmers. Other applied research institutes exist in France, but Arvalis is the largest one in terms of employees and studied crops (cereals, maize, potatoes, protein-rich crops, flax, forage). Various fields of research are undergone by Arvalis (innovation for agricultural equipment, innovation in plant protection, cultivation practices). However the main task for Arvalis is to evaluate crop varieties.

Agriculture in France faces many challenges as in the rest of the developed countries. Main concerns are global warming and its consequences, increasing costs of nitrogen fertilizers, and the legislation changes towards reduced use of pesticides. In order to address the concerns of farmers, Arvalis has deeply modified the way it evaluates varieties by adapting phenotyping methods to address new challenges. We perform the evaluation of genotypes in a national network of experiments under optimal plant protection and fertilization in order to approximate the highest potential of each new released variety. In addition, we evaluate also the same varieties under stressful environments (e.g., drought, frost, fungal attacks, and nitrogen depletion).

Global yield is not a sufficient indicator to monitor the potential of a variety under stress. We try to follow the elaboration of yield throughout the successive phenological phases. This allows us to better understand the interactions between genotype and the various limiting factors. The use of high-throughput phenotyping tools and crop models are now unavoidable. Twenty years ago our institute first proposed the use of shading facilities to control rainfall, and we are now developing more innovative techniques to better control the environment (the PHENOME project). Recent advances in the field of robotics and miniaturisation of devices have opened the door for innovative phenotyping techniques under field conditions. Arvalis plays a major role in France in the developing and testing of new phenotyping tools (e.g., Phenoblé, Racine, and Phenomobi). We progressively integrate those facilities into the phenotyping platforms and we hope to achieve major breakthroughs in that field.

Nondestructive, high-throughput phenotyping to study cereals under stress conditions.

Bettina Berger [1] and M. Tester [1,2].

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Genetics and genomics are powerful tools for gene discovery. However, the ability to phenotype at high-throughput plant growth and function is forming a bottleneck in discovering the role of genetic loci in plants. Increasingly, efficient transgenic technologies are generating large numbers of GM crop plants; and the genotyping of mapping and mutant populations is now highly efficient. However, the ability to quantitatively phenotype these populations is limiting progress in plant science. The increasing power of digital imaging and computational technologies offers the opportunity to relieve this phenotyping bottleneck. The Plant Accelerator® is a new, 4,500-m^2 growth facility that provides -omic-scale shoot phenotyping of large populations of plants using automated plant handling and digital imaging. Current projects use the phenotyping capacity to analyze cereals under various stress conditions, including water deficit and salinity. First results and the experimental approach taken will be discussed.

Poster abstracts

Poster 1. The International Wheat Genome Sequencing Consortium (IWGSC).

The International Wheat Genome Sequencing Consortium. IWGSC, 5207 Wyoming Road, Bethesda, MD 20816, USA.

Bread wheat, the staple food for 35% of the world's population and the most widely produced crop, is one of the most important crop species. Genomics offers powerful tools for understanding the molecular basis of phenotypic variation as well as accelerating gene cloning, marker-assisted selection, and more efficient exploitation of genetic diversity. In 2005, a group of growers, breeders, and plant scientists launched the International Wheat Genome Sequencing Consortium (IWGSC) with the goal of securing a high quality, reference sequence of the bread wheat genome. The IWGSC facilitates and coordinates research projects and funding efforts at the national and international levels; develops and supports the design of research proposals; provides a framework for the establishment of common guidelines, protocols, and resources; and organizes scientific meetings and workshops. The IWGSC is governed by six co-chairs, a Coordinating Committee, and an executive director. General membership is open to any individual, laboratory, or entity with an active interest in meeting IWGSC objectives. The mission, goals, organizational structure, projects, and online membership registration are available at http://www.wheatgenome.org. IWGSC activities are guided by a milestone-based strategy coupled with short- and long-term roadmaps designed to provide breeders access to an increasing array of tools and resources without having to wait for the completed sequence. To reduce the complexity of the allohexaploid, highly repetitive, 17-Gb bread wheat genome, the IWGSC follows a chromosome-specific approach to develop physical maps, low coverage sequencing, and high quality sequencing of the Minimum Tiling Paths before moving towards a gold standard reference sequence. Physical maps have been completed or are underway for all 21 chromosomes of the reference cultivar, Chinese Spring. To facilitate anchoring, marker development, and to gain a first insight into the gene space composition, the IWGSC launched an internationally coordinated survey sequencing initiative that is providing breeders with survey sequences and the virtual gene order for all 21 chromosomes. High quality, BAC-by-BAC sequencing of chromosome 3B was completed in 2011 and sequencing of other chromosomes is under way. IWGSC activities and results will be presented.

Poster 2. The IWGSC Chromosome-Based Survey Sequencing Initiative.

The International Wheat Genome Sequencing Consortium. IWGSC, 5207 Wyoming Road, Bethesda, MD 20816, USA.

Applying advanced genomics to wheat breeding will play a central role in securing affordable and nutritious food. Bread wheat (*Triticum aestivum*) has one of the largest (~17 Gb) and most complex (allohexaploid, 6n=42, AABBDD) genomes. Although genome size varies in grasses, gene order, generally, is conserved along large chromosomal segments enabling comparative methods between related species. The IWGSC aims to establish a high quality, reference sequence

of the wheat genome (cv. Chinese Spring) that is anchored to the genetic and phenotypic maps to provide high resolution linkages between the traits and the underlying variations in sequence and polymorphisms. A first goal has been to obtain physical maps of the individual chromosomes/arms. To anchor the physical contigs, survey sequences were achieved using NGS technologies for a majority of the Chinese Spring chromosomes. The IWGSC then launched a short-term initiative to provide survey sequences of all 21 chromosomes. Sponsored by industry and government partners, the aim is to generate sequence and virtual order for most wheat genes. The sequences generated for each chromosome arm are assembled using the latest software tools. A first pass annotation is implemented over the draft assembly sequences where comparative genomics and colinearity with other grass genomes is used to derive a virtual gene order with an account of non-syntenic genes and pseudogenes. As sequences are generated independently for each chromosome, a second aim of this project is to characterize genomic variation between gene homoeologues (ie orthologous genes placed in different subgenomes), regulatory elements, and repeat content. An update of this initiative will be presented.

Poster 3. TriAnnot: a high performance pipeline for the automated structural and functional annotation of plant genomes - new developments.

Philippe Leroy [1], Nicolas Guilhot [1], Isabelle Lesur-Kupin [4], Patricia Faivre [5], Sébastien Theil [1], Frédéric Choulet [1], Hiroaki Sakai [3], Michael Alaux [2], Takeshi Itoh [3], Hadi Quesneville [2], Christophe Plomion [4], and Catherine Feuillet [1].

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A versatile, easy-to-use, online, automated annotation pipeline, TriAnnot (Leroy et al. 2012. Frontiers in Plant Sciences 3:1-14; http://www.clermont.inra.fr/triannot), has been developed under the umbrella of the International Wheat Genome Sequencing Consortium, the TriticeaeGenome, and 3BSEQ projects to obtain a reference sequence of the bread wheat genome. Its modular architecture allows for the annotation and masking of transposable elements, the structural and functional annotation of protein-coding genes with an evidence-based quality indexing, and identification of conserved noncoding sequences and molecular markers. The performance of TriAnnot was evaluated in terms of sensitivity and specificity using curated reference sequence sets from rice (IRGSP build5, August 2010) and wheat (Choulet et al. 2010. Plant Cell 22:1686-1701). In less than 8 hours, TriAnnot was able to predict more than 83% of the 3,748 CDS from rice chromosome 1 with a fitness of 67.4%. On a set of 12 reference Mb-sized contigs from wheat chromosome 3B, TriAnnot predicted and annotated 93.3% of the genes among which 54% were perfectly identified in accordance with the reference annotation. It also allowed the curation of 12 genes based on new biological evidences, increasing the percentage of perfect gene predictions to 63%. TriAnnot systematically showed a higher fitness than other annotation pipelines that are not improved for wheat. The TriAnnot pipeline is parallelized on a 712 CPU computing cluster that can run a 1-Gb sequence annotation in 26 hours. It is accessible through a web interface for small-scale analyses and/or through a server for large scale annotations. For the later, the pipeline is launched automatically using a PERL script. After completion, the structural and functional annotation can be viewed through an online GBrowse and can be manually curated using Artemis and GenomeView graphical editors. As it is easily adaptable to the annotation of other plant genomes, TriAnnot is currently improved for other plant genomes annotations such as barley, rice, maize, and oak species, and should become a useful resource for the annotation of large and complex genomes in the future. Release 3.5 is on-line since January 2012, and a new release 3.6 is underway.

Poster 4. Current status of physical mapping on wheat chromosome 6B.

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For the purpose of better understanding the genome structure in wheat and accelerating the development of DNA markers for gene isolations and future breeding, the Japanese research consortium, as a member of IWGSC, is now conducting a project for the physical mapping and genomic sequencing of Chinese Spring chromosome 6B (914 Mb). With a

sequence-based finger printing method (Whole Genome Profiling; Amplicon Express, KeyGene Inc.), physical maps for the short and long arms of chromosome 6B have been successfully established using the 6BS- and 6BL-specific BAC libraries, respectively, which consist of 2,667 and 1,842 BAC contigs. The estimated chromosomal coverage is more than 80% of both arms. Overlap analysis between the neighboring clones within the BAC contigs resulted in a total number of 5,079 and 4,889 MTP (Minimal Tiling Path) BACs on 6BS and 6BL, respectively. For the confirmation and the chromosomal assignment of the BAC contigs onto their corresponding genomic regions, we currently are developing a large number of 6B-specific DNA markers using the public marker resources, available EST databases, and the 6B survey sequence. To date, among 3,743 markers tested for their availability, 2,480 were found to be useful for the PCR screening of BACs, and 451 BAC contigs has been anchored by 627 markers to the specific genomic regions on chromosome 6B presumed on the basis of genetic maps, deletion maps and/or Genome Zipper. In parallel, a high-resolution, radiation hybrid map and a genetic map using recombinant inbred lines are under development for further improvement of physical maps. This work was supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics Agricultural Innovation; KGS-1001 and KGS-1003) and funding from Nisshin Flour Milling Inc.

Poster 5. The distribution, duplication, pseudogenization, and expression of noncollinear genes of wheat chromosome 3B.

Natasha Glover, Frédéric Choulet, Sébastien Theil, Josquin Daron, Lise Pingault, Etienne Paux, and Catherine Feuillet. INRA-University Blaise Pascal Joint Research Unit 1095 Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand 63100, France.

3BSeq is a large, ANR (Agence Nationale de la Recherche)-funded project developed under the umbrella of the IWGSC. It aims to sequence and characterize the largest wheat chromosome (3B) at the molecular level. The complete chromosome sequence is being produced by combining Roche/454 sequencing of BAC pools with whole chromosome shotgun using Illumina technology. Preliminary studies showed that about 50% of the genes identified in wheat are not collinear with the other grass genomes, raising several questions about their origin, fate, and expression in the wheat genome.

A set of 3,005 collinear genes and 2,432 noncollinear genes was defined using the annotation of chromosome 3B and orthologous genes from *Brachypodium*, rice, and sorghum. While waiting for the establishment of the 3B pseudomolecule, deletion bin information was used to determine the gene density per deletion bin and the relative gene order along the chromosome. Collinear genes show a 2-fold increase in gene density from the centromeric regions to the telomeric regions, whereas noncollinear genes have five-fold increase. In addition, noncollinear genes are more likely to be resulting from gene duplication events, with approximately 10% more of the noncollinear genes having duplicates on 3B in comparison to the collinear genes. The same pattern was found for pseudogenes. RNASeq data, collected from 15 different RNA samples, was used to determine that a higher percentage of collinear genes are expressed in comparison to noncollinear genes.

These results provide evidence that noncollinear genes have a higher probability than collinear genes to be duplicated, revert to pseudogenes, and subsequently become nonfunctional. The primary mechanism for this gene duplication and movement on 3B remains to be determined. We found 50% more CACTA superfamilies in the regions surrounding noncollinear genes than collinear genes, suggesting a major role for transposable elements in noncollinear gene movement. These results will shed some light on the evolutionary forces involved in the shuffling of the gene content on 3B and more generally in the accelerated evolution of the wheat genome. Functional analyses are underway to determine the fate of noncollinear duplicated genes in wheat.

Poster 6. Survey of the wheat 5AS chromosome synteny in Triticum species with different ploidy levels.

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In the frame of the project 'Physical mapping of wheat chromosome 5A', we have analyzed markers collinearity in the short arm of chromosome 5A (5AS) considering different species of the *Triticum* genus (*T. aestivum*, AABBDD, *T. turgidum* subsp. *durum* (AABB), and *T. monococcum* (AA) characterized by different ploidy levels and evolutionarily separated on a time scale in order to get insights into possible chromosomal rearrangements occurred during evolution. In detail, we relied on four mapping populations: (1) Chinese Spring (CS, *T. aestivum*) x Renan (*T. aestivum*), (2) CS x CS disomic substitution line for chromosome 5A (*T. turgidum* subsp. *dicoccoides*), (3) Latino (*T. turgidum* subsp. *durum*) x MG5323 (*T. turgidum* subsp. *dicoccum*), and (4) DV92 (*T. monococcum*) x G3116 (*T. monococcum*). Several categories of molecular markers, including SSRs (simple sequence repeat), SSR–EST (SSR-expressed sequence tags), TE junction (transposable elements), and COS (conserved ortholog set) were used to obtain high density genetic maps for the short arm of wheat chromosome 5A. The specificity of each marker for chromosome 5AS was assessed with nullitetrasomic lines derived from the reference cultivar Chinese Spring, and deletion lines were used to assign the physical position of the developed markers to deletion bins of 5AS. The evaluation of syntenic blocks and nonconserved regions among the homologous segments of different *Triticum* species is reported, while the mapping of EST-based markers allowed identification of syntenic regions in the rice genome. Identification of possible rearrangements in the different 5AS genetic maps of wheat is providing valuable information for the subsequent steps of BAC contigs anchoring, while the consensus map deriving from the integration of these four maps will provide a fundamental tool to link the genetic and physical maps.

Poster 7. Physical mapping resources for large plant genomes: Radiation hybrids for the D-genome of Chinese Spring and Aegilops tauschii accession AL8/78.

Ajay Kumar [1], Vijay K. Tiwari [2], Thomas Drader [3], Kristin Simons [1], Muhammad J. Iqbal [1], Monika M. de Jimenez [1], Filippo M. Bassi [1], Farhad Ghavami [1], Omar Al-Azzam [4], Lingli Dong [3], Yi Wang [3], Ming-Cheng Luo [5], Yong Q. Gu [3], Anne Denton [4], Gerard Lazo [3], Jeffrey M. Leonard [2], Oscar Riera-Lizarazu [2], and Shahryar F. Kianian [1].

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Due to the lack of recombination in certain regions of the chromosomes, genetic mapping alone is not sufficient to develop a high quality marker scaffold for sequence ready physical maps. Radiation hybrid (RH) mapping, which uses radiation induced chromosomal breaks to identify physical marker linkages, has proven to be a valuable tool for generating physical maps for complete genome assembly in animal systems. In order to construct high-resolution, RH-based physical maps of the wheat D-genome chromosomes that integrate the BAC based contigs, we developed D-Genome Radiation Hybrid (DGRH1) panels for the wheat D-genome donor *Aegilops tauschii* accession AL8/78 (1,510 DGRH1) and the reference hexaploid wheat cultivar Chinese Spring (2,565 DGRH1). Characterization of these DGRH1s with a set of molecular markers evenly spanning the entire genome indicates a homogenous marker loss (2.1%) across the chromosomes. Four different marker systems used in this study, mostly detected unique deletions suggesting that a combination

of marker systems will yield a better contiguous map. The mapping resolution of these RH panels estimated on the basis of markers spanning known distances was <140 kb. Two sets of informative lines carrying breaks in multiple D-genome chromosomes were selected from Ae. tauschii DGRH1s (399 lines) and Chinese Spring DGRH1s (300 lines). First generation RH maps based on 178 lines and 676 markers (641 DArT and 35 SSR) showed a 17:1 map ratio cR/cM when compared with the genetic maps. A NimbleGen array has been designed and tested for high-throughput mapping, and a total of ~30,000 retro-junction markers and ~6, 000 gene-based markers, specific to the D genome were identified. The selected DGRH1 lines currently are undergoing genotyping with this array and, once analyzed, will provide a very dense scaffold for the assembly of the D genome of wheat. This research also provides valuable resources for fine mapping and map based cloning studies of genes present on the D genome along with an unprecedented view into the evolution of grass genomes (http://avena.pw.usda.gov/RHmapping/).

Poster 8. Construction of a radiation hybrid map for chromosome 6B of common wheat.

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It is well known that recombination events in wheat are not evenly distributed along the length of chromosomes. This is the case with chromosome 6B, which is the target chromosome assigned to Japan by the International Wheat Genome Sequencing Consortium. The genetic maps of chromosome 6B so far constructed are heavily populated by markers in the pericentromeric region and scarcely in the telomeric regions. This represents that recombinations mostly take place in the telomeric ends of the chromosome. Therefore, we would face problems in determining orientation of contigs in the pericentromeric region if we solely depend on the genetic maps. Our objective of the current study is to establish a radiation hybrid (RH) mapping panel that can be useful in determining marker orders in the recombination-poor, pericentromeric region of chromosome 6B. We crossed nullisomic 6B-tetrasomic 6A plants of Chinese Spring (CS) wheat with the pollen freshly irradiated by γ-ray (15 Gy). We sowed 2,171 M0 seeds and extracted genomic DNA from 461 (21.2%) surviving plants. Additionally, we used 12 6B deletion lines (five deletions in 6BS and seven deletions in 6BL; obtained from NBRP-Wheat, Japan). We scored the presence or absence of 21 previously reported 6B-specific SSR markers and four newly developed EST-based markers. We analyzed the data by CarthaGene software to construct a RH map with the default setting. The resulting RH map consisted of two linkage groups, corresponding to the short and long arms. The gap between the linkage groups may be due to the absence of markers in a pericentromeric bin (6BS-CEN-0.25). The marker order is consistent with that of bin mapping and largely with a previously reported genetic map. All the RH-map markers occupied individual loci. Two pairs of markers genetically mapped to the same loci in the pericentromeric region of 6B were completely separated from each other in the RH map. This result indicates that RH mapping with our panel has better resolution in proximal region of 6B.

Poster 9. A NimbleGen comparative genomic hybridization array for high-throughput physical mapping of genome specific repeat junction and gene-based markers in the D genome of hexaploid wheat.

Thomas Drader [1], Lingli Dong [1,4], Yi Wang [1,4], Ajay Kumar [2], Vijay K. Tiwari [3], Muhammad Iqbal [2], Jeff M. Leonard [3], Gerard Lazo [1], Anne Denton [2], Shahryar Kianian [2], Ming-Cheng Luo [4], and Yong Gu [1].

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Mapping and map-based cloning of agriculturally and economically important traits remain a great challenge in complex highly repetitive genomes such as the grass tribe, Triticeae. This limitation is primarily based upon the availability of polymorphic markers and frequency of genetic recombination. Most markers are gene-based, derived from polymorphisms within coding regions. Non-gene-based markers, such as repeat junction markers, are derived from the noncoding intergenic space. Repeat junction markers take advantage of the repetitive nature of the wheat genome, providing random and equal distribution of these markers throughout the genome and can facilitate mapping efforts. Repeat junction markers are designed upon the junction of nested repetitive elements, and approximately 90% of these markers have been determined to behave as a single copy locus and are genome specific. Repeat junction markers were designed from 454 genome sequences of the wheat D-genome progenitor, *Aegilops tauschii*. Mapping of *Ae. tauschii* repeat junction

markers to deletion bins within the D genome of reference polyploid wheat (Chinese Spring) will allow for the construction of a physical marker scaffold that will aid in genome sequence completion and future mapping and cloning studies. To design an optimal marker array, we tested hybridization temperature, oligo length, and different statistical analysis methods. After determining appropriate marker design and experimental conditions we screened a pool of 206,486 repeat junction markers as well as 26,800 gene markers representing 6,700 genes. Screening results provided 46,224 markers in total that were selected for design of a final mapping array. These 46,224 markers are comprised of 30,900 repeat junction markers and 15,324 gene markers representing 6,330 genes. This final mapping array is being used to construct a high-resolution, physical map using D-genome deletion lines and radiation hybrid panels. Here we present our methods for design and analysis of the NimbleGen comparative genomic hybridization arrays constructed from *Ae. tauschii* repeat junction markers, the construction of a NimbleGen repeat junction array using selected markers, and its use in the development of a physical map for the D genome of hexaploid wheat.

Poster 10. Genome-wide characterization of transposable element repeat junctions in barley and their application in marker development for chromosome 3H.

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Transposable elements (TEs) account for about 70% of the barley (*Hordeum vulgare* L.) genome. TEs move in the genome by inserting to new regions through copy-and-paste or cut-and-paste mechanisms. Insertion of TEs in DNA regions generates unique junctions between the TEs and the sequences in which they land. We investigated the uniqueness of these junctions throughout the barley genome and their potential application for marker development. The 10-Gb survey sequencing data of the seven barley chromosomes was searched with the 'RJPrimers' pipeline to estimate the frequency of repeat junctions (RJs). We found 988,750 RJs distributed evenly among the chromosomes with an average of 1 RJ per 10 Kb. Repeat junction markers (RJMs) for each chromosome were designed based on detected repeat junctions. Each RJM consists of one primer that spans unique insertion site of TE, whereas the second primer is designed from any region within 1 Kb of the junction. We randomly chose 36 RJMs from chromosome 3H to amplify five barley cultivars (Betzes, Golden Promise, Bowman, and Haruna Mugi) and two wild barley (*H. bulbosum* L.) accessions. Out of 36 RJMs, 28 (68%) amplified a single band and 21 (58%) were polymorphic. Based on scoring data of 21 RJMs, we were able to separate all tested barley accessions and cluster them into two main groups. The abundance of RJMs makes them an ideal marker for genome saturation. Further, the high level of polymorphism makes them ideal for molecular breeding applications.

Poster 11. Best practices for RNA-Seq differential expression analysis in barley.

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Barley (*Hordeum vulgare*) being a member of the grass family is one of the most important large-genome cereals and a close relative of wheat. It has a complex diploid genome of 5.1 Gb and is being extensively used for genetic studies. As a part of the Barley Genome Sequencing Project, RNA-Seq experiments were performed using eight different tissues representing different developmental stages, including 4-day embryos, roots, and shoots from seedlings, young developing inflorescences (5-mm and 10–15 mm stages), at the six-leaf stage, and from the developing grain (5 and 15 days post anthesis). These datasets have been used to improve the barley gene model predictions and to detect differentially expressed transcripts. Here we discuss the results of the expression analysis that we implemented using different available

software packages. In the first instance, we processed the raw reads by filtering them for low quality and then proceed to map them to the latest barley genome reference using Bowtie (Langmead et al. 2009) and Tophat (Trapnell et al. 2009) to consider spliced reads. These mapped reads were used for a downstream expression analysis study using the Cufflinks packages (Cufflinks, Cuffcompare, and Cuffdiff) (Trapnell et al. 2010) and R bioconductor packages (DESeq, NOISeq, and edgeR) (Anders and Huber 2010; Tarazona et al. 2011; Robinson et al. 2010). A comparison based on the results of these approaches indicated differences in the sets of genes reported with significant fold changes. Identification of the biological function of the nonoverlapping genes produced by the different analyses has been undertaken to explore the impact of using any one of these analyses in isolation.

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Poster 12. A triticale (x Triticosecale Wittm.) reference transcriptome.

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Triticale produces high biomass and grain yields and is resistant to many abiotic and biotic stresses. Hexaploid triticale (AABBRR) has a large genome similar to that of common wheat (AABBDD), and possesses large numbers of repeated sequences, making it difficult to sequence. Little is known about coordination of gene expression of rye and wheat genomes in this interspecific hybrid but understanding this interaction is crucial for improving its agronomic traits. We have produced cDNAs from four different triticale lines/cultivars; AC Certa, Alta, line 797, and line 1308, from different tissues including leaf, stem, and reproductive tissues at different developmental stages or exposed to different abiotic treatments (salt and drought). Reads were cleaned, and we assembled 6.7 M 454 reads and 276. 3 M Illumina reads together and identified, using a stringent assembly protocol, a total of 72,218 contigs with N50 and N90 values of 1,658 and 588 bp, respectively. Of these 72,218 contigs, 50,524 transcripts appeared to be full length after comparison to the sequences obtained from the four monocot sequenced genomes (rice, maize, sorghum and Brachypodium). The noncoding sequences appear to represent long, noncoding cDNAs, short ORFs, and precursors of microRNAs. At least 31,230 of these transcripts appear to be translated. In addition, we determined that approximately 20% of the genes had transcripts with alternative splice variants. Furthermore, nearly 60% of these transcripts were electronically annotated. Compared to our previous results with development of a rye transcriptome, the transcripts corresponding to the R genome in triticale were segregated from the sequences originating from genome A and B. A detailed molecular characterization of the transcripts into different functional groups as well differential expression of the rye and wheat transcripts under control and stressed conditions will be presented. We also will report on the utilization of fractionated DNA to further extend the 5' region of transcripts. Therefore, using next generation sequencing to de novo assemble a comprehensive reference transcriptome for triticale was possible. This approach would be useful for species with large, unsequenced genomes.

Poster 13. De novo assembly and characterization of wheat root transcriptome.

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Root systems primarily provide plants water, nutrients and anchorage. While much progress has been made in understanding root development and growth in the model plant *Arabidopsis*, little is known in most crops such as wheat (*Triticum aestivum* L.). As the first step of wheat root genomics and genetics, we are sequencing, assembling, and annotating the wheat root transcriptome in reference cultivar Chinese Spring (CS). Messenger RNA was purified from the root tips at 4 d after germination and sequenced using 454/Roche Titanium platform. A total of 818,038 quality reads were assembled into 24,492 contigs with average contig length of 752 bp, N50 of 798, and a total assembly size of 16.2 Mbp using Newbler. These 24,292 Newbler-contigs were further assembled with 26,849 CS root ESTs deposited in NCBI using the CAP3 program. This hybrid assembly generated 27,852 transcripts (>100 bp) with average transcript length of 730.75 bp, N50 of 771, and a total assembly of 20.36 Mbp. Approximately 87% of the transcripts had BLASTX hits in NCBI nr protein database, of which 78% of the total transcripts were assigned with gene ontology (GO) terms and 18% were assigned with enzyme commission (EC) annotation. Of the 19,196 transcripts with GO assignments, top eight GO classes identified in biological process include cellular process (34.85%), metabolic process (34.3%), localization (6.26%), response to stimulus (5.37%), cellular component organization (5.34%), biological regulation (4.84%), cellular component biogenesis (4.38%), and signaling (1.71%). Important GO classes identified in molecular function include nucleotide binding (3.17%), transcription factor (0.5%), sequence specific transcription factors (0.17%), DNA binding (1.91%), transporter activity (1.28%), kinase activity (0.88%), and receptors (0.22%). Although the majority of the root transcripts also were found in the aboveground organs, putative root-specific transcripts account for ~12% of the assembled root transcriptome. More than 9,000 SSRs were identified comprising di- (11.48%), tri- (57.75%), tetra- (19.37%), penta- (5.35%), and hexa-nucleotide (6.05%) motifs. In addition, a very small fraction of the root transcriptome was found to contain transposable elements, mainly MITES. Assembly and annotation of wheat root transcriptome will lay a foundation for molecular biology to understand wheat root development and improve wheat tolerance to soil-derived abiotic stresses.

Poster 14. BREEDWHEAT: Breeding for economically and environmentally sustainable wheat varieties: an integrated approach from genomics to selection.

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Wheat represents a major renewable resource for food, feed, and industrial materials and is the most widely grown crop worldwide. With its high-yielding wheat production (~70 q/ha), France is a major producer and exporter (fifth producer and exporter) and wheat production contributes significantly to the French economy with a positive balance of more than 4 billion €. To face the challenge of delivering safe, high-quality, and health-promoting food and feed in an economical, environmentally sensitive, and sustainable manner while maintaining yield and stability across environments affected differently by climatic change, a paradigm shift is needed in wheat breeding. BREEDWHEAT is conceived to support the competitiveness of the French breeding sector as well as answer the societal demand for sustainability, quality, and safety. BREEDWHEAT gathers the best public and private partners (26) in wheat research and breeding in France to ensure that the knowledge, resources, and methods resulting from the project are translated rapidly into products and varieties. In an unprecedented effort, BREEDWHEAT proposes to break barriers that have thwarted the translation of knowledge and molecular resources into breeding as well as the exploitation of genetic resources to enlarge the genetic diversity of the wheat gene pool. BREEDWHEAT will not only provide a breakthrough in technological development of markers and phenotypes, but it uniquely will integrate high throughput genotyping, phenotyping, and modeling studies to decipher the molecular and ecophysiological basis of important traits. This long-term project (9 years) will include sequencing of a wheat chromosome (1B), detection of new structural polymorphisms, large-scale SNP production, genetic and physical mapping of those SNPs, 48,000 phenotyping trials, and the generation of 33 million genotyping data points for association genetics studies. Moreover, 5,000 wheat lines from INRA genetic stocks will be extensively characterized and used to identify new alleles to support a pre-breeding program aimed at developing varieties that can be directly exploited by the breeders. The efficiency and economic impact of various selection schemes will be assessed in a farm-scale breeding program. Finally, a robust bioinformatics platform enabling efficient association analyses and breeder friendly access to the data will also be established.

Poster 15. Molecular breeding in wheat: findings from an international survey.

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Molecular breeding for crop improvement has the target to create gene-to-phenotype knowledge for breeding objectives and to use this knowledge in product development and deployment. Molecular techniques can impact every stage of the breeding process from parental characterization and selection for cross prediction to introgression of known genes and population enhancement via allele enrichment or gene stacking. It is often reported that molecular breeding through gene transfer and marker-assisted selection has been successfully applied especially in the private sector and that its wider use especially in the public sector institutions in the developing world is still limited due to various bottlenecks. Tangible information on the application of molecular breeding tools in public sector wheat programs is however fragmented. During the preparation of the 21st ITMI in Mexico City in 2011, we distributed a survey to the participants and CIMMYT collaborators addressing this subject. The survey included questions regarding the magnitude of application of molecular markers in the respective breeding programs, the form of deployment and trait target, bottlenecks in the case molecular markers are not used, and questions regards logistic and molecular marker systems. A total of 220 responses form 58 countries were received. The outcome of the survey will be presented.

Poster 16. Imprints of selection in CIMMYT wheat lines targeted to irrigated and rain-fed environments.

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CIMMYT international yield trials sent to national collaborators globally and targeted to diverse growing environments have been analyzed, but genotypic characterization of this elite material has not been extensively carried out. Twenty-three Elite Spring Wheat Yield Trials (ESWYT) and 17 Semi-Arid Wheat Yield Trials (SAWYT) together with recent CIMMYT elite lines were therefore genotyped with DArT and partly GBS markers. The high-throughput genotyping platforms were able to assign the lines in both yield trials into various but mainly two germplasm groups according to their targeted environment reflecting the establishment of diverse germplasm pools due to breeding for improved adaptation. Constant genetic diversity was observed across the years of trial distribution. The average genetic distance was slightly higher in the ESWYT than in the SAWYT and significantly increased in both trials with a growing difference in distribution years, suggesting a systematic change in allele frequencies during the breeding process. Observed frequencies for each marker allele, and haplotypes with a four-marker, sliding window, were determined for each of the ESWYT and SAWYT to further identify regions in the genome under selection. Markers and haplotypes displaying a significant change in allele frequency across years were identified and interpreted as an indicator for constant selection. Markers identified were partly linked to traits under CIMMYT breeder's selection and point to key genomic regions for further investigation.

Poster 17. Deriving a hard red winter wheat prebreeding population suitable for marker-facilitated recurrent mass selection.

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A new hard red winter wheat pedigree breeding program is being established at NDSU. As a result, there is an urgent need to develop improved breeding stock that can be used for making elite crosses. The most important demand is to develop breeding parents with high levels of cold-hardiness coupled with effective resistance against the major diseases, which include Fusarium head blight, leaf and stem rust, tan spot, and the *Septoria* complex. Backcrosses of resistance sources to a winter-hardy variety, such as Jerry, can only partially solve the problem as it restricts overall genetic variability. Recurrent selection is a population improvement strategy that is ideally suited to the development of improved germplasm as it maximizes opportunities for genetic recombination and gene pyramiding and allows for shorter genera-

tion cycles and more rapid progress while not imposing yield or genetic diversity ceilings typical of backcrossing. The effectiveness of a recurrent selection program can furthermore be greatly enhanced by using genetic male sterility and by supplementing phenotypic selection with marker-aided selection strategies.

A highly diverse, prebreeding base population is being produced. It incorporates a wide range of native and exotic resistance and adaptation genes, most of which derive from either spring wheat or less cold-hardy winter wheats from the southern United States. These will be involved in a complex cross that will eventually combine genes from approximately 150 diverse genotypes contained within five parental populations. While making the cross, the *Ms3* (dominant male sterility) gene is simultaneously being established within the hybrid population such that the final F_1 will segregate 1:1 for male sterility/ fertility. This final F_1 will be randomly intercrossed for two further cycles to fully disperse the target genes before the onset of selection. The intermediate and final hybrid populations will furthermore be evaluated to confirm the genetic diversity that it contains by employing phenotypic and/or marker-based evaluations of disease resistance, adaptation and performance. Concurrent with the development of the program, a procedure for random, large-scale intercrossing of individual plants through the use of *Ms3* will be optimized.

Poster 18. Creation of a new resource for fine mapping in wheat: a nested association mapping population.

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Linkage mapping using recombinant inbred lines usually achieves high power of QTL detection, but suffers from relatively poor accuracy of QTL location. Association or linkage disequilibrium (LD) mapping using panels of unrelated lines enables high mapping accuracy, depending on the range of LD, but is prone to several sources of false positive results if the panel is genetically structured. Nested association mapping (NAM) has been recently proposed as a method that combines the advantages of both linkage and LD mapping. It consists of developing a set of recombinant populations which are 'connected' by at least one common parent. The parental lines (founders) must be genotyped with a high marker density; the accuracy of QTL detection will rely on the short range LD among the founders. Then the recombinant progeny will be genotyped at low density. The small number of recombination events from founders to progeny enables large LD blocks to be conserved. The high density markers can therefore be imputed in the progeny from the information of low density markers thus keeping the high detection accuracy. On the other hand, recombination is sufficient to reshuffle the genetic background, thus limiting the source of spurious association with unlinked markers. Roughly, a NAM population achieves nearly the same accuracy as an unrelated association panel of similar size, while high-density genotyping or resequencing is only needed on a few dozen of founders. Simulations results suggest an optimal number of founders of 80-100 for a total sample size of 2,500–5,000.

In a cooperative program between Biogemma and INRA (ARN-08-GENM-005), we have developed a NAM population of RILs, and DHs, by producing recombinant progeny from a 'star' mating design. The pivot parental line was Altigo, a high-yielding, modern French cultivar. The other founders were taken either in the elite European cultivars or in a worldwide core collection built up at INRA GDEC Clermont-Ferrand France (http://www4.clermont.inra.fr/umr1095_eng/Teams/Technical-and-Experimental-Platforms/Genetic-Resources-Centre). Seed increase of the 5,000 progenies is now in progress. This new resource will be available soon for collaborative programs.

Poster 19. Association analysis in a panel of eastern U.S. winter wheat lines.

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We report our results from the first year of a multi-year study to discover new disease and insect resistance genes within a diverse panel of 449 eastern U.S. winter wheat lines. The panel is comprised of both landrace and elite cultivars of current or historic importance to eastern U.S. wheat breeding programs. Lines were submitted by eastern U.S. wheat breeders for inclusion in the panel. The diseases of primary focus are *Blumeria graminis* f. sp. *tritici* (powdery mildew), *Puccinia triticina* (leaf rust), and *Stagonospora nodorum* (glume blotch). The insect of primary focus is *Mayetiola destructor* (Hessian fly). These diseases and insects can significantly decrease yields of winter wheat in the eastern U.S. While resistance genes do currently exist for each of these diseases or insects, novel genes are needed to continue to incorporate into breeding programs as current resistance begins to break down. During the 2011–12 season, this panel was grown in head rows and screened for disease resistance in three reps at three locations in North Carolina. Disease screening at each location was with *B. graminis* f. sp. *tritici*, *S. nodorum*, or *P. triticina*, with one disease per location. Disease pressure for powdery mildew and leaf rust was naturally very high at the respective sites and no inoculations were performed. Inoculation with *S. nodorum* was done by spreading wheat straw over seedlings in January, 2012. Additionally, Hessian fly screening was done in the greenhouse using biotype L. These phenotypic data were used in an association analysis with SNP data on these lines, which was obtained from the Illumina 9k wheat chip. Population structure, seedling vigor, maturity at rating, and heading date were all used as covariates in the analysis. We anticipate the use of our results in helping to characterize the genetics of resistance to these pests and pathogens and also in providing breeders with additional tools for marker assisted selection.

Poster 20. Genome-wide characterization and capture of exotic alleles for increased yield from primary synthetic bread wheat.

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To address the loss of genetic diversity due to domestication and breeding of modern wheat cultivars, exotic alleles from bread wheat progenitors, *Triticum turgidum* subsp. *dicoccum* and *Aegilops tauschii* have been captured in primary synthetic bread wheat lines developed by the CIMMYT Wide Cross Program. The primary synthetics carry resistance and tolerance to a range of biotic and abiotic stresses. We analyzed selected high-yielding lines from double haploid (DH) populations between an elite CIMMYT cultivar (Opata) and six different primary synthetics, Opata x Synthetic recombinant inbred lines (RILs), and synthetic-derived, CIMMYT semi-arid wheat yield trial (SAWYT) breeding lines. Grain yield, agronomic, and physiological measurements were collected at CIMMYT over several years in a high-yield potential, heat and drought-stressed environment. We observed several synthetic derived lines outperforming the elite parent Opata in all environments, indicating that the primary synthetics contribute alleles increasing yield. Genotyping-by-sequencing (GBS) will be used to generate whole genome profiles and identify yield promoting genomic regions inherited form the primary synthetics. We will apply specifically developed genomic selection (GS) models to rapidly introgress valuable alleles and develop high-yielding breeding lines. In a next step, allelic predictions and GS models will be validated on derivatives of these DH, RIL, and SAWYT lines. New populations derived from crosses between the highest yielding lines in the existing DH populations are under development and will be evaluated under optimal and stress conditions in yield trials at CIMMYT. Testing of multiple types of synthetic derivatives including DH lines and breeding lines will provide rigorous validation of alleles for increased yield and the predictive power of our GS models. This pioneering study takes a whole genome approach to characterize, improve, and utilize exotic germplasm to increase yield.

Poster 21. A genomewide SNP scan of the diversity, population structure, and linkage disequilibrium in East African wheat (Triticum aestivum L.).

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For over a century, wheat has been a valued food crop in the East Africa region, but production is threatened by drought, insect pests, and a recurrence of rust disease epidemics as characterized by the recent highly virulent race Ug99 of stem rust. Recent effort is dedicated to genomewide association mapping (GWAM) for resistance loci and their deployment into the commercial cultivars. The objectives of this study were to dissect the diversity of the East African wheats and explore the nature of population structure and extent of linkage disequilibrium (LD), both of which have implications on the power and resolution of GWAM. A panel of 300 lines was assembeled, 90% of which are past and present East African cultivars as well as a few landraces and breeder lines. The material was genotyped using the 9000 SNP chip and a genome-wide set of 6,488 informative SNPS successfully called. An implementation of the model-based cluster analysis revealed a relatively strong population structure identifying five genetically distinct subpopulations (denoted as CIMMYT1, CIMMYT2, North America, Landraces, and Mixed). These were enriched with lines known to have initially originated from the international center for wheat and maize improvement (CIMMYT) and North America, consistent with known pedigree history, plus recognized landraces and a mixed group. The number of polymorphic loci was relatively high, ranging between 87% and 97%. Differences among the subpopulations were observed in the number of alleles, expected heterozygosity, and polymorphic information content (PIC). Estimates of relative kinship revealed a complete spectrum of values, with about 40% of the lines scoring above 0.5. The Level of LD (r^2) was higher and LD extend persisted longer in the CIMMYT1 subpopulation both in the A (13cM) and D (11cM) genomes. In the B genome, the LD extended longest (18 cM) in the North America subpopulation. This work hints at the suitability of the assembled panel for GWAM, if supported by a sufficient control of population structure and kinship.

Poster 22. Sequence-based, SNP genotyping in durum wheat.

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The availability of Single Nucleotide Polymorphism (SNP)-based platforms is highly desirable for the mapping and selection of loci (genes and QTL) of breeding value. As compared to other crops, SNP discovery and validation in durum wheat is lagging behind and only recently a number of SNPs were described and validated (Trebbi et al. 2011 Theor Appl Genet 123:555-569). A novel SNP discovery and genotyping technology, developed by KeyGene and called random Sequence Based Genotyping (rSBG), was tested and validated in both durum and bread wheat. This technology enables sequence-based SNP discovery and genotyping in a single assay, which is cost-effective (no separate costs for SNP discovery and genotyping) and robust (no marker conversion required). Within the EU FP7 BioExploit project, the rSBG technology was adjusted to highly repetitive, polyploid genomes and optimized on the durum wheat parental lines Colosseo (CLS) and Lloyd (LLD). This protocol was subsequently used to genotype 91 'CLS x LLD' recombinant inbred lines (RILs) in a single GAII run. A total of 10,761 putative SNPs in 10,729 loci were identified between the parents, using stringent SNP mining rules. Out of these, 1,038 were mapped with high confidence (two-point LOD > 6) to a pre-existing framework map containing 709 markers (SSRs, DArT® markers, SNPs from CRoPS® technology). The relatively low percentage of SNPs available for mapping was mainly due to lack of sequencing depth. Nevertheless, this rSBG experiment allowed for the genotyping of the RILs at a density of approximately one SNP marker every 2.8 cM. It is expected that an increased sequencing output will generate a higher number of genetically informative SNPs. Because the SNPs are associated with unique sequences, we will explore the possibility of linking them to the reference conserved orthologous sets from the grass genomes, which could add additional value to the rSBG SNP set. The rSBG and CRoPS® technologies are covered by patents and patents owned by Keygene N.V. CRoPS is a registered trademark of Keygene N.V. Other (registered) trademarks are the property of their respective owners.

Poster 23. SNP mapping in a doubled haploid, hexaploid wheat population.

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A total of 294 SNP (single nucleotide polymorphisms) markers were developed via a KASP assay (KBioscience®) on a DH hexaploid population comprising 182 individuals generated from a cross between parental cultivars 'RL 4452/AC Domain'. Of these 294 SNP markers, 225 were polymorphic between both parents, and the remaining 69 were either monomorphic or found unsuitable for mapping. These 225 SNP markers were meant to be integrated into an existing 'RL 4452/AC Domain' map comprising 652 SSR, DArT, and EST markers. The 225 polymorphic SNPs were mapped on 163 of the 182 DH progeny using MapDisto, resulting in the final assignment of 211 SNP markers to 18 of the 21 chromosomes. The majority of markers (55%) mapped to B-genome chromosomes, and the remaining 94 markers were assigned to chromosomes belonging to the A (27%) and D (18%) genomes. On a per chromosome basis, 5B had the most SNPs (15%), followed by 1B (12%) and 1D (9%). None of the SNP markers mapped to chromosomes 3D, 4D, and 7D. The integration of SNP markers into existing 'RL 4452/AC Domain' maps resulted in an overall reduction in the map sizes of 13 of the 18 chromosomes, with reduced map lengths varying between 0.5 cM and up to12.3 cM. No correlation could be established between reduced map size and number of SNP markers per chromosome. Most of the SNP markers either flanked or co-segregated with existing SSRs/QTL for several important genes/traits, making them useful for further fine mapping studies.

Poster 24. Study of pre-anthesis development in barley (Hordeum vulgare L.).

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Optimal anthesis time is one of the important strategies used in breeding programs to acclimatize crops to environment and subsequently achieving high yield potential. The pre-anthesis phase is one of the most important phases and has direct impact on yield potential and final grain yield. Maximum number of spikelet primordia per spike (maximum yield potential) and its survival are the major events during this phase. The pre-anthesis phase can be divided into three sub-phases: 1) leaf initiation, 2) spike initiation, and 3) spike growth phases. The lengths of these phases are affected by environmental conditions such as photoperiod and vernalization as well as genotypes. Such factors directly contribute to reach the final time of anthesis and yield potential. Hence, genetic analysis of pre-anthesis phases in cereals is necessary at this time point to a better understand the role of these phases in increasing yield. We are interested to use molecular-genetic approaches to explain the role of pre-anthesis development in barley for yield potential and final grain yield. The present study of pre-anthesis development using molecular markers and genetic associations may identify QTL for growth and developmental traits. Through synteny between barley and other grass species (rice, Sorghum, and Brachypodium), we aim to deduce candidate genes underlying QTL for growth and developmental traits by mapping and sequencing.

Poster 25. Sequencing of vrs1 and int-c loci shows that labile barleys (Hordeum vulgare convar. labile) have a six-rowed genetic background.

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Labile-barleys (*Hordeum vulgare* L. convar. *labile* (Schiem.) Mansf.) are found in the highlands of Ethiopia, Eretria, and north India–Pakistan districts. They represent a distinct spike form showing row-type alterations even within individual spikes of the same genotypes. Variation at the *six-rowed spike 1* (*vrs1*) locus is sufficient to control barley lateral spikelet fertility, which is also modified by alleles at the *intermedium-c* (*int-c*) locus. This study aimed at resequencing these two loci in 221 supposedly *labile*-barley accessions from Ethiopia to investigate whether these *labile*-barley accessions have a two-rowed genetic background, resulting in increased lateral spikelet fertility, or show reduced lateral fertility if they

possess a six-rowed genetic background. The resequencing results of *Vrs1* revealed 13 accessions with two novel *vrs1.a1* haplotypes. Following the current nomenclature of *vrs1* haplotypes, the new haplotypes were named as haplotypes 66 and 67. Resequencing at the *int-c* locus showed that 118 of the *labile*-barleys possessed the previously described *Int-c.a* allele, but only one accession was found having a novel *Int-c.a* haplotype in the homozygous state (termed *Int-c.a haplotype1*; *Hap_1*). Interestingly, 101 *labile*-barleys carried the *Int-c.a* allele and *Int-c.a haplotype1* simultaneously, suggesting maintained heterozygosity or recent gene duplication at this locus. Only one accession had a two-rowed haplotype (*Vrs1.b3*, *int-c.b1*), and one accession possessed the *Vrs1.t* (*deficiens*) and *Int-c.a* alleles (six-rowed). These two accessions were considered as misclassified *labile* genotypes and not included in further analysis. On the other hand, the phenotypic data obtained from the *labile* accessions and their comparison to the observed allele/haplotypes combinations showed that, in spite of the presence of *vrs1.a* and *Int-c.a* (genotypically six-rowed alleles) in the large majority of the analyzed accessions, the observed phenotypic data did not support the expected six-rowed phenotype in *labile*. The *labile*-barley spike phenotype displays a variable number of fertile lateral spikelets (from 0 to 2) at each rachis node. Thus, our analysis demonstrated that all of the 219 *labile* accessions studied in this work showed six-rowed alleles at *vrs1* and *int-c* but reduced lateral spikelet fertility. This reduction is most likely caused by the recessive *labile* (*lab*) locus which we are in the process to characterize further.

Poster 26. A large-scale, mutant panel of einkorn wheat developed by heavy-ion beam mutagenesis and its application for flowering-time mutant screening.

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Mutation analysis is a powerful tool for investigation of gene function. Heavy-ion beam mutagenesis has been recognized to be an effective method of producing mutations because of its high linear energy transfer (LET). High-LET radiation effectively induces DNA double-strand breaks than other mutagenic methods. We have been constructing a large-scale, mutant panel of diploid einkorn wheat (*Triticum monococcum*) using heavy-ion beam mutagenesis for 12 years. Seeds of the einkorn wheat strain KU104-1, KU104-2, or DV92 were treated with 50–58 Gy of N or C ion beam with LET of 30 ke/Vμm and then sown in the field. The spikes of M_1 plants were bagged and the harvested selfed seeds of each spike were used to produce the M_2 lines. Every year, we obtained about 1,000 M_2 lines, eventually developing a mutant panel with a sum of 10,000 M_2 lines. We are using this mutant panel for screening mutation of reproductive growth, especially for flowering-time mutants. We have identified several flowering-time mutants of great interest; nonflowering mutants (maintained vegetative phase), late-flowering mutants, and early-flowering mutants. In the late-flowering mutants, for example, we identified a mutation that had an abnormally large number of nodes; we termed this mutation *fushi-darake* (*fdk*), which means too many nodes in Japanese. The *fdk* mutant plants have increased numbers of nodes and leaves. WT plants show spiral phyllotaxy; however, *fdk* mutants have 1/2 alternate phyllotaxy with a shortened plastochron. Each tiller in the *fdk* plants branches at the upper part of the culm. A small spike sometime appears from the tip of culm in main tiller. The SEM analysis of developing SAMs indicated that transformation of spikelet meristems into vegetative shoot meristems in the *fdk* plants. Based on the phenotype, we concluded that the *fdk* mutant has a heterochronic nature, i.e., both reproductive and vegetative programs are simultaneously in operation during the reproductive phase, resulting in a shortened plastochron and transformation of reproductive spikelets into vegetative shoots.

Poster 27. Disruption of circadian clock caused by the earliness per se 3Am locus (Eps-3Am) contributes to early flowering in wheat (Triticum sp. L.).

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In temperate grasses, such as wheat and barley (*Hordeum vulgare* L.), earliness *per se* is understood as the intrinsic difference in flowering time of fully vernalized plants grown under long day conditions. In the current study, two einkorn wheat lines (*Triticum monococcum* L. x *T. boeoticum* Boiss.), RIL25 (early) and RIL71 (late) were selected from a RILWA1 population to generate a new F_2 population for fine mapping of the *Eps3A* locus. About 650 F_2 individuals were screened for genetic recombinations, and four new markers were added utilizing the physical map from barley chromosome 3H. This way, the locus could be delimited to approximately 350 kb and contained only two putative genes. Moreover, both genes were found to be deleted in the mutant parent of the RILWA1 population KT3-5 (*T. monococcum*) as well as in RIL25. The deletion of *TmLUX* (*lux arrhythmo*, an evening clock element) caused clock distortion and misexpression of circadian clock-related genes. Both effects were detectable by using delayed fluorescence measurements as well as a time-course qRT-PCR experiment on two key nuclear clock genes *TmTOC1* (*timing of CAB2 expression 1*) and *TmLHY* (*late elongated hypocotyl*). On the other hand, sequences of the *TmPUMILIO* (RNA-binding protein) and *TmLUX* were subjected to screen a barley TILLING population of the cultivar Barke, resulting in 34 confirmed mutations for *HvPUM* (including one nonsense mutation) and 39 putative mutations for *HvLUX*. Future analysis may reveal the phenotypes of the putative TILLING mutants in barley. The better candidate, *TmLUX* also was resequenced in a collection of 96 diploid and tetraploid wheats revealing a single A-genome-specific haplotype with a unique 21nt deletion found in the MYB domain. The Chinese cultivar Yunnan possessing this mutation was heading relatively early, thus supporting *TmLUX* as a sensible candidate for *Eps-3A^m*. Time course qRT-PCR on this accession as well as on transgenic putative knock-down lines will be performed to verify the *TmLUX*–circadian clock–earliness *per se* connection.

Poster 28. The pleiotropic effects of the master regulator Q and its homoeologous loci in polyploid wheat.

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The *Q* gene on chromosome 5A is of major importance because it governs the free-threshing character, and also pleiotropically affects many other traits associated with wheat development and domestication. To precisely investigate the function of *5AQ*, we developed three *Q* gene near-isogenic lines (NILs) in the background of the hexaploid wheat cultivar Bobwhite (BW), which consist of the BW wild type *5AQ*, an EMS-induced *5AQ* mutant (*5AQm*) with a premature stop codon, and the recessive *5Aq* allele from *Triticum turgidum* subsp. *dicoccoides*. Phenotypic analyses confirmed the previously identified traits controlled by the *Q* gene, such as spike shape, threshability, glume toughness, plant height, and flowering time. In addition, the grain yield per plant of the *5AQ* NIL was significantly higher than the *5AQm* and *5Aq* NILs due to differences in several yield component traits. Microscopic analysis of spike tissue revealed obvious differences in cell morphology among the NILs, which likely underlies the differences in glume toughness and rachis disarticulation attributed to *Q*. To investigate potential downstream genes of *5AQ* responsible for these traits, we analyzed the expression of wheat genes homologous to known development-associated genes, such as *AGAMOUS*, *FLOWERING LOCUS T* (*FT*), *CONSTANS*, and *SHATTERPROOF*. RT–PCR showed a dramatic change in the expression of

these genes in *5AQ* compared to *5AQm* and *5Aq*, demonstrating the role of *Q* in regulating gene networks associated with wheat development. Homoeologous copies of *Q* exist on chromosomes 5B and 5D, but their functions are less understood. Phenotypic analysis of genetic stocks with various combinations of *Q* homoeoalleles indicated that *5Bq* and *5Dq* also contributed to the suppression of speltoid characters and glume toughness, but to a lesser degree than does the *5AQ*. An RQ-PCR study showed that *5Bq* and *5Dq* are transcriptionally active, but *5Bq* is a pseudogene. Combined phenotypic and expression analysis indicated the presence of complex interactions among the homoeoalleles. The evolution of the *Q*/*q* loci in polyploid wheat resulted in the hyperfunctionalization of the master regulator *5AQ*, pseudogenization of *5Bq*, and subfunctionalization of *5Dq*, but all are finely tuned in a coordinated manner to govern domestication and development in polyploid wheat.

Poster 29. Molecular mapping of the brittle rachis (Br-A1) gene in Triticum timopheevii.

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Free shattering at maturity is an important strategy in plants for seed dispersal in nature. Domestication of wheat in the Bronze Age occurred mainly due to two important evolutionary changes, namely the loss of rachis fragility at maturity and evolution of free threshability. The *Brittle rachis* (*Br1*) gene located on homoeologous group-3 chromosomes controls rachis fragility in wheat and barley. In *Triticum timopheevii* subsp. *armeniacum* (A^tA^tGG genomes, 2n=4x=28), characteristic wedge-type disarticulation occurs due to the formation of abscission zone at the base of the spikelets making them spear shaped dispersal units at maturity. Mapping of the A^t-genome, brittle rachis gene (*Br-A^{t}1*) was done in a recombinant inbred line (RIL) population of tetraploid *T. timopheevii* subsp. *armeniacum* and *T. timopheevii* subsp. *timopheevii*. The RIL population segregated 62 brittle and 73 tough rachis, fitting the monogenic segregation ratio of 1:1. Nine out of a total of 72 BAC-end derived markers from wheat 3AS BAC library and 8/24 SSRs from the publically available databases were found to be polymorphic between the parents. The gene was mapped to a 7.4-cM region on short arm of chromosome $3A^t$ flanked by microsatellite markers *Xgwm2* and *Xbem44*. Previously this gene was located in a 35-cM region on chromosome $3A^tS$ between RFLP markers *XksuA6* and *Xpsr1196*. The *T. timopheevii* subsp. *armeniacum Br-A^{t}1* gene may be orthologous to the hexaploid wheat *Br1* locus. Five recombinants were found in the RIL population for these two markers, and 30 recombinants for the closest markers were found in a set of 355 individuals of $F_{2:3}$ families segregating for *Br-A^{t}1*. Heterogenous inbred families from the recombinants for the closest flanking markers are being used for fine mapping. Primers are being designed, tested, and mapped from syntenous regions of rice chromosome 1 and *Brachypodium* chromosome 2. Resources available for chromosome 3A sequencing will be used for the fine mapping and cloning.

Poster 30. Metabolic, physiological, and molecular characterization of cuticular variation in wheat.

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All the aerial organs of land plants are covered with a layer of hydrophobic lipids, termed the cuticle. It plays important roles in development, growth, and defense against biotic and abiotic stresses. Structurally, the cuticle consists of a cutin frame, with intracuticular wax embodied in and an epicuticular wax overlaid on them. A field of densely distributed, epicuticular wax crystals is visible as bloom or glaucousness. In wheat, variation of glaucousness is mainly controlled by wax-production genes *W1* and *W2*, and wax-inhibition genes *Iw1* and *Iw2*. *W1* and *Iw1* are located on the short arm of chromosome 2B (2BS), and *W2* and *Iw2* are on 2DS. Although early field studies showed glaucousness played an important role in drought and heat tolerance, little is known about the molecular mechanisms and metabolic products of these wax genes. We are characterizing a set of six wax near-isogenic lines (NILs) in an S-615 background by combining the genetic, metabolic, physiological, and molecular approaches. Wax profiling by GC-MS indicated that the wax load and composition vary greatly among the NILs. The *Iw2*-NIL has highest wax load, followed by *Iw1*-NIL, S-615, *W2*-NIL, *W1*-NIL, and *w*-NIL. Wax profiles suggested that (1) gene *W2* plays an important role in decarbonylation pathway for n-

heptacosane formation; (2) *Iw2* inhibits fatty acid chain elongation from C_{24} to C_{26} and C_{28} and leads to accumulation of tetracosan-1-ol and absence of n-heptacosane; and (3) carbon chain length of wax species is important for glaucousness formation. Physiological studies showed that the nonglaucous NILs (*w*-NIL, *Iw1*-NIL, and *Iw2*-NIL) showed significantly higher rate of water loss and chlorophyll bleaching than S-615, suggesting the wax composition rather than wax load is important in determining cuticle permeability and glaucousness. Expression profiling of 32 genes involving cuticle biosynthesis, transport, and transcription regulation indicated that (1) *Iw1*, *Iw2*, *W1*, and *W2* employ different molecular mechanisms affecting cuticle biosynthesis are different; and (2) interactions between *W1* and *W2* are either additive or nonadditive. Combined analysis of metabolic and expression profiles suggested that several wheat homologs, including *CER4* and *WSD1*, are functionally different from their *Arabidopsis* counterparts most probably due to gene duplication and subsequent subfunctionalization.

Poster 31. Difference in vernalization duration requirement in U.S. soft winter wheat is associated with variation in VRN1 genes.

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In winter wheat (*Triticum aestivum* L.), the timing of flowering is a crucial trait that allows the plant to fill grain during favorable conditions of spring. The timing of flowering initiation is governed by the action two main, environmentally controlled groups of genes; vernalization that defines a plant's requirement for a prolonged exposure to cold temperatures and photoperiod sensitivity defining the need for a long days to initiate floral transition. Genetic variation in both vernalization and photoperiod sensitivity allow wheat to be grown in extremely diverse environments. In the United States, winter wheat occupies more than 70% of the wheat acreage and is grown in environments with large differences in mean temperatures during winter, as well as day length at flowering. Vernalization-induced flowering is controlled by the *VERANALIZATION1* (*VRN1*) genes located on chromosomes 5A, 5B, and 5D and dominant alleles confer spring habit. Variations in *vrn-A1* were reported to be associated with early stem elongation during the floral transition. In this study, we evaluated the effect of vernalization duration (8 and 4 weeks) on flowering time of 130 recombinant inbred lines of a population generated from a cross between two soft winter wheat cultivars (carrying *vrn-A1b*, late stem elongation allele), AGS2000 and Neuse. After vernalization treatments, plants were grown in a controlled environment under long photoperiod. We identified a major locus for flowering time in the 4-week vernalization treatment in the region where *vrn-B1* resides in chromosome 5B. This region did not have a significant effect on flowering time in plants that were fully vernalized (8-week treatment). QTL for early spring growth and heading date were observed in the *vrn-B1* region when the population was evaluated in the field during 2012. Therefore, we conclude that variation in the recessive *vrn-B1* allele is important for adaptation of winter wheat to diverse environments.

Poster 32. Development of high throughput KASPar assays for the grain color loci in wheat.

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Grain color of is an important trait in wheat that is one of the defining characteristics of market class and end-use. Wheat seed color is controlled by three homoeologous genes, *R-A1*, *R-B1*, and *R-D1*, located on the long arms of chromosomes 3A, 3B, and 3D, respectively. The *R* loci interact epistatically with red color being dominant and increased number of red alleles leading to darker red color. White kernels result when the recessive alleles are present at all three loci. Wheat having both red and white grain is considered of mixed market class; therefore, purity of seed color is an important objective of wheat breeders and seed producers. However, it can be difficult to visually distinguish the red and white grain as seed

color can be influenced by environment. We developed high-throughput, KASPar assay for genotyping the *R-A1*, *R-B1*, and *R-D1* loci. The sequence information of the *Tamyb 10-A1* gene (Genbank no. AB599721 / AB191458), *Tamyb 10-B1* gene (Genbank no. AB599722 / AB191459), and dominant *Tamyb10-D1* gene (Genbank no. AB191460) were used from NCBI for designing allele-specific primers for the KASPar assay. These assays were applied to 672 U.S. wheat cultivars and breeding lines from different market classes. Complete agreement was found when genotypes determined with the new KASP assays were compared with results of the previously reported, gel-based markers and/or visual assessment of grain color as either red or white. All white wheat cultivars were determined with the KASP markers to have recessive alleles at all three loci, and the frequency of white wheats was 32%. The frequency of one-, two-, and three-gene red alleles was calculated for the red wheat lines. The most common red allele was *R-D1b*, which was present in 43% of the lines, followed *R-A1b* and *R-B1b*, present in 35% and 28% of the lines, respectively. Only 8% of cultivars had dominant alleles at all three loci. In conclusion, this diagnostic KASPar assay for scoring one-, two-, or three-gene red seed color genotypes provides a great advantage in marker-assisted selection over gel-based PCR markers by reducing cost and time in evaluating thousands of individuals along with low error rate.

Poster 33. Quantitative trait loci mapping of transgressive agronomic and quality traits in an elite by elite wheat recombinant inbred line population.

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In this study, we investigated a population of 129 recombinant inbred lines (RIL), developed from a cross between the cultivar Steele-ND and an elite line ND 735, to identify QTL controlling several yield and quality traits. Phenotypic data were collected from four North Dakota environments for days-to-heading, plant height, spike density, spike length, grain yield, grain volume weight, 1,000-kernel weight, kernels per spike, kernel size distribution, protein, flour extraction, kernel hardness, kernel diameter, and mixograph peak time. Strong transgressive segregation was observed for all traits, with some RILs outperforming the best commercial varieties. Using a linkage map of 392 markers, composite interval mapping (CIM) identified a total of 13 environment-specific QTL. All QTL explained large phenotypic variation (R^2 = 16–44%) as expected for loci determining transgressive segregation. Two major QTL, one each on chromosome 5A and 6B, affected three yield-related traits and provided up to 252 kg/ha additional yield. Many QTL have been identified before for yield and quality, but their polygenic nature has often prevented successful marker-assisted selection. In this study, we employed directly the identified QTL for within population selection, in an extension of the concept of 'map as you go'.

Poster 34. A major QTL for gluten strength in eurum wheat (Triticum turgidum L. var. durum).

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Gluten strength is an important characteristic in determining the end product quality of durum wheat semolina. In order to identify the genetic basis of gluten strength in North Dakota durum wheat cultivars, a doubled-haploid mapping population was developed from the cross of the low-gluten cultivar Rugby and the high-gluten cultivar Maier. A framework linkage map consisting of 228 markers was constructed and used with phenotypic data on gluten strength (measured by sedimentation volume) to conduct single- and two-locus QTL analyses. Only one consistent QTL (*QGs.ndsu-1B*), contributing up to 90% of the phenotypic or 93% of the genotypic variation, was detected on 1BS. No 'QTL×QTL' or 'QTL × environment' interactions were observed. *QGs.ndsu-1B* was flanked by two DArT markers that were converted to STS markers and used along with SSR and EST–SSRs to develop a map of 1BS. QTL analysis delineated *QGs.ndsu-1B* in a 7.3-cM region flanked by an STS marker locus, *Xsts-wPt2395*, and an SSR marker locus, *Xwmc85*. The adapted back-

ground of this material and availability of PCR-based markers closely associated with this locus represent invaluable resources for marker-assisted introgression of gluten strength into other durum wheat varieties. A single QTL segregating in this population also makes it an ideal target for map-based cloning.

Poster 35. Towards positional cloning of QYLD.IDW-3B, a major QTL for grain yield in durum wheat.

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In durum wheat, a major QTL (*QYld.idw-3B*) for plant height, peduncle length, stay-green, leaf greenness, 1,000-kernel weight, and grain yield *per se* (i.e., not due to difference in flowering time) across a broad range of soil moisture regimes was identified in an RIL population derived from Kofa and Svevo (Maccaferri et al. 2008. Genetics 178:489-511), two high-quality, elite cultivars well adapted to Mediterranean environments. The fine mapping of *QYld.idw-3B* is underway in the framework of the FP7 TriticeaeGenome project (http://www.triticeaegenome.eu). In this regard, three pairs of NILs with contrasted parental haplotypes at the target region were crossed to produce approximately 7,500 F_2 plants that were screened for the identification of recombinants within the 11-cM interval between *Xgwm389* and *Xgwm493* that flanked the *QYld.idw-3B* peak. In 2011, 233 informative, homozygous $F_{4:5}$ segmental isolines were evaluated in the field and profiled molecularly. To increase the map resolution in the target region, new polymorphic markers were identified by exploiting the sequence information produced from the assembly of the chromosome-3B physical map of bread wheat. A total of 50 new markers (BAC ends-derived SSR, ISBP, and SNP markers) have been added to the target interval. All markers were anchored to the Chinese Spring physical map of chromosome 3B, thus allowing us to identify the BAC contigs that span the QTL region. A high-resolution map has been obtained with an average marker distance of approximately 0.25 cM. *QYld.idw-3B* has been confined to a 1-cM interval spanned by contig 954 of Chinese Spring, which contains 10 genes. The functional characterization via transcriptomics of these genes is underway. The haplotype at this target region is being investigated in a collection of 189 elite genotypes suitable for association mapping studies (Maccaferri et al. 2011. J Exp Bot 62:409-438).

Poster 36. Association studies of Ppo-A1 and Ppo-D1 genes and polyphenol oxidase activity using an Argentinean hexaploid wheat panel.

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Polyphenol oxidases (PPOs) are enzymes involved in the browning of fruits, vegetables, and cereal products, which negatively influence their marketing value. These enzymes are very important to the wheat industry because they are implicated in the detrimental and time dependant browning of various products especially Asian noodles and Middle East flat breads. In this work, we determined the genetic variability of the *Ppo-A1* and *Ppo-D1* genes using functional markers and evaluated its effect on the polyphenol oxidase activity using an Association Mapping (AM) approach with a panel of 97 Argentinean bread wheat cultivars. The AM panel was grown at Marcos Juarez, Argentina (32º 41' S and 62º 09' W) in the 2009–10 and 2010–11 growing seasons using hill plots in a completely randomized block design with two replications. PPO enzymatic activity was measured in each cultivar using the L-DOPA standard assay. *Ppo-A1* and *Ppo-D1* variability was determined using functional markers previously described. To minimize spurious associations, a mixed lineal model (Q+K) was used to account for population structure and kinship relatedness of individuals among 97 entries. A genetic structure (Q) and kinship matrix (K) among the 97 cultivars was inferred using 17 nonlinked, gene-based, molecular markers and HMW-glutenin storage proteins (three loci). The genetic structure was determined using STRUCTURE-2.3 and Structure Harvester with a number of subpopulations estimated in three (Q=3). The relative

kinship matrix (K) was calculated using SPAGeDi-1.3c. Analysis of *Ppo-A1* and *Ppo-D1* variability showed 76.28% of cultivars carrying the *Ppo-A1a* allele and the remaining 23.71% with *Ppo-A1b*. In the case of *Ppo-D1*, 31.95% of the cultivars showed the *Ppo-D1a* allele and 68.05% presented *Ppo-D1b*. This is the first characterization of the genetic variability of *Ppo* genes in Argentinean hexaploid wheat germplasm. The association analysis showed that variation at the *Ppo-A1* locus was significantly related with PPO activity ($P = 0.0011$), with *Ppo-A1b* allele producing lower PPO activity (9.72 U) than *Ppo-A1a* (13.76 U). The *Ppo-D1* locus did not show significant association with the PPO activity in this AM panel ($P = 0.0943$). These results positioned *Ppo-A1b* allele as a valuable genetic tool to reduce PPO activity in the Argentinean bread wheat breeding programs.

Poster 37. Characterization of the effect of single and double GPC-A1 and GPC-D1 mutations in hexaploid wheat.

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Plant senescence is a tightly regulated process designated to minimize the loss of minerals by maximizing remobilization to developing organs (e.g., seeds). The individual mechanisms and regulatory networks that define senescence are still poorly understood. In wheat, most of the nitrogen accumulated in the grain was already present in the plant at anthesis and is remobilized to the grains during maturation. Therefore, attempts to understand nutrient remobilization must consider senescence as an integral part of this complex process. Recently, the existence of a close connection between these two processes was shown through the map-based cloning of a wheat *GPC* (*Grain Protein Content 1*) gene. The *GPC-B1* gene encodes a NAC transcription factor associated with earlier senescence and increased grain protein, iron and zinc content in wheat. Recombinant inbred lines (RILs) of durum wheat carrying the functional allele from wild emmer wheat senesced 4–5 days earlier and had 5–10% higher grain protein, iron and zinc concentrations. In the current research, we have identified 'loss of function' ethyl methane sulphonate (EMS) mutants for the two homeologous genes, *GPC-A1* and *GPC-D1*, in hexaploid wheat. The mutants and control lines were grown under field conditions at four locations in Israel and characterized for their senescence patterns, *GPC*, and yield components. Our results showed a delay of senescence in both *gpc-A1* and *gpc-D1* mutants and a greater effect in the double mutant, *gpc-A1*/*gpc-D1*. Complete senescence of the single *gpc-A1* and *gpc-D1* mutants was delayed 10–20 days relative to the wild type control, and the difference increased to almost 70 days in the *gpc-A1*/*gpc-D1* double mutants. Grain protein content measurements in all mutants were lower then in wildtype plants, whereas grain yield was the same for all the tested genotypes suggesting the existence of different gene regulation for the accumulation of carbohydrates and minerals in the grain.

Poster 38. Development of Thinopyrum distichum-based, hexaploid tritipyrums.

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Thinopyrum distichum (Thunb.) Löve ($2n = 28 = J_1^dJ_1^dJ_2^dJ_2^d$) is a highly salt-tolerant, perennial grass that is indigenous to the shoreline of Southern Africa where it grows within the spring high-tide zone. It is rhizomatous, exhibits facultative apomixis, and occupies highly saline coastal sands with low fertility, limited soil water, and high pH. Due to its adaptation to adverse environmental conditions, the grass has previously been targeted for gene mining and transfer to durum and common wheat, rye and triticale. It is furthermore a segmental autotetraploid and, in partial polyhaploids ($-J_1^dJ_2^d$), its two genomes show a high degree of meiotic pairing. Two lineages of plants with 2n=42 chromosomes that are presumed to have the genomic composition AABBJJ, were selected from segregating generations of crosses among primary and secondary *Triticum turdidum* subsp. *durum* / *Th. distichum* amphiploids. The J genome in each lineage is assumed to consist of seven *Thinopyrum* chromosomes. Because the plants are well developed, highly fertile, and produce well-developed seeds, their J genomes probably comprise full sets of homoeologous chromosomes, with each individual chromosome having been derived from either of the J_1^d or J_2^d genomes and altered through recombination with its homoeologue. In seedling salt-tolerance tests, the hybrids had high levels of salt tolerance comparable to those of the *Th. bessarabicum*-based tritipyrums that were developed at the John Innes Centre. One of the selections is free-threshing and without the brittle rachis trait.

Poster 39. Mapping QTL related to drought tolerance in durum wheat (Triticum turgidum L. subsp. durum).

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Durum wheat (*Triticum turgidum* L. subsp. *durum*) is largely grown in Mediterranean environments where drought stress affects grain yield and yield stability. Mapping quantitative trait loci (QTL) in segregated populations allows the detection of chromosome regions controlling traits of agronomic interest with the opportunity to dissect complex traits. To understand the genetic basis of quantitative traits involved in drought stress tolerance, 130 F_6 recombinant inbred lines (RILs) , derived from a cross between the cultivar Zardak and genotype 249 (a local variety and a genotype of the Kermanshah province, Iran, respectively) were analyzed with a total of 85 microsatellite markers. Most of the markers were mapped to the 13 linkage groups (83.5%). Interval mapping was employed for QTL detection using a linkage map sequence repeat (SSR) markers. Chromosomal regions with QTL were identified for several traits related to drought tolerance. The identified genomic regions controlling wheat traits can be targeted during further studies for their genetic dissection.

Poster 40. Expression analysis of genes involved in gibberellin biosynthesis under drought stress in wild emmer wheat.

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Drought tolerance mechanisms play an important role in plant adaptation to arid environments. Aiming to identify candidate genes for drought resistance, we recently have compared the transcriptome of drought resistant (R) vs. susceptible (S) genotypes of wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*) under water stress. These studies revealed differential expression of genes involved in metabolism and signaling of absicic acid (ABA), auxin (IAA), and gibberellin (GA) in response to drought stress. However, while ABA- and IAA-related genes had a similar pattern of expression in leaves and roots, some of the GA-related genes showed opposite expression pattern in the two tissues. GA is an essential hormone involved in many aspects of plant development, but it is not recognized as an important hormone in relation to plant stress response. In order to clarify whether modulation of specific genes in the GA metabolic pathway play a role in adaptation to drought stress, we studied the expression pattern of additional genes encoding for enzymes in the GA biosynthetic pathway. Gene expression was tested in leaves and roots of the R genotype after 3, 5, and 7 days of withholding water and in well-watered plants. The analysis showed complex pattern of gene expression, in which genes functioning at the beginning and at the end of the pathway were differentially expressed under drought in the roots, confirming the idea of different GA roles in leaves and roots. In addition, the promoter regions of the GA-related genes and GA-receptor gene (*GID1*) were scanned for abundance of transcription factors binding sites (TFBSs). The promoter regions of these genes were obtained by BLAST of the cDNA sequences against the survey sequence of wheat (IWGSC Survey Sequencing Initiative). Interestingly, promoters of the differentially expressed genes were enriched with TFBSs related to abiotic stress (e.g., DREB/CBF/ERD/COR). Our results are in agreement with recent publications showing that the cross-talk of GA with ABA and IAA contributes to developmental plasticity of root systems under water stress. These processes result in morphological changes that extend the water-absorbing surface area of the roots, including the increase in rooting depth.

Poster 41. Transcript profiling identifies novel transcripts with unknown functions as a primary response components to dehydration stress in wheat (Triticum aestivum L.).

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Dehydration is among the major abiotic stresses that adversely impacts crop productivity and plants often display cultivar-dependent response. For understanding the molecular mechanism underlying differential dehydration tolerance of two wheat (*Triticum aestivum* L.) cultivars with contrasting stress adaptability, we compared transcriptomic changes occurring at early onset of dehydration and identified 107 nonredundant transcripts. Of these, most had unknown functions (~30%), signifying existence of putative novel wheat-specific genes reported here for the first time. Through macroarray analysis, ~64% clones were shown to differentially express (≥ 3-fold) in response to dehydration and some highly upregulated known or unknown function transcripts was further confirmed by quantitative RT–PCR. Upon comparing with previous transcriptomic studies, 40% of these 107 dehydration stress-specific transcripts were found not to be documented. These new transcripts may therefore signify unexplored gene sources for specific responses to short-term dehydration stress in wheat. Expression analysis of the unknown function transcripts also revealed tissue- and other stress-specific differentially regulation. Comparative *in silic*o mapping of 107 wheat transcripts against the available mapping data of rice (40; ~37%), maize (34; ~32%) and sorghum (33; ~31%) revealed wheat orthologous sequences to be present in the respective cereals. This study provides interesting account of several unknown genes that in addition to genes with known functions may regulate stress dynamics and thus may be used as future candidates to improve stress adaptability through genetic manipulation.

Poster 42. Association mapping of Russian wheat aphid resistance in barley as a method to identify diversity in the National Small Grains Collection.

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Russian wheat aphid (RWA) infestations of barley cause chlorotic leaf spotting and streaking, and prevent unrolling of leaves which traps spikes and reduces grain yield. Resistant accessions identified in the NSGC were used to develop adapted, resistant germplasm and cultivars. This study identified loci affecting RWA resistance and diversity in the NSGC using association mapping. Resistant and susceptible accessions, breeding lines, and cultivars were genotyped with DArT markers and phenotyped for RWA responses. A core set of nine markers explained 83% of the variation for chlorosis. Most resistant and susceptible accessions had opposite genotypes at each of these markers. The six susceptible adapted cultivars were exceptions and shared the haplotype of the resistant accessions. Variability at four additional loci associated with resistance did not sufficiently explain phenotypic variability between resistant accessions and susceptible cultivars. Examining subsets of the data identified six additional markers associated with RWA response, which discriminated between resistant accessions and susceptible cultivars. Additional investigation is necessary to better understand the genetics of RWA resistance. However, this study provided useful information on diversity in the NSGC, and suggested that RWA resistance is a complex trait that may share physiological components with other characteristics that were selected during domestication.

Poster 43. High-density mapping of a Russian wheat aphid resistance gene: chromosome survey sequences in use.

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The Russian wheat aphid (RWA), *Diuraphis noxia*, has become a serious world invasive pest of small grain cereals. Several *D. noxia* strains (biotypes) varying in virulence have spread in all wheat- and barley-growing areas with the exception of Australia. Numerous genes contributing to RWA resistance were found in various wheat lines. A dominant gene, *Dn2401*, identified in CI 2401 resistant to both *D. noxia* U.S. biotypes 1 and 2 was mapped on the short arm of the wheat chromosome 7D (7DS). Development of tightly linked markers and isolation of the resistance gene will facilitate marker-assisted breeding and/or direct gene transfer by molecular methods. To facilitate positional cloning of this gene in a complex polyploid wheat genome, we employ chromosome-based resources such as 7DS-specific BAC library, a 7DS physical map, and survey sequences of wheat group-7 chromosomes. Furthermore, a synteny-based tool GenomeZipper enabling virtual ordering of cereal genes as well as a newly constructed high-density *Ae. tauschii* linkage map assist us in a highly focused saturation of the map within a 2.5-cM interval delimited by available microsatellite markers. Moreover, annotated syntenic build of 7DS (http://www.wheatgenome.info) facilitates searching for candidate genes within the region of interest. This work has been supported by Czech Science Foundation (grant award P501/12/2554), Internal Grant Agency PrF-2012-001 and Australian Research Council (grant award LP0882095).

Poster 44. Towards fine mapping and cloning of the Hessian fly resistance gene, H13.

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Wheat (*Triticum aestivum* L.) is a widely adopted crop planted all over the world and provides nearly 55% of the carbohydrates consumed. The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a major pest of wheat worldwide and causes 5–10% loss in wheat production. Hessian fly larvae cause damage to wheat by feeding between leaf sheath. Major symptoms include stunted growth, weak stems, reduced grain fill, and reduction in yield. Resistance genes in wheat have been the most effective and primary source for controlling Hessian fly damage. *H13* is a dominant resistance gene, which confers stable level of antibiosis against a wide range of Hessian fly biotypes. The *H13* gene is derived from KU2076 (*Aegilops tasuchii* (TA2452)). In previous studies, *H13* was mapped to the distal arm of chromosome 6DS, proximal to the breakpoint of del 6DS-6 (FL 0.99) and was found to be co-segregating with marker *Xcfd132* and flanked by *Xgdm36* at 2.7 cM (Liu et al. 2005. Theor Appl Genet 111:243-249). We have developed a high-resolution mapping population of 1,368 F_2 individuals derived from the cross between PI372129 (*Dn4*) and PI562619 (Molly, *H13*). The population was genotyped with linked co-dominant microsatellite markers *Xcfd132* and *Xgdm36* (2.7 cM distal to *H13*). Eighty-nine recombinants were observed. The F_2 plants from which the DNA was extracted for marker studies were self-pollinated to produce F_3 seeds. Around 30 F_3 seeds of each recombinant F2 plant along with Molly, Newton, *Dn4*, Karl 92, and Cladwell as controls were evaluated for phenotypic reaction following Hessian fly infestation. At the 1.5 leaf stage, seedlings were infested and 3 weeks post infestation, susceptible and resistant plants were characterized based on stunting in the compatible interactions and normal growth in the incompatible interactions respectively. *H13* was flanked by *Xcfd132* at 1.53 cM and *Xgdm36* at 2.2 cM. Three-dimensional BAC pools developed from minimal tiling path of a chromosome 1D, 4D, and 6D FPC assembly were screened for flanking markers (*Xcfd132*, *Xcfd213*, and

Xgdm36). Three independent BAC contigs were identified and shotgun sequence from selected BACs in these contigs is being used to develop new markers for fine mapping *H13*.

Poster 45. Towards map-based cloning of Hessian fly-resistance gene H26 derived from Aegilops tauschii.

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Hessian fly (*Mayetiola destructor* (Say)) is one of the most important insect pests of wheat. Deployment of resistance genes in wheat cultivars is the most effective measure to control Hessian fly. Among the 33 *H* genes that confer resistance to Hessian fly, *H26* derived from *Aegilops tauschii*, is highly effective against several of the world's most virulent Hessian fly populations. This gene was previously mapped to the wheat chromosomal deletion bin 3DL3-0.81-1.00 in a synthetic hexaploid wheat. This study attempted to isolate *H26* through map-based cloning. In this research, we developed a mapping population of approximately 3,000 F_2 individuals derived from the cross between the *Ae. tauschii* accession CIae 25, having a resistance allele at the *H26* locus, and the *Ae. tauschii* accession AL8/78, having a susceptible allele at this locus. We conducted high-resolution mapping of *H26* in this population and developed several markers within 0.1 cM from *H26*. Using a pair of flanking markers, we identified the *Brachypodium* genomic region that is collinear with the region harboring *H26*. An *Ae. tauschii* BAC contig was identified by blasting the *Ae. tauschii* AL8/78 BAC library with the 20-kb collinear *Brachypodium* sequence. Six BAC clones in the middle of the contig were further analyzed using two flanking markers. One BAC clone that was positive for the two flanking markers was identified and it is currently being sequenced. The *H26* locus will be delimited using the markers developed based on the BAC sequence, and will be confirmed by transformation and expression analysis.

Poster 46. Introgression of crown rot resistance from hexaploid wheats into durum wheats.

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Triticum turgidum subp. *durum* (tetraploid durum) germplasm is very susceptible to crown rot, caused by the fungus *Fusarium pseudograminearum*. Partial resistance to this disease has been identified in a number of *T. aestivum* (hexaploid wheat) lines, such as 2-49 and Sunco. As these two wheat species are closely related, genes can be transferred between them. This study discusses the introgression of partial crown rot resistance from hexaploid wheat into durum wheat. Results will be presented on the cytogenetics of these crosses and the screening of the progeny for crown rot resistance. A number of different *T. aestivum* x *T. turgidum* crosses were investigated using DArT markers to determine the inheritance of parental A-, B-, and D-genome material in subsequent generations derived from these crosses. Significant variation was observed among individual crosses in the proportions of A-, B-, and D-chromosomal segments inherited from the hexaploid parent. In particular, while several early generation populations retained a significant proportion of D-genome material from the hexaploid parent, other equivalent populations from different crosses contained only tetraploid lines entirely lacking D-genome segments. Seven derived tetraploid lines showing improved resistance to crown rot over F_5, F_6, and F_7 generations were backcrossed to a range of durum parents. Results will be presented for two BCF_2 populations screened for crown rot resistance in the field during 2011.

Poster 47. QTL analysis of Fusarium head blight resistance in two durum wheat backcross derived inbred line populations.

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Host–plant resistance is recognized as the most effective means of controlling Fusarium head blight (FHB) infection. Resistant FHB varieties in hexaploid wheat have been released; however, the progress toward the same goal in durum (*Triticum turgidum* subsp. *durum* (Desf.) MacKey) wheat has been limited. Sources of resistance in durum wheat are few and transferring the resistance genes from hexaploid wheat have met with limited success. The new sources of resistance in Tunisian durum wheat show a promising amount of resistance compared to hexaploid wheat sources. To incorporate the new sources of FHB resistance, two populations of 174 and 171 backcross-derived, inbred lines (BC_1F_6) were developed by crossing Tun108 with durum wheat cultivars Ben and Lebsock. Both populations were evaluated for type-II FHB resistance for two seasons in the greenhouse and two seasons in a field nursery. The analysis of variance for type-II FHB resistance showed significant effects for different environments, different genotypes, and also the 'genotype x environment' interactions (GxE). We observed transgressive segregation for FHB resistance genes in both populations and some progenies were even better than the well-known, resistant hexaploid wheat Sumai 3. A total of 280 polymorphic DArT markers were used for genotyping 168 BC_1F_7 lines of a 'Tun108/Ben//Ben' population. At a minimum LOD of 3, a total of 274 markers were mapped to 201 unique loci belonging to all 14 chromosomes. These markers representing 201 loci covered a genetic distance of 1,555.4 cM with an average distance between any two marker loci being 7.74 cM. Six different QTL were identified in 'Tun108/Ben//Ben' population located on Chromosome 1B, 5A, 5B, 7A, and 7B. The QTL on 5A and 7A were both effective in the field and greenhouse and explain ~9% of total phenotypic variation and 22.5% of genetic variation in the 'Tun108/Ben//Ben' population. One of the QTL from chromosome 1B was inherited from Ben, and the other QTL were from the resistant parent Tun108.

Poster 48. Use of generation acceleration to enhance the transfer of Fusarium head blight resistance into hard red winter wheat.

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A new, winter wheat breeding program has been established at North Dakota State University in response to a growing need for better-performing, regionally adapted cultivars. Economic losses from winter wheat disease susceptibility impact North Dakota annually with leaf rust, Fusarium head blight (FHB), and tan spot ranking among the most damaging diseases. This situation can be offset through breeding tolerant or resistant cultivars.

This project aims to speed up the transfer of FHB resistance QTL from spring wheat by first developing intermediate, semidwarf, FHB-resistant winter wheat inbred lines with reasonable levels of cold-hardiness. Such lines can then be used in crosses with well-adapted, winter-hardy, elite germplasm. Starting material were the F_1 of crosses between winter wheat and ten spring wheat varieties/lines carrying one or more of the FHB resistance QTL: *Fhb1*, *Fhb2*, *Ofhs.ifa-5A*, a Frontana-derived locus on 3A, and two 5A loci derived from PI277012. Doubled-haploid (DH) and/or modified single-seed descent (SSD) inbred lines are being developed for each of the crosses. The material is being selected for winter type, presence of the semidwarfing gene, *Rht-B1b*, and the targeted FHB resistance gene(s) during inbreeding (SSD) or at the completion of the process (DH). Marker-aided selection will be applied where possible to enhance the identification of suitable progenies. The F_5-derived, F_6 inbred lines and DH populations will then be field-tested to identify those with elevated levels of cold-hardiness, better phenotype, and FHB resistance. Selected lines will be used as parents in the pedigree breeding program. Compared to backcrossing, this approach could be quicker and simultaneously avoid a narrow genetic base inherent to the use of a small number of recurrent parents.

Generation acceleration methodology will be developed in parallel to speed up SSD inbreeding. Factorial experiments to determine which stresses and/or hormonal treatment combinations can be applied to limit and hurry plant development are being executed and the results applied to the SSD procedure. The ultimate aim is to achieve three generations of winter wheat per year. Stresses will include, but not limited to small soil volume, extension of daylight hours, elevated temperature, premature harvesting and drying, and prematurely breaking seed dormancy.

Poster 49. QTL mapping of adult-plant resistance to Stagonospora nodorum leaf blotch in bread wheat line 'Shanghai-3/Catbird'.

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Leaf blotch, caused by *Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*), is a severe disease on wheat in Norway and other wheat-producing areas with a temperate and rainy climate. Insufficient resistance in the currently grown cultivars causes severe grain shriveling and reduced yield in years with favorable conditions for the disease. As an alternative to fungicide applications there is a need to improve the levels of resistance. The bread wheat line 'Shanghai-3/Catbird', from the wheat breeding program at CIMMYT, which is currently utilized as a source of non-Sumai 3 resistance to Fusarium head blight, was identified in our field nurseries to exhibit high levels of resistance to *Stagonospora nodorum* leaf blotch. The objective of the present study was to identify the main genetic factors behind the resistance in 'Shanghai-3/Catbird'.

A population of 168 recombinant inbred lines (RILs) from the cross of 'Shanghai-3/Catbird' with the susceptible spring wheat cultivar Naxos was tested in hillplot trials naturally infected with *S. nodorum* across two locations in southeastern Norway during the 2009, 2010, and 2011 growing seasons. Leaf blotch severity was scored as percentage of diseased leaf area based on the whole canopy. To avoid confounding effects of earliness, the severity data was regressed against days to maturity and the resulting maturity-corrected leaf blotch severities were used for the QTL analysis. Preliminary QTL mapping results indicate that 'Shanghai-3/Catbird' carries major disease-reducing alleles on chromosomes 2DL and 7DS, and Naxos contributes resistance alleles on 3BL and 5BL. Work is currently under-way to saturate the linkage map with markers surrounding the known toxin-sensitivity loci *Tsn1*, *Snn1*, *Snn2*, *Snn3*, and *Snn4*, in order to determine their potential involvement in the leaf blotch resistance segregating in this population.

Poster 50. Identification and genomic mapping of three new Stagonospora nodorum blotch susceptibility genes in wheat.

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Stagonospora nodorum is a necrotrophic fungal pathogen of wheat that causes the disease Stagonospora nodorum blotch (SNB). The wheat–*S. nodorum* pathosystem involves pathogen-produced necrotrophic effectors (NEs) (formerly known as host-selective toxins) that are recognized by corresponding host genes to confer disease susceptibility. To date, five host gene–NE interactions have been reported in the wheat–*S. nodorum* system, all of which play significant roles in the development of SNB. Here, we present the identification and mapping of three additional wheat genes that confer sensitivity to different NEs produced by *S. nodorum*. One NE sensitivity gene (temporarily designated *Snn4B*) was identified in the durum wheat variety Lebsock and mapped to the long arm of chromosome 4B in a population of doubled haploids. Evaluation of SNB in this population indicated that the *Snn4B* locus explained as much as 53% of the variation demonstrating that *Snn4B* is a major SNB susceptibility gene. The second NE sensitivity gene (*Snn6A*) was identified in the hexaploid variety Opata 85 and mapped to the long arm of chromosome 6A in the ITMI population. This gene accounted for 20% of the variation in SNB development. Finally, a third NE sensitivity gene (*Snn5D*) was discovered in the hexaploid landrace Chinese Spring and mapped to the long arm of chromosome 5D using chromosome deletion lines. Further characterization, analysis, and marker development for these susceptibility genes is underway. This research broadens our knowledge of the wheat–*S. nodorum* pathosystem and will lead to the efficient development of SNB resistant wheat varieties.

Poster 51. Genomic analysis and fine-mapping of two homoeologous wheat genes conferring susceptibility to Stagonospora nodorum blotch.

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The necrotrophic fungal pathogen *Stagonospora nodorum* produces multiple necrotrophic effectors (NEs), also known as host-selective toxins, which interact with corresponding wheat genes in an inverse gene-for-gene manner to cause the disease *Stagonospora nodorum* blotch (SNB). In previous research, we showed that the homoeologous wheat genes *Snn3-B1* and *Snn3-D1*, located on wheat chromosome arms 5BS and 5DS, respectively, both recognize the NE SnTox3 to confer effector-triggered susceptibility. Here, we describe genome analysis and mapping results from ongoing efforts to clone the two *Snn3* genes. Saturation mapping of the genes in relatively small F_2 populations using SSRs and EST-derived markers followed by comparative analysis with the rice and *Brachypodium* genomes revealed that both the *Snn3-B1* and *Snn3-D1* regions were highly conserved with regions of rice chromosome 12 and *Brachypodium* chromosome 4. This colinearity allowed us to develop numerous additional markers to further saturate the *Snn3-B1* and *Snn3-D1* regions. Subsequent fine-mapping of both genes in large F_2 populations resolved some co-segregating markers and delineated the genes to small intervals. BAC contigs identified with flanking markers were anchored to the *Snn3-D1* genetic map. The ratio of physical to genetic distance in the *Snn3-D1* region was estimated to be 500–800 kb/cM. Because these two NE sensitivity genes are homoeologous, we can work towards cloning them in parallel and, once cloned, we can study their evolutionary history and investigate their functional roles in mediating recognition of SnTox3.

Poster 52. Characterization of natural variation in the Tsn1 gene in Aegilops speltoides.

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The *Tsn1* gene confers sensitivity to the necrotrophic effector (NE) ToxA, which is produced by the pathogens that cause tan spot and *Stagonospora nodorum* blotch on wheat. Although *Tsn1* is a susceptibility gene, it contains resistance gene-like features such as protein kinase, nucleotide binding (NB), and leucine-rich repeat (LRR) domains. Previous research indicated that *Tsn1* arose in the diploid B-genome progenitor of polyploid wheat. However, nucleotide variation in *Tsn1* is nearly nonexistent among polyploids. Here, accessions of *Aegilops speltoides* (SS genome), a close relative of the B-genome progenitor, were studied to further characterize the structure, function, evolution, and diversity of *Tsn1*. Multiple plants from each of 123 accessions were evaluated for reaction to ToxA and genotyped for presence of *Tsn1*. A total of 95 accessions were insensitive to ToxA and null for *Tsn1*, whereas the remaining 28 harbored *Tsn1* alleles and were either sensitive or insensitive to ToxA. Comparative sequence analysis of the 4,473-bp coding region from 15 sensitive *Ae. speltoides* plants revealed numerous single nucleotide polymorphisms (SNPs) compared to the *Tsn1* allele in the durum wheat variety Langdon. Among *Ae. speltoides* accessions, there were approximately the same number of nonsynonymous and synonymous mutations, but none of the nonsynonymous changes occurred within the protein kinase, NB, or LRR domains indicating the importance of these domains for *Tsn1* function. The diversity in *Ae. speltoides* allowed us to gain a better understanding of the evolution of *Tsn1*, and further studies will enhance our understanding of *Tsn1*-ToxA interactions.

Poster 53. Unraveling the complexity of the net form net blotch resistance locus on barley chromosome 6H.

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Net form net blotch (NFNB) in barley, caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* (Ptt), has a negative impact on barley production throughout the world. Resistance sources from diverse barley varieties collected around the world have been characterized and many mapped to a common locus near the centromeric region of barley chromosome 6H. The 6H locus is complex containing multiple resistance and/or susceptibility genes. Two recessive NFNB resistance genes, *rpt.r* and *rpt.k*, from the barley lines Rika and Kombar confer resistance against isolates 15A and 6A, respectively. Previous research delineated the genes to an ~3.3-cM region of barley chromosome 6H in a 'Rika/Kombar' double-haploid population consisting of 118 individuals. Utilizing genome synteny between barley chromosome 6H and *Brachypodium distachyon* chromosome 3, a ~1-Mb *Brachypodium* sequence was identified spanning the *rpt.r*/*rpt.k* region. Predicted *Brachypodium* genes were utilized to develop PCR-based molecular markers. The markers were used to saturate the *rpt.r*/*rpt.k* region in a high-resolution population developed by screening 2,973 recombinant gametes. The *rpt.r* and *rpt.k* genes were delimited to an ~0.13-cM region representing ~150 kb of *Brachypodium* sequence. Cultivar Morex BAC clones were identified using barley probes from the *rpt.r*/*rpt.k* region, and a physical map of the locus is under construction. Two orthologous barley genes with high homology to a family of *Brachypodium* leucine-rich repeat (LRR)-like genes were identified (*rpt.cg1* and *rpt.cg2*) that cosegregate with *rpt.r* and *rpt.k*, respectively, in the high-resolution map. The sequence of the *rpt.k* candidate gene (*rpt.cg2*) from Kombar has been obtained by a tailed PCR method and sequencing of the *rpt.r* candidate gene is under way. The nucleic acid sequence was determined from the full-length *rpt.k* cDNA and used to predict the protein structure. The protein contains a transmembrane domain near the N-terminus and an imperfect leucine-rich repeat region near the C-terminus. These candidate genes may support the inverse gene-for-gene model where dominant susceptibility factors interact with necrotrophic effectors that initiate cell death and necrosis resulting in susceptibility.

Poster 54. Searching via association mapping for novel sources of resistance to Ug99 and other Ethiopian stem rust races in durum wheat.

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Puccinia graminis f. sp. *tritici*, the causative agent of stem rust in wheat, is known to rapidly evolve new virulence to resistance genes. Although more than 50 stem rust resistance (*Sr*) loci have been identified in wheat, only a few remain effective, particularly against the highly virulent race Ug99 (TTKSK race) and a mixture of durum-specific races. Association mapping was deployed on 183 elite durum wheat accessions tested in four seasons under artificial inoculation in order to identify quantitative trait loci (QTL) for resistance to Ug99 and durum-specific races under field conditions. The panel was genotyped with 1,250 SSR and DArT markers (Mantovani et al. 2008. Mol Breed 22:629-648). Twelve QTL-tagging markers with R^2 values up to 11.3% were significantly associated with stem rust resistance across three to four seasons. Although some markers were linked to known *Sr* genes (e.g., *Sr9*, *Sr13*, and *Sr14*), other significant markers were located in chromosome regions where no *Sr* genes have been previously reported. The allelic variation identified at these novel QTL provides additional opportunities to deploy marker-assisted selection to improve resistance to stem rust in durum wheat grown under field conditions.

Poster 55. Construction of low-coverage, non-gridded BAC libraries for isolation of a genomic region involved in resistance to the stem rust in Sinvalocho wheat variety.

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Plant genomes are complexes regarding many aspects, their large size that often reach gigabases, their polyploidy due to their multiple hybrid origins and the high percentage of repetitive elements that may represent the majority of the genome size. Next-generation DNA sequencing (NGS) technologies have revolutionized the genomic research in several domains, as it offers the capacity to obtain large amount of sequences in a short time. However, this approach is not sufficient to decipher the high complexity of plant genomes because of their size (the wheat genome is 40 times the rice genome, 17Gb), their level of polyploidy (the wheat genome is hexaploïd) and their high percentage in transposable elements (80 % in the wheat genome). Bacterial Artificial Chromosome (BAC) libraries are still invaluable tools for plant genome analysis. They allow physical mapping, map-based cloning, and sequencing projects. They facilitate gene cloning and contribute to rapidly identify homologous genes in polyploid species. During the last decade, BAC libraries from many plant species have been constructed world wide.

The French Plant Genomic Resource Center (Centre National de Ressources Génomiques Végétales–CNRGV) is in charge of more than 9 million unique BAC samples belonging to more than 100 model and crop plant genomic libraries (http://cnrgv.toulouse.inra.fr/en/Library) and is a leader in the development of approaches involving BAC libraries to study plant genomes. In order to focus directly on a genomic region of interest in specific genotypes and rapidly isolate BAC clones spanning a genomic region, we have developed a non-gridded BAC library approach. This method avoids time and cost expensive steps of BAC clones re-arraying and screening, and may give an efficient access to sequence diversity among plant cultivars in specific genomic region. This strategy has proven to be an efficient way to identify and sequence region of interest and will be illustrated with the characterization of the region of wheat variety Sinvalocho responsible for the resistance to the stem rust disease.

Poster 56. Simultaneous transfer, genomic localization and introgression of genes for resistance to stem rust race Ug99 from the wheat D-genome progenitor species, Aegilops tauschii, to cultivated wheat, Triticum aestivum.

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The diploid, D-genome species, *Aegilops tauschii*, has provided numerous genes for resistance to fungal pathogens and insect pests of hexaploid wheat, *Triticum aestivum*. Wheat production is currently threatened by widely virulent races of the wheat stem rust fungus, *Puccinia graminis* f.sp. *tritici*, that are part of the Ug99 lineage. Screening of a large set of *Ae. tauschii* germplasm for resistance to TTKSK (Ug99) identified potentially novel sources of resistance.

To expedite TTKSK resistance from *Ae. tauschii*, we established a direct-hybridization protocol that integrates gene transfer, mapping and introgression into one process. Direct crossing of *Ae. tauschii* accessions with an elite wheat breeding line combines the steps of gene transfer and introgression while development of mapping populations during gene transfer enables the identification of closely linked markers. Direct crosses were made using TTKSK-resistant *Ae. tauschii* (2n=2x=14,DD) accessions as a male and a stem rust susceptible *T. aestivum* (2n=6x=42, AABBDD) breeding line as a female. Embryo rescue enabled recovery of F_1 (2n=28, ABDD) plants that were backcrossed as females to the hexaploid recurrent parent. Stem rust-resistant BC_1F_1 plants from each *Ae. tauschii* donor source were used as males to generate BC_2F_1 mapping populations. A bulked-segregant analysis of BC_2F_1 genotypes at 70 SSR loci across the D genome identified the chromosome locations of stem rust resistance genes and facilitated genetic mapping. Using this approach, three genes for resistance to TTKSK, located on chromosomes 1DS, 6DS, and 7DS, have been transferred from *Ae. tauschii* to *T. aestivum* and are present in genetic backgrounds suitable for stem rust resistance breeding.

Poster 57. Stem rust resistance in Jagger winter wheat.

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Jagger has been utilized widely in hard red winter wheat varieties throughout the southern Great Plains, yet the genetic basis of its stem rust resistance remains unresolved. Marker analysis and resistance to leaf rust and stripe rust demonstrate that Jagger has the chromosome 2AS *Aegilops ventricosa* segment containing resistance genes *Sr38*, *Lr37*, and *Yr17*. However, Jagger's stem rust infection types are inconsistent with the presence of *Sr38*. Seedling tests with Jagger and the stem rust differential line with *Sr38*, Trident, are similar for all races except TPMKC and TTTTF. Jagger has a high infection type to TPMKC and the *Sr38* differential has a low infection type, whereas Jagger is low and *Sr38* is high when inoculated with TTTTF. A BC_1F_3 population was developed and screened with race TTTTF. Genotyping of bulked resistant and susceptible samples was conducted using the 9,000 SNP IlluminaiSelect Bead Chip. Linkage mapping is currently underway to identify chromosomal regions associated with resistance.

Poster 58. Identification and mapping of genes expressing and suppressing resistance to stripe rust in synthetic hexaploid wheat.

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating foliar diseases of wheat resulting in 5–25% losses worldwide. Use of resistant cultivars is the best strategy to combat this disease. *Aegilops tauschii*, the D-genome donor of hexaploid wheat, is a rich source of resistance genes to different diseases. To identify potential new stripe rust resistance genes, six accessions from the WGGRC gene bank, Manhattan, KS, were evaluated for resistance to stripe rust race PSTv-46 at the seedling stage under controlled conditions. Four amphiploids were synthesized by crossing resistant *Ae. tauschii* accessions with extracted tetraploids Prelude or Thatcher. All four amphiploids, the donor *Ae. tauschii* accessions, and tetraploid Prelude and Thatcher were evaluated as seedlings for resistance to race PSTv-46. All lines, except the donor *Ae. tauschii* accessions, were susceptible to race PSTv-46. Resistance of *Ae. tauschii* was not expressed in synthetic hexaploid wheat, suggesting the presence of suppressor gene/s in the A and/or B genomes of tetraploid Prelude and Thatcher. For further genetic analysis of suppression of resistance, the amphiploid TA4161-L4, from cross between *Ae. tauschii* accession TA2435 and tetraploid Thatcher, was crossed to the wheat cultivar Lal Bahadur to produce a segregating F_2 population. Evaluation of the F_1 resulting from cross between TA4161-L4 and Lal Bahadur, 134 F_2 individuals and parents was conducted for resistance to race PSTv-46 of stripe rust at seedling stage. The F_1, TA4161-L4, and Lal Bahadur were susceptible, whereas the F_2 population segregated for resistance in 13S:3R ratio indicating presence of dominant suppressor in A and/or B genomes of tetraploid Thatcher. From evaluation of 99 $F_{2:3}$ families of this population at seedling stage with stripe rust race PSTv-46, we identified 10 families segregating for resistance gene in 3R:1S ratio with postulated genotype *Rrss*, 14 families segregating for suppressor gene in 3S:1R ratio with postulated genotype *RRSs*, 19 families segregating for the resistance gene and suppressor gene in 13S:3R ratio with postulated genotype *RrSs*, 3 homozygous resistant families with postulated genotype *RRss*, and 53 completely susceptible families with genotypes either *RRSS*, *RrSS*, *rrSS*, *rrSs*, or *rrss*. Mapping of the resistance and suppressor genes using bulked-segregant analysis is in progress.

Poster 59. Haplotype analysis of the leaf stripe resistance locus Rdg2a in different barley genotypes.

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Leaf stripe disease on barley is caused by the seed-transmitted hemi-biotrophic fungus *Pyrenophora graminea* (anamorph *Drechslera graminea*). Race-specific resistance to leaf stripe is controlled by two known *Rdg* (*Resistance to Drechslera graminea*) genes, the *H. spontaneum*-derived *Rdg1a*, mapped to chromosome 2HL, and *Rdg2a*, identified in *H. vulgare* and mapped on chromosome 7HS and cloned in the resistant cultivar Thibaut. The *Rdg2a* locus contains a gene cluster of three sequence-related coiled-coil, nucleotide-binding site, and leucine-rich repeat (CC–NB–LRR) encoding genes. However, only one gene conferred resistance to isolate Dg2, against which *Rdg2a* is effective, when the susceptible cultivar Golden Promise was transformed with the *Rdg2a* candidates. The high level of sequence similarity between the three genes most likely contributed to significant rearrangements during evolution, probably derived from unequal crossing-over resulting in sequence exchange between paralogs and in the generation of recombinant genes, as well as in expansion/contraction of gene copy number. To examine the haplotype variation at the *Rdg2a* locus, the sequencing of the allelic *Mrdg2a* (*Morex rdg2a*) locus of the Dg2-susceptible cultivar Morex was carried out and revealed large rearrangements including two deletions that generated an *Rdg2a*-homolog gene. This gene most likely derived from an un-equal crossing-over between the *Rdg2a* ancestor(s) and its paralog *Nbs2-Rdg2a*. PCR analyses performed with informative markers at eight loci within the *Rdg2a* locus identified eight different haplotypes. The Thibaut haplotype was observed to be largely conserved in Dg2-resistant barley cultivars. The resequencing of the *Rdg2a* gene in barley genotypes showing the same Thibaut haplotype or the same resistant phenotype revealed high sequence similarity to Thibaut *Rdg2a*, demonstrating the widespread conservation of the gene. Nonetheless, some sequence variation were identified in at least two barley genotypes that were verified for possible differences, with respect to *Rdg2a*, in the range of resistance specificities towards different leaf stripe isolates.

Poster 60. Identification of a novel major leaf rust resistance QTL in the Swiss winter wheat cultivar Forno.

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Leaf rust (*Puccinia triticina*) is a major wheat disease which can cause significant yield losses globally. Rapidly evolving pathogen races increase the demand for more durable and race-nonspecific resistance genes. So far only four (*Lr34*, *Lr46*, *Lr67*, and *Lr68*) such durable leaf rust resistance genes have been identified. The identification and characterization of new sources of durable leaf rust resistance is important to ensure food security in the future. We earlier identified two major leaf rust resistance loci on chromosomes 1BS and 7DS in the Swiss winter wheat cultivar Forno. The resistance on chromosome 7DS was conferred by a single gene named *Lr34* that encodes ABC transporter gene.

In this study we further characterized the second resistance locus on chromosome 1BS, named *QLrP.sfr-1BS*. This locus initially explained 28–32% of the phenotypic variance using 240 recombinant inbred lines (RILs) of the cross 'Arina/Frono'. We grouped these 240 RILs into the following categories: *+QLrP.sfr-1BS/–Lr34*, *–QLrP.sfr-1BS/+Lr34*, *+QLrP.sfr-1BS/+Lr34*, *–QLrP.sfr-1BS/–Lr34*, each having 21, 26, 22, and 18, lines respectively. The selected lines were phenotyped for the three traits, area under disease progress curve for the percentage of leaf area infected infection type, and leaf tip necrosis (LTN). The average length of LTN of only the *QLrP.sfr-1BS* group was significantly lower than the group having *Lr34*, indicating that unlike *Lr34*, *QLrP.sfr-1BS* is not associated with LTN. The groups only containing either *QLrP.sfr-1BS* or *Lr34* showed the same level of resistance, whereas the group containing both *QLrP.sfr-1BS* and *Lr34* show significantly higher level of resistance, demonstrating an additive effect of the two genes. A genetic linkage map for chromosome 1BS spanning a distance of 190 cM was constructed using SSRs and RFLP markers. Composite interval mapping detected *QLrP.sfr-1BS* with a LOD score of 17, flanked by markers wmc230 and gwm18. By adding

more polymorphic SSR markers, the target interval was reduced from 40 cM to 8 cM. To further enrich the linkage map we explore the syntenic information of the reference genomes of *Brachypodium* and wheat.

Poster 61. Fine mapping of the leaf rust resistance Lr14 locus in durum wheat.

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Leaf rust is a main disease that affects durum wheat production. Resistance to this fungal pathogen is therefore a main objective for durum wheat breeding. The leaf rust resistant allele *Lr14*-Creso from the durum wheat cultivar Creso and its derivative Colosseo is one of the most important leaf rust resistance sources present in the modern durum germplasm, and it has been located in the distal portion of chromosome 7BL (Maccaferri et al. 2008. Theor Appl Genet 91:731-738), with the identification of linked SSR markers (gwm146 and gwm344) suitable for marker-assisted selection. Our target is to fine map and eventually clone *Lr14*-Creso. To this end, a set of ~100 recombinant $BC_2F_{3:4}$ isolines were developed. Additional BC_3 isolines were developed in order to confirm and to further study the phenotypic effects of *Lr14*-Creso. New SSRs and 13 conserved orthologous sequence (COS-SNP) derived markers (UBW) were developed and mapped within an interval of 8 cM that includes the QTL peak. The COS-SNP markers have been obtained by exploiting the conserved collinearity between the most distal portions of rice chromosome 6, *Brachypodium* chromosome 1, and wheat chromosome arm 7BL. Using the coding sequence of the rice and *Brachypodium* collinear genes, the corresponding wheat orthologs were retrieved, specific PCR assays (~1 kb) targeting the intron/exon boundaries of the genes were designed, amplified on the genomic DNA of the parents Colosseo and Lloyd and the amplicons cloned in pGEM®-T Easy Vector. Sequencing of the amplicons allowed for the identification of the SNPs differentiating the two homeologous copies of each gene (genome-specific SNPs) as well as the varietal-SNPs between Colosseo and Lloyd. These SNPs were then used to develop markers that, at the same time, were 7B-specific and polymorphic between the two parents. The detailed synteny analysis and the map of the region including the newly developed markers will be reported. The results are supported by an independent association mapping study carried out using a panel of 183 elite accessions (Maccaferri et al. 2010. Mol Breed 26:189-228), which allowed us to validate the presence of *Lr14* and to further improve mapping resolution.

Poster 62. Marker development for the wheat leaf rust resistance gene Lr16 using a comparative genomic approach.

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Wheat leaf rust, caused by the fungus *Puccinia triticina*, is one of the serious diseases of wheat worldwide. *Lr16* is a widely deployed leaf rust resistance gene that is effective against the North American *P. triticina* population when in combination with *Lr34*. Previous studies mapped this gene on the distal end of wheat chromosome bin of 2BS (fraction length (FL) 0.84–1.00). In the current work, we integrated a flanking marker and additional markers that are within a close proximity to the gene. Orthologous conserved markers were developed from bin-mapped expressed sequence tags (ESTs) and from ESTs identified based on colinearity with the *Brachypodium* genome. ESTs with orthologous genes in these collinear regions were used to develop new conserved markers for saturating the region. Seventy-three pairs of primers were developed, and ~30% showed polymorphism in the segregating RIL/DH populations. Efforts are now underway to integrate these polymorphic markers into genetic maps.

Poster 63. A genomic study of homoeologous recombinants of the Lr19 (T4) translocation in wheat.

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Wheat is an important global food crop and is of major importance for international food security. Wheat leaf rust, caused by *Puccinia triticina*, can result in significant yield losses. The use of resistance genes is the most economical and environmentally friendly way to combat the cereal rusts. These genes have had great impact on stabilizing wheat production globally. Numerous genes have been identified in the hexaploid wheat gene pool and used in breeding for new resistant cultivars. However, the constant evolution of pathogens to overcome new sources of resistance is a major threat to sustained wheat production. Overuse of the primary gene pool of wheat, coupled with the narrow genetic base of common wheat has left the crop vulnerable to diseases, pests, and changes in the environment. The wild relatives possess numerous resistance genes that can be exploited in wheat breeding. Various wheat–*Thinopyrum ponticum* (*Lr19*) translocations involving wheat chromosome 7DL were produced in the 1960s and 70s. Unfortunately, these translocations could not be used for breeding in many countries, due to the presence of a linked gene (*Y*) for yellow endosperm pigmentation. As a result, lines with white endosperm have been derived through homoeologous recombination or mutation. One such attempt involved the T4 translocation and produced several 7BL recombinants that lacked both the *Y* and *Sr25* genes. The latter modified translocations have not been thoroughly characterized and mapped to determine the actual alien chromatin amounts. This study employed fluorescent genomic in situ hybridization (FGISH) and mapped simple sequence repeat markers to confirm the earlier conclusions and to determine the physical sizes of the remaining alien chromosome fragments in the shortest recombinants. An integrated cytogenetic and linkage map has been constructed for the recombinant chromosomes through FGISH and marker analyses. The recombinants with smallest alien fragments are being characterized for their agronomic usefulness and are simultaneously being backcrossed into the NDSU winter wheat breeding populations.

Poster 64. Enhancement of Lr34 function by its over-expression in transgenic wheat.

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Lr34 is a resistance gene that is mainly used against leaf rust caused by *Puccinia triticina* Eriks and stripe rust caused by P*uccinia striiformis* Westend. f. sp. *tritici* Eriks. A susceptible, *Lr34* allele is caused by a single nucleotide polymorphism in exon 11 or exon 12 that resulted in an alteration of an amino acid residual in the *Lr34* protein or a point mutation in exon 22, which produced a nonfunctional form of the *Lr34* protein due to the presence of a premature stop codon. A resistant *Lr34* allele should be transcribed into initial pre-mRNA transcript in the transcription, during which an interrupted intron between two neighboring exons is removed, and retained exons are concomitantly joined to make up a matured mRNA. In a recent study, however, we have found that even though a wheat cultivar carries a resistant *Lr34* allele, the majority of *Lr34* transcripts in this cultivar were mis-spliced due to intron retention (a complete or partial intron was not spliced out) or exon skipping (a complete or partial exon was mistakenly spliced out). These mis-splicing or alternative splicing events have resulted in nonfunctional forms of the *Lr34* protein. We are testing to determine if the plant resistance to leaf rust and stripe rust could be significantly enhanced when a complete and functional *Lr34* cDNA is over-expressed in transgenic wheat using a cultivar carrying the resistant *Lr34* allele.

Poster 65. Identification and mapping of a new leaf rust resistance gene derived from Triticum turgidum var. dicoccum.

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Leaf rust, caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*) is one of the most damaging foliar pathogens of wheat, causing significant yield losses annually in many wheat growing regions of the world. Sources of genetic resistance are valuable to increase the sustainability of cereal production, from both economic and environmental standpoints. If the genetics of leaf rust resistance has long been studied in hexaploid wheat (*Triticum aestivum* L.), a detailed analysis of the genetic bases in durum wheat has been undertaken only recently.

In order to investigate the genetic basis of leaf rust resistance, we developed a genetic linkage map on a RILs population (122 F_9 lines) derived from a cross between the susceptible durum wheat cultivar Latino and the resistant accession MG5323 of *T. turgidum* var. *dicoccum*. The infection type and the percentage of infected leaf area (DS) were evaluated on the RIL population by means of artificial inoculation with two *P. triticina* isolates (Villamarique de la Condesa and 12766). A total of 486 molecular markers well distributed on the whole genome were used to search polymorphisms between the two parents of the mapping population out of which 70% were polymorphic. A genetic linkage map for QTL analysis was developed using of 320 markers distributed within 16 linkage group and spanned greater than 1,400 cM. Using both simple interval mapping (SIM) and the Multiple QTL Model mapping functions (MQM), we identified three chromosomal regions specifically associated with leaf rust resistance. The largest and the most consistent leaf rust resistance locus was identified by a DS score (LOD 12, $R^2 = 38\%$) on the short arm of chromosome 1B flanked by SSR markers *Xgwm413* and *Xgwm448*. Two additional minor QTL were identified and were located on chromosome arms 5AS and 7BL. The major QTL is localized in a genomic region where no previously identified leaf rust resistance genes (*Lr* genes) have been positioned and suggests the identification of a new resistance gene to leaf rust in the durum wheat genetic background.

Poster 66. Utility of EMS mutants in rust resistance studies and map-based cloning in wheat.

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Seeds of cultivars AC Domain and Kenyon were mutagenized with ethyl methanesulfonate (EMS), planted and grown to maturity (M_1 plants). M_2 seed of each cultivar were harvested and bulked separately. A total of ~12,000 M_2 seeds of AC Domain and Kenyon were planted in equal quantities in root trainers and inoculated 11–12 days later with a leaf rust pathotype avirulent on *Lr16*. Both AC Domain and Kenyon are known to carry *Lr16*. Two weeks later, seedlings were scored as resistant, intermediate or susceptible. The plants were selfed and grown to maturity for M_3 seed. Disease phenotyping resulted in the identification of 47 putative *Lr16* mutant plants; 29 AC Domain and 18 Kenyon. Next, genomic DNA was extracted from individual plants and screened with SSRs that were linked to *Lr16*. The SSR profiles of individual mutants were compared and correlated with their respective disease phenotypes, which was done to identify off-types and enable selection of true *Lr16* mutants with SSR profiles that were identical to either of their respective non-EMS-treated parental cultivars, AC Domain or Kenyon. As a result, a total of 27 true *Lr16* mutant plants, 17 AC Domain and 10 Kenyon, were identified. Furthermore, SSR markers from across the genome were screened on the select mutants. Although M_3 mutant seed stocks for cultivars AC Domain and Kenyon are a valuable resource for ongoing efforts in map-based cloning of important genes, they also would serve as a tilling platform for future functional genomic studies.

Poster 67. Gene expression differences in wheat induced by six Puccinia triticina races.

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Next generation sequencing provides a unique opportunity to understand gene expression. Solexa RNAseq was used to look into gene expression differences that occur in the wheat variety Thatcher when infected with different races of leaf rust. Six races were selected from the North American 5 linage, single pustule purified, and inoculated individually onto 2–3 leaf stage wheat seedlings. At six days post inoculation, leaves were sampled and total RNA was isolated and sent to Cofactor Genomics. A single lane of paired end read Illumina GAIIx sequencing was performed for each race. Each race averaged 27.4 million reads and 3.295 million base pairs. Reads were aligned to the Puccinia triticina genome and a wheat EST data set. Pairwise comparisons were made. Thirty-one genes were found to have expression differences. These genes are currently being verified by real-time PCR.

Poster 68. Functional analysis and localization of SnTox1, a necrotrophic effector produced by the wheat pathogen Stagonospora nodorum.

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Stagonospora nodorum, the causal agent of wheat Stagonospora nodorum blotch, is known to produce multiple necrotrophic effectors, each of which specifically interacts directly or indirectly with a corresponding host gene product to induce disease. SnTox1/*Snn1* was the first fungal effector–host gene pair to be identified in this pathosystem by using ITMI population. We recently cloned the fungal gene encoding for SnTox1 and found it to be a highly cysteine-rich protein that can trigger PCD-like reactions in sensitive plants as well as having a significant role in fungal penetration. In the present work, we are investigating the mode of action and cellular localization of SnTox1. Using yeast culture filtrates containing SnTox1, we have demonstrated that widespread necrotic lesions form after directly spraying SnTox1 onto the leaf surface of lines harboring the corresponding sensitivity gene *Snn1*. However, this is not the case for SnToxA or SnTox3, suggesting a unique mode of action for SnTox1. Furthermore, the recognition of SnTox1 on the leaf surface takes place within a few minutes, which was demonstrated by SnTox1 application to the leaf surface followed by washing at different time points. Based on a Prosite motif search of SnTox1, multiple predicted sites including a putative chitin-binding domain were targeted for site-directed mutagenesis. SnTox1 activity was significantly reduced when mutations were produced at a casein kinase II phosphorylation site and a predicted helical region where lysine residues are abundant. Using a fungal strain expressing an SnTox1GFP fusion protein, we examined the location of the SnTox1 protein during fungal growth and infection. SnTox1 was observed in higher concentration on parts of several fungal structures, including the surface of conidia and mycelium, hyphal septa, and hyphal tips. The accumulation of SnTox1GFP is particularly obvious at hyphal regions where new hyphae are arising. *In planta*, SnTox1 is highly expressed in the hyphopodia where the penetration is initiated, providing further evidence that SnTox1 plays a role in penetration. Currently, we are using immunolocalization methods to determine if SnTox1 is internalized into the plant cell.

Poster 69. Dissecting the cytoplasmic component of plant-pathogen interaction pathways.

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The interactions between genomes present in the cytoplasm and the nucleus are critical to all eukaryotic organisms. These interactions trigger changes in gene expression, production of ATP, and control a large number of morpho-physiological functions. Wheat alloplasmic lines are plants where the cytoplasmic genomes of one wheat species (*Triticum*) are substituted by those of a wild relative (*Triticum* or *Aegilops*), while the original nucleus is maintained. Under these con-

ditions, the interaction between the nucleus and different cytoplasm can be evaluated at the morphological and molecular level. Cytoplasmic organelles (mitochondria and chloroplast) have been implicated in multiple plant-pathogen interaction pathways.

Our project is to measure differential responses of various alien cytoplasms in a specific nuclear background to various pathogens to provide a better understanding on the effect of nuclear-cytoplasmic interactions on biotic stress tolerance. In this study, we analyzed the nuclear donors of 56-1 (tetraploid), and Chris and Selkirk (both hexaploid), alloplasmic lines. Fifty selected alloplasmic lines were tested for their diseases response to *Pyrenophora tritici-repentis* (Ptr) isolates BR15 (produces ToxA, B, and C) and Pti2 (produces ToxA and C), one of the more virulent isolates in our collection. Results indicated that *Aegilops bicornis* cytoplasm provided reduced sensitivity to isolate BR15 as identified in alloplasmic lines of Selkirk and Chris, whereas *Ae. variabilis* cytoplasm provided reduced sensitivity to isolate BR15 as identified in alloplasmic lines 56-1 and Chris. Selkirk alloplasmic lines showed a similar reaction to the euplasmic parent to isolate Pti2. These alloplasmic lines, and others that showed significant increases in resistance or susceptibility, are being screened with additional Ptr isolates. Further investigations determining the mechanism of increased resistance or sensitivity by measuring the changes in the production of reactive oxygen species in those selected alloplasmic lines are underway. In conclusion, cytoplasm variability can improve resistance to plant diseases.

Poster 70. The function of scs, Rf, and vi for proper compatibility of durum wheat nucleus in alien cytoplasms.

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Interactions between alien cytoplasms and particular species cytoplasm specific, *scs*, genes from *Triticum timopheevii* or common wheat (*T. aestivum*) transferred to the nuclear genome of durum wheat (*T. turgidum*) produce male-sterile, A lines that are maintained by crossing to normal counterparts (B lines). Our objective was to study the function of fertility restorer (*Rf*), *scsti*, and *vitality* (*vi*) genes in alloplasmic lines of durum wheat to provide partial (sterile) and full compatibility (fertile) between alien cytoplasm and nucleus. We crossed A lines of durum wheat with cytoplasms from different related species, including *T. timopheevii* (Tt), *T. araraticum* (Ta), and *Aegilops speltoides* (Spt) to the (*Ae. longissima*) double-ditelosomic 1B ((lo) dDt 1B) having *scsti scsti* and *vi vi* and the durum lines having *scsti scsti* or *vi vi*. The crosses of (lo) dDt 1B with the *scsti scsti* and *vi vi* gene pairs produced fertile F_1s, showing that *scsti* and *vi* produce fertile F_1s, whereas crosses to durum lines having a *scsti scst*i or *vi vi* produced male sterile F_1s, showing that *scsti* or *vi* alone do not produce fertility in A lines with alien cytoplasm. Also, crosses of (lo) *scsti*–durum to the R lines having *Rf* genes and cytoplasm of Tt, Ta, spt, or other related species produced plump and viable seeds having *scsti* and *Rf* and fertile F_1s, but seeds having *Rf* genes alone were shriveled and inviable, like those from a cross to control durum. In summary, the (lo) *scsti vi* or (lo) *scsti Rf* produced plump seeds and fertile F_1,whereas (lo) *vi* produced plump seeds with greatly reduced fertility and plant vigor, and *Rf* produced shriveled and inviable seeds.

Poster 71. Homoeoallelic relationship of two speciation genes involved in the evolution of allopolyploid wheat.

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The evolution of wheat is a very complex process involving both nuclear and extranuclear genomes. Here we present work on homoeologous relationship between two speciation genes affecting nuclear-cytoplasmic (NC) interactions, critical in the development of allopolyploid wheat. Disruption of such NC interactions leads to multiple incompatibilities, such as lack of seed viability or low vigor. These incompatibilities create genetic barriers, playing an important role in the speciation process, and preclude the commercial production of hybrid wheat and the full exploitation of the secondary and tertiary gene pools in breeding of *Triticum* ssp. The species-cytoplasm specific (*scs*) genes restore adequate compatibility between durum wheat nucleus and *Aegilops longissima* (S^1S^1; 2n=2x=14) cytoplasm. Classical mapping using

5,932 F_2s and radiation hybrid (RH) mapping using 237 RH1 semi-durum lines, treated with 150 Gy gamma rays, were employed to position the *scsti* and *scsae* loci, respectively. The two compatibility genes were mapped, with ten common markers (out of 49 EST-based markers used), onto homoeologous chromosomes 1D (introduced to durum from *T. aestivum* (AABBDD; 2n=6x=42) and 1A (with the *scsti* locus introduced from *T. timopheevii* (A^tA^tGG; 2n=4x=28). The *scsti* locus was mapped within 0.3-cM region of chromosome 1A, whereas the region of scsae was narrowed to ~6.9 centi Rads (cR; units of RH maps). Wheat Zapper, an online application developed to localize orthologous relationships with model species, was used to compare wheat and rice sequence in the *scs* region. A conserved colinearity between wheat and chromosomes 10 and 5 of rice (*Oryza sativa*) was observed. The syntenic relationship between two *scs* genes and the fact that both loci restore identical phenotypes in the same alloplasmic line provide firm evidence for their homoeoallelic identity. Our observations also support the 'Maan hypothesis', which states that each species had pre-established unique compatible NC interactions before undergoing evolutionary polyploidization and cytoplasm exchange with other species. The map-based cloning of the *scs* genes will allow the employment of multiple NC genes into wheat cultivars to produce interspecific hybrids and to provide breeders with a novel set of tools to adapt wheat to the fast changing environment.

Poster 72. Re-evolution of wheat mitochondria.

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Mitochondria are centers of energy metabolism in the cell. The cross talk between the genomes present in the mitochondria and nucleus in a cell are critical to all eukaryotes. Thousands of phenotypic variations in plants and other organisms are known to be the result of changes in nuclear-mitochondrial (NM) communication. Recent data indicates that a mixture of more than one type of the mitochondrial genome exists within a cell (namely heteroplasmy), which adds a level of complexity to NM communication. Because plants cannot escape from adverse environmental conditions, a high level of heteroplasmy and changes in frequencies of different mitotypes could play an important role in stress tolerance. Analyzing the NM interaction in an alloplasmic line of wheat (line with alien cytoplasm), having *Aegilops longissima* cytoplasm but a *Triticum durum* nucleus, revealed the presence of a particular nuclear encoded species cytoplasm specific (*scs*) gene is critical for proper NM communication required for several developmental processes. High-resolution mapping of *scsti* (from *T. timopheevii*) and *scsae* (from *T. aestivum*) by a combination of radiation-hybrid and genetic mapping, located these genes on the same region of chromosome 1A and 1D of wheat, respectively. Because both loci restore identical phenotypes in the same alloplasmic line, the two genes seems to be homoeologous genes playing an important role in Triticeae speciation. Detailed analysis of the mitochondrial genome composition of the same alloplasmic line revealed alteration of the mitochondrial genome and gene expression patterns relative to parental lines. These changes are dramatic considering the timeframe. We also identified a high level of heteroplasmy in addition to the new mitotypes in this alloplasmic line leading to increased variation of this organelle, possibly because of an alien nuclear genome. Our analysis also indicates differential response of various alloplasmic wheat lines with the same nucleus to the fungal pathogen *Pyrenophora tritici-repentis* (tan spot) providing additional evidence on the importance of cytoplasm in response to biotic stresses. Re-evolution of wheat by introducing a new cytoplasm and a compatible *scs* gene in the nucleus may provide a further mechanism for tolerating biotic and abiotic stresses.

Poster 73. Accelerated, mitochondrial genome evolution of a Triticum alloplasmic line.

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Making an alloplasmic line (a line with alien cytoplasm) of durum wheat (*Triticum turgidum*) with *Aegilops longissima* cytoplasm is impossible, resulting in nonviable seeds unless the species cytoplasm specific, *scs*, gene regulating the nuclear–cytoplasmic compatibility is introduced to the nucleus of alloplasmic line. To find out the possible changes in the mitochondrial genome of *Ae. longissima* in the alloplasmic conditions, the mitochondrial genomes of the alloplasmic wheat and its parents were sequenced and described. All mitochondrial genes characterized previously in the *T. aestivum* genome were present in the sequenced mitochondria. The genome comparison showed major differences in the *atp6*, *nad9*, *nad6*, and *rps19-p* genes. We were able to identify species specific ORFs, e.g., orf113, as one of chimeric ORFs found only in alloplasmic line. We recognized that the orf359 in alloplasmic line is the most polymorphic region when compared to *T. turgidum* and that it is entirely missing in *Ae. longissima*. Nucleotide polymorphism across the genomes indicated possible mitochondrial heteroplasmy. Structural differences between three *Triticum* mitochondrial genomes were observed where conserved gene blocks and gene pairs remained together among species but were rearranged within the genome. Three possible recombination events in gene blocks, I, V, and VI, were found. Significant differences in the alloplasmic line mitochondrial genome were observed when compared to its donor based on gene sequence, ORFs, single nucleotide polymorphism, and overall synteny. These results indicate accelerated evolution of the mitochondrial genome as a result of nuclear genome substitution and may have significant evolutionary and genetic implications in terms of adaptation and stress tolerance.

Poster 74. Changes in gene expression of mitochondrial genes in alloplasmic wheat lines carrying restorer genes.

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The replacement of a particular nucleus with the nucleus of another species in alloplasmic lines of wheat is an unique approach to analyze nuclear–cytoplasmic (NC) communication events. Usually these replacements produce non-viable, sterile, or weak progenies due to the incompatibility in NC interaction. The incompatibility in (lo) durum wheat, where the nucleus of *Aegilops longissima* has been replaced with that of *Triticum turgidum*, can be improved by the addition of the *scs* (species cytoplasm specific) and *Vi* (vitality) gene pairs. These two genes can recover plant vigor and male fertility in (lo) durum lines, respectively. In many cases, cytoplasmic male sterility is associated with the expression of particular mitochondrial orfs. Therefore, the relative expression level of several ORFs and mitochondrial genes were analyzed in (lo) durum lines having different combinations of *scs* and *Vi* genes as well as normal lines using $2^{-\Delta\Delta Ct}$ method with SYBR green real-time PCR. Our preliminary analysis revealed higher expression of several orfs in (lo) durum lines compared with the cytoplasm donor (*Ae. longissima*) and nuclear donor (*T. turgidum*). These data support the hypothesis that the expression of mitochondrial orfs is suppressed by gene(s) in the nucleus of the normal plants. It appears the normal suppression mechanism are affected in alloplasmic conditions increasing the expression of certain orfs and eventually leads to decrease in male fertility and/or plant vigor. Based on our data, the nuclear *scsti* (*T. timopheevi*-derived *scs*) gene product can down regulate the expression of orf48–orf25 in (lo) durum line. The mechanism of this down regulation is still unclear and needs more investigation.

Poster 75. Androgenic response of Nebraskan winter wheat (Triticum aestivum L.) varieties to isolated microspore culture for doubled haploid plant production.

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Isolated microspore culture based androgenesis is being used for production of haploid (H) and doubled haploid (DH) plants in wheat. This technique can facilitate the efficiency of wheat breeding and genetic mapping as well as research in functional genomics and gene expression. Until now there was no report of microspore culture in Nebraskan winter wheat varieties. The objectives of the present report were to (1) study the androgenic response of Nebraskan winter wheat varieties and (2) establish efficient procedure for green DH plant regeneration. Three Nebraskan winter varieties (Anton, Antelope, and Camelot) were used. The spikes were collected from greenhouse-grown plants when microspores were at mid-late to late-uninucleate stage. For each batch of pretreatment, anthers from 16 spikes were pretreated in solution B at 25°C for 4–5 days followed by microspores isolation (no cold pretreatment). For cold pretreatment, anthers were incubated at 4°C for additional five days. The numbers of embryogenic microspores, multicellular, and embryo-like structures were recorded and analyzed. Compared to no cold pretreatment, a cold pretreatment increased the number of embryogenic microspores significantly in Anton by two fold, but no significant differences between the two pretreatments were observed in Camelot and Antelope. *In vitro* development of microspores into multicellular and embryo-like structures were quicker in Camelot than Anton and Antelope. The green plants were regenerated in all three varieties following both cold and no cold pretreatment. The number of regenerated green plants per batch of pretreatment was four (no cold) and eight (cold) in case of Antelope. However, for Anton and Camelot, there was one green plant per batch in both the cold and no cold treatments. It seems that higher number of embryogenic microspores due to cold pretreatment in Anton was not regenerated into proportionate number of green plants. An experiment is under progress to determine a similar response in Camelot and Antelope. This is the first report of androgenic response of Nebraskan winter wheat varieties. We believe that this method will be a beneficial tool in our wheat breeding efforts. However green plant regeneration frequency needs to be increased for cost-effective use.

Poster 76. Chromatin state affects the DNA breakage/repair mechanism in wheat.

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Meiotic recombination, the basis of genetic mapping is not uniformly distributed across the genome. The regions of high and low recombination that result in uneven map resolution across the chromosomes are evident across many grass genomes including wheat (*Triticum aestivum* L.). Recombination is believed to be linked to the double-stranded DNA break and repair, a phenomenon highly dependent on the chromatin state. Radiation hybrid (RH) maps have been proposed to provide i) higher, ii) be more uniform resolution than genetic maps, and iii) to be independent from recombination constraints. We generated an RH panel for mapping of wheat chromosome 3B and used it to test these three assumptions. Our RH map contains 541 markers anchored to chromosome 3B BAC contigs. Detailed comparisons with a genetic map of similar quality confirmed that i) the resolution of the RH map was 10.5X higher and ii) six times more uniform. We identified a strong interaction ($r = 0.879$ at $p = 0.01$) between the DNA repair mechanism in mitotic cells and the distribution of crossing-over events in meiotic cells. We could explain this finding only by admitting the possibility that the DNA repair mechanism is affected by the chromatin state in a way similar to the effect that chromatin state

has on recombination frequencies. Our RH data support for the first time *in vivo* the hypothesis of noncasual interaction between recombination hot-spots and DNA break/repair. This means that since the initial RH application 37 years ago, we were able to show now for the first time that the third hypothesis of RH mapping (iii) might not be entirely correct.

Poster 77. Molecular characterization and protein-DNA interaction analysis of a WRKY transcription factor in wheat (Triticum aestivum L.) showing differential spatiotemporal expression during leaf-rust pathogenesis.

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The plant WRKY proteins are one of the largest families of transcription factors involved in physiological processes including biotic and abiotic stresses. These transcription factors have a recognition sequence (T)(T)TGAC(C/T), known as the W-box, found in the promoter region of *WRKY* and other defense-related genes. These transcription factors have a strictly conserved 60 amino acid region containing the WRKYGQK peptide sequence and zinc finger-like motifs. The *WRKY* gene family is subdivided into three different groups (I, II, and III) based on the number of WRKY domains and features associated with the zinc finger like motifs, group I and group II have finger motif C_2H_2, whereas group III contains a C_2HC motif. Very little information on WRKY transcription factors of wheat are available and, hence, the present study was undertaken to characterize a wheat WRKY transcription factor and decipher its role during leaf-rust infection. *WRKY*-specific, wheat consensus sequences were obtained from NCBI to design primers to amplify genomic and cDNA sequences from leaf-rust resistant and susceptible near isogenic wheat lines. The sequences were analyzed using multiple sequence alignment and a conscientious phylogenetic tree was constructed to study the relationship with WRKY transcription factors of other plants. Various catalytic domains were identified using ScanProsite and other bioinformatic softwares. *In silico* docking between a 26-bp oligonucleotide containing the W-box sequence and a 74 amino acid sequence having the conserved 60 amino acids including the core WRKYGQK domain was performed at Haddock server. The optimized peptide and DNA models were able to form a complex that showed interaction between the W-box and the conserved WRKY domain. The protein–DNA binding was validated by observing shifts in electrophoretic mobility shift assay using heterologously produced recombinant WRKY proteins. Temporal and spatial gene expression profiling was done by quantitative real-time PCR using universal probe library based, WRKY-specific probe and primers with RNA isolated at different time points from resistant and susceptible plants that were either mock inoculated or infected with virulent leaf-rust pathogen. During pathogenesis, maximum expression of *WRKY* gene was observed at 24-hours post-inoculation in both compatible and incompatible interaction.

Poster 78. Transferring maize transposable elements to bread wheat through 'wheat x maize' crossing.

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Transposon tagging and insertional mutagenesis are important reverse genetic tools in eukaryotes. These are widely used for understanding gene function and targeted manipulation of genes in crop improvement. Although no characterized transposable element system is available in wheat, the maize systems have proven to be very successful for gene tagging not only in maize but also in other plant species including tobacco, *Arabidopsis*, peas, and barley. Normally, genetic transformation is used to introduce these active transposable elements (TE) into nonhost plants. In the case of wheat, however, genetic transformation is technically challenging, variety specific, and the frequency is usually very low. We attempted a simpler method based on direct 'wheat x maize' crossing to transfer well-characterized maize transposable elements into wheat. Wheat doubled haploids are routinely produced by 'wheat x maize' crossing where the resulting zygotes undergo uniparental chromosome elimination giving rise to haploid wheat plants. The maize chromosomes are present during the few initial cell cycles during zygote differentiation, giving enough time for the transposition of TEs. Different maize lines harboring Activator (*Ac*) and Mutator (*Mu*) transposable elements were crossed with bread wheat lines and the haploid embryos were rescued. Regenerated wheat plants were treated with colchicine for chromosome

doubling. These plants were screened by PCR amplification using multiple maize TE specific primers. Three of the 64 plants from the Louise/Maize Activator crosses showed a maize-specific, PCR fragment amplified using *Ac*-specific, PCR primers. Similarly, four of the 40 plants derived from PBW 621/Maize Mutator crosses showed a maize-specific ,PCR fragment amplified using *Mu*-specific PCR primers. Amplified fragments were sequenced to confirm their specificity. All the fragments were matching up with their corresponding maize transposable element sequences. DNA gel blot analysis and inverse PCR are underway to confirm the integration and subsequent transposition activities. The most current status of the project will be presented at the meeting.

Poster 79. The wheat meiotic cohesin gene TtRec8 and its role in haploidy-dependent, unreductional meiotic cell division.

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Meiosis is a specialized cell division that halves chromosomes and generates haploid gametes in eukaryotes. It ensures genomic integrity and generates genetic variability. Variation of the meiotic process leads to aneuploidy and polyploidy. Unreductional meiotic cell division (UMCD) was observed in the polyhaploid of the tetraploid wheat cultivar Langdon (LDN) (*Triticum turgidum* L.) and its interspecific hybrid with *Aegilops tauschii*. This haploidy-dependent UMCD gives rise to unreduced gametes, leading to polyploidy. It has been considered a major mechanism of polyploidization in wheat. The meiotic cohesin gene *Rec8* has been proven to play a significant role in kinetochore orientation and chromosome segregation at meiosis I in model species. In the present study, we attempted to understand the function of the *Rec8*-like gene in the meiotic cell division of wheat and determine the role of this gene in the onset of the haploidy-dependent UMCD. We cloned the *Rec8* homologue in LDN, designated *TtRec8*, and developed the polyclonal antibody against the TtRec8 protein in rabbit. *TtRec8* exhibited an expression pattern similar to *Rec8* in model species as revealed by real-time PCR and Western blotting throughout the meiotic process in anthers. In addition, the TtRec8 protein exhibited analogous kinetics of the meiotic cohesin *Rec8* as revealed by the immunolocalization of the cohesion protein on chromosomes over the meiotic stages. Two homoeoloci of *TtRec8* were identified on chromosome 1A and 1B. We have been determining the map location of this gene on the chromosome in a doubled haploid population of tetraploid wheat. Meanwhile, we have been investigating the expression profiles of *TtRec8* and the kinetics of the TtRec8 protein in an LDN haploid as well as the interspecific hybrid of LDN with *Ae. tauschii*. This will provide new insights into the role of this meiotic cohesin gene in the onset of the haploidy-dependent UMCD in wheat.

ITEMS FROM AZERBAIJAN

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Resistance in Azerbaijani durum and bread wheat accessions to leaf and stem rust.

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World food security will depend on increased production of cereal crops — wheat, maize, barley, and rice. Of these, wheat is of greater importance, in terms of tonnage if not in financial value. One significant constraint to increased wheat production is the variety of rust diseases attacking this crop. Cereal rusts have no doubt been present and evolving during domestication of cereal crops as a major segment of agriculture. Kislev reported archaeological evidence of Puccinia graminis on wheat lemma fragments dated at 1400-1200 B.C. Savile and Urban reviewed the evolution of cereal rusts relative to human-guided evolution of cereal crops. Sources of resistance to these diseases are known, and have been utilized by wheat breeders for a long time. Zhukovsky (1959, 1961) postulated that the home of wheat is Transcaucasia, the central and western parts of Asia Minor, the eastern Mediterranean areas, and the western part of Iran. These regions abound in endemic wild and cultivated wheat and store the variation potential of the genera *Triticum*, *Aegilops*, and *Secale*. Vavilov (1939) maintained that in the mentioned regions there is the world's richest concentration of wild relatives of small grains. Azerbaijan is also one of the primary gene centres of speciation of the genus Triticum. Zhukovsky (1961) and Vavilov (1939) also ascertained the epicenters of wheat are also the homeland of the most destructive wheat rust parasites, *Puccinia triticina*, *P. striiformis*, and *P. graminis*. Therefore the screening for resistance genes to biotic and abiotic stresses in wheat germplasm available in centers of origin is of paramount importance.

Materials and methods. For this study, 121 durum and bread wheat accessions representing different botanical varieties (germplasm) and differing for some morphological traits (spike, awn and seed color, and hairiness) and 36 modern Azerbaijani durum and bread wheat varieties from the Azerbaijan Genebank were studied (Table 1, p. 65).

Phenotype screening. Seedlings were grown in '20 x 20 x 5'-cm aluminum pans in Metromix 360, a peat/perlite/bark ash growing mix, with 30 entries of five to six seedlings each. Each pan contained the cultivar Morocco as a susceptible control. Plants were grown in the greenhouse at 20±4°C and ambient light. Spores were thawed and heat-shocked for 6 min in a water bath at 42°C just prior to use. Spores were suspended in Soltrol 170 isoparaffin oil at a concentration of approximately 5 x 10^6/mL and sprayed onto seedlings at the two-leaf stage. The oil was allowed to evaporate for at least 10 min, and then plants were placed in a dew chamber at 20±1°C with 100% relative humidity in the dark for approximately 16 hours. For stem rust, the dew chamber was illuminated for the last hour to stimulate infection. Plants were moved to a growth chamber at 20±1°C with a 16 hour photoperiod and light intensity of 300–400 μmol/m^2/sec for symptom development. Infection types (ITs) were recorded using the Stakman 0 to 4 scale at 12 days (leaf rust) or 14 days (stem rust) post-inoculation. Leaf rust races BBBDB (avirulence – 1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 17, 30, B, 10, 18, 21, 28, 39, 42 / virulence – 14a), MFBJG (avirulence – 2a, 2c, 9, 16, 3ka, 11, 17, 30, B, 18, 21, 39, 42 / virulence – 1, 3, 24, 26, 10, 14a, 28), TTRSD (avirulence – 17, 18, 21, 28, 42 / virulence - 1, 2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 30, B, 10, 14a, 39), and MRDSD (avirulence – 2a, 2c, 24, 3ka, 11, 30, 18, 21, 28, 42 / virulence – 1, 3, 9, 16, 26, 17, B, 10, 14a, 39) were used. Plants were inoculated with stem rust races MCCFC (avirulence – 21, 9e, 11, 6, 8a, 36, 9b, 30, 9a, 9d, 24, 31, 38 / virulence – 5, 7b, 9g, 17, 10, Tmp, McN), TPMKC (avirulence – 6, 9b, 30, 9a, 24, 31, 38 / virulence – 5, 21, 9e, 7b, 11, 8a, 9g, 36, 17, 9d, 10, Tmp, McN) and RKQQC (avirulence – 9e, 11, 30, 17, 10, Tmp, 24, 31, 38 / virulence – 5, 21, 7b, 6, 8a, 9g, 36, 9b, 9a, 9d, McN).

Table 1. Characteristics of the *Triticum turgidum* subsp. *durum* and *T. aestivum* subsp. *aestivum* lines used in the study.

Number of lines	Variety	Spike color	Awn color	Seed color	Hairiness	% low IT	
						leaf rust	stem rust
***T. turdigum* subsp. *durum* accessions**							
14	v. *leucurum*	white	white	white	absent	86	21
1	v. *affine*	white	white	red	absent	100	0
9	v. *leucomelan*	white	black	white	absent	44	66
5	v. *melanopus*	white	black	white	present	40	40
2	v. *reichenbachii*	white	black	red	absent	100	100
11	v. *hordeiforme*	red	red	white	absent	45	18
3	v. *murciense*	red	red	red	absent	33	66
6	v. *apulicum*	red	black	white	present	17	17
3	v. *erytromelan*	red	black	white	absent	0	2
1	v. *niloticum*	red	black	red	present	1	0
1	v. *libycum*	black	black	red	present	1	1
2	v. *boeffi*	black/white background	black	white	present	100	100
2	v. *alboprovinchiale*	black/white background	black	white	absent	50	0
2	v. *coerulescens*	black/red background	black	white	present	0	0
2	v. *obscurum*	black/red background	black	white	absent	0	0
***T. aestivum* subsp. *aestivum* accessions**							
5	v. *graecum*	white	white	white	absent	0	100
2	v. *meridionale*	white	white	white	present	50	50
7	v. *erythrospermum*	white	white	red	absent	14	71
3	v. *hostianum*	white	white	red	present	0	100
5	v. *albidum*	white	awnless	white	absent	20	80
6	v. *lutescens*	white	awnless	red	absent	50	100
1	v. *velutinum*	white	awnless	red	present	0	50
2	v. *triticum*	red	red	white	present	0	100
4	v. *barbarossa*	red	red	red	present	0	100
9	v. *ferrugineum*	red	red	red	absent	0	89
5	v. *alborubrum*	red	awnless	white	absent	0	20
7	v. *milturum*	red	awnless	red	absent	0	71
1	v. *leucospermum*	white	awnless	white	present	0	50
5	v. *graecum*	white	white	white	absent	0	100
2	v. *meridionale*	white	white	white	present	50	50

Genotype screening. DNA extraction from young leaves was done using a BioSprint96 robot, and 16 markers were used for screening for 16 resistance genes. Fragment analysis was used an ABI DNA 3730 analyzer. A (+) and (–) control was used for each marker, and the results were analyzed by GeneMarker software.

Phenotyping results. *Leaf rust*. Eighteen of 82 durum wheat accessions were resistant to leaf rust, 23 were moderately resistant, and 41 were susceptible. A majority of the resistant accessions (61%) belonged to var. leucurum, i.e., they had white spikes, awns, and seed, and the spikes lacked hair. White spikes were observed in 83.3% of resistant genotypes. Moreover, among the 23 moderately resistant genotypes, 14 (60.9%) also had white spikes. On the contrary, in 70.7% of the 41 wheat genotypes that were rated susceptible, the spike color was red. Therefore, using leucurum varieties and/or white spikes may accelerate breeding for leaf rust resistance. Most bread wheat accessions (61) are susceptible to leaf rust; we have selected four highly resistant and nine moderately resistant bread wheat accessions.

Stem rust. In contrast to leaf rust, a majority of bread wheats were resistant or moderately resistant to stem rust. Our results showed that 29 of the bread wheats were highly resistant and 33 were moderately resistant to stem rust. Only 13 bread wheat genotypes were classified as susceptible. Among the durum wheats, 14 were resistant and 12 moderately resistant to stem rust. Material identified as leaf and stem rust resistant in these experiments will be grouped into germ-

plasm pools and incorporated into our breeding program as potential sources of resistance or tested for yield performance in target environments.

Genotyping results. Sixteen markers were used to screen for 16 genes (*Lr21*, *Lr24*/*Sr24*, *Lr34*/*Yr18*, *Lr37*/*Sr38*/*Yr17*, *Lr39*, *Lr46*/*Yr29*, *Lr50*, *Sr36*, HGPC/*Yr36*, T1RS·1BL (*Lr26*/*Sr31*/*Yr9*), and T1RS·1AL (*Sr1A.1R*)). *Lr34* was recorded in 12 bread wheat accessions, most of which (eight accessions) were highly resistant only to the BBBDB race. *Lr46* was found in six durum wheats and 55 bread wheats; most were susceptible to leaf rust at the seedling stage. Results of the molecular screening revealed the presence of the T1RS·1BL rye translocation in nine bread wheat accessions (Fig. 1); all were highly resistant to stem rust and most were highly or moderately resistant to leaf rust.

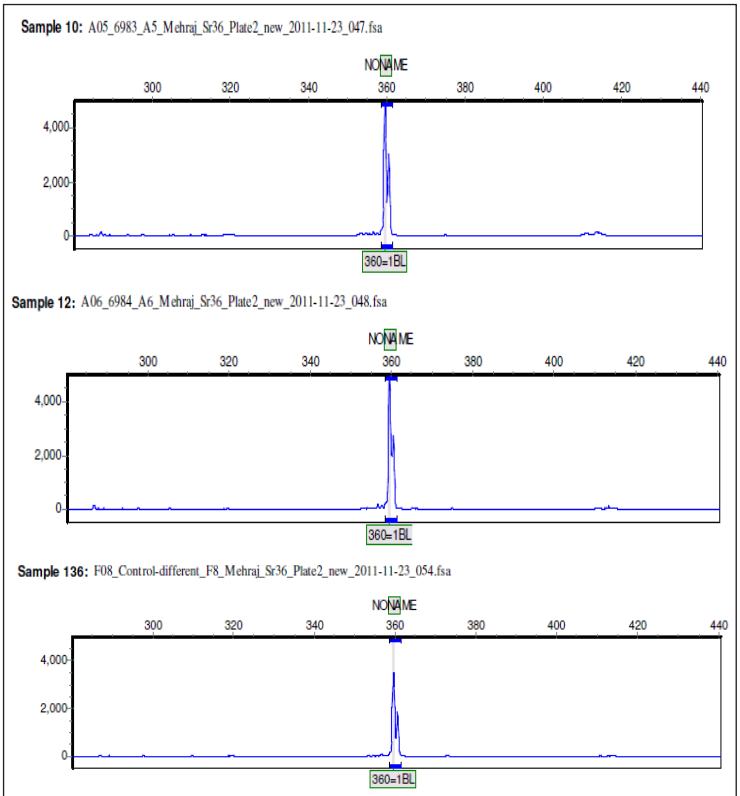

Fig. 1. T1RS·1BL translocation in two bread wheat accessions (top two) and in the control (bottom).

Conclusions. Bread wheats are more resistant to stem rust than to leaf rust. Compared with bread wheats, durum wheats are resistant to leaf rust and susceptible to stem rust. Additional tests are planned with durum-specific races of leaf rust. Spike color and leaf rust resistance are correlated in durum wheats. T1RS·1BL translocation carrying *Lr26* and *Sr31* has an important role in rust resistance in this collection of bread wheats. All accessions with T1RS·1BL have white spikes, seeds and awns. *Lr34*/*Yr18* was present in both old and new bread wheat cultivars from Azerbaijan but not the durums. Highly resistant accessions can be useful in breeding programs.

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ITEMS FROM BRAZIL

BRAZILIAN AGRICULTURAL RESEARCH CORPORATION — EMBRAPA
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Wheat in Brazil – the 2011 crop year.

Eduardo Caierão, Pedro L. Scheeren, Márcio Só e Silva, Ricardo Lima de Castro, Adeliano Cargnin, and Edina Moresco.

In the 2011 crop year, Brazilian wheat production was about 6 x 10^6 tons (Conab 2012), which is enough to supply 50% of the domestic demand (Table 1). The deficit in production makes Brazil the largest country that imports wheat. The south region, comprised of the states of Rio Grande do Sul, Santa Catarina, and Paraná, accounts for 94,6% of the national production. Nonetheless, due to the characteristics of the cultivation system, the average grain yield is not the highest in the country.

Table 1. Area of cultivation, total production, and grain yield of wheat in Brazil in 2011 (Source: CONAB, 2012).

Region	Area (ha x 1,000)	Production (t x 1,000)	Grain yield (kg/ha)
North	—	—	—
Northeast	—	—	—
Central–West	45.3	109.0	2,406.0
Southeast	70.0	200.8	2,869.0
South	2,050.9	5,478.8	2,671.0
Brazil	**2,166.2**	**5,788.6**	**2,632.0**

In 2011, the wheat area cultivated was higher than that in 2010 (2,166.2 against 2,149.8). However, the total production and average grain yield/ha achieved in 2011 were about 1.6% smaller than those of 2010. The grain yield average in the Southern Region of Brazil in the 2011 crop season was one of the highest in the history. Low temperatures during the vegetative stage and grain filling associated with sunny days contributed to the high productivity. The grain quality was good as well.

Reference.

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Development of wheat germ plasm to biotic and abiotic stresses.

Adeliano Cargnin, Flávio Santana, Luciano Consoli, Marcos Vinicios Fabris, Eduardo Caierão, Pedro Luiz Scheeren, Marcio Só e Silva, Edina Moresco, Ricardo Lima de Castro.

Global warming, based on climate change predictions, demands genetic progress and improvements in production systems in order to explore crop potential by reducing losses caused by biotic and abiotic features. High rainfall from the heading to harvest, which is usual in Brazil, can trigger wheat to sprout and lead to losses for farmers, disparaging the product for the bakery trade (industrial quality). The high level of crop diseases in Brazil, especially Fusarium head blight and wheat blast, work as barriers against an increase in the national wheat production. One strategy to improve the grain production is to exploit the genetic resources and make them available to breeding programs. Hence, our goal is to develop new wheat lines resistant/tolerant to the main biotic (Fusarium head blight and wheat blast) and abiotic stresses (wheat sprouting). The backcross method will be used to transfer resistant/tolerant alleles to potential recurrent parents (cultivars or elite wheat lines). Because the donors will be mainly germ plasm from the wheat core collection, which has been characterized for their resistance/tolerance, as well other genotypes from the Germplasm Bank known for carry other desired features, especially from synthetic wheats of related species (*Aegilops* and *Agropyron*). Resistant/tolerant plants will be selected from each generation for total of three generations of backcrosses (BC_1 to BC_3), following by another three generations of self pollination (BC_3F_1 to BC_3F_3). The four, best BC_3F_3 lines with regard to agronomic features will be genotyped by microsatellite markers. The conversion index of the new, resistant/tolerant lines should vary. As a consequence, only that one with the highest index will become an advanced line. Therefore, these new lines will represent new options for growing or even a new, genetically diverse, wheat genotype resistant/tolerant to biotic and abiotic stresses available to wheat-breeding programs.

Inoculant promotes wheat yield increase.

Ricardo Lima de Castro, Giandro Duarte Teixeira, Thiago Mignoni de Lima, Eduardo Caierão, and Adeliano Cargnin.

Azospirillum brasiliense is a facultative, endophytic bacteria capable of fixing nitrogen from the atmosphere, providing part of the N required to the associated plant. The bacteria also may induce plant hormones, which stimulate the growth of plant roots, improves water and nutrient absorption, and increases chlorophyll content of the leaves and tolerance to stress, especially that caused by drought. Field experiments at Fepagro Nordeste, Vacaria, with five wheat cultivars from the state of Rio Grande do Sul, Brazil, evaluated the effect of *A. brasiliense* inoculant on wheat yield. Wheat seed inoculated with *A. brasiliense* increased grain yield from 165 to 555 kg/ha (3–15%). Considering the statistical analysis, in 67% of the experiments, the grain yield average from the inoculated treatments were higher than that from the non-inoculated treatments (Tukey Test, $p \leq 0.05$). This technology may reduce the economic and environmental costs related to the production, transport, and use of nitrogen fertilizers for the wheat crop. As a follow-up step ro these studies, a core collection of genotypes from the active germ plasm bank of Embrapa Trigo are under testing to observe the response to inoculation with *A. brasiliense*.

ITEMS FROM GERMANY

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Haplotype analysis of molecular markers linked to stem rust resistance genes in Ethiopian durum wheat cultivars and landraces.

Wheat is one of the most important cereals cultivated in Ethiopia. In the country, more than 70 bread and 30 durum wheat cultivars have been released for production since the 1940s. However, the national average yield of wheat is still about 1.4 tons/ha. Even though over 30 fungal diseases of wheat have been identified in Ethiopia, stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* (Pgt), is a major production constraint in most wheat-growing areas and causes up to 100% yield losses in epidemic outbreaks. The recent emergence of wheat stem rust race Ug99 (TTKSK) and related strains threaten Ethiopian as well as world wheat production because they overcome widely used resistance genes that had been effective for many years. The major cause that aggrivates the ineffectiveness of Ethiopian wheat cultivars against stem rust is the narrow genetic base on which breeding for resistance has been founded, however, little is known about the resistance genotypes of Ethiopian tetraploid wheat cultivars and landraces.

Our objective was to identify the stem rust resistance genes that are present in the Ethiopian tetraploid wheat cultivars and landraces using molecular markers and assess which genes are effective for current Ethiopian stem rust races of Pgt including Ug99. A total of 58 tetraploid wheat accessions consisting of 22 Ethiopian cultivars released during from 1966–2009, four ICARDA cultivars, and 27 landraces were genotyped using 17 molecular markers (SSR, EST, and InDel) linked or diagnostic for stem rust resistance genes *Sr2*, *Sr13*, *Sr22*, and *Sr35*. Haplotype analysis indicated that many of the Ethiopian durum wheat cultivars carried *Sr13*. The resistant cultivar Sebatel showed a haplotype for *Sr2* and *Sr22* and cultivar Boohai for *Sr22*. However, further evaluation for the diagnostic value of these haplotypes is needed. This study is the first report on the presence of stem rust resistance genes in Ethiopian durum wheat cultivars and tetraploid landraces based on linked or associated molecular markers and may help to identify cultivars carrying resistant

alleles, which will provide valuable genetic material for the development of new, improved cultivars in further breeding programs.

Rht genes – agronomic comparison under the climate of Southeastern Europe.

Sets of *Rht* NILs in four genetic backgrounds (April Bearded, Bersée, Maris Huntsman, and Maris Widgeon) were grown in a 4-year field experiment in Sofia, Bulgaria (42o41′N, 23o19′E). Plant height and yield components were genetically variable due to both cultivar background and *Rht* genic effects and were significantly influenced by the growing season, accounting for the climatic fluctuations. Averaged over all cultivars and years, plant height reductions relative to the tall control (*Rht-B1a*+-*D1a*) were in the order *Rht-B1b* ≈ *Rht-D1b* < *Rht-B1b*+-*D1b* < *Rht-B1c* < *Rht-B1c*+-*D1b* and amounted to 22, 53, 58, and 68%, respectively. Tillering was consistently greater in the *Rht-B1c* isolines, and the two double dwarfs. Although the spike was longer only in *Rht-D1b* and *Rht-B1b*+-*D1b* isolines, all alleles increased the spikelet number/spike up to 5%. *Rht-B1b* and *Rht-D1b* increased grain number/spike by 16% and 20%, respectively, and significantly reduced the grain mass per plant by 10% and 20%, respectively. *Rht-B1c*, *Rht-B1b*+-*D1b*, and *Rht-B1c*+-*D1b* reduced considerably both the grain number and grain mass per spike and per plant. All *Rht* alleles reduced the 50-grain mass within the range from 12% (*Rht-B1b*) to 20% (*Rht-B1c*).

Linkage between the red coleoptile (Rc-1) and purple pericarp (Pp-1) color genes.

We scored coleoptile color in durum wheat of F_3 families of the cross 'TRI 15744/TRI 2719' used previously for mapping *Pp-B1*. Among 113 F_3 families from this cross, 26 were homozygous red, 59 heterozygous, and 28 homozygous white, consistent with a monogenic 1:2:1 segregation ($\chi^2 = 0.292$, $P > 0.80$). The genetic distance between *Rc-B1* and *Pp-B1* was 7.6 cM (*Rc* proximal to *Pp*).

In bread wheat, an allelism test showed that the *Rc* genes determining dark-red coleoptile color in the Purple Feed and Purple lines are allelic to the *Rc-D1* gene of Novosibirskaya 67. In a 'Saratovskaya 29/Purple' cross, we distinguished F_3 families having plants with dark-red coleoptiles (97 F_3 families) from those having light red (from Saratovskaya 29) or noncolored coleoptiles (30 F_3 families), consistent with a monogenic 3:1 segregation ($\chi^2 = 0.129$; $P > 0.70$). The genetic distance between *Rc-D1* and *Pp-D1* was 2.5 cM (Rc proximal to Pp).

Thus, bread wheat did not inherit purple glumes from durum wheat as was thought earlier, but obtained only one of the two complementary *Pp* genes from durum wheat. The other gene came to bread wheat with the D genome of *Ae. tauschii*. Close linkage between *Rc-1* and *Pp-1* and similar function (regulation of anthocyanin biosynthesis) suggest that they are likely duplicated from a single locus.

Susceptibility to wheat midge infestation.

A panel of 96 winter wheat accessions originating from 21 countries were investigated in 2011 with the aim of finding genotypes resistant to the orange (*Sitodiplosis mosellana* (Géhin)) and yellow (*Contarinia tritici* (Kirby)) wheat midges. The accessions were highly variable in their phenotype with respect to growth pattern and coloration. In addition, there was variation for ear morphology and hairiness of different organs. We evaluated three flowering times, early, intermediate, and late.

Wheat midges were surveyed using pheromone traps, white water traps, and evaluation of insects in the spike samples. The pheromone traps were activated at BBCH 45 at a distance of 15 m in the experimental plots and took off at BBCH 75. The flight activity of the orange wheat midge was investigated weekly (nine times) by counting the orange midge males on the adhesive surfaces. To evaluate the larval infestation of wheat ears, six samples/plot were collected at three periods (flowering, milky, and late milky stages).

The results from the pheromone traps at the Gatersleben site showed a good activity of males of orange wheat midge; the maximum record was 59/trap/week. There was a weak coincidence between the main flight period of wheat midge and the optimum wheat stage of winter wheat for laying eggs (BBCH 47-60), because the weather conditions in 2011 were not suitable for wheat midge development. The results from the white traps were subjected to a genetic as-

sociation mapping study and analyzed with the STRUCTURE and TASSEL programs. Highly significant marker–trait associations for both wheat midge species were detected on different chromosomes.

Seed longevity and dormancy.

A total of 183 wheat accessions maintained in the cold store of the germ plasm repository at IPK–Gatersleben since 1974 were tested for viability in 2008. The mean germination in 1978 for this collection was 87%, which dropped to 56% after 34 years of storage. Seeds investigated in 2010 after regeneration exhibited a mean germination of 86%. Longevity of the 2010 seed was studied using artificial ageing (AA) and controlled deterioration (CD) tests. AA reduced the mean germination of the seed to 66%. Relative germination after AA was 77%. The mean germination after controlled deterioration was 59%, whereas relative germination after CD was 68%. The 2010 seed also were investigated for dormancy and preharvest sprouting (PHS). Mean percentages of dormant seed at 10°C and 20°C were 12% and 76%, respectively. Dormancy index reached a mean value of 33. Preharvest sprouting showed the opposite trend in relation to dormancy. The mean score for PHS was 4.0.

Association mapping analyses revealed 14 marker trait associations (MTA) for germination after long-term cold storage on chromosomes 1DC, 2AS, 2BL, 3AL, 4AL, 5BL, 6BS, and 7D. There were 14 MTAs recorded after AA of 2010 seed on chromosomes 1AS, 1BL, 2BS, 4BS, 5BS, 5BL, 6AC, 6BS, 7AS, 7BS, and 7D. Similarly, CD revealed 18 MTA for longevity on chromosomes 2AL, 2BS, 3BS, 4AL, 4B, 5B, 6BL, and 7BL. For dormancy and PHS, 23 and 30 MTA, respectively, were recorded. The MTA for dormancy were located on chromosomes 1DS, 2AS, 2BL, 2D, 3AL, 3BC, 3BL, 4AL, 4BL, 5AS, 5BS, 5BL, 6BL, and 7BL, whereas for PHS, they were located on chromosomes 1AS, 1BL, 1DL, 2BL, 3AL, 3BS, 3BC, 3BL, 4AL, 5AS, 5BL, 6AS, 6BS, 6BL, 7BC, and 7BL.

Genetic analysis of hybrid dwarfness aroused in crosses of common wheat with rye.

A set of 101 rye inbred lines originating from the Peterhof rye genetic stock collection of the Laboratory of Plant Genetics (St. Petersburg State University, Russian Federation) and selected from the rye cultivars Vyatka, Steel, Heine, Petkus, and Volkhova, was used to pollinate bread wheat cultivar Chinese Spring. Two unrelated self-fertile lines, V1 and V10, gave rise wheat–rye hybrids with a dwarf phenotype. The development of the dwarf, wheat–rye plants stopped at the stage of three leaves and the plantlets died at 6 weeks. For genetic analysis of the hybrid dwarfness, interline F_1 rye hybrids between lines L4 and L7 and V1 and V10 were produced. Interline hybrids F_1 (V1/L4), F_1 (L4/V1), and F_1 (L7/V1) were used as pollinators for crosses with Chinese Spring and Priekulskaya 421 wheat. For all cross combinations under investigation, a 1:1 segregation for the presence of normal vs. dwarf plants in the wheat–rye hybrids was obtained, as expected, with a total ratio of 212:196. Therefore, we concluded that hybrid dwarfness in wheat–rye crosses is determined in rye by one gene having two alleles. Rye lines V1 and V10 carry the allele preventing the development of hybrid plants in the seedling stage. This gene was named *Hdw* (Hybrid dwarfism). Because we cannot yet determine whether the allele for hybrid dwarfism is dominant or recessive, we designate the allele determining the production of wheat–rye hybrids with normal development (normal or wild-type) *Hdw-R1a* and allele determining dwarfism as *Hdw-R1b*.

Overcoming embryo lethality in wheat–rye hybrids.

Embryo lethality in crosses of common wheat with rye could be the result of complement interaction between the incompatible *Eml-R1b* rye allele and the *Eml-A1* gene in wheat. This kind of postzygotic barrier cannot be overcome by *in vitro* embryo rescue. Analysis of hybrid embryos revealed morphological differences at the age of 16 days after pollination (DAP). We found that the interaction of wheat and rye incompatible alleles arrests the formation of the shoot meristem but had no influence on root meristem formation. A method for overcoming such a hybrid embryo lethality was developed. The percent of embryos that produce embryogenic callus compose 73.3–100.0% for 14 DAP embryos and 92.3–100.0% for 16 DAP embryos. The numbers of green adventive buds with leaf primordial at one embryogenic callus were 5.2–8.0/callus and 3.2–5.9/callus for embryos at ages 16 DAP and 14 DAP, respectively. The number of regenerative plants per embryogenic callus were 2.8–5.4 and 2.3–4.9 for 16 DAP and 14 DAP embryos, respectively. A more complex parameter is the total number regenerative events per embryogenic callus, which was 9.7–12.0/callus for 16 DAP and 6.3–10.6/callus for 14 DAP embryos. Thus, our experiments showed that embryo lethality caused by complement interaction of incompatible wheat and rye alleles could be successfully overcome via somatic embryogenesis.

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ITEMS FROM HUNGARY

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www.agrar.mta.hu

Institute reorganization. As part of the reform of its institute network, the General Assembly of the Hungarian Academy of Sciences voted on 5 December, 2011, to establish a Centre for Agricultural Research (HAS CAR), which came into existence on 1 January, 2012. In the course of the reorganization, the Veterinary Medical Research Institute, the Plant Protection Institute, and the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences merged with the Agricultural Research Institute and no longer exist as independent, publicly financed institutions.

The new Centre for Agricultural Research (www.agrar.mta.hu) has its headquarters in Martonvásár (2462 Martonvásár, Brunszvik u. 2), where the Agricultural Institute is located. The other institutes making up the Centre for Agricultural Research are the Institute for Veterinary Medical Research (1143 Budapest, Hungária krt. 21), the Plant Protection Institute (1022 Budapest, Herman Ottó út 15 and 2094 Nagykovácsi, Nagykovácsi út 26–30), and the Institute for Soil Sciences and Agricultural Chemistry (1022 Budapest, Herman Ottó út 15). The latter institute continues to maintain its experimental stations in Őrbottyán (2162 Őrbottyán, Kvassay telep) and Sárhatvan (7019 Sárhatvan, Ötvenkilencpuszta). These institute addresses also serve as invoice addresses.

The acting Director-General of the Centre for Agricultural Research is Zoltán Bedő (+36-22-569570, bedo.zoltan@agrar.mta.hu), who also is the director of the Agricultural Institute. The Financial Management of the Research Centre is also located in the Martonvásár headquarters. The acting Financial Director is Dr Ágnes Gaál (+36-22-569570, gaal.agnes@agrar.mta.hu).

Tibor Magyar (+36-1-467-4060, magyar.tibor@agrar.mta.hu) has been appointed as the director of the Institute for Veterinary Medical Research, and Levente Kiss (+36-1-4877521, kiss.levente@agrar.mta.hu) as the director of the Plant Protection Institute. He also acts as deputy to the Director-General. The director of the Institute for Soil Sciences and Agricultural Chemistry is Attila Anton (+36-1-2122265, anton.attila@agrar.mta.hu).

As the legal successor of the above research institutes, the Centre for Agricultural Sciences carries out basic and applied research and innovation activities, participates in the dissemination of professional and scientific knowledge in the field of both education and agriculture, and works in coöperation with all social organisations whose activities contribute to the sustainable development of agriculture, the food industry, rural development, and environment protection.

Wheat season. After the extremely wet 2010 (>1,000 mm rain), 2011 proved to be the dryest year in the last several decades (262 mm) compared to the average of 550 mm. Due to the dry conditions, no serious disease epidemics occurred. The national wheat average reached only 4.04 t/ha, which is equal to the average of the last 20 years. The quality of harvested wheat was good with relatively low protein content.

Breeding.

Z. Bedő, L. Láng, O. Veisz, G. Vida, M. Rakszegi, I. Karsai, K. Mészáros, S. Bencze, and Cs. Kuti.

New releases. Three Martonvásár winter wheat and two winter durum wheat cultivars were registered in Hungary in 2011.

Mv Karéj (Mv 18-08) is an early maturing cultivar with high yield potential and outstanding protein quality. The origin of Mv Karéj is 'F29K4-22211/F30K2/3/MVMA/MV12//F2098W2'. The cultivar has very good winter hardiness and good lodging resistance. Grains are hard red, wet gluten content is 30–31%, farinograph quality A1, farinograph

stability is 17–18 min, the alveograph W value is 380–440, and extensograph energy (at 135 min) is 170–180. Mv Karéj is moderately resistant to powdery mildew and leaf rust and moderately susceptible to stem rust.

Mv Lepény (Mv 05-08) is a high-yielding, early maturing, soft red winter wheat. Mv Lepeeny has a very low (20–24%) wet gluten content, low water uptake, and poor farinograph stability, alveograph, and extensograph properties. This cultivar possibly could be used for bisquit or beer production. The winter hardiness is reliable and it is resistant to leaf rust and moderately resistant to powdery mildew, but is susceptible to stem rust races used in artificial inoculation.

Mv Sobri (Mv 23-08) is an early maturing, bread wheat cultivar. Selected from the cross 'MV111-88/F2076//KARL92/3/MVMA/MV8//F2098W2-21/4/UKRAINKA', Mv Sobri is resistant to stem rust and has good field resistance to powdery mildew and leaf rust. The gluten content exceeds 30%; gluten quality is good, but not outstanding.

Mv Pennedur (MvTD33-08) isa high yielding, winterhardy, durum wheat. Selected from the cross 'GKD75/Aisberg//GK Bétadur', this cultivar possesses a good combination of high yellow pigment content and strong gluten, as measured by the gluten index method.

Mv Hundur (MvTD05-08) is a winter durum cultivar with a high wet gluten content. According to the results of the official state trial, the 3-year mean protein content of Mv Hundur was 15.5% and the yellow pigment content was 8.0 mg/kg, which were the highest values in case of both quality characteristics. This cultivar has outstanding vitriousness stability among the winter durum wheats.

Breeding for quality traits. Organoleptic, nutritional, and end-use properties of the breeding lines and populations developed under WP3-6 of the SOLIBAM project were studied in the frame of WP7. This study included 750 wheat, 196 durum, 57 barley, and 72 *T. monococcum* samples. Properties were studied on plants growing at different agroclimatic conditions using different breeding techniques and crop management systems. Nutritional properties, such as antioxidants, protein, and starch or amylose content, were measured to evaluate the health-related effects of the organically bred lines. At the same time, the impact of crop management and breeding (crop rotation, mulching, and irrigation) was studied on the end-use quality (breadmaking and pasta making) of the lines, especially taking into consideration the requirements of the production of special local products.

Breeding of wheat with high dietary fiber content. An exotic, Chinese wheat cultivar, Yumai-34, contains extremely high quantities of water-extractable arabinoxylans in the flour compared to wheat cultivars in commercial use in Europe and other parts of the world today. This property was utilized in a breeding program begun in order to produce wheat cultivars with good adaptability and a high dietary fiber content. The breeding lines produced were analyzed not only for dietary fiber, but also for protein and alkylrezorcinol content, in order to select genotypes having several beneficial properties by taking into consideration a multidimensional matrix affect. The F_4–F_6 generations already had been analyzed and outstanding breeding lines were selected for further study and seed multiplication. Oats has another component of dietary fiber in outstanding quantity related to other cereals, β-glucan. In order to improve the dietary fiber content of oat, the variability of β-glucan content was studied in several winter and spring oat genotypes and outstanding lines were used for breeding purposes. We found that the β-glucan content depended on the year and environmental factors, but did not depend on the seasonal type of the cultivar. This project requires the effective coöperation among different areas, plant breeding, cereal chemistry, quality control, nutrition, technology, and food safety. Clinical studies also were applied to strengthen the authenticity of our results for consumers and for the society.

Genotypes with different arabinoxylan and gluten content were selected, and a 'GxE' experiment was set up. The agronomical, biochemical, rheological, and other breadmaking quality-traits were analyed according to protocols developed previously. Correlations of the biochemical traits and the rheological properties, grain hardness, falling number and waterabsorption were studied. The effect of the genotype and the environment were also determined to those properties.

Forty-two genotypes were selected for this study including old and modern Hungarian wheat cultivars using Glenlea and Bezostaja-1 as controls. The old cultivars, TF-Kompolt, TF-Gyulavári, TF-Tiszatarján, and Szekacs 1055-4-1, contained outstanding quantities of water-extractable arabinoxylane, whereas Mv Suba and Mv Marsall were outstanding from the modern wheat cultivars. HMW- and LMW-glutenin composition and quantities were analyzed by LOC, MALDI, and SE- and RP-HPLC methods. Some of the measurements were made in Australia in collaboration with Ferenc Békés. We found that both the HMW- and the LMW-glutenin alleles significantly affected the Zeleny sedi-

mentation, farinograph dough development time, and stability. Alveographic W and the gluten index were determined mainly by the HMW-glutenins, whereas gluten spread, alveograph P/L, and farinograph dough softening were influenced by the LMW-glutenin content. No correlation between the water absorption, falling number, and the protein allele composition of the cultivars was observed. From the quantitative traits, the unextractable polymeric protein (UPP%) content correlated most significantly with most of the breadmaking quality properties (Zeleny, GI, W, stability, and QN). A new mathematical model will be developed based on this knowledge of the relationship of breadmaking and genetic and biochemical properties. At the same time, a useful tool for breeding practices also will be developed to monitor any selelcted genotypes.

Disease resistance studies.

Fusarium head blight resistance. The 228 RILs of the population of 'Ning8331(resistant)/ Martonvásári 17 (moderately susceptible)', established for studying the genetic background of Fusarium head blight resistance, were screened with 17 AFLP primer combinations. The evaluation of the gel images revealed 366 different types of polymorphism. Together with the 97 polymorphic SSR markers identified earlier, a total number of 463 loci were evaluated with the JoinMap4 software and linkage groups were created.

The QTL mapping of the 'Bánkúti 1201-9086/Mv Magvas' population, developed to analyze the Fusarium head blight resistance of the old Hungarian wheat cultivar Bánkúti 1201, was continued by testing the parental lines with a total of 140 microsatellite markers. The whole population was tested with 33 SSR markers and 24 different AFLP primer combinations. A preliminary linkage map was created with JoinMap 5.0 software using 319 polymorphic markers.

Leaf rust resistance. Genotypes carrying designated leaf rust resistance genes were tested for infection in an artificially inoculated nursery. In 2011, genes *Lr9*, *Lr19*, *Lr25*, *Lr28*, and *Lr29* provided effective protection against leaf rust in Martonvásár. Resistance genes (*Lr9*, *Lr24*, *Lr25*, *Lr29*, *Lr35*, *Lr37*, *Pm21*, and *Stb2* were introduced into wheat cultivars adapted to Hungary. By jointly incorporating several resistance genes (pyramiding), winter wheat genotypes carrying new *Lr* gene combinations were developed.

Stem rust resistance. Among the genotypes carrying designated *Sr* genes, lines with *Sr28*, *Sr30*, or *Sr36* were highly resistant to stem rust and, additionally, some more major resistance genes (A, B, C, and D alleles of *Sr9*, *Sr13*, *Sr18*, *Sr31*, and *Sr33*) provided effective resistance againts the spread of the pathogen.

Powdery mildew virulence survey. Studies on the composition of the wheat powdery mildew population showed that race 77 was present in the highest ratio (47.6%) in 2011, followed by race 76 (40.2%). Race 51, which infected all the cultivars tested for differentiation, appeared in an even lower frequency than in the previous year (3.9%). The virulence complexity of the pathogen population was 5.46, which represented a decline compared to previous years.

Impact of elevated atmospheric CO_2 level on disease resistance. Susceptible wheat varieties were found to become more susceptible to leaf rust and stem rust when grown at doubled atmospheric CO_2 level. Resistant cultivars were, however, unaffected by high CO_2. The severity of Fusarium head blight infection in susceptible wheat cultivars also was enhanced under high CO_2, despite that the establishment of the pathogen was delayed in some cases.

Abiotic stress resistance studies. The role of the biotic and abiotic stresses in the antioxidant enzyme activities of wheat cultivars was determined. Based on the results of phytotron and field studies, we found that the activity of peroxidases was outstandingly high in winter, whereas catalase played a role in mitigating the harmful environmental effects in the early summer. The levels of glutathione reductase, glutathione-S-transferase, and guaiacol peroxidase were found to correlate positively with water shortage.

A preliminary molecular genetic study on 759 wheat cultivars, either bred in Martonvásár or originating from various parts of Europe, was carried out. The allele compositions of the major genes regulating vernalization and day-length sensitivity (*VRN-A1*, *VRN-B1*, *VRN-D1*, and *PPD-D1*) were determined in the test sortiment. Based on the genetic diversity, wheat genotype subgroups with even population structures were established (262 genotypes) and drawn into further thorough field and laboratory analyses. The date of anthesis and the grain yield components were determined in the first field experiment with the subgroups.

A mapping population created with the SSD method was tested with SSR markers of known chromosome localization for the background of heat tolerance. Twenty-nine SSR markers were mapped on 323 lines of the wheat population. We selected 282 lines to investigate DArT to create a molecular marker map of the population.

The water use efficiency (WUE) of small grain cereals was investigated at optimum water supply level and under water withdrawal. We found that the water use of cereals was the highest during heading. The greatest amount of water during the entire vegetation period was used by winter barley, irrespective of the water supply level. The requirement of oats was a little lower, whereas water uptake was considerably less for wheat genotypes. The mean values of WUE (m^3 water/kg grain yield) at normal water supply level and under water stress, respectively, were 0.54 and 0.716 for the wheat cultivars, 0.73 and 1.28 for winter barley, and 1.2 and 2.02 for winter oats.

According to a field experiment on the drought tolerance of a biparental dihaploid genetic population, the plants grown under precipitation shortage, without irrigation, headed significantly earlier than those that received irrigation. Significant decreases in the population in plants receiving no irrigation were found in the mean values for plant height parameters, including the length up to the flag leaf, and to the base and top of the spike, but also in the total number of nodes, length of the last internode, and the number of productive tillers. A considerable reduction in the foliage during the vegetation period also was observed.

A comprehensive tool to manage and analyze phenotypic and molecular breeding data.

In recent years, an information system has been elaborated and constantly improved in Martonvásár, making it possible to handle the 3–4 x 10^6 identifications, observations, measurements, pedigree, and other data generated for a total of nearly 120,000 experimental plots each year. The data were grouped according to subject, leading to different data structures within the data model: (1) breeding databases, which are renewed from one year to the next; (2) a genealogical database; (3) a seed exchange database; and (4) a genebank database.

For the data to be available within the information system, special applications were designed to support various research tasks. These applications are based on the technological description of the task in question. Approximately 50 applications are included in the 'Breeder' user interface, providing uniform availability to breeders and other agricultural staff. The uniform framework of the various modules contains menus, submenus, and a quick launch toolbar for the most frequently used program modules such as crossing, selection, or plot design.

When crosses are made, the program automatically creates the new pedigree, selection history, and saves major data related to the various crossing programs in crossing lists.

The selection module records the origin of the genotype (previous year: experiment, plot), number of rows planted, automatically develops selection history, and provides links with variety maintenance and frost and resistance testing. The program automatically records grain weight and it is possible to automatically select genotypes according to previously adjusted scoring values or other previously established conditions.

The plot design module is able to automatically add checks or elaborate experiments of a given size. When the plot order is finalized, the breeder has the option of distinguishing between particular spike types, arranging the lines in order of heading, or grouping related lines within genotypes.

The seed exchange module stores information on all the incoming and outgoing seed lots for Hungarian and foreign breeding programs.

The gene bank module was designed to store data on genetic resources and has links to the breeding and genealogical data.

A uniform pedigree model, capable of creating a unique identification code for each genotype and handling the homonyms and synonyms, is one of the cornerstones of the information system. The pedigree data-handling module automatically assign two identification codes to each pedigree; PID identifies the combination and SID differentiates between sister lines arising from the same crossing combination. The name of each genotype appears only once across the whole system, and genealogical information can be attached from here to the records of individual years or experiments.

No special statistical packages are required for the basic statistical analysis of experiments sown at one or more locations, the statistical evaluation of replicated single-factor and multi-factor experiments was incorporated into the system.

Automated data collection is used for instant and automatic capturing of data and information from various sources and is based on automatic data identification. Automatic data identification is based on barcodes, so a module designed to generate and print barcodes was incorporated into the system. With the help of the module, combining a number of data fields into a single barcode and printing them onto self-adhesive or plastic labels directly from the database is possible.

Later, a separate data structure was introduced to accommodate molecular data elements, such as gene source, primer bank, primer combinations, markers, genes, and alleles, but also data management tools and a standalone software interface to combine both molecular and phenotypic data.

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Association between pericentromeric SSR bands and intergenomic translocation breakpoints in natural populations of *Aegilops biuncialis* and *Ae. geniculata*. Repetitive DNA sequences are thought to be involved in the formation of intergenomic translocations, which is an important step in allopolyploid speciation. The allotetraploids *Ae. biuncialis* and *Ae. geniculata*, wild, genetic resources for wheat improvement, are excellent objects to study the role of microsatellite clusters in the formation of intergenomic translocations. The chromosomal localization of (ACG)n and (GAA)n microsatellite sequences in *Ae. biuncialis* and *Ae. geniculata* and their diploid progenitors *Ae. comosa* and *Ae. umbellulata* was investigated by sequential *in situ* hybridization with SSR probes and repeated DNA probes (pSc119.2 Afa family and pTa71) and by dual-color GISH. Thirty-two *Ae. biuncialis* and 19 *Ae. geniculata* accessions were screened by GISH for translocations, which were further identified by FISH and GISH. Single pericentromeric (ACG)n signals were localized on most U- and on some M-genome chromosomes, whereas strong pericentromeric and several intercalary and telomeric (GAA)n sites were observed on the *Aegilops* chromosomes. Three *Ae. biuncialis* accessions carried $T7U^b–7M^b$ reciprocal translocations and one had a $T7U^b–1M^b$ rearrangement, and two *Ae. geniculata* accessions carried $T7U^g–1M^g$ or $T5U^g–5M^g$ translocations. Conspicuous (ACG)n and/or (GAA)n clusters were located near the translocation breakpoints in eight out of the ten translocated chromosomes analyzed, SSR bands and breakpoints being statistically located at the same chromosomal site in six of them. SSR clusters seem to be involved in the formation of intergenomic translocations between the constituent genomes of allopolyploid *Aegilops* species. The (ACG)n and (GAA)n SSR motifs serve as additional chromosome markers for the karyotypic analysis of UM-genome *Aegilops* species.

Chromosome isolation by flow sorting in *Ae. umbellulata* and *Ae. comosa* and their allotetraploid hybrids *Ae. biuncialis* and *Ae. geniculata*. An international cooperation with Jaroslav Doležel (Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic) was carried out to study the potential of flow cytometry for chromosome sorting in the wild wheats *Ae. umbellulata* (2n=2x=14, UU) and *Ae. comosa* (2n=2x=14, MM) and in their natural hybrids *Ae. biuncialis* (2n=4x= 28, $U^bU^bM^bM^b$) and *Ae. geniculata* (2n=4x=28, $U^gU^gM^gM^g$). Histograms of fluorescence intensity (flow karyotypes) obtained after the analysis of DAPI-stained chromosomes were characterized and the chromosome content of the four peaks on flow karyotypes was determined for the first time for these *Aegilops* species. Chromosomes sorted onto microscope slides were identified after FISH with probes pSc119.2, Afa repeats, and pTa71. Only chromosome 1U could be discriminated by the peak I on flow karyotypes of *Ae. umbellulata* and *Ae. biuncialis* with the standard karyotype. Microscopic evaluation of the sorted fractions showed that chromosome 1U could be sorted at purity >95%. Remaining chromosomes formed composite peaks and could be sorted only as groups. Distribution of chromosomes among the peaks of flow karyotypes reflected different size modifications for the chromosomes 4U, 1M, 2M, 3M, 4M, and 6M in the *Ae. biuncialis* and *Ae. geniculata*. Twenty-four wheat SSR markers were used to map the U- and M-genome chromosomes by the use of DNA amplified from the sorted chromosome fractions and wheat–*Ae. geniculata* addition lines. Ten SSR markers located unambiguously on the *Aegilops* chromosomes supported the chromosome content of sorted fractions and confirmed the suitability of flow-sorted chromosomes for physical mapping. These SSR markers are suitable tools for the marker-assisted production of wheat–*Aegilops* introgression lines. These results open the way for the construction and sequencing of large-insert,

chromosome 1U-specific, DNA libraries in *Ae. umbellulata* and *Ae. biuncialis*, which would greatly promote the targeted isolation of molecular markers and the discovery of novel genes for the wheat improvement.

Characterization of a new T4BS·7HL wheat/barley translocation line using GISH, FISH, and SSR markers and its effect on the β-glucan content of wheat. A spontaneous interspecific Robertsonian translocation was revealed by GISH in the progenies of a monosomic 7H addition line originating from a new wheat/barley, 'Asakaze komugi/Manas' hybrid. FISH with repetitive DNA sequences Afa family, pSc119.2, and pTa71 allowed identification of all wheat chromosomes, including wheat chromosome arm 4BS involved in the translocation. FISH using barley telomere- and centromere-specific repetitive DNA probes (HvT01 and AGGGAG) confirmed that one of the arms of barley chromosome 7H was involved in the translocation. SSR markers specific to the long and short arms of barley chromosome 7H identified the translocated chromosome segment as 7HL. Further analysis of the translocation chromosome clarified the physical position of genetically mapped SSRs within 7H, with a special focus on its centromeric region. The presence of the HvCslF6 gene, responsible for (1,3;1,4)-β-D-glucan production, was revealed in the centromeric region of 7HL. An increased (1,3;1,4)-β-D-glucan level also was detected in the translocation line, demonstrating that the HvCslF6 gene is of potential relevance for the manipulation of wheat (1,3;1,4)-β-D-glucan levels.

Constructing a detailed mcFISH karyotype and studying chromosome polymorphisms of *Elytrigia elongata* E genome using highly repetitive and rDNA sequences. *Elytrigia elongata* (= *Agropyron elongatum*, *Thinopyrum elongatum*, 2n=2x=14, EE) has long been used as a source of various resistance for wheat improvement, and numerous transfers have been made. However, despite heavy use, the species has never enjoyed cytological attention it deserves and no high resolution karyotype exists, perhaps because its chromosomes do not C-band well and the genome is rather symmetrical. As the interest in *E. elongata* for wheat improvement does not appear to abate, and alien transfers are now routinely detected and followed using the techniques of *in situ* probing with labelled DNA, a detailed FISH karyotype of the E genome was generated and verified in several accessions. The karyotype itself was generated using highly repetitive DNA sequences and sequential GISH–mcFISH; chromosome identification was using the complete *E. elongata* disomic chromosome addition series and 11 ditelosomic addition lines in Chinese Spring wheat. The E-genome chromosomes in a wheat background were detected by probing with the total genomic DNA of *E. elongata*, followed by mcFISH with five repetitive DNA probes. Of these, two clones failed to hybridize to the E-genome chromosomes. Based on the successful mcFISH, each complete chromosome and each telocentric studied was unambiguously identified. Validation of the karyotype in four *E. elongata* accessions with different geographical origins showed extensive variation of the probe hybridization patterns but this did not prevent positive chromosome identification. We believe that this karyotype will be useful in quick identification of potential donor chromosomes in wheat improvement programs so that proper alien transfer approaches can be selected and implemented.

Development of synthetic amphiploids based on different *Triticum turgidum* × *T. monococcum* crosses to improve the adaptability of cereals. Cultivated einkorn (*T. monococcum* subsp. *monococcum*) is an excellent source of resistance against several wheat diseases and quality parameters. Semidwarf, einkorn lines with good crossability were identified in order to produce '*T. turgidum* × *T. monococcum*' synthetic amphiploids. Two combinations were used to develop the amphiploids: 'durum × einkorn' and 'emmer × einkorn'.

After the genome duplication of the F_1 seed, highly fertile amphiploids were developed. The A^uBA^m genome structure of the progenies was confirmed by GISH. Lines derived from 'durum × einkorn' and 'emmer × einkorn' crosses were studied for agronomic performance, disease resistance, and genetic variability. Both amphiploid combinations showed excellent resistance against certain wheat diseases (leaf rust and powdery mildew), but not against Fusarium. The durum-based synthetic amphiploid lines showed a higher level of phenotypic diversity. The newly produced '*T. turgidum* × *T. monococcum*' synthetic hexaploids are promising genetic resources for wheat breeding. Selected 'durum × einkorn 'lines are currently used in bread wheat improvement to transfer the useful properties of einkorn into cultivated hexaploid wheat via bridge crosses.

Martonvásár Cereal Gene Bank and Organic Plant Breeding. The Cereal Gene bank activity mainly concentrated on the characterization of the different resistance sources such as *T. monococcum* and *T. timopheevii* accessions, and their possible use in the development of new synthetic amphiploids. New, highly crossable, semidwarf, *T. monococcum* lines have been developed, and crossed with selected *T. turgidum* subsp. *durum* and *T. timopheevii* accessions or selected breeding lines. Triploids were identified in the F_1 generation, and treated with colchicine to double the chromosome number of the interspecific hybrids. Fertile hexaploids were selected in the C_2 generation and multiplied under greenhouse conditions. The new synthetics will be used in wheat-improvement programs and are target of independent breeding line development.

Our organic breeding program is highly focused on the development of new, organic cultivars of alternative or underutilized cereals such as einkorn and emmer. A new, organically bred, einkorn cultivar, **Mv Menket**, was released last year. This is the first semidwarf einkorn cultivar in the market, with elevated yield potential, and excellent resistance against most of the wheat diseases, except Fusarium. Mv Menket is an organic cultivar, because it is highly sensitive against all herbicides used in the Hungarian farming practice, and there is no possibility to use it in traditional agricultural practices.

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ITEMS FROM INDIA

DIRECTORATE OF WHEAT RESEARCH
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A study of floral biology traits in bread wheat.

S.K. Singh and Dharmendra Singh.

Summary. Three floral biology traits, anther length, stigma length, and anther extrusion, were investigated in 92 elite wheat germ plasm lines. A wide range of variability was observed for these floral traits and promising genotypes were identified for their further utilization as parents for hybrid wheat development.

Wheat productivity levels in the present genotypes have reached a saturation level, which limits higher production to meet the targets for food security. Newer, innovative techniques can be promising approaches in order to break yield barriers and, in this regard, hybrid wheat development through exploitation of heterosis may be a potential tool. Wheat production in the Northwest Plains Zone has reached peak yields. Knowledge about the variability and character association between floral characteristics is crucial to identify suitable male or female parental lines for further utilization in hybrid development programs. Our objective was to study the extent of variability present in some wheat genotypes for various floral characters and establish correlations between them.

Materials and methods. A total of 92 elite genotypes maintained by the Hybrid Wheat Programme were used in the study. These entries included elite selections from international nurseries and trials from CIMMYT; genetic stocks;

advanced lines for yield, abiotic stress tolerance, and disease resistance; and yield component lines. The experiment was laid out in randomized block design with three replications with plot size of double row of 2.5-m in length. All agronomical practices for raising a good crop were adopted. Wheat is a self pollinated crop and, therefore, floral traits promoting out crossing were investigated. The genotypes were evaluated for three floral traits, anther length, stigma length, and anther extrusion in order to get basic information about their floral behavior. Observations were taken on 10 randomly selected plants from each replication. Anther length was measured in mm and recorded as the average length of three anthers belonging to the lateral florets of the two central spikelets of a spike. Stigma length also was measured in mm and recorded as the average length of two stigmas of the lateral florets from two central spikelets of a spike. Anther extrusion was measured as a percent after counting the extruded anthers of two lateral florets of five central spikelets from 10 spikes/replication. The data were analyzed (Panse and Sukhatme 1967) to work out the range, mean, and correlations among the traits and to identify promising genotypes for further utilization.

Results and discussion. Mean values for various traits in different genotypes indicated a very wide range for all three traits under study (Table 1). Anther length ranged from 3.20 to 5.60 mm with a mean value of 4.53 mm. Stigma length ranged from 2.20 to 4.30 mm with a mean value of 3.10 mm. Anther extrusion ranged from 15.17% to 63.07% with an average value of 33.27%. Similar findings also were reported by Hucl (1996), Singh and Joshi (2003), and Singh (2006). The association between these floral traits indicated a high correlation between anther and stigma length.

Table 1. Range, mean, and character association for floral traits in wheat.

Floral trait	Range	Mean	Correlation coefficient	
			Stigma length	Anther extrusion
Anther length (mm)	3.20–5.60	4.53	0.36	0.08
Stigma length (mm)	2.20–4.30	3.10		–0.03
Anther extrusion (%)	15.17–63.07	33.27		

The frequency of genotypes in the different floral traits classes also was recorded (Fig. 1). Most of the genotypes showed an anther length of 4.51–5.00 mm, a stigma length of 2.51–3.00 mm, and anther extrusion of 25.1–45.0%.Twelve genotypes had an anther length of more than 5.0 mm. Genotype HT 97 had longest anthers, at 5.60 mm, followed by Giant 3 and KRL 237 (5.50 mm). Other genotypes with promising anther lengths were 25 SAWSN 3034, 25 SAWSN 3178, DWR 39, GW 273, HD 2009, HUW 34/LR 19, KRL236, LOK 62, and NIAW 1275. HI 1077 showed the highest anther extrusion of 63.07%, followed by 18 HRWSN 2066 (55.91%) and 15 HRWYT 222 (55.63%). NIAW 1275 was the only genotype with a stigma length (4.30 mm) of more than 4.0 mm.

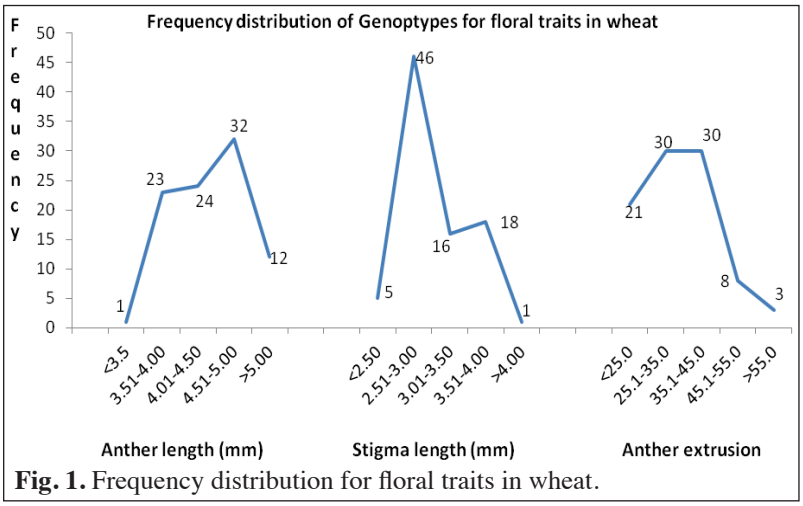

Fig. 1. Frequency distribution for floral traits in wheat.

Based on the performance for various floral traits, promising genotypes with high values were identified (Table 2, p. 81). Among these, 15 HRWYT 222, 25 SAWSN 3034, GW 273, HD 2329, KRL 236, and NIAW 1275 were found promising for two or more floral traits. These can be further utilized in conversion into CMS and restorer lines for hybrid wheat development.

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Table 2. Promising genotypes for floral traits.

Genotype	Anther length (mm: >5.00)	Stigma length (mm: >3.75)	Anther extrusion (%: >40.0)
15 HRWYT 222	5.00	3.90	55.63
18 HRWSN 2066	4.00	2.60	55.91
25 SAWSN 3034	5.20	3.50	47.36
25 SAWSN 3169	5.00	4.00	24.79
25 SAWSN 3178	5.20	2.70	37.75
DWR 39	5.10	3.10	25.34
FLW 8	5.00	4.00	23.59
GIANT 3	5.50	3.50	27.43
GW 273	5.20	2.50	45.13
GW 411	5.00	4.00	20.15
HD 2009	5.30	2.50	38.08
HD 2329	5.00	4.00	40.03
HI 1077	5.00	2.90	63.07
HP 1296	5.00	3.90	36.11
HT97	5.60	3.70	51.23
HUW 34/LR 19	5.20	2.50	22.13
KRL 237	5.50	3.00	23.33
KRL236	5.30	3.80	29.89
LOK 62	5.30	2.60	21.46
NIAW 1275	5.20	4.30	36.84
PHR 1005	4.50	3.00	53.55
UAS 323	5.00	3.90	22.05

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Molecular and phenotypic diagnostics applied to verify the presence of rust resistance genes Lr24, Yr15, and Sr2 in a high-yielding bread wheat genotype suited for cultivation in Central India.

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Wheat area, production, and productivity in the Central Zone (CZ) of India is steadily increasing and this increase in area is attributed to a slow shift from rainfed to limited irrigated areas with the advent of modern irrigation facilities adopted by farmers. This shift leads to an increase in the demand for cultivars suitable for irrigated, timely sown conditions. A bread wheat genotype developed at the IARI, Regional Station, Wellington, and designated as HW 5207, has the potential to meet this requirement, because it has shown consistent yield under conditions prevailing in the CZ. The proposed cultivar carries the durable stem rust resistance gene *Sr2* and leaf rust resistance gene *Lr24* (resistant to all Indian pathotypes of leaf rust) providing high degree of resistance to stem and leaf rusts and *Yr15* for yellow rust resistance. Thus, the identification of HW 5207 as a suitable genotype for cultivation in the CZ will provide an alternative as well as add to the desired genetic variability in terms of yield and rust resistance in the CZ.

Materials and methods. The wheat genotype HW 5207 (HW stands for Hybrid Wellington) was developed by the pedigree method from the cross 'HW 3029//*Yr15* (V763-2312)' and tested under multilocation yield trials in the CZ conducted under the aegis of All India Coordinated Wheat and Barley Improvement Project (AICW&BIP) coordinated by the Directorate of Wheat Research (DWR), Karnal (Haryana) for irrigated, timely sown conditions. Trials were conducted

over three years, starting with 2007–08, to evaluate yield ability (DWR 2007a, 2008a, 2009a). Yield potential was evaluated comparatively with popular wheat cultivars of the CZ, GW 322, GW 366, Lok 1 ,and HI 1544 (checks). Yield trials were conducted in the states of Gujarat, Madhya Pradesh, Rajasthan, Chhattisgarh, and Uttar Pradesh on selected representative locations. Rust resistance under artificial epidemics was evaluated simultaneously for three years beginning in 2006–07 by entering the genotype in the multilocational Initial Plant Pathological Screening Nursery (IPPSN) and Plant Pathological Screening Nursery (PPSN) conducted by the DWR, Karnal (DWR, 2007b, 2008b, 2009b). After establishing artificial epidemics at multilocations following the method of Joshi et al (1982), the rust scores were determined as Average Coefficient of Infection (ACI) calculated by a formula (Joshi et al. 1982). Reaction to rusts also were recorded under natural conditions at all the test locations following the Peterson scale (Peterson et al. 1948). Only the highest score observed under natural conditions among multilocations was considered. Seedling resistance tests also were made during 2008–09 and 2009–10 at the DWR, Regional Station, Flowerdale, Himachal Pradesh (Prashar et al. 2009). Seedling reactions produced with Indian rust pathotypes were recorded by following the standard methods described by Nayar et al. (1997).

Validation of rust resistance genes Sr2, Lr24 and Yr15 applying molecular markers. Stem rust resistance gene *Sr2* was confirmed by the symptoms of pseudo blackchaff on the glumes of this genotype (Fig. 1). The presence of *Sr2* was further confirmed by applying the microsatellite marker *Xgwm533* that amplified at 120 bp (Fig. 2). Leaf rust resistance gene *Lr24* in HW 5207 was speculated on the basis of pedigree details indicating HW 3029 as one of the parents developed through backcross program at the IARI, Regional Station, Wellington, Tamil Nadu, which has a rust resistance from *Thinopyrum elongatum* (Sivasamy and Brahma 1999). The presence of *Lr24* also was evidenced by seedling resistance data showing HW 5207 as resistant to all Indian pathotypes of leaf rust (Prashar et al. 2009). *Lr24* was further validated by applying the SCAR marker *SCS1302-607bp*. Similarly, the presence of another resistance gene, *Yr15*, also was confirmed by applying the SSR marker *Xgwm273-175bp*. In both cases, detection was done in F_4 lines developed out of the cross 'HW 3029 (*Lr24*)//V763-2312 (*Yr15*)'.

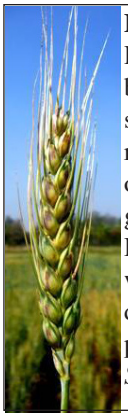

Fig. 1. Pseudo black-chaff symptoms recorded on the glumes of HW 5207, which indicates the presence of *Sr2* gene.

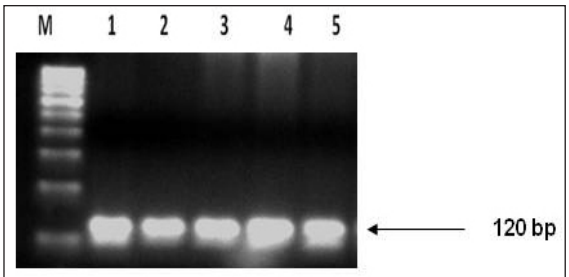

Fig. 2. Molecular confirmation of the presence of *Sr2* gene in HW 5207. The marker *Xgwm533* is linked to *Sr2*. Lane M = 100-bp ladder, lane 1 = Kingbird (positive control), lanes 2–5 = HW 5207.

Results and discussion. ***Yielding ability of HW 5207***. Genotype HW 5207 has a significantly higher mean grain yield (48.97 q/ha) over all the checks and the recently released HI 1544; it is equal with GW 322. HW 5207 has a yield potential of 51.3 q/ha. This genotype also has ranked 20 times in the first nonsignificant group out of 43 trials conducted at different locations over three years of testing, indicating a wide adaptability and stability in its performance.

Rust resistance of HW 5207. HW 5207 exhibited a high degree of resistance to stem, leaf, and stripe rusts when tested at multiple locations under both artificial (PPSN sites) and natural epidemics (Yield trial sites in the states of Gujarat, Madhya Pradesh, Rajasthan, Chhatisgarh, and Uttar Pradesh). HW 5207 also showed a resistant reaction to all the pathotypes of stem and leaf rust pathogens when tested at the seedling stage at DWR, Regional Station, Flowerdale. For stripe rust, data of only one year, 2009–10, were available.

Validation of genes Sr2, Lr24, and Yr 15 using molecular markers. The stem rust resistance gene *Sr2* present in this study has provided durable, broad-spectrum, adult-plant resistance to the fungal pathogen of stem rust. This resistance gene tends to be nonspecific and is currently effective against all isolates of *P. graminis* throughout wheat-growing regions of the world (Sunderwith and Roelfs 1980; McIntosh et al. 1995). The tightly linked, microsatellite marker *Xgwm533* with specific bands at 120 bp confirmed the presence of *Sr2* (Fig. 2). The HW 5207 genotype also has leaf rust resistance gene *Lr24* introgressed by backcrossing and stripe rust resistance gene *Yr15* (introgressed from Avocet/*Yr15*). Negative and positive controls also were included in our study to confirm molecular markers for *Lr24* and *Yr15*. Both genes were in the background of the wheat HW 3029 (Sivasamy and Brahma 1999). The presence of *Lr24* was confirmed by specific amplification of a single fragment of 607 bp in HD2687+*Lr24* and HW 5207, whereas no amplified product was detected in other genotypes; HD 2687 was used as a negative control (Fig. 3A, p. 83). Similarly, PCR with SSR primers of rust resistance gene *Yr15* resulted in the amplification of a 156-bp fragment in HW 5207 and the positive control 'Avocet/*Yr15*' only (Fig. 3B, p. 83); no amplified product was observed in the negative control genotype

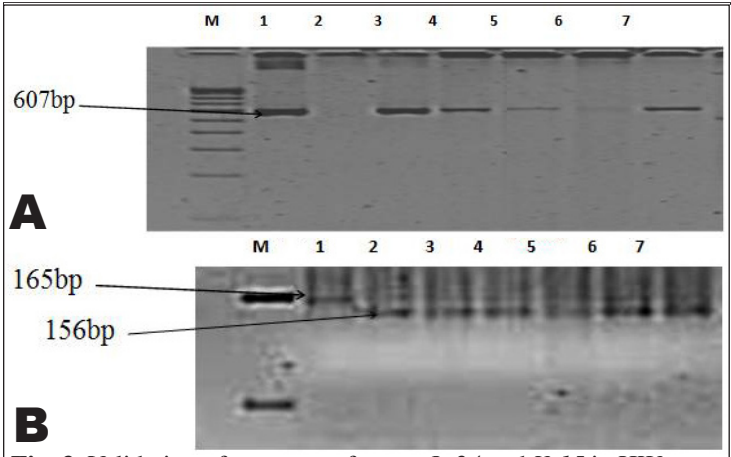

Fig. 3. Validation of presence of genes *Lr24* and *Yr15* in HW 5207 through molecular markers. A. Marker *Xscs1302* linked to *Lr24* (Lane M = 100-bp ladder, lane 1 = HD 2687+*Lr24* positive control, lane 2 = HD 2687 negative control, and lanes 3–7 = HW 5207). B. Marker *Xgwm273* linked to *Yr15* (Lane M = 100-bp ladder, lane 2 = HD 2687 negative control, lane 2 = Avocet*6/*Yr15* postitive control, and lanes 3–7 HW 5207).

HD 2687. The leaf rust resistance genes *Lr24* was introgressed from *Th. elongatum*, but the amplified fragment was detected only in the genotypes possessing the *Lr24* gene.

The first line of defence against wheat rusts in India obviously is the cultivation of disease resistant cultivars. Therefore, controlling wheat rust in India depends upon evolving genotypes with major genes conferring high resistance to the existing races of the rust pathogens. When rust epidemiology was investigated in India, the existence of a *Puccinia* path was evident (Nagarajan and Joshi 1980). This path is divisible into subzones depending on the time and mode of arrival of the primary inoculum. Because of a congenial temperature and October sowing, leaf rust appears early in central India, and this area acts as secondary focus of infection for the wheat crop of the North West Plain Zone (NWPZ) including other areas of the Indo-Gangetic plains. By arresting epipdemtic development of leaf rust in central India, the wheat crop can be kept free of rusts to a larger extent in the entire country. Therefore, deploying resistance genes in central India will help curtail the spread of southern Indian inoculum further to northern India (Nagarajan and Joshi 1985). Different resistance genes can be deployed along the *Puccinia* path, which necessitates deployment of strong major genes capable of restricting inoculum build up of the pathotypes at transition areas so as to avoid their further spread to other wheat zones in the country.

Bread wheat cultivar HW 5207 was developed at the IARI, Regional Station, Wellington, by crossing 'HW 3029 (*Lr24*)/V763-2312'. One of the parents, HW 3029, was developed at this station by incorporating the alien gene *Lr24* derived from *Th. elongatum* into a well-adapted wheat cultivar PBW 343 (Sivasamy and Brahma 1999). HW 5207 carries a high degree of stem and leaf rust resistance due to the presence of resistance genes *Sr2* and *Lr24*. *Sr2*, an important source of durable resistance to the wheat stem rust pathogen, is linked to the presence of pseudo-black chaff. Despite the appearance of pseudo-black chaff on the glume, the tightly linked microsatellite marker *Xgwm533* has greatly enhanced the confirmation of this gene (Fig. 2). This gene will prevent the epidemics of rust, thereby curtailing the inoculum load that might spread northward and infect the wheat crop in the NWPZ and other areas of Indo-Gangetic plains. Hence, this genotype will act as an effective genetic barrier for Nilgiri-borne leaf rust inoculum. Gene *Lr24* was molecularly confirmed using SCAR markers. Molecular tests with a SCAR marker revealed the presence or absence of *Lr24* (Fig. 3A). The *Lr24* gene is known to be linked to the gene *Sr24* conferring resistance to stem rust, which is apparently effective against all races of stem rust (Knott 1989) except race 40-1 (62G29-1) in India (Bhardwaj et al. 2010). The selection of genotypes with the molecular marker for *Lr24* gives an additional advantage of selection for *Sr24*. *Lr24* isreported to be resistant to all the races of brown rust in India (Bhardwaj et al. 2010).

Although yellow rust is not a serious problem in the CZ, it has been sporadically observed in areas of Rajasthan and Uttar Pradesh adjoining to Madhya Pradesh. Thus, the presence of *Yr15* was confirmed molecularly (Fig. 3B) in HW 5207 is a desirable and added advantage. HW 5207 is the first genotype developed in the country to have *Yr15*, which is resistant to all pathotypes of the stripe rust pathogen prevailing in India. This additional characteristic of yellow rust resistance in this genotype may extend its utility for direct cultivation in yellow rust prone areas in the NWPZ or as an effective genetic stock for breeding yellow rust resistant cultivars in India.

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Leaf rust resistance gene Lr34 recognized in some Indian bread wheat accessions.

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A total of 2,200 germ plasm accessions of *Triticum aestivum* (bread wheat) were planted at the IARI, Regional Station, Wellington, Tamil Nadu (11°22' N 76°46' E, elevation 1,817 m, rainfall 1,500 mm) during summer (May to September) of 2011. Genotypes were sown in single, 1-m rows with 23 cm spacing maintaining one infector/spreader line after every 20th genotype along with border rows of the universally susceptible bread wheat cultivar Agra Local. Recommended cultural practices were followed until harvest.

Leaf rust survives at Wellington year round through the agencies of self sown/stray plants, breeding materials planted regularly by Indian wheat breeders for resistance evaluation, and a green bridge maintained at the station for the regular supply of rust inoculum to the breeding program. The leaf rust pathotypes 77A(109R31), 77-5(121R63-1), 77-7(121R127), 77-8(253R31), and 17(61R24) were identified from random leaf rust samples collected from fields planted with accessions and analyzed following Nagarajan et al. (1985). Rust severity was recorded following the modified Cobb scale (Peterson et al. 1948) after accessions had completed the growth stage 87 (Zadoks et al. 1974). Simultaneous observations also were made on the presence of leaf-tip necrosis (LTN) on flag leaf. Scores of 40 and below were considered resistant and those above 40 as susceptible. Three types of LTN symptoms, designated as low, medium, and high, were categorized to determine their individual effect on the terminal severity of leaf rust in the accessions. These categories of LTN were distinguished following an earlier description that symptoms of leaf tip necrosis include 2 to 3 cm of necrosis at the end of the leaves, which extend an additional 2 to 4 cm on the edge of the leaves (Singh 1992). The effect of three different categories of LTN on the terminal severity of leaf rust was visualised by counting accessions showing various severities under each category. Genotypes categorised in different classes of LTN are presented (Table 1, pp. 85-88).

Table 1. Accessions grouped according to leaf-tip necrosis type (LTN) and their reaction to leaf rust (*Puccinia trticina*). Rating of % severity after Peterson et al. 1948).

LTN type	Rust reaction	Number	Accession name
Low	Trace	150	IC542566, IC542567, IC536168, IC536188, EC573567, EC573571, EC573575, EC573576, EC573577, EC573579, EC573582, EC573583, EC573584, EC573587, EC573589, EC573599, EC573606, EC573629, EC573640, EC573641, EC573644, EC573645, EC573647, EC573649, IC536236, IC536238, IC536256, IC536259, EC573762, EC573766, EC573767, EC573768, EC573777, EC573779, EC573780, EC573783, EC573784, EC573785, EC573787, EC573788, EC573792, EC573820, EC573831, EC573833, EC573834, EC573837, EC573838, EC573841, EC573842, EC573843, EC573844, EC573845, EC573847, EC573853, EC573863, EC573864, EC573868, EC573888, EC573895, EC573896, EC573903, EC573905, EC573907, EC573920, EC573924, EC573925, EC573926, EC573927, EC573960, EC573970, EC573971, EC573974, EC573983, EC573994, EC574016, EC574017, EC574019, EC574020, EC574027, EC574028, EC574037, EC574047, EC574068, EC574069, EC574074, EC574077, EC574078, EC574079, EC574082, EC574086, EC574087, EC574088, EC574091, EC574092, EC574093, EC574094, EC574096, EC574097, EC574104, EC574106, EC574107, EC574108, EC574109, EC574120, EC574121, EC574122, IC536348, IC536364, IC536365, IC536366, IC536394, IC536403, IC536406, IC536510, IC536511, IC536512, IC536521, IC536524, IC535353, IC542418, IC542429, IC542430, IC542432, IC542438, EC574277, EC574313, EC574339, EC574340, EC574341, EC574347, EC574348, EC574384, EC574395, EC574396, EC574436, EC574626, EC574634, EC574749, EC574756, EC574760, EC574762, EC574763, EC577429, EC577431, EC577437, EC577440, EC577443, EC577464, EC577465, EC577486
	5	75	IC542570, IC542573, IC536131, IC536156, EC573586, EC573607, EC573626, EC573632, EC573642, EC573652, IC536246, EC573800, EC573803, EC573852, EC573859, EC573875, EC573910, EC573930, EC573942, EC573957, EC574075, EC574081, EC574102, EC574113, IC536398, IC536401, IC536405, IC536409, IC542395, IC542438, EC574193, EC574231, EC574234, EC574279, EC574283, EC574289, EC574294, EC574300, EC574302, EC574305, EC574306, EC574337, EC574344, EC574345, EC574365, EC574366, EC574391, EC574394, EC574400, EC574402, EC574405, EC574406, EC574419, EC574433, EC574434, EC574480, EC574481, EC574482, EC574483, EC574502, EC574579, EC574625, EC574662, EC574667, EC574668, EC574748, EC574853, EC574854, EC574934, EC574989, EC575077, EC575079, EC575081, EC575082, EC575090
	10	321	IC542568, IC542569, IC542572, IC542574, IC536125, IC536128, IC536129, IC536130, IC536136, IC536140, IC536142, IC536147, IC536158, IC536192, IC536199, IC536201, IC536202, EC573553, EC573555, EC573556, EC573557, EC573563, EC573564, EC573573, EC573574, EC573588, EC573594, IC536205, IC536208, IC536209, IC536212, IC536215, IC536226, IC536227, EC573604, EC573608, EC573609, EC573610, EC573612, EC573616, EC573617, EC573618, EC573619, EC573620, EC573622, EC573623, EC573625, EC573627, EC573646, EC573650, EC573651, EC573655, IC536237, IC536241, IC536250, EC573781, EC573790, EC573791, EC573796, EC573797, EC573798, EC573802, EC573804, EC573805, EC573806, EC573812, EC573814, EC573815, EC573824, EC573825, EC573826, EC573851, EC573854, EC573855, C573860, EC573866, EC573867, EC577530, EC577531, EC573871, EC573877, EC573878, EC573879, EC573880, EC573884, EC573886, EC573887, EC573894, EC573897, EC573899, EC573900, EC573901, EC573902, EC573908, EC573917, EC573918, EC573921, EC573938, EC573939, EC573952, EC573955, EC573956, EC573958, EC573972, EC573979, EC573980, EC573992, EC573993, EC574012, EC574018, EC574023, EC574024, EC574025, EC574026, EC574031, EC574032, EC574033, EC574041, EC574042, EC574053, EC574061, EC574062, EC574063, EC574064, EC574083, EC574099, EC574103, EC574119, EC574125, EC574126, EC574127, EC574128, EC574177, IC536349, IC536350, IC536352, IC536392, IC536523, IC535518, IC536321, IC536322, IC536324, IC536334, IC536336, IC536337, IC536338, IC536346, IC542386, IC542392, IC542397, IC542398, IC542411, IC542412, IC542416, IC542420, IC542421, IC542422, IC542423, IC542426, IC542427, IC542431, IC542433, IC542434, IC542436, EC574194, EC574195, EC574203, EC574204, EC574209, EC574230, EC574241, EC574246, EC574258, EC574278, EC574280, EC574285, EC574288, EC574292, EC574301, EC574307, EC574308, EC574309, EC574311, EC574322, EC574338, EC574343, EC574350, EC574351, EC574352, EC574356, EC574358, EC574362, EC574363, EC574369, EC574370, EC574372, EC574373, EC574374, EC574384, EC574392, EC574401, EC574404, EC574405, EC574406, EC574407, EC574408, EC574418, EC576116, EC574431, EC574432, EC574435, EC574477, EC574576, EC574577, EC574582, EC574592, EC574596, EC574612, EC574613, EC574614, EC574628, EC574629, EC574630, EC574633, EC574634, EC574637, EC574647, EC574648, EC574649, EC574650, EC574651, EC574652, EC574653 ,EC574656, EC574657, EC574658, EC574661, EC574669, EC574671, EC574711, EC574720, EC574721, EC574723, EC574737, EC574738, EC574743, EC574744, EC574745, EC574746, EC574778, EC574786, EC574793, EC574793, EC574795, EC574805, EC574809, EC574838, EC574839, EC574849, ,EC574851, EC574852, EC574856, EC574857, EC574863, EC574877, EC574879, EC574883, EC574884, EC574885, EC574886, EC574887, EC574895, EC574914, EC574915, EC574916, EC574917, EC574918,EC574919, EC574921, EC574987, EC574988, EC574992, EC574993, EC574997, EC574998, EC575001, EC575013, EC575015, EC575016, EC575028, EC575029, EC575032, EC575033, EC575036, EC575039, EC575049, EC575050, EC575058, EC575063, EC575066, EC575067, EC575068, EC575069, EC575070, EC575071, EC575072, EC575074, EC575075, EC575084, EC575085, EC575086, EC575087, EC575088, EC575089, EC575091, EC575092, EC575093, EC575094, EC575095, EC577490, EC575364

Table 1. Accessions grouped according to leaf-tip necrosis type (LTN) and their reaction to leaf rust (*Puccinia trticina*). Rating of % severity after Peterson et al. 1948).

LTN type	Rust reaction	Number	Accession name
Low	20	169	IC542575, IC536118, IC536126, IC536132, IC536133, IC536149, IC536154, EC573545, EC573546, EC573552, EC573565, EC573570, EC573581, EC573590, EC573591, EC573593, EC573597, IC536213, IC536224, IC536225, EC573618, EC573621, EC573624, EC573635, IC536245, IC536254, IC536255, EC573793, EC573807, EC573810, EC573822, EC573823, EC573848, EC573869, EC573870, EC573873, EC573874, EC573883, EC573885, EC573889, EC573890, EC573891, EC573909, EC573923, EC573934, EC573940, EC573947, EC573949, EC573953, EC573961, EC573963, EC573964, EC573965, EC573966, EC573967, EC573969, EC573982, EC573991, EC574001, EC574002, EC574004, EC574005, EC574006, EC574011, EC574013, EC574015, EC574021, EC574045, EC574049, EC574060, EC574070, IC536359, IC536516, IC536517, IC536518, IC535487, IC535488, IC535499, IC536311, IC536339, IC536340, IC536341, IC536342, IC536347, IC542380, IC542396, IC542399, IC542408, IC542415, IC542424, IC542425, IC542428, EC574202, EC574226, EC574227, EC574240, EC574247, EC574248, EC574286, EC574287, EC574303, EC574371, EC574403, EC574479, EC574571, EC574573, EC574574, EC574578, EC574581, EC574584, EC574588, EC574591, EC574597, EC574598, EC574599, EC574613, EC574617, EC574632, EC574635, EC574636, EC574646, EC574696,EC574717, EC574719, EC574757, EC574774, EC574777, EC574791, EC574796, EC574798, EC574800, EC574806, EC574815, EC574816, EC574836, EC574837, EC574842, EC574843, EC574850, EC574855, EC574858, EC574859, EC574861, EC574878, EC574980, EC574981, EC574983, EC574985, EC574986, ,EC574991, EC574994, EC574995, EC575002, EC575007, EC575010, EC575037, EC575053, EC575164, EC575287, EC577430, EC577432, EC577433, EC577434, EC577488, EC577489, EC577492, EC575409, EC575503, EC575504
	40	101	IC536121, IC536127, IC536145, IC536148, IC536150, IC536153, IC536157, EC573542, EC573544, EC573596, EC573598, IC536206, IC536207, IC536214, EC573614, EC573630, EC573638, EC573643, EC573799, EC573809, EC573817, EC573827, EC573828, EC573850, EC573892, EC573893, EC573935, EC573946, EC573950, EC573951, EC573959, EC573962, EC573995, EC573996, EC574003, EC574007, EC574009, EC574035, EC574036, EC574039, EC574055, EC574056, EC574059, IC536360, IC536361, IC536362, IC536363, IC536384, IC535356, IC535358, IC535556, IC536323, IC536345, IC542381, IC542382, IC542383, IC542384, IC542388, IC542400, IC542401, IC542407, IC542413, IC542414, EC574585, EC574589, EC574595, EC574602, EC574779, EC574841, EC574898, EC574913, EC574969, EC574975, EC574976, EC574977, EC574978, EC574979, EC575000, EC575003, EC575004, EC575005, EC575006, EC575009, EC575011, EC575018, EC575019, EC577441, EC577466, EC575391, EC575400, EC575413,EC575423, EC575426, EC575449, EC575456, EC575457, EC575466, EC575490, EC575491, EC575496, EC575505
	60	23	IC536116, IC536152, EC573543, EC573914, EC574038, EC574043, EC574044, IC535273, IC535289, IC535484, IC536317, IC542385, EC574563, EC577436, EC577442, EC577444, EC577445, EC577446, EC577487, EC575487, EC575510, EC575511, EC575512

The effect of the three different LTN categories on terminal severity of leaf rust also was visualised by correlating the extent of LTN expression with disease severity, and it was found to have no impact on final reduction of leaf rust. The three different types of LTN do not behave differently in their effect on the terminal severity of leaf rust supporting the earlier findings of Singh (1992), wherein a range of leaf-tip necrosis was observed but without any individual effect on terminal severity in breeding populations. Many cultivars with different resistant genes have been developed and deployed to reduce the effect of leaf rust on wheat yield (Roelfs 1988; Mc Intosh 1995). However, most of these cultivars carrying major seedling resistant genes have followed the boom-and-bust cycle. For a durable solution, breeders are putting more emphasis on adult-plant resistant genes. One such gene is *Lr34*, which has been widely used as a buffer in case of the immediate breakdown of other major genes. *Lr34* is recognized by the presence of leaf-tip necrosis, and Dyck (1991) associated this trait with *Lr34*. This gene is present in a large number of cultivars either singly, or in combination, and is providing effective and durable resistance against leaf rust (Roelfs 1988; Dyck 1991).

Our observations lead us to conclude that accessions bearing LTN suffered less from leaf rust infection compared to those without this trait. Because leaf-tip necrosis has proved to be genetically linked with *Lr34* (Singh 1992; Dyck 1991), the Indian wheat germ plasm studied here can be assumed to possess this useful gene of resistance for leaf rust. These accessions may find their utility as genetic stocks for breeding varieties with durable resistance to leaf rust.

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Table 1. Accessions grouped according to leaf-tip necrosis type (LTN) and their reaction to leaf rust (*Puccinia trticina*). Rating of % severity after Peterson et al. 1948).

LTN type	Rust reaction	Number	Accession name
Medium	Trace	62	EC573540, EC573541, EC573560, EC573568, EC573580, EC573611, EC573636, EC573648, EC573653, IC536231, IC536233, IC536234, IC536235, IC536239, EC573769, EC573856, EC573904, EC573922, EC574072, EC574100, EC574123, IC536356, IC536399, IC536408, IC536412, IC536453, IC536496, IC536506, IC542391, IC542439, EC574199, EC574200, EC574201, EC574210, EC574211, EC574212, EC574213, EC574214, EC574215, EC574216, EC574218, EC574219, EC574221, EC574229, EC574233, EC574244, EC574245, EC574257, EC574265, EC574266, EC574274, EC574282, EC574290, EC574326, EC574327, EC574413, EC574623, EC574756, EC577427, EC577428, EC577435, EC577438
	5	86	IC536123, IC536159, IC536185, IC536200, IC536219, IC536221, EC573603, EC573613, EC573633, EC573654, IC536230, IC536240, IC536247, IC536257, IC536260, EC573906, EC573929, EC573937, IC542451, IC536351, IC536371, IC536377, IC536380, IC536386, IC536444, IC536467, IC536474, IC536477, IC536483, IC536311, IC542386, IC542440, IC542441, IC542442, EC574196, EC574197, EC574205, EC574222, EC574223, EC574224, EC574235, EC574238, EC574259, EC574260, EC574264, EC574267, EC574281, EC574312, EC574316, EC574317, EC574319, EC574321, EC574325, EC574328, EC574336, EC574354, EC574355, EC574361, EC574383, EC574417, EC574441, EC574442, EC574451, EC574452, EC574580, EC574624, EC574643, EC574644, EC574663, EC574664, EC574665, EC574666, EC574698, EC574794, EC574845, EC574846, EC574847, EC574889, EC575044, EC575045, EC575046, EC575047, EC575064, EC575078, EC575080, EC575083
	10	213	IC536124, IC536141, IC536144, IC536155, IC536180, IC536186, IC536195, IC536198, IC536203, EC573550, EC573561, EC573562, EC573566, IC536206, IC536210, IC536211, IC536217, IC536223, EC573601, EC573628, EC573634, IC536229, IC536232, IC536242, IC536252, EC573786, EC573789, EC573794, EC573801, EC573813, EC573816, EC573821, EC573830, EC573913, EC573916, EC573931, EC573932, EC573954, EC573973, EC573978, EC573986, EC573990, EC574029, EC574030, EC574064, EC574067, EC574101, EC574177, IC536358, IC536369, IC536373, IC536376, IC536400, IC536407, IC536413, IC536440, IC536465, IC536473, IC536497, IC536505, IC536516, IC535289, IC535518, IC542379, IC542380, IC542383, IC542384, IC542388, IC542389, IC542402, IC542415, IC542422, IC542423, IC542435, IC542437, EC574208, EC574225, EC574236, EC574237, EC574239, EC574242, EC574249, EC574251, EC574252, EC574253, EC574254, EC574255, EC574261, EC574262, EC574293, EC574298, EC574310, EC574314, EC574318, EC574320, EC574323, EC574324, EC574329, EC574330, EC574331, EC574332, EC574333, EC574334, EC574335, EC574342, EC574359, EC574393, EC574430, EC576115, EC574439, EC574440, EC574466, EC574467, EC574490, EC574541, EC574583, EC574611, EC574615, EC574616, EC574618, EC574619, EC574622, EC574640, EC574645, EC574654, EC574655, EC574659, EC574660, EC574670, EC574673, EC574674 EC574675, EC574676, EC574677, EC574678, EC574679, EC574680, EC574681, EC574682, EC574683, EC574684, EC574685, EC574686, EC574695, EC574697, EC574708, EC574709, EC574710, EC574722, EC574731, EC574732, EC574733, EC574734, EC574739, EC574740, EC574741, EC574742, EC574747, EC574751, EC574758, EC574772, EC574788, EC574797, EC574801, EC574802, EC574803, EC574804, EC574811, EC574812, EC574813, EC574840, EC574844, EC574848, EC574852, EC574862, EC574863, EC574864, EC574867, EC574871, EC574875, EC574880, EC574881, EC574882, EC574888, EC574890, EC574891, EC574900, EC574901, EC574902, EC574903, EC574904, EC574905, EC574910, EC574911, EC574912, EC574920, EC574990, EC574996, EC575034, EC575035, EC575042, EC575043, EC575059, EC575060, EC575061, EC575062, EC575065, EC575076, EC575288, EC575289, EC577456, EC575365, EC575411
Medium	20	86	IC536122, IC536138, IC536162, IC536170, IC536182, IC536191, IC536193, EC573547, IC536228, EC573639, IC536243, IC536249, EC573808, EC573829, EC573876, EC573936, EC573941, EC573984, EC574000, EC574014, EC574022, IC536357, IC536368, IC536370, IC536378, IC536385, IC536404, IC536454, IC536475, IC536482, IC536502, IC536515, IC535358, IC536322, IC536323, IC536324, IC542396, IC542408, EC574206, EC574207, EC574220, EC574228, EC574346, EC574386, EC574493, EC574537, EC574557, EC574558, EC574586, EC574587, EC574590, EC574593, EC574601, EC574610, EC574621, EC574687, EC574688, EC574689, EC574718, EC574759, EC574782, EC574783, EC574784, EC574785, EC574810, EC574814, EC574833, EC574834, EC574835, EC574868, EC574869, EC574870, EC574873, EC574874, EC574876, EC574892, EC574897, EC574999, EC575017, EC575051, EC575052, EC575495, EC575497, EC575498, EC575501, EC575502
	40	50	IC536190, EC573551, EC573631, EC573881, EC573882, EC573943, EC573944, EC573945, EC573948, EC573968, EC574008, EC574034, EC574057, EC574058, EC574084, IC536375, IC536387, IC536471, IC536484, EC574776, EC574865, EC574866, EC574893, EC574894, EC574982, EC575008, EC577460, EC577483, EC575363, EC575401, EC575419, EC575429, EC575438, EC575442, EC575446, EC575447, EC575448, EC575452, EC575462, EC575464, EC575465, EC575488, EC575492, EC575493, EC575494, EC575499, EC575500, EC575521, EC575522, EC575523
	60	13	IC536164, EC573549, EC573559, EC574494, EC574781, EC577475, EC575404, EC575514, EC575515, EC575516, EC575517, EC575520, EC575527

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Table 1. Accessions grouped according to leaf-tip necrosis type (LTN) and their reaction to leaf rust (*Puccinia trticina*). Rating of % severity after Peterson et al. 1948).

LTN type	Rust reaction	Number	Accession name
High	Trace	20	IC536139, IC536168, EC573572, IC536220, EC573975, EC573976, EC573977, EC574115, IC536433, EC574273, EC574390, EC574426, EC574427, EC574437, EC574438, EC574444, EC574445, EC574446, EC574454, EC574627
	5	37	EC573569, IC536222, IC536431, EC574291, EC574412, EC574421, EC574428, EC574450, EC574458, EC574459, EC574460, EC574461, EC574462, EC574463, EC574475, EC574476, EC574478, EC574484, EC574485, EC574503, EC574504, EC574642,EC574690, EC574691, EC574700, EC574701, EC574702, EC574704, EC574705, EC574706, EC574707, EC574789, EC574828, EC574829, EC574830, EC574831, EC574847
	10	102	2IC536161, IC536167, IC536174, IC536176, IC536178, IC536181, IC536183, IC536187, IC536196, IC536197, IC536204, IC536216, EC573911, EC573912, EC573987, EC573988, EC573989, EC573999, IC536475, IC536503, IC536508, EC574217, EC574268, EC574271, EC574367, EC574368, EC574387, EC574397, EC574398, EC574399, EC574409, EC574410, EC574411, EC574414, EC574415, EC574422, EC574423, EC574424, EC574429, EC574447, EC574448, EC574453, EC574455, EC574456, EC574457, EC574459, EC574460, EC574464, EC574465, EC574469, EC574470, EC574471, EC574473, EC574474, EC574487, EC574488, EC574489, EC574491, EC574492, EC574495, EC574496, EC574498, EC574570, EC574594, EC574603, EC574639, EC574642, EC574690, EC574691, EC574693, EC574694, EC574712, EC574713, EC574714, EC574715, EC574716, EC574724, EC574725, EC574726, EC574727, EC574728, EC574729, EC574730, EC574735, EC574736, EC574790 , EC574817, EC574818, EC574819, EC574820, EC574821, EC574822, EC574823, EC574824, EC574825, EC574832, EC574906, EC574907, EC574908, EC574909, EC575040, EC575041
	20	15	IC536218, EC574387, EC574443, EC574468, EC574476, EC574497, EC574499, EC574501, EC574567, EC574568, EC574620, EC574826, EC574827, EC574899, EC575048
	40	2	EC573997, IC536383
	60	1	EC574569

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Resistance to cereal soilborne mosaic virus in durum wheat is recessive.

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The type-member of the *Furovirus* genus, soil-borne wheat mosaic virus (WSBMV), was first identified in the U.S. about 80 years ago and, thereafter, reported in most of the wheat-growing areas of the world including Italy. In 2005, following the results of sequence and alignment analyses, the soilborne mosaic virus isolates prevalent in North America, Europe, and far-eastern Asia were subdivided into three distinct species within the *Furovirus* genus denominated by, respectively, soil-borne wheat mosaic virus, soil-borne cereal mosaic virus (CSBMV), and Chinese wheat mosaic virus (CWMV).

According to various reports, resistance to WSBMV and CWMV in common (hexaploid) wheat is governed by 1–3 major genes, whereas CSBMV resistance in durum (tetraploid) wheat is controlled by major genes as well by a plethora of genes with small effects. Indeed, as many as nine minor genes contributed by both parents have been detected in RILs from a single durum wheat cross. In hexaploid wheat, resistance to WSBMV and to CWMV generally is believed to be inherited as a dominant trait, and this view has been implicitly extended to CSBMV-resistance in durum wheat. Quite unexpectedly, observations by the senior author on F_1 durum wheat plants grown outdoors near Rome for

general breeding purposes suggested that at least some durum wheat cultivars carry either recessive or co-dominant CSBMV-resistance genes. In fact, F_1 plants derived from crosses between CSBMV-resistant and CSBMV-susceptible durum wheat cultivars often showed severe CSBMV symptoms even under mild disease pressure.

A six-parent, diallel cross without reciprocals was set up to verify this hypothesis. The parents included cultivars Ionio (resistant = R), Neodur (R), Duilio (moderately resistant = MR), Cirillo (susceptible = S), Valnova (S), and Simeto (moderately susceptible = MS). Cultivars Neodur and Ionio, both derived from the cultivar Edmore, are known to carry a major CSBMV-resistance gene or gene-block located on the short arm of chromosome 2B. Duilio also is known to carry one or more major CSBMV-resistance factors on 2BS, possibly the same as Neodur and Ionio (Maccaferri et al. 2011; Russo et al. in press). Based on their response in previous trials and on the results of recent genetic and molecular marker studies, all the cultivars intercrossed, including the susceptible ones, presumably carry minor resistance genes. The six parental cultivars were grown during 2008–09 in a field free of CSBMV near Foggia and intercrossed in all combinations excluding reciprocals. In the the following season, the resulting 15 F_1s were seeded on 29 October, along with their parents, in a naturally CSBMV-infected field near Bologna in plots consisting of single 1.5-m rows. Twenty seeds were sown in each row. Plots were distributed according to a randomized-block design with three replicates. Symptom-severity was rated on 7 April on a whole-plot basis using a 0–4 scale. DAS ELISA was performed on extracts from a bulk of the basal portions of the two youngest fully expanded leaves collected on 9 April from 10 plants/plot.

CSBMV-pressure was severe, as testified by the high mean symptom scores recorded for the susceptible parents (Table 1). Symptom scores and ELISA values were significantly correlated (r = 0.887; P 0.001). The nine F_1s derived from crosses between resistant and susceptible parents manifested a clearly susceptible reaction in terms of symptom severity (range = 2.5–3.4), in all cases significantly higher than that recorded for any of the three resistant parents (range = 0.6–1.0). Moreover, ELISA values for the 'R/S' F_1s (ELISA range = 0.70 – 1.12) were much closer to those recorded for the susceptible parents (ELISA range = 1.06–1.15) than for the resistant ones (range = 0.03–0.47). The noticeable difference (2.2) between the mean symptom score recorded for the 'R/S' F_1s and for the three resistant parents closely corresponds to the effect estimated for the major CSBMV-resistance QTL identified in recent studies on RILs derived from the durum wheat crosses 'Meridiano / Claudio' and 'Neodur / Cirillo'.

Table 1. Mean CSBMV symptom score (on a 0–4 scale) and mean DAS-ELISA value for the parents and F_1 hybrids of a six-parent diallel cross without reciprocals between durum wheat cultivars.

Genotype	Mean symptom score (April 7)	Mean DAS-ELISA value (April 9)
Resistant parents (3)	0.8	0.18
Resistant/Resistant F_1s (3)	0.5	0.34
Resistant/Susceptible F_1s (9)	3.0	0.87
Susceptible/Susceptible F_1s (3)	3.6	1.01
Susceptible parents (3)	3.7	1.09

The 'R/S' F_1s showed a somewhat greater degree of CSBMV-resistance than the susceptible parents both in terms of symptom severity and ELISA value, suggesting that the minor genes for CSBMV-resistance contributed by the parental cultivars were prevalently dominant. This hypothesis, however, was not validated by the response of single 'R/S' F_1s, which was quite erratic, nor by that of the 'R/S' F_1s, which was pratically identical to that of their susceptible parents.

Based on the above results, we concluded that the durum wheat cultivars Ionio, Neodur, and Duilio carry a recessive (or incompletely recessive) major CSBMV-resistance gene or gene-block, and that the six parental cultivars carry dominant, as well as recessive, modifiers that interact in disparate ways to induce small and, as yet unpredictable, modifications in the final expression of resistance in F_1 plants.

Given the close affinity between durum (genome AABB) and common wheat (genome AABBDD) as well as between WSBMV, CSBMV, and CWMV, our results on CSBMV-resistance in durum wheat are difficult to reconcile with the dominance generally reported for WSBMV and CWMV in hexaploid wheat. In this respect, it should be noted that some of the papers thus far published on the genetics of WSBMV and CWMV resistance contain obvious contradictions, and that they are all quite vague in relation to both the phenotyping criteria adopted and to the disease pressure encountered. We are presently conducting further experiments on the inheritance of CSBMV resistance using a different set of durum and common wheat cultivars of various origins.

References.

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Response of 32 durum wheat cultivars to cereal soilborne mosaic virus in 2009.

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Cereal soil-borne mosaic virus in Italy was first detected in the Po Valley in 1960 and now is known to be widespread throughout most of the country, particularly in the northern and central regions. Thirty-two durum wheat cultivars were grown during 2008–09 in a field with CSBMV at Cadriano, near Bologna, and evaluated for resistance on the basis of symptom severity, DAS-ELISA value and agronomic performance. The cultivars, planted on 20 November, 2008, were grown in 10-m^2 solid-seeded plots distributed in the field according to a randomized block design with three replicates. Symptom severity was evaluated on three dates (1, 9, and 14 April) using a 0–4 scale. DAS-ELISA was performed on extracts from a bulk of the basal half of the second and third youngest leaves of 10 randomly chosen plants/plot collected on 6 April, 2009.

Cereal soil-borne mosaic virus pressure during the 2008–09 season was relatively low, as testified by the mild symptom scores recorded for cultivars known for their susceptibility. The data collected, in any case, indicated that some of the cultivars assayed for the first time, particularly Canova, Karur, Liberdur, Trionfo, and Tripudio, are susceptible to CSBMV (Table 2, continued on p. 91).

Table 2. Response to cereal soil-borne mosaic virus of 32 durum wheat cultivars grown near Bologna, Italy, in 2008–09. Items with the same letter(s) are statistically similar.

Cultivar	Symptom severity score (0–4 scale)				ELISA value	Grain yield (t/ha)	Plant height (cm)	Days-to-heading (from 1 April)	Kernel weight (g)	Test weight (kg/hL)
	1 April	9 April	14 April	Mean	6 April					
Achille	2.1 ad	2.8 ab	2.7 ab	2.53	0.795 bf	5.04 bh	80.0 bf	42 bd	38.4 jk	78.6 a
Alemanno	1.1 cg	0.6 fg	0.5 eh	0.74	0.008 f	5.79 af	89.0 a	39 hj	50.4 a	75.9 ch
Anco Marzio	2.5 ab	2.7 ac	2.7 ab	2.61	0.626 cf	5.18 bg	82.7 ad	40 ef	39.2 jk	77.5 ac
Arnacoris	0.6 eg	0.1 g	0.1 gh	0.28	0.017 f	6.16 ae	81.3 be	39 hj	43.4 ei	74.7 fj
Artemide	0.9 dg	1.4 bg	1.4 bh	1.25	0.399 ef	5.27 bg	74.3 ei	40 fh	44.6 dg	74.5 gj
Biensur	0.2 g	0.1 g	0.1 gh	0.13	0.009 f	5.77 af	74.7 ei	42 bc	38.5 jk	75.0 ej
Casanova	2.3 ad	2.7 ac	2.4 ac	2.44	0.855 af	4.91 ch	77.3 ch	38 ik	50.3 a	74.7 fj
Ciccio	2.2 ad	2.5 ad	2.7 ab	2.44	1.460 ae	3.61 h	69.0 i	38 il	38.8 jk	75.5 di
Ciclope	2.4 ac	2.2 af	2.5 ac	2.34	0.953 af	4.09 gh	76.7 dh	39 gi	42.8 fi	69.8 m
Claudio	1.9 ae	2.0 af	2.3 ac	2.06	0.872 af	4.72 dh	85.7 ab	40 eg	43.5 eh	78.8 a
Creso	1.5 bg	1.1 cg	1.5 bh	1.36	0.783 bf	5.58 ag	75.3 ei	42 b	45.9 bf	77.0 ad
Duilio	0.6 eg	0.8 eg	0.1 gh	0.47	0.279 f	5.40 ag	81.0 be	38 jl	48.2 ac	76.4 bf
Dylan	0.2 g	0.1 g	0.0 h	0.09	0.043 f	6.91 a	84.0 ac	41 ce	44.4 dg	74.6 fj
Imothep	1.8 ae	1.4 bg	1.4 bh	1.56	0.008 f	6.01 ae	83.0 ad	37 l	45.3 bg	77.9 ab
Iride	1.2 bg	1.2 bg	1.1 ch	1.14	0.313 ef	6.51 ab	77.3 ch	38 il	42.1 gj	75.1 ej
Isildur	0.7 eg	0.8 dg	1.2 bh	0.89	0.273 f	5.29 bg	79.0 bg	38 jl	46.3 bf	76.7 be
Karur	2.2 ad	2.3 ae	2.1 ad	2.19	1.980 a	5.27 bg	78.3 ch	45 a	40.1 hk	72.7 kl
Latinur	0.9 dg	0.8 dg	0.7 dh	0.81	0.882 af	5.89 af	72.7 gi	41 df	43.6 eh	75.5 di
Levante	1.3 bg	1.1 cg	1.3 bh	1.24	0.305 ef	6.31 ac	80.7 be	41 ce	44.5 dg	76.2 bg
Liberdur	2.9 a	3.3 a	3.3 a	3.19	1.871 ab	4.35 fh	73.3 fi	44 a	39.1 jk	72.3 l
Minosse	1.8 ae	2.3 af	2.4 ac	2.17	0.507 df	4.94 ch	79.0 bg	38 ik	44.2 eg	78.0 ab
Neolatino	1.8 ae	1.1 cg	1.5 bh	1.47	0.632 cf	4.89 ch	78.3 ch	38 il	48.3 ac	76.3 bg

Table 2. Response to cereal soil-borne mosaic virus of 32 durum wheat cultivars grown near Bologna, Italy, in 2008–09. Items with the same letter(s) are statistically similar.

Normanno	0.4 fg	0.7 eg	0.3 fh	0.46	0.457 ef	6.27 ad	78.0 ch	40 fh	46.8 be	75.2 ej
Orobel	2.3 ad	2.7 ac	1.8 ae	2.25	1.712 ac	4.41 fh	77.3 ch	45 a	44.8 cg	73.4 jl
Pr22d89	0.9 dg	0.7 eg	1.3 bh	0.94	0.314 ef	4.97 bh	78.0 ch	37 kl	48.6 ab	77.1 ad
Principe	1.5 bg	1.7 bg	1.6 bg	1.58	1.709 ac	4.95 ch	82.7 ad	38 jl	47.9 ad	73.9 il
Saragolla	1.2 bg	1.0 cg	0.7 dh	0.96	0.017 f	5.52 ag	74.7 ei	38 jl	40.4 hj	75.7 ci
Severo	1.4 bg	1.2 bg	1.7 bf	1.42	0.395 ef	5.78 af	84.0 ac	40 eg	36.7 k	75.0 ej
Simeto	2.2 ad	2.1 af	2.3 ac	2.17	1.899 ab	4.82 ch	71.7 hi	39 gi	48.3 ac	73.5 jl
Tirex	0.7 eg	0.2 g	0.4 eh	0.42	0.017 f	5.86 af	80.7 be	38 ik	42.7 fi	77.9 ab
Trionfo	1.8 af	2.1 af	2.3 ac	2.06	1.718 ac	4.69 eh	78.0 ch	42 b	39.9 ik	74.3 hk
Tripudio	1.8 af	2.1 af	2.7 ab	2.17	1.657 ad	5.02 bh	77.0 ch	41 df	40.3 hj	75.9 ch
Mean	1.47	1.49	1.53	1.49	0.743	5.32	78.6	39.8	43.7	75.5
Minimum	0.17	0.05	0.00	0.09	0.008	3.61	69.0	37.0	36.7	69.8
Maximum	2.92	3.33	3.33	3.19	1.980	6.91	89.0	44.7	50.4	78.8

Mean symptom severity score was significantly correlated with ELISA value and grain yield, but not with the other plant characters measured (Table 3). Regression analysis indicated that the 12 cultivars showing symptom scores between 2.0 and 3.0 suffered a 25% mean grain yield loss.

Table 3. Simple correlation coefficients between mean symptom severity, mean ELISA value, and various agronomic characters for 32 durum wheat cultivars grown in a field with cereal soil-borne mosaic virus near Bologna, Italy, during 2008–09. Items with ** are significantly correlated; all others are nonsignificant.

	ELISA value	Grain yield	Plant height	Heading date	Kernel weight	Test weight
Symptom severity	0.729**	–0.751**	–0.248	0.314	–0.276	–0.188
ELISA value	—	–0.663**	–0.407**	0.500**	–0.160	–0.473**

Response of 33 durum wheat cultivars to cereal soilborne mosaic virus in 2010.

C. Rubies-Autonell and C. Ratti, (DiSTA, Bologna), A. Sarti (ASTRA, Faenza), R. Canestrale (CRPV, Imola), and V. Vallega (CRA–QCE, Rome).

Thirty-three durum wheat cultivars were grown during 2009–10 in a field with CSBMV at Cadriano, near Bologna, and evaluated for resistance on the basis of symptom severity, DAS-ELISA value, and agronomic performance. The cultivars, planted 30 October, 2009, were grown in 10-m^2 solid-seeded plots distributed in the field according to a randomized block design with three replicates. Symptom severity was evaluated on three dates (17 and 25 March and 7 April) using a 0–4 scale. DAS-ELISA was performed on extracts from a bulk of the basal half of the second and third youngest leaves of 10 randomly chosen plants/plot collected on 26 March and 9 April, 2010.

The cultivars Dylan and Biensur showed very mild symptoms and relatively low ELISA values; both produced high grain yields, superseeded only by those recorded for the moderately resistant cultivar Levante (Table 4, p. 92). Relatively low symptom scores and ELISA values, as well as relatively high grain yields, were recorded also for cultivars Duilio, Saragolla, Alemanno, Pharaon, Meridiano, and Svevo.

Mean ELISA value and mean symptom severity score were significantly correlated (0.736**), and both resistance parameters were significantly correlated with all the agronomic traits considered except test weight (Table 5, p. 92). Regression analysis indicated that cultivars showing symptom scores between 3.0 and 3.8 (Table 6, p. 93) suffered a 53% mean grain yield loss, as well as severe reductions in plant height (31%) and kernel weight (16%).

Table 4. Response to cereal soil-borne mosaic virus of 33 durum wheat cultivars grown near Bologna, Italy, in 2009–10. Items with the same letter(s) are statistically similar. Symptom severity was rated on a 0–4 scale and are the mean of three dates.

Cultivar	Symptom severity score			ELISA value	Grain yield (t/ha)	Plant height (cm)	Days-to-heading (from April 1)	Kernel weight (g)	Test weight (kg/hl)
	26 March	9 April	Mean						
Achille	3.78	0.981 ad	1.160 a	1.071	2.77 ik	60.0 jl	44 ab	36.0 pq	75.5 ag
Alemanno	1.36	0.915 ae	0.310 il	0.613	4.31 bh	85.0 a	37 hl	52.1 a	73.3 di
Anco Marzio	3.44	1.085 ad	1.066 ab	1.076	2.44 jk	61.7 hl	41 cd	39.4 mp	72.4 hi
Arnacoris	2.82	0.751 af	0.358 il	0.555	3.53 ej	75.7 be	39 ei	43.9 ek	72.3 hi
Aureo	3.16	0.611 cf	0.559 ek	0.585	2.58 jk	82.0 ab	40 dg	40.3 jo	73.0 fi
Biensur	0.69	0.723 af	0.384 hl	0.554	5.36 ab	79.0 ac	41 cd	40.0 lo	74.7 bh
Cannavaro	3.35	1.127 ab	1.156 a	1.142	3.28 gk	68.3 fh	44 ab	48.0 bd	72.0 hi
Ciccio	3.17	1.099 ac	1.095 a	1.097	2.28 k	65.0 gk	36 jl	38.0 oq	73.7 ch
Claudio	3.61	1.062 ad	0.912 af	0.987	0.87 l	49.7 m	45 ab	41.8 ho	73.0 fi
Creso	3.44	1.023 ad	0.957 ae	0.990	2.42 jk	58.3 kl	44 ab	45.4 bh	76.7 ab
Duilio	1.04	0.638 bf	0.259 il	0.449	4.92 bd	82.7 ab	35 km	48.4 bc	74.7 bh
Dylan	0.89	0.605 cf	0.195 jl	0.400	5.19 ac	81.7 ab	40 cf	44.7 ci	73.8 ch
Grazia	3.32	1.128 ab	1.130 a	1.129	2.41 jk	66.3 gj	41 ce	35.2 q	72.9 fi
Ignazio	2.87	0.641 bf	0.623 ci	0.632	4.15 bh	78.7 ad	40 dg	49.1 ab	76.8 ab
Imhotep	3.14	0.961 ad	0.548 ek	0.755	3.96 di	76.3 be	35 lm	45.6 bh	77.6 a
Iride	2.33	0.581 df	0.391 gl	0.486	4.39 bh	74.3 cf	35 km	42.4 gn	78.0 a
Karur	3.12	1.173 a	1.096 a	1.135	3.92 di	60.3 il	46 a	41.4 io	73.5 dh
Latinur	2.12	1.028 ad	0.806 ag	0.917	3.28 gk	65.0 gk	38 fj	41.4 io	70.6 i
Levante	1.31	0.395 f	0.001 l	0.198	6.24 a	82.0 ab	39 dh	44.9 ci	73.9 ch
Liberdur	3.62	1.144 ab	1.047 ab	1.096	3.53 ej	57.0 l	44 ab	40.1 ko	76.3 ac
Meridiano	1.89	0.873 af	0.447 gk	0.660	4.57 bf	83.0 ab	35 km	47.5 bf	76.0 ad
Minosse	2.70	0.983 ae	0.784 ah	0.884	2.52 jk	67.3 fi	36 il	42.9 gm	73.2 ei
Neolatino	2.17	0.839 af	0.667 bi	0.753	3.19 hk	79.3 ac	35 lm	47.7 be	75.8 ae
Normanno	2.73	0.958 ae	0.532 fk	0.745	3.43 fk	71.7 dg	39 ei	43.9 ej	73.2 ei
Pharaon	1.53	0.454 ef	0.179 kl	0.317	4.52 bg	78.3 ad	37 il	45.2 ci	73.8 ch
Pr22d89	2.61	1.110 ac	0.969 ad	1.040	4.10 ch	70.3 eg	37 gk	44.7 ci	76.4 ac
Saragolla	1.22	0.909 ae	0.601 dj	0.755	4.71 be	75.7 be	35 km	44.1 di	72.7 gi
Severo	3.67	0.704 af	1.060 ab	0.882	3.56 ej	58.7 kl	42 bc	38.9 np	78.0 a
Simeto	2.56	1.186 a	1.018 ac	1.102	3.59 ej	68.0 fh	38 fj	45.9 bg	73.2 di
Svevo	1.94	0.751 af	0.431 gk	0.591	4.38 bh	84.7 a	33 m	44.1 di	75.7 af
Tirex	2.36	1.037 ad	0.504 fk	0.771	4.53 bg	79.7 ac	35 lm	46.2 bg	76.8 ab
Trionfo	3.12	1.110 ac	1.093 a	1.102	3.56 ej	66.0 gj	41 cd	40.0 lo	75.7 af
Tripudio	2.93	1.139 ab	1.086 a	1.113	3.65 ej	68.3 fh	41 cd	43.8 fl	77.0 ab
Mean	2.54	0.901	0.710	0.806	3.70	71.5	39	43.4	74.6
Minimum	0.69	0.395	0.001	0.198	0.87	49.7	33	35.2	70.6

Table 5. Simple correlation coefficients between mean symptom severity, mean ELISA value, and various agronomic characters for 33 durum wheat cultivars grown in a field with cereal soil-borne mosaic virus near Bologna, Italy, during 2009–10. Items with ** are significantly correlated; all others are nonsignificant.

	ELISA value	Grain yield	Plant height	Heading date	Kernel weight	Test weight
Symptom severity	0.736**	–0.779**	–0.757**	0.535**	–0.470**	–0.139
ELISA value	—	–0.669**	–0.777**	0.491**	–0.420*	–0.047

Table 6. Estimated mean effects of cereal soil-borne mosaic virus on 33 durum wheat cultivars with different disease severity grown in a field near Bologna, Italy, during 2009–10.

Disease score	Number of cultivars	Grain yield loss		Plant height reduction		Kernel weight reduction		Heading delay
		t/ha	%	cm	%	g	%	
0.00–1.00	2	0.82	13	11.6	13	6.2	13	7
1.01–2.00	7	1.29	21	10.3	11	1.9	4	2
2.01–3.00	11	2.43	40	19.4	21	3.8	8	4
3.01–3.80	13	3.20	53	28.1	31	7.8	16	8

Response of 31 durum wheat cultivars to cereal soilborne mosaic virus in 2011.

V. Vallega (CRA–QCE, Rome), C. Rubies-Autonell and C. Ratti, (DiSTA, Bologna), A. Sarti (ASTRA, Faenza), and R. Canestrale (CRPV, Imola).

Thirty-one durum wheat cultivars were grown during 2010–11 in a field with CSBMV at Cadriano, near Bologna, and evaluated for resistance to this pathogen on the basis of symptom severity and DAS-ELISA readings. Seven of the cultivars (Dorato, Ismur, Kanakis, Ramirez, Sculptur, Torrese, and Yelowdur) had not been assayed for CSBMV-resistance before. The cultivars were planted 9 November, 2010, in 10-m^2 solid-seeded plots distributed in the field according to a randomized block design with three replicates. DAS-ELISA was performed on extracts from a bulk of the basal half of the first fully developed leaf of 10 randomly chosen plants/plot collected on 24 April, 2011. Because plant stunting was neglible and foliar mosaic symptoms became severe only towards the end of March, symptom severity was rated late in the season (4 and 24 April) and solely on the basis of foliar mosaic. Due to fund scarcity, grain yield and other agronomic characters were not measured.

ELISA value and mean symptom severity score were closely correlated (0.569**) but far less than in seasons characterized by an early appearance of severe visible CSBM-symptoms. As a matter of fact, some cultivars, particularly Anco Marzio, Creso, and Imhotep, exhibited a resistant response to CSBMV in terms of ELISA value yet a susceptible or moderately susceptible reaction in terms of visible symptoms (Table 7). These three cultivars were classified as susceptible or moderately susceptible in previous trials. Cultivars Sculptur and Yelodur, assayed for the first time, also showed discrepancy in response to severity of visible symptoms and ELISA value. Among the other cultivars tested for the first

Table 7. Response to cereal soil-borne mosaic virus of 31 durum wheat cultivars grown near Bologna, Italy, in 2010–11. Symptom severity was rated on a 0–4 scale. Values with the same letter(s) are statistically similar.

Cultivar	Symptom severity score			ELISA value
	4 April	24 April	Mean	24 April
Achille	2.33 ag	2.33 ag	2.34 af	0.619 be
Anco Marzio	3.08 ac	2.17 ai	2.63 ae	0.007 e
Arnacoris	1.58 ei	2.17 ai	1.88 dg	0.168 de
Biensur	0.33 j	0.00 k	0.17 i	0.032 e
Ciccio	2.17 bh	2.92 ac	2.54 ae	1.126 ad
Claudio	2.58 ae	1.92 bj	2.25 af	0.667 be
Creso	2.83 ad	2.33 ag	2.59 ae	0.263 ce
Dorato	3.33 ab	2.83 ad	3.08 ab	0.528 be
Duilio	1.75 dh	2.49 af	2.12 bf	0.014 e
Dylan	2.00 ch	2.25 ah	2.13 bf	0.002 e
Grazia	3.00 ac	3.08 a	3.04 ac	1.375 ab
Imhotep	2.83 ad	2.67 ae	2.75 ad	0.007 e
Iride	1.75 dh	1.50 fj	1.63 eh	0.070 e
Ismur	3.42 a	3.00 ab	3.21 a	1.747 a
Kanakis	1.25 gj	1.50 fj	1.38 fh	0.006 e
Karur	2.17 bh	1.92 bj	2.05 cf	0.929 ae
Latinur	2.50 af	2.42 ag	2.46 ae	0.601 be
Levante	2.58 ae	1.67 ej	2.13 bf	0.001 e
Liberdur	3.08 ac	3.08 a	3.09 ab	1.702 a
Meridiano	1.08 hj	1.00 j	1.04 gh	0.064 e
Neolatino	1.33 fj	1.92 bj	1.63 eh	0.075 e
Normanno	1.67 di	1.08 ij	1.38 fh	0.604 be
Pharaon	2.17 bh	1.67 ej	1.92 dg	0.003 e
Ramirez	0.58 ij	1.17 hj	0.88 hi	0.002 e
Saragolla	2.08 ch	1.83 cj	1.96 dg	0.047 e
Sculptur	2.42 ag	1.75 dj	2.09 bf	1.183 ac
Simeto	3.00 ac	2.08 aj	2.55 ae	0.877 ae
Svevo	2.00 ch	2.08 aj	2.04 cf	0.008 e
Tirex	2.00 ch	1.75 dj	1.88 dg	0.009 e
Torrese	2.08 ch	1.33 gj	1.71 eh	0.008 e
Yelodur	2.58 ae	2.00 aj	2.29 af	0.001 e
Mean	2.18	2.00	2.09	0.411
Minimum	0.33	0.00	0.17	0.001
Maximum	3.42	3.08	3.21	1.747

time, Kanakis, Ramirez, and Torrese showed high levels of CSBMV resistance, whereas Ismur and Dorato were susceptible.

All cultivars classified as resistant or moderately resistant in previous trials exhibited comparable reactions in 2011, and consistent responses also were observed for all those previously classified as susceptible or moderately susceptible except for Anco Marzio, Creso, and Imhotep, which expressed a susceptible reaction only in terms of symptom expression.

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CEREAL QUALITY RESEARCH UNIT (CRA) OF THE ITALIAN RESEARCH COUNCIL
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Comparison between bread wheat and barley in the inner hillside of south-central Italy.

Mauro Fornara, Massimiliano Camerini (University of Molise, Department S.A:V.A), Michelina Colonna (ARSIAM–Molise), Carlo Rossi, Ferdinando Sereni, and Fabrizio Quaranta.

In 2009 and 2010, field experiments were carried out in Colletorto, a location in the Molise region (41°40' N), an inner hill environment (515 masl) surrounded by the Central Apennine Mountain Range. In this location, 14 bread wheat cultivars and 19 barley cultivars for livestock feeding are tested yearly. Trials are in a randomized complete block design with three replications. Bread wheat cultivars are catalogued according with the Synthetic Quality Index method (Indice Sintetico di Qualità, ISQ), from the strongest type, FF (frumento di forza, improver wheat), particularly used for manufacturing products with a strong and well-leavened structure, to the weakest type, FAU (frumento per altri usi, wheat for other purposes). The intermediate wheat categories, FPS (frumento panificabile superiore, superior bread making wheat) and FP (frumento panificabile, ordinary bread making wheat), present properties suitable for ordinary bread making. The average yields for the period were similar for both crops (4.15 t/ha for bread wheat and 4.19 t/ha for barley (Table 1, p. 95)). An overall yield reduction in 2009, compared to 2010, was observed for both barley and bread wheat. Five bread wheat genotypes (Epidoc, Exotic, Blasco, Genesi, and Adelaide) reached a yield greater than 4.5 t/ha with yield indices higher than 100 in every year. Among these cultivars, Blasco and Adelaide showed a very interesting test weight average (84.8 kg/hL and 82.1 kg/hL, respectively), a character very important for milling industry.

Seven barley cultivars (Estival, Oleron, Shangrila, Mattina, Campagne, Calanque, and Aldebaran) exceeded a yield of 4.5 t/ha, with yield indicies higher than 100 in each year. Among these, only Calanque exceeded a 70 kg/hL average test weight value.

Table 1. ISQ class, growth cycle, grain yield, and test weight of 14 Italian bread wheat cultivars and 19 Italian barley cultivars tested during a 2-year period (2010–11) in the Molise region of Italy.

Cultivar	ISQ class	Heading date (days after 1 April)	Yield			Test weight		
			Index		Mean (t/ha)	Index		Mean (kb/hL)
			2009	2010		2009	2010	
Bread wheat								
Bologna	FF	48	63	96	3.45	102	101	81.4
Valbona	FF	39	99	91	3.90	103	100	81.7
Adelaide	FPS	44	124	101	4.58	103	101	82.1
Blasco	FPS	47	120	110	4.73	105	105	84.8
Antille	FP	53	102	88	3.89	99	98	79.5
Aubusson	FP	51	94	95	3.94	100	99	79.8
Colledoro	FP	52	100	90	3.92	101	101	81.5
Epidoc	FP	51	125	118	5.02	99	98	79.1
Exotic	FP	50	127	107	4.78	97	98	78.4
Genesi	FP	50	117	106	4.60	100	101	80.9
Lilliput	FP	48	111	88	4.04	101	101	81.4
Mieti	FP	46	66	70	2.83	99	99	80.0
PR22R58	FP	51	105	91	4.02	100	100	80.4
Sollario	FAU	51	97	99	4.08	103	99	81.1
Mean		**48**	**3.37**	**4.94**	**4.15**	**79.0**	**82.1**	**80.5**
Barley								
Cultivar	**Height (cm)**	**Heading date (days after 1 April)**	**Yield**			**Test weight**		
			Index		**Mean (t/ha)**	**Index**		**Mean (kb/hL)**
			2009	**2010**		**2009**	**2010**	
Aldebaran	77	40	108	108	4.51	92	94	63.7
Amillis	82	38	73	79	3.24	99	103	69.1
Calanque	79	40	105	110	4.54	103	104	70.8
Campagne	82	40	124	101	4.57	100	98	67.4
Cometa	78	42	105	95	4.12	99	103	69.1
Estival	76	37	111	124	5.02	98	99	67.4
Explora	78	37	115	89	4.11	98	96	66.6
Ketos	79	40	96	97	4.06	99	101	68.4
Laverda	84	42	72	104	3.88	95	96	65.3
Lutece	80	38	97	100	4.15	98	97	66.6
Marjorie	84	38	98	105	4.30	107	104	72.0
Mattina	80	42	110	114	4.72	99	101	68.5
Meseta	75	38	115	80	3.87	104	105	71.8
Nure	80	38	95	86	3.74	102	103	70.2
Oleron	78	38	114	117	4.84	95	92	64.0
Rodorz	90	43	102	100	4.21	103	100	69.2
Sfera	80	40	87	103	4.07	104	105	71.5
Shangrila	80	39	123	109	4.78	101	98	67.9
Siberia	76	38	80	103	3.97	96	95	65.5
Mean	**80**	**40**	**2.98**	**5.39**	**4.19**	**67.9**	**68.9**	**68.4**

Conventional bread wheat: results of the 2010 – 2011 varietal evaluation in Latium region (Central Italy).

Mauro Fornara, Gaetano Bentivenga, Pierino Cacciatori, Vincenzo Miozzi,Valerio Vecchiarelli (University of Perugia), and Fabrizio Quaranta.

The Research Unit for Cereal Quality of the Italian Agricultural Research Council (CRA–QCE, Rome) performs a varietal evaluation of bread wheat cultivars in different experimental fields in Central Italy. In the 2-year period between 2010 and 2011, field experiments were carried out in Latium region (Central Italy), in four locations (Montelibretti, Rieti, Rome and Tarquinia) . In every study site, trials were performed according with a RCBD with 3 replicates.

The tested cultivars are catalogued according with the Synthetic Quality Index method (Indice Sintetico di Qualità, ISQ), from the strongest type FF (frumento di forza, improver wheat), particularly used for manufacturing products with a strong and well leavened structure, to the weakest type FB (frumento da biscotto, wheat for biscuits), more appropriate to lend friableness to the products. The intermediate wheat categories, FPS (frumento panificabile superiore, superior bread making wheat) and FP (frumento panificabile, ordinary bread making wheat), present properties suitable for ordinary bread making.

The agronomic performance of the 17 bread wheat genotypes is summarized (Table 2). The overall average yield during the two-year period reached 6.20 t/ha. Eight cultivars reached yields exceeding 6.30 t/ha, with yield indexes not lower than 100 in every year. Among these, Masaccio, Altamira, Arabia, Tiepolo, and Anforeta, also showed test weights higher than 80 kg/hL. Blasco (class FPS) was characterized by the highest test weight (83.6 kg/hL); on the contrary, its productive performance (6.00 t/ha) resulted slightly below the average grain yield.

Table 2. ISQ class, grain yield, and test weight of 17 Italian bread wheat cultivars tested during a 2-year period (2010–11) at four locations in the Latium Region of central Italy.

		Yield			Test weight		
		Index		Mean	Index		Mean
Cultivar	ISQ class	2010	2011	t/ha	2010	2011	kg/hL
Bologna	FF	95	87	5.61	103	100	80.7
Apoteosi	FPS	84	94	5.51	102	102	81.1
Arrocco	FPS	95	99	6.01	100	100	79.7
Blasco	FPS	96	98	6.00	106	105	83.6
Tiepolo	FPS	106	102	6.46	101	100	80.0
Altamira	FP	111	103	6.64	100	102	80.1
Andana	FP	87	93	5.58	102	101	80.5
Anforeta	FP	100	104	6.36	102	103	81.4
Aubusson	FP	95	97	5.97	97	96	76.8
Bandera	FP	113	100	6.60	100	100	79.4
Masaccio	FP	112	105	6.74	101	101	80.0
Mieti	FP	91	84	5.40	99	99	78.5
PR22R58	FP	113	102	6.63	100	100	79.1
Sirtaki	FP	106	107	6.61	97	96	76.6
Solehio	FP	99	116	6.70	100	98	78.4
Arabia	FB	106	103	6.47	102	101	80.5
Artico	FB	101	95	6.08	95	96	75.5
Location		**Mean (t/ha)**			**Mean (kg/hL)**		
		5.85	**6.54**	**6.20**	**77.4**	**81.2**	**79.3**
Montelibretti		6.08	7.99	7.03	78.2	83.5	80.8
Roma		4.91	6.67	5.79	74.3	80.5	77.4
Rieti		6.40	4.94	5.67	76.3	79.2	77.8
Tarquinia		6.03	6.57	6.30	80.9	81.6	81.2

Conventional durum wheat: results of the 2009–11 Italian National Network Trials.

Andreina Belocchi, Maria Grazia D'Egidio, Mauro Fornara, Ester Gosparini, Valerio Mazzon, and Fabrizio Quaranta.

The Research Unit for Cereal Quality of the Italian Agricultural Research Council (CRA-QCE, Rome) coördinates national network trials for the evaluation of the overall performance of durum wheat cultivars in conventional farming. From 2009 to 2011, field experiments were performed at 50 locations, grouped into six main geographical and pedoclimatic areas (Sicily, southern Italy, Sardinia, Thyrrenic–central Italy, Adriatic–central Italy, and northern Italy). Several cultivars were evaluated; some were tested in all the environments, whereas others were considered suitable only for a part. Trials were in a randomized complete block design with three replications.

A synthesis is shown of the agronomic performance of the 13 genotypes evaluated in all the areas for the entire period between 2009 and 2011 (Table 3. pp. 97-98). Such cultivars represents a considerable amount of the durum wheat seed commercialized in Italy. The average national yield for the period reached 4.83 t/ha; the highest production was achieved in Adriatic–central Italy (6.13 t/ha) and the lowest in Sicily (3.87 t/ha). Three of the cultivars, Claudio, Tirex, and Normanno, reached yield indexes greater than or equal to 100 in all six environments. The medium-maturing cultivar Claudio confirmed an excellent yield stability, as in the previous three-year period (2007–09).

Table 3. Yield, grain protein content and test weight of 13 italian durum wheat cultivars tested during a three-year period (2009–11) in six areas of Italy. For length of growing cycle: E = early; ME = medium early; M = medium; ML = medium late, and L = late.

		Yield							
		Index (yield/column mean*100)							**t/ha**
Cultivar	**Cycle**	**Sicily**	**Sardinia**	**South**	**Thyrrenic–central**	**Adriatic–central**	**North**	**Mean**	**Mean**
Ciccio	E	94	92	89	88	87	84	88	4.26
Duilio	E	102	103	102	101	98	100	101	4.87
Imhotep	E	103	104	99	99	95	96	99	4.77
Iride	ME	106	109	109	105	99	101	105	5.05
Saragolla	ME	103	108	107	107	99	100	104	5.02
Tirex	ME	107	105	108	109	101	104	106	5.11
Simeto	ME	98	93	95	89	89	89	92	4.43
Anco Marzio	ME	109	106	98	103	99	103	102	4.94
Latinur	M	99	102	98	92	102	95	97	4.70
Claudio	M	109	106	108	106	104	107	107	5.15
Normanno	M	100	105	102	107	102	102	103	4.98
Dylan	ML	107	98	104	108	105	107	105	5.09
Creso	L	87	83	88	90	99	91	90	4.36
Mean (t/ha)		**3.87**	**5.82**	**3.90**	**4.85**	**6.13**	**5.42**	**100**	**4.83**
		Grain protein content							
		Index (grain protein content/column mean*100)							**% DM**
Ciccio	E	96	99	100	101	104	102	101	13.2
Duilio	E	99	97	99	100	101	99	99	13.0
Imhotep	E	96	95	95	97	99	98	97	12.7
Iride	ME	97	95	95	96	97	95	96	12.6
Saragolla	ME	98	97	96	97	97	97	97	12.7
Tirex	ME	98	98	100	100	101	101	100	13.2
Simeto	ME	102	102	105	107	109	107	106	13.9
Anco Marzio	ME	100	99	99	99	100	98	99	13.0
Latinur	M	100	102	103	103	102	102	102	13.4
Claudio	M	97	98	100	100	101	101	100	13.1
Normanno	M	101	101	99	99	101	100	100	13.1
Dylan	ML	102	100	100	98	98	100	100	13.1
Creso	L	104	106	104	102	101	102	103	13.5
Mean (%DM)		12.4	12.7	12.7	13.5	13.9	13.5	100	13.1

Table 3. Yield, grain protein content and test weight of 13 italian durum wheat cultivars tested during a three-year period (2009–11) in six areas of Italy. For length of growing cycle: E = early; ME = medium early; M = medium; ML = medium late, and L = late.

		Test weight							
		Index (test weight/column mean*100)							kg/hL
Ciccio	E	101	101	101	100	100	100	100	79.6
Duilio	E	100	100	100	99	100	99	99	78.9
Imhotep	E	101	101	101	101	101	101	101	80.2
Iride	ME	100	99	100	100	100	99	100	79.0
Saragolla	ME	100	99	99	99	99	98	99	78.3
Tirex	ME	103	102	102	102	101	102	102	80.9
Simeto	ME	97	97	98	97	96	96	97	77.0
Anco Marzio	ME	102	102	102	102	102	101	102	80.8
Latinur	M	102	101	101	100	101	101	101	80.2
Claudio	M	102	102	103	103	103	103	103	81.4
Normanno	M	99	100	100	99	100	99	99	78.9
Dylan	ML	101	101	101	101	101	102	101	80.4
Creso	L	102	102	101	102	101	101	101	80.5
Mean (kg/hL)		81.5	80.6	79.6	79.0	78.9	77.8	100	79.4

The nationwide average of grain protein content was 13.1%. The greatest value was detected in Adriatic–central Italy (13.9%), and the lowest in Sicily (12.4%). Simeto and Creso, were greater than the average in every area, but this was not coupled with satisfactory grain yields. Tirex, Claudio, Normanno, and Dylan were a satisfactory compromise between high yields and good protein levels.

The national test weight mean value was 79.4 kg/hL; the best results were in Sicily (81.5 kg/hL) and Sardinia (80.6 kg/hL). As in the previous 3-year study period, the grain samples collected in northern Italy (77.8 kg/hL) had lower values. Good qualitative values were achieved by all the tested genotypes, excluding Simeto, whose average test weight for the period was 77.0 kg/hL; other values ranged from 81.4 kg/hL (Claudio) to 78.3 kg/hL (Saragolla). Claudio, Tirex, Dylan, and Anco Marzio, whose average national test weight was greater than 80 kg/hL, exceeded the local average in every area, at the same time reaching satisfactory grain yields.

Organic durum wheat: results of the 2009–11 Italian National Network Trials.

Fabrizio Quaranta, Andreina Belocchi, Massimiliano Camerini (University of Molise, Department S.A:V.A), Mauro Fornara, Sahara Melloni, Stefano Pucciarmati, and Maria Grazia D'Egidio.

The Research Unit for Cereal Quality of the Italian Agricultural Research Council (CRA–QCE, Rome) coördinates a national network trials for the evaluation of the overall performance of organically

Table 4. Yield, grain protein content, and test weight of 15 organically managed, italian durum wheat cultivars tested during a three-year period (2009–11) in three main cropping areas of Italy. For length of growing cycle: E = early; ME = medium early; M = medium; ML = medium late, and L = late.

		Yield				
		Index (yield/column mean*100)				t/ha
Cultivar	Cycle	South	Thyrrenic–central	Adriatic–central and north	Mean	Mean
Ciccio	E	102	99	88	96	3.06
Svevo	E	101	102	102	101	3.22
Duilio	E	105	105	105	105	3.34
Simeto	ME	102	98	90	97	3.09
Saragolla	ME	102	107	100	102	3.25
Neolatino	ME	99	97	94	97	3.08
Meridiano	ME	110	105	110	109	3.47
Anco Marzio	ME	98	115	108	105	3.34
Vinci	M	96	100	98	98	3.10
Claudio	M	107	106	105	106	3.37
Colosseo	M	105	96	104	103	3.28
San Carlo	M	97	97	101	99	3.14
Normanno	M	101	98	106	103	3.26
Dylan	ML	90	98	98	94	2.99
Creso	L	90	89	93	91	2.89
Mean (t/ha)		**3.08**	**2.85**	**3.51**	**100**	**3.18**

Table 4. Yield, grain protein content, and test weight of 15 organically managed, italian durum wheat cultivars tested during a three-year period (2009–11) in three main cropping areas of Italy. For length of growing cycle: E = early; ME = medium early; M = medium; ML = medium late, and L = late.

		Grain protein content				
		Index (grain protein content/column mean*100)				%DM
Ciccio	E	99	105	105	102	12.3
Svevo	E	105	106	105	105	12.6
Duilio	E	98	98	97	98	11.8
Simeto	ME	105	108	107	106	12.8
Saragolla	ME	95	93	93	94	11.3
Neolatino	ME	104	103	103	104	12.5
Meridiano	ME	96	95	96	96	11.6
Anco Marzio	ME	100	97	97	98	11.8
Vinci	M	99	98	97	98	11.8
Claudio	M	99	98	101	100	12.0
Colosseo	M	101	100	99	100	12.0
San Carlo	M	101	103	101	101	12.2
Normanno	M	99	96	97	98	11.7
Dylan	ML	99	98	98	99	11.9
Creso	L	103	103	100	102	12.2
Mean (%DM)		**11.6**	**12.0**	**12.7**	**100**	**12.0**
		Test weight				
		Index (test weight/column mean*100)				**kg/hL**
Ciccio	E	101	100	100	100	80.2
Svevo	E	101	101	101	101	80.6
Duilio	E	100	99	99	99	79.2
Simeto	ME	99	98	97	98	78.2
Saragolla	ME	97	98	98	97	77.9
Neolatino	ME	100	100	100	100	80.1
Meridiano	ME	98	99	98	98	78.1
Anco Marzio	ME	101	102	102	101	80.8
Vinci	M	99	99	99	99	78.8
Claudio	M	101	101	101	101	80.9
Colosseo	M	101	101	100	101	80.6
San Carlo	M	101	101	102	101	80.8
Normanno	M	99	99	99	99	79.2
Dylan	ML	101	100	101	101	80.3
Creso	L	101	101	100	101	80.4
Mean (kg/hL)		**79.9**	**80.6**	**79.3**	**100**	**79.9**

managed durum wheat cultivars, in collaboration with diverse national agencies and universities.

Between 2009 and 2011, 15 durum wheat genotypes were evaluated in 17 experimental sites, grouped in three geographical macroareas (southern Italy, Thyrrenic–central Italy, and Adriatic–central–northern Italy). Trials were carried out in a randomized complete block design with three replications.

The agronomic performance of the tested genotypes are given (Table 4, pp. 98-99). The average grain yield of these cultivars for the period 2009–11 was 3.18 t/ha. The highest value was recorded in the Adriatic central–northern Italy region (3.51 t/ha); yields were lower in the Southern (3.08 t/ha) and Thyrrenic–central Italy (2.85 t/ha) areas. Five cultivars, Meridiano, Claudio, Duilio, Saragolla, and Svevo, achieved yield indexes not lower than 100 in all three areas. Meridiano, Claudio, and Duilio, as in the previous three-year period (2007–09), had indexes that were remarkably above average in every growing area.

Grain protein content reached an average of 12.0%. Once again, the Adriatic central–northern Italy region was the highest (12.7%). Low grain protein content was detetcted in grains from Thyrrenic–central Italy (12.0%) and most of all from southern Italy (11.6%), despite the reduced grain yield in these areas. Svevo had a good protein content level in every environment, associated with good productivity. The overall good performance achieved by Simeto in southern Italy is worth highlighting.

The nationwide average test weight was 79.9 kg/hL, confirming that this technological parameter reaches satisfactory values in organically managed durum wheat also. Cultivars Claudio, Anco Marzio, and Svevo gave the highest values for test weight, associated with huge yields.

Triticale: results of 2010–11 cultivar trials in central Italy.

Massimiliano Camerini (University of Molise, Department S.A:V.A.), Mauro Fornara, Federico Malagesi, Sahara Melloni, Alberto Sestili, and Fabrizio Quaranta.

The Research Unit for Cereal Quality of the Italian Agricultural Research Council (CRA–QCE, Rome) carries out field trials for the evaluation of the performance of triticale cultivars in central Italy. The aim of these trials is to provide useful informations about the quali-quantitative traits of triticale cultivars, testing at the same time their suitability to specific agroclimatic conditions. In the 2-year period between 2010 and 2011, triticale field experiments were carried out in Rome (41°58'04''N), where fields are in a tight river plain. The trials were made in a randomized complete block design with three replicates.

The agronomic performance of 19 triticale genotypes is given (Table 5). In 2011, the overall performance of all the tested cultivars exceeded that in 2010, basically due to reduced emergence rates. The average yield for the period was 4.83 t/ha. Amarillo and Trimour, two cultivars with medium growth cycles, reached yields that remarkably exceeded the average in every year; test weight values were slightly below the mean of the period. The highest test weight was in Forricale, a cultivar with a very early growth cycle, which also reached yields higher than the field average in both years.

Table 5. Growth cycle, grain yield, and test weight of 19 Italian triticale cultivars tested during a two-year period (2010–11) in central Italy.

Cultivar	Heading date (days after 1 April)	Yield (t/ha)			test weight (kg/hL)		
		Index			Index		
		2010	2011	Mean	2010	2011	Mean
Rigel	8	99	86	4.38	100	98	68.2
Forricale	9	110	102	5.06	109	106	73.7
Trica	10	97	82	4.23	97	96	66.2
Catria	11	96	83	4.24	94	91	63.5
Oceania	12	82	96	4.39	93	91	63.2
Agrano	18	83	76	3.80	97	98	66.9
Bienvenu	18	150	93	5.46	104	101	70.2
Trimour	18	115	116	5.58	99	98	67.8
Wilfried	18	114	91	4.78	101	99	68.7
Amarillo	20	133	124	6.14	100	99	68.5
Altair	22	90	95	4.50	95	97	65.8
Universal	22	82	116	5.03	103	103	70.5
Magistral	24	90	86	4.23	98	99	67.7
Maximal	24	99	108	5.06	105	103	71.3
Costant	25	86	85	4.11	105	104	71.8
Quark	27	98	93	4.58	96	96	65.9
Tulus	28	60	114	4.57	98	101	68.3
Isotop	28	72	123	5.08	102	99	69.3
Talentro	28	30	112	4.01	100	103	69.9
Mean	**19**	**3.42**	**6.24**	**4.83**	**67.2**	**70.2**	**68.7**

ITEMS FROM MEXICO

NATIONAL INSTITUTE FOR FORESTRY, AGRICULTURE, AND LIVESTOCK RESEARCH (INIFAP–CIRNO)
Campo Experimental Valle del Yaqui, Apdo. Postal 155, km 12 Norman E. Borlaug, entre 800 y 900, Valle del Yaqui, Cd. Obregón, Sonora, México CP 85000.

Characteristics and description of phenotypic components of Villa Juárez F2009, a new bread wheat cultivar for southern Sonora, Mexico.

Víctor Valenzuela-Herrera, Guillermo Fuentes-Dávila, Pedro Figueroa-López, Gabriela Chávez-Villalba, José Luis Félix-Fuentes, Miguel Alfonso Camacho-Casas, and José Alberto Mendoza-Lugo.

Introduction. Bread wheat production in Mexico was 3.9 x 10^6 tons in year 2010, which was not enough to fulfill the national demand, so 3.3 x 10^6 tons had to be imported to suffice consumption needs (OEIDRUS 2011). In order to assure minimum strategic reserves with the objective of reducing the risk of depending on fluctuating international market prices, several years ago the regional industry reacted by implementing 'agriculture by contract' with wheat producers (Melis-Cota 2008).

Despite the problems caused by Karnal bunt at the beginning of the 1980s (SARH 1987), 220,409 ha were sown with bread wheat in 1990–91, which represented 89% of the area grown with wheat in the state of Sonora; however, since wheat season 1994–95, durum wheat has reached more area than bread wheat in the state. The area grown with wheat in 2010–11 in Sonora was 292,247 ha (Table 1); 87% (254,531 ha) corresponded to the southern region (OEIDRUS 2011). The predominating durum wheat cultivars were CIRNO C2008 and Átil C2000, with more than 40% of the total area. Bread wheat cultivars occupied 30% of the area.

Leaf rust, caused by the fungus *Puccinia triticina*, and Karnal bunt, by *Tilletia indica*, are the most important diseases in southern Sonora (Figueroa-López et al. 2011). However, since 2000, the incidence of stripe or yellow rust (*P. striiformis*) has increased in the region with the appearance of new races of the fungus. Tacupeto F2001 was the most grown bread wheat cultivar in 2009–10 in southern Sonora (Table 2, p. 102) and in 2010–11 in the state. Despite its quality attributes for the milling industry, Tacupeto F2001 has shown susceptibility to stripe rust (up to 80% severity) and moderate susceptibility to leaf rust (up to 40% severity), levels that make chemical application necessary and increase production costs. A collaborative project between the Mexican National Institute for Forestry, Agriculture, and Livestock Research (INIFAP) and the International Maize and Wheat Improvement Center (CIMMYT) with support by the farmer´s union (PIEAES) of the Yaqui Valley, has the objective of improving the

Table 1. Area (ha) grown with wheat during the 2010–11 agricultural season in Sonora, Mexico.

Cultivar	Area (ha)	Percent of total wheat area
Durum wheat		
CIRNO C2008	88,235	30.5
Átil C2000	50,236	17.3
Sáwali Oro C2008	14,353	4.9
Patronato Oro C2008	11,753	4.0
Júpare C2001	10,069	3.4
RSM Imperial C2008	7,149	2.4
CEVY Oro C2008	6,197	2.1
Río Colorado	5,111	1.7
Samayoa C2004	4,905	1.6
Rafi C97	1,806	0.6
RSM Chapultepec C2008	1,650	0.5
Others	1,210	0.4
Platinum	1,173	0.4
Aconchi C89	752	0.2
TOTAL		70.0
Bread wheat		
Tacupeto F2001	36,819	12.6
Kronstad F2004	18,681	6.4
Roelfs F2007	10,358	3.6
Navojoa M2007	8,046	2.8
RSM Norman F2008	4,499	1.5
Cachanilla F2000	3,493	1.2
Rayón F89	2,576	0.9
Abelino F2004	1,355	0.5
Palmerín F2004	964	0.3
Others	538	0.1
Oasis F86	319	0.1
TOTAL	87,648	30.0

cost/benefit and competiveness of bread wheat by producing advanced lines with better quality, yield, resistance to diseases, and better water use efficiency, that could be released as commercial cultivars (Figueroa-López et al. 2011).

Pedigree, history selection, and description of Villa Juárez F2009. After evaluations of grain yield carried out since the 2008–09 agricultural season at the Norman E. Borlaug Experimental Station (CENEB), we proposed to release the experimental bread wheat line 'WBLL1*2/BRAMBLING' as cultivar Villa Juárez F2009 (Valenzuela-Herrera et al. 2012). Villa Juárez F2009 is a spring-type bread wheat cultivar, which originated from hybridizations made in the Bread Wheat Breeding Program of CIMMYT. The cross number and history selection is CGSS01B00062T-099Y-099M-099M-099Y-099M-12Y-0B. Shuttle breeding was carried out between the experimental stations of El Batán, state of Mexico (B) (19°30'N and 2,249 msnm), San Antonio Atizapán, state of Mexico (M) (19°17'N and 2,640 msnm), and the Yaqui Valley (Y) (27°20'N and 40 msnm), in Sonora (Table 3, p. 103).

The most important phenotypic characteristics of Villa Juárez F2009, according to the International Union for the Protection of New Varieties of Plants (UPOV 1994), are given in Table 4 (p. 103). This cultivar has an average of 72 days to heading with a range of 67 to 74, a biological cycle with an average of 114 days to physiological maturity; however, the cycle may be shortened due to the lack of cold hours if planting is late (Wardlaw and Moncur 1995), and may average 106 days when sowing is done at the end of December. Villa Juárez F2009 has an average height of 91 cm (Fig. 1), a maximum of 100, and minimum of 80. Plant growth habit is semi-prostrate and shows a high frequency of recurved flag leaves. Ear shape in profile view is parallel sided, density is lax, and the length excluding awns is very long. Ear glaucosity is strong and at maturity it becomes white. The width of the lower glume is absent or very narrow (spikelet in mid-third of ear), shoulder shape is sloping, and the beak length is short and slightly curved. Grain shape is semi-elongated (Fig. 2, p. 103), and grain coloration when treated with phenol is lacking or very light.

Acknowledgements. The authors wish to thank the International Maize and Wheat Improvement Center (CIMMYT), for providing the advanced lines from which Villa Juárez F2009 originated.

Table 2. Area (ha) grown with wheat during the agricultural season 2009–10 in Sonora, Mexico.

Cultivar	Area (ha)	Percent of total wheat area
Durum wheat		
Átil C2000	81,777	33.07
Júpare C2001	53,164	21.50
Samayoa C2004	23,318	9.43
Sáwali Oro C2008	4,761	1.93
CIRNO C2008	3,256	1.32
CEVY Oro C2008	3,233	1.31
Platinum	2,655	1.07
Patronato Oro C2008	2,325	0.94
Aconchi C89	1,019	0.41
RSM Imperial C2008	980	0.40
Banámichi C2004	826	0.33
RSM Chapultepec C2008	499	0.20
Rafi C97	351	0.14
Río Colorado	296	0.12
Nácori C97	241	0.10
Altar C84	105	0.04
TOTAL		
Bread wheat		
Tacupeto F2001	40,552	16.40
Kronstad F2004	25,021	10.12
Abelino F2004	736	0.30
RSM-Norman F2008	659	0.27
Rayón F89	636	0.26
Tarachi F2000	384	0.16
Roelfs F2007	248	0.10
Navojoa M2007	235	0.10
Monarca F2007	4	0.00
TOTAL	68,475	

Fig. 1. Bread wheat cultivar Villa Juárez F2009 has an average height of 91 cm. Plants are semi-prostrate and present a high frequency of rcurved flag leaves.

Fig. 2. Grain of the bread wheat cultivar Villa Juearez F2009 is semi-elongated. Grain color after treatemtn with phenol is lacking or very light.

Table 3. History selection and localities where cultivar Villa Juárez F2009 was evaluated. Planting dates for the INIFAP yield trials were 15 and 30, November, 15 December, and 1 January. For season, F–W = fall–winter and S–S = spring–summer; for irrigation conditions RR = regular rainfed, NI = normal irrigation, and RI = reduced irrigation.

Activity	Locality	Season	Irrigation conditions
Simple genetic cross	El Batan, Mexico	S–S/2001	RR
F_1 Generation	Cd. Obregon, Sonora	F–W/2001–02	NI
F_2 Generation	Atizapan, Mexico	S–S/2002	RR
F_3 Generation	Atizapan	S–S/2003	RR
F_4 Generation	Cd. Obregon	F–W/2003–04	NI
F_5 Generation	Atizapan	S–S/2004	RR
F_6 Generation	Cd. Obregon	F–W/2004–05	NI
F_7 Generation	El Batan	S–S/2005	RR
Yield trials by CIMMYT	Cd. Obregon	F–W/2005–06	NI
		F–W/2006–07	NI
Yield trials by INIFAP	Cd. Obregon	F–W/2007–08	NI and RI
		F–W/2008–09	NI and RI

Table 4. Characteristics and description of phenotypic components of cultivar Villa Juárez F2009.

Structure	Characteristic	Description
Coleoptile	Anthocyanin coloration	Absent or very weak
First leaf	Anthocyanin coloration	Absent or very weak
Plant	Growth habit	Semi-prostrate
	Frequency of plants with recurved flag leaves	High
Ear	Time of emergence	Early
Flag leaf	Glaucosity of blade	Strong
Ear	Glaucosity	Strong
Straw	Pith in cross section (halfway between base of ear and stem node below)	Thin
Culm	Glaucosity of neck	Medium
Plant	Length (stem, ear, and awns)	Medium
Lower glume	Shape of shoulder	Sloping
	Shoulder width	Absent or very narrow
	Length of beak	Short
	Shape of beak	Slightly curved
	Hairiness on external surface	Medium
Ear	Length (excluding awns)	Very long
	Hairiness of margin of first rachis segment	Absent or very weak
	Color (at maturity)	White
	Shape in profile view	Parallel sided
	Density	Lax
Grain	Shape	Semi-elongated
	Coloration with phenol	None or very light
Plant	Seasonal type	Spring

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Reaction of elite bread wheat lines and cultivars to Karnal bunt under artificial inoculation, during the crop season 2009–10 in the Yaqui Valley, Mexico.

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Introduction. *Triticum aestivum* is the most susceptible plant species to Karnal bunt. Under artificial inoculation, some lines may show more than 50% infeted grain (Fuentes-Dávila et al. 1992, 1993). Although the fungus *Tilletia indica* (Mitra 1931) (syn. *Neovossia indica*) may affect durum wheat and triticale (Agarwal et al. 1977), the levels of infected grain are generally low. Control of this pathogen is difficult because teliospores are resistant to physical and chemical factors (Krishna and Singh 1982; Zhang et al. 1984; Smilanick et al. 1988). Chemical control can be accomplished by applying fungicides during flowering (Fuentes-Dávila et al. 2005),

Table 5. Elite bread wheat lines and commercial cultivars artificially inoculated with Karnal bunt (*Tilletia indica*) in the field at three planting dates, during the fall–winter 2009–10 crop season, in the Yaqui Valley, Sonora, Mexico.

Entry	Pedigree and selection history
1	Tacupeto F2001
2	Kronstad F2004
3	Navojoa M2007
4	Roelfs F2007
5	Toba97/Pastor CMSS97M05756S-040M-020Y-030M-015Y-3M-1Y-3M-0Y
6	Kamb1*2/Brambling CGSS01B00069T-099Y-099M-099M-099Y-099M-20Y-0B
7	Betty/3/Chen/*Ae. tauschii*//2*Opata CMSW00WM00150S-040M-040Y-030M-030ZLM-3ZTY-0M
8	WBLL1*2/Brambling CGSS01B00062T-099Y-099M-099M-099Y-099M-12Y-0B
9	Babax/LR42//Babax*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ CGSS01B00045T-099Y-099M-099M-099Y-099M-26Y-0B
10	Babax/LR42//Babax/3/ER2000 CMSA01Y00176S-040P0Y-040M-030ZTM-040SY-24M-0Y-0SY
11	PFAU/MILAN/3/Babax/LR42//Babax CMSS02M00056S-030M-28Y-0M-040Y-25ZTB-0Y-01B-0Y
12	Thelin/2*WBLL1 CGSS02Y00079T-099B-099B-099Y-099M-6Y-0B
13	PBW343//CAR422/ANA/3/Elvira CMSS02M00409S-030M-1Y-0M-040Y-10ZTB-0Y-02B-0Y
14	Babax/LR42//Babax/3/ER2000 CMSA01Y00176S-040P0Y-040M-030ZTM-040SY-30M-0Y-0SY
15	TC870344/GUI//TemporaleraA M87/AGR/3/2*WBLL1 CMSA01Y00725T-040M-040P0Y-040M-030ZTM-040SY-10M-0Y-0SY
16	ROLF07/YANAC//Tacupeto F2001/Brambling CGSS05B00121T-099TOPY-099M-099NJ-4WGY-0B
17	Waxwing*2/Kronstad F2004 CGSS04Y00020T-099M-099Y-099ZTM-099Y-099M-3WGY-0B
18	Whear/Kronstad F2004 CGSS04Y00106S-099Y-099M-099Y-099M-9WGY-0B
19	KEA/TAN/4/TSH/3/KAL/BB//TQFN/5/Pavon/6/SW89.3064/7/Sokoll CMSS04Y00153S-099Y-099ZTM-099Y-099M-5WGY-0B
20	CAL/NH//H567.71/3/Seri/4/CAL/NH//H567.71/5/2*KAUZ/6/WH576/7/WH542/8/Waxwing CMSS04Y00364S-099Y-099ZTM-099Y-099M-2WGY-0B
21	Becard CGSS01B00063T-099Y-099M-099M-099Y-099M-33WGY-0B
22	Whear/Sokoll CMSS04Y00201S-099Y-099ZTM-099Y-099M-11WGY-0B
23	PFAU/Milan//Trost/3/PBW65/2*Seri.1B CMSS04M01426S-0TOPY-099ZTM-099Y-099M-3RGY-0B
24	Whear/Kronstad F2004 CGSS04Y00106S-099Y-099M-099Y-099M-3WGY-0B
25	Chewink CGSS03B00074T-099Y-099M-099Y-099M-6WGY-0B-3B
26	Norman F2008

however, this measure is not feasible when quarantines do not allow tolerance levels for seed production. Resistant wheat cultivars are the best means to control this disease. Our objective was to evaluate 21 elite, advanced, bread wheat lines and five commercial cultivars for resistance to Karnal bunt.

Materials and methods. Twenty-one elite, advanced, bread wheat lines and five commercial cultivars, Tacupeto F2001, Kronstad F2004, Navojoa M2007, Roelfs F2007, and Norman F2008 (Table 5, p. 104) were evaluated for resistance to Karnal bunt during the fall–winter 2009–10 crop season in block 910 in a clay soil with pH 7.8, in the Yaqui Valley, Sonora, Mexico. Planting dates were 19, 25, and 30, November 2009, using a 1-m bed with two rows. Inoculum was prepared by isolating teliospores from infected kernels, followed by centrifugation in a 0.5% sodium hypochlorite solution, and plating on 2% water-agar Petri plates (Fig. 3). After teliospore germination, fungal colonies were transferred and multiplied on potato-dextrose-agar. Inoculations were by injecting 1 mL of an allantoid sporidial suspension (10,000/mL) during the boot stage (Fig. 4) in ten heads from each line and cultivar. High relative humidity in the experimental area was provided by a fine spray of water with back-pack manual sprayers. Harvest was done manually, and the counting of healthy and infected grains was done visually to determine the percentage of infection. Evaluated lines originated from the collaborative project between the International Maize and Wheat Improvement Center (CIMMYT) and the National Institute for Forestry, Agriculture and Livestock Research in Mexico (INIFAP).

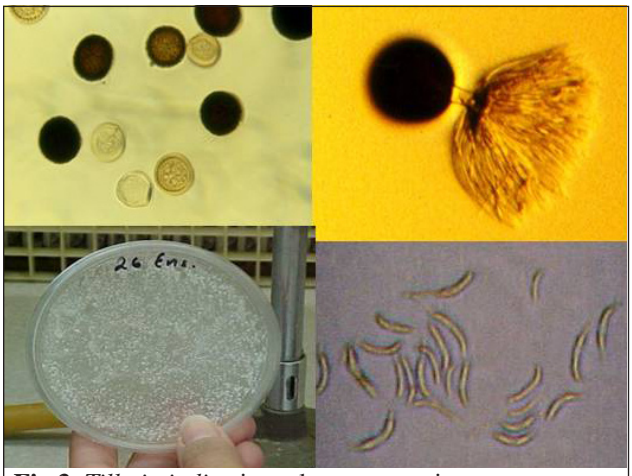

Fig 3. *Tilletia indica* inoculum preparation.

Fig. 4. Artificial inoculation by boot injection.

Results and discussion. The range of infection for the first planting date (19 November) was 0.00 to 30.68%, with a mean of 10.01; one line did not have any infected grains (Fig. 5). The range of infection for the second planting date (25 November) was 0.00 to 32.20%, with a mean of 9.10. Two lines did not have any infected grains For the third planting date (30 November), the range of infection was 0.00–45.26 with a mean of 17.09. The frequency of lines in the different infection categories at the three dates is shown (Fig. 6, p. 106). The suceptible check KBSUS 1 had 100% infection.

Overall, three lines fell within the 2.6–5.0 infection category, nine in the 5.1–10.0 category, and 14 in the 10.1–30.0 infection category (Fig. 7, p. 106). Lines with less than 5% infection are considered resistant (Fuentes-Dávila and Rajaram

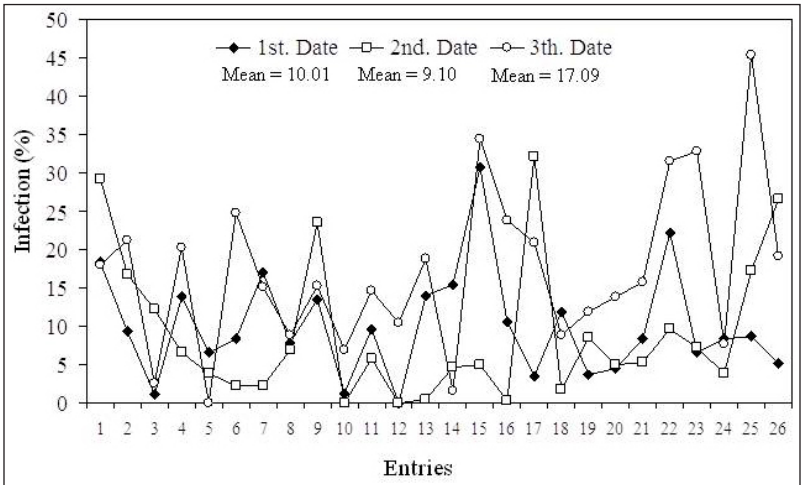

Fig. 5. Percent of infection with Karnal bunt (*Tilletia indica*) of 21 elite, advanced, bread wheat lines and five cultivars artificially inoculated in the field during the 2009–10 crop season at three dates in the Yaqui Valley, Sonora, México.

1994). Fifty-three percent of the entries were moderately susceptible to susceptible, including the cultivar Tacupeto F2001 and lines 'TC870344/GUI//Temporalera M 87/AGR/3/2*WBLL1', 'Whear/Sokoll', and 'Chewink'. Cultivar Navojoa M2007 and eight lines were within the 5.1–10.0% infection category; this cultivar had a mean of 5.25%. Cultivars Roelfs F2007, Kronstad F2004, Norman F2008, Tacupeto F2001, and ten lines were within the 10.1–30.0% infection category; the mean percentage of infection of these cultivars was 13.54, 15.73, 16.92, and 21.86, respectively. Cultivars with the highest levels of infection were Tacupeto F2001 with 29.22% and Norman F2008 with 26.53%. Lines with the highest percent infection were 'Chewink' (45.26%) and 'TC870344/GUI//TemporaleraM87/AGR/3/2*WBLL1' (34.41%), whereas lines with the lowest percent infection were 'Babax/LR42//Babax/3/ER2000' (2.76%), 'Toba97/Pastor' (3.47), and 'Thelin/2*WBLL1' (3.50%). Tacupeto F2001 has been and is the leading bread wheat cultivar in Sonora; the area grown with this cultivar in 2009–10 in the southern part of the state was 40,552 ha and in 2010–11 was 36,819 ha in the entire state (OEIDRUS 2011). Tacupeto F2001 complies with the quality requirements of the milling industry, however, susceptibility to stripe rust and moderate susceptibility to leaf rust and to Karnal bunt make necessary the application of fungicides; therefore, it is important to look for other cultivars that have been released by INIFAP, as well as experimental lines such as 'Babax/LR42//Babax/3/ER2000', 'Toba97/Pastor', and 'Thelin/2*WBLL1', which are considered resistant and good prospects for commercial release.

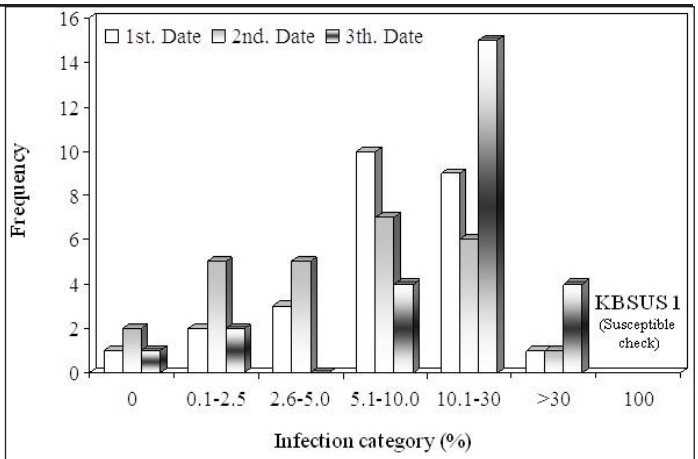

Fig. 6. Results of artificial field inoculatin of 21 elite, advanced, bread wheat lines and five cultivars with Karnal bunt (*Tilletia indica*) on three dates during the 2009–10 crop season in the Yaqui Valley, Sonora, México. The level of infection of KBSUS 1 is the mean of the three highest infection scores.

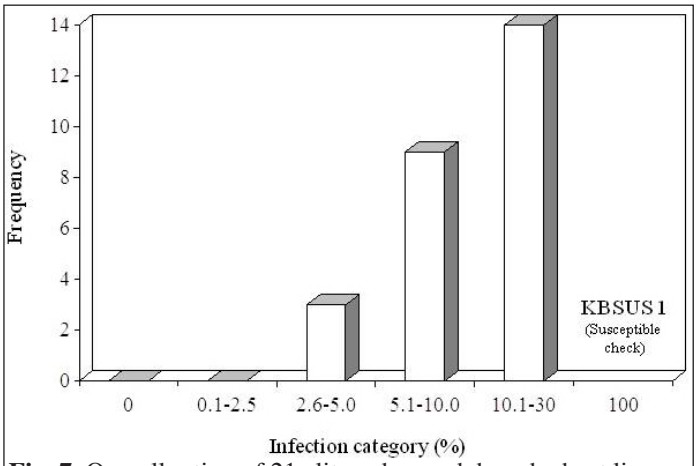

Fig. 7. Overall rating of 21 elite, advanced, bread wheat lines and five cultivars artificially inoculated with Karnal bunt (*Tilletia indica*) on three dates during the 2009–10 crop season in the field in the Yaqui Valley, Sonora, México. The level of infection of KBSUS 1 is the mean of the three highest infection scores.

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CO_2 flux in soils cultivated with wheat with and without tillage and with tillage in beds burning residues in the Yaqui Valley, Sonora, Mexico.

Juan Manuel Cortés-Jiménez, Teresa de Jesús Ruiz-Vega, Alma Angélica Ortiz-Ávalos, and Guillermo Fuentes-Dávila.

Summary. CO_2 flux was quantified during a 10-year period in soils cultivated with wheat under no till or direct sowing and under tillage in beds burning residues. The evaluation was carried out at the Norman E. Borlaug Experimental Station located in block 910 in the Yaqui Valley, Sonora, in a clay soil. A dynamic, closed-chamber system was used. A greater CO_2 flux was observed in soils under no till or direct sowing with a difference of 0.030 g/m^2/h over tillage in beds burning crop residues. Direct sowing or no till treatments reduced the temperature, but increased significantly, the soil moisture content at a depth of 0–10 cm.

Introduction. Currently, there is an increasing interest in agricultural practices that reduce carbon losses in soils (Regina and Alakukku 2010). The intensive soil tillage has been a constant in management of agricultural soils. The main reasons for soil tillage have been the control of weeds, pests and diseases, sowing preparation, the increase of water storage, and the improvement of natural fertilization of plants. The adoption of conservation tillage practices has not only allowed greater control of the erosion, but also the reduction in losses of organic matter as a consequence of the intensive tillage of soils (Fuentes et al., 2010). Carbon sequestration can be one the most profitable agricultural activities in order to reduce the process of global warming and contribute to environmental security (Reicosky and Archer, 2005). The objective of the study was to quantify the CO2 flux in soils cultivated with wheat under no till or direct sowing, and under tillage in beds burning crop residues in the Yaqui Valley, Mexico.

Materials and methods. The evaluation was carried out at the Norman E. Borlaug Experimental Station, which belongs to the Mexican National Institute for Forestry, Agriculture, and Livestock Research located in block 910 in the Yaqui Valley, Sonora (27º22´3.01" N and 109º55´40.22" W) in a clay soil. CO_2 flux was quantified during 2011 in soils subjected during 10 years to conservation tillage under the scheme of no till or direct sowing, and tillage in beds burning residues; both with a summer fallow and wheat during the fall–winter season. The wheat crop was established over straw from the previous crop season for the no till and direct sowing treatments. Weeds that emerged after rain during the summer season were controlled with herbicides. Fertilization was broadcast. For the tillage in beds treatment, residues were burned after harvest, and the beds from the previous season were reutilized. The CO_2 flux was determined for each agricultural management during the period January–October 2011, with a total of 11 readings with six replications for each tillage method. The dates for the period of evaluation were 29 January, 5 February, 17 February, 11 March, 25 March, 15 April, 20 April, 29 April, 30 June, 1 July, 4 August, 4 October, and 5 October. The period of evaluation, from 29 January to 20 April, coincided almost entirely with the vegetative development of the wheat crop. To quantify CO_2 flux in the soil, a dynamic, closed-chamber system was used (Pumpanen et al. 2004), which consisted of an infrared gas analyzer (IRGA, LI-820, Licor, Lincoln NE, USA) connected to a console CR23X (Cambell Sci, Logan UT, USA) and to a pneumatic pump in order to circulate air through the system and a flux regulator. The air that circulates through the system is filtered in order to prevent impurities into the system; a thermopar monitors the temperature within the chamber and a pressure release valve in the top of the chamber (0.2 cm internal diameter and 1.8 cm height), and peripheral sensors of soil moisture (HH2 Moisture Meter, Delta-T Devices, Cambridge, England) and soil temperature (Fig. 8). The chamber has a cylindrical shape with an internal diameter of 10 cm and a height of 12.8 cm, which cover a volume of 1.44 x 10^{-3} m^3. The flux speed was adjusted

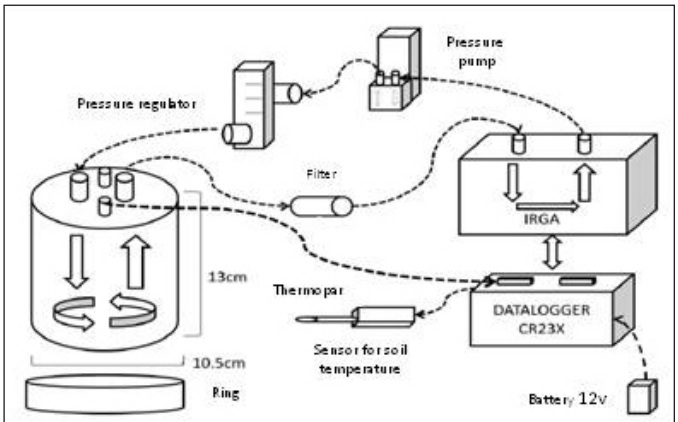

Fig. 8. Conceptual diagram of the soil respiration chamber (Cueva-Rodriquez 2011).

to a constant speed of 500 ml/min. The system measures the increment of CO_2 inside the chamber during 150 seconds. To calculate the flux, the sampling point and the slope of the relation time vs CO_2 concentration are used (Cueva-Rodríguez 2011). A completely randomized block design was used with six replications and 11 evaluation dates. Data were analyzed in Statgraphics Plus 5.1. Mean comparison was done with Tukey´s test (0.01).

Results and discussion. Greater CO_2 fllux was observed in soils where wheat was subjected to no till or direct sowing, with an increase of 0.030 g/m^2/h over tillage in beds burning crop residues (Fig. 9); however, this difference was not statistically significant (Table 6). The highest CO_2 flux under no till was attributed to the straw layer, which reduced the temperature and increased the moisture content in the soil. A high negative correlation (r = –0.95) was observed between temperature and CO_2 flux. The regression coefficient indicated that CO_2 flux decreased 0.007 g/m^2/h for each centigrade increase in soil temperature and, as an average, the no till treatment decreased the temperature by 1.3ºC at 0–10 cm depth and increased the moisture content by 2.6%. In relation to the soil moisture content, the highest percentage of flux was between 23.64 and 25.27%; a higher or lower moisture content than the interval indicated caused a reduction in the value of the CO_2 flux. Therefore, a greater CO_2 flux was observed with direct sowing due to the temporal variability of the moisture content (Fig. 10), and a lower CO_2 flux for tillage in beds burning crop residues by the effect of a higher temperature observed during the months of the evaluation (Fig. 11, p. 109). The greatest CO_2 from soil under the two tillage systems was observed during the vegetative development of the wheat crop, when complementary irrigations were applied. We assumed that during this time, root respiration is more active. CO_2 flux decreased significantly after the month of March and primarily after the wheat harvest. From a study carried out in the central part of the valley in México about the effect of different types of tillage on carbon distribution and emissions during a crop season in plots with residues of maize and wheat, Fuentes et al. (2011) reported that wheat residues emit more CO_2 than maize residues because the decomposition rate of wheat residues is faster.

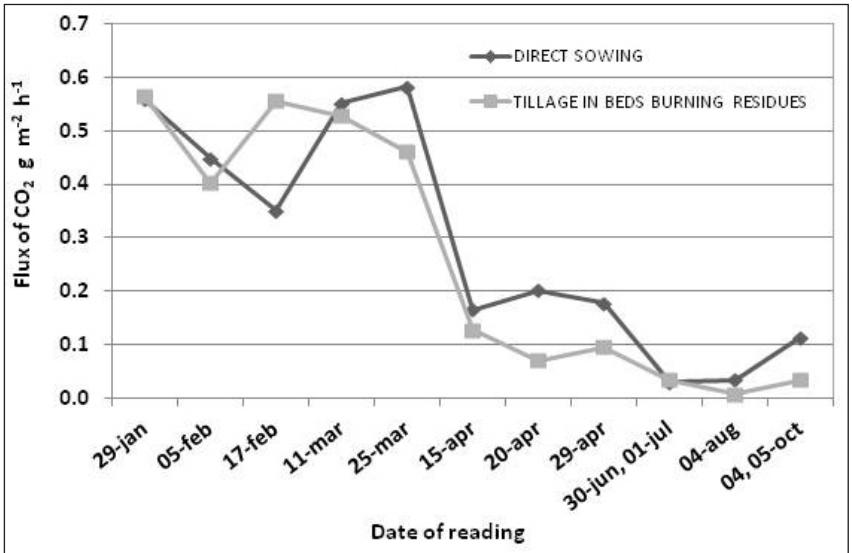

Fig. 9. Temporal variation of CO_2 flux in soils with two tillage systems

Table 6. Effect of two tillage systems upon CO_2 flux, temperature, and soil moisture, during a period January–October, 2011.

Treatment	CO_2 flux (g/m^2/h)	Moisture (%)	Soil temperature (°C)
Direct sowing	0.292	16.9 a	30.9 a
Tillage in beds with burn residue	0.262	14.3 b	32.2 b
		Tukey 0.01=1.4081	Tukey 0.01=1.08695

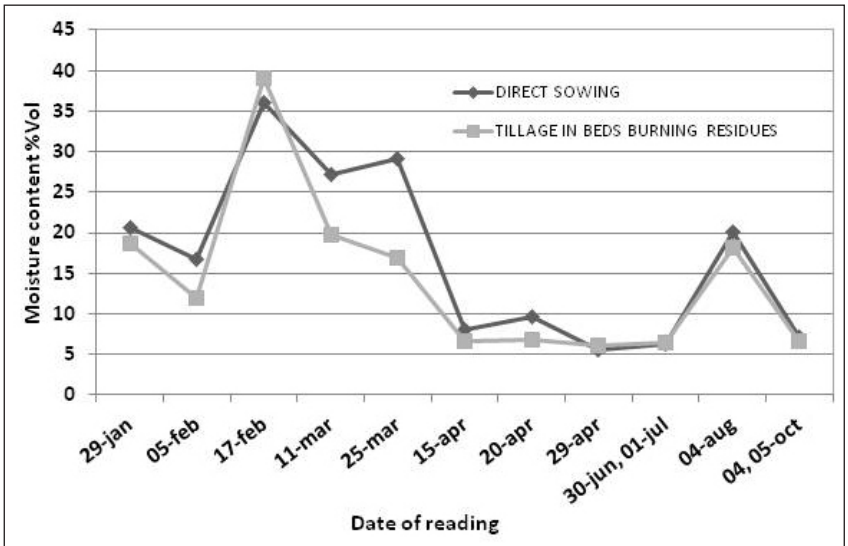

Fig. 10. Temporal variability of the soil moisture content under two tillage systems

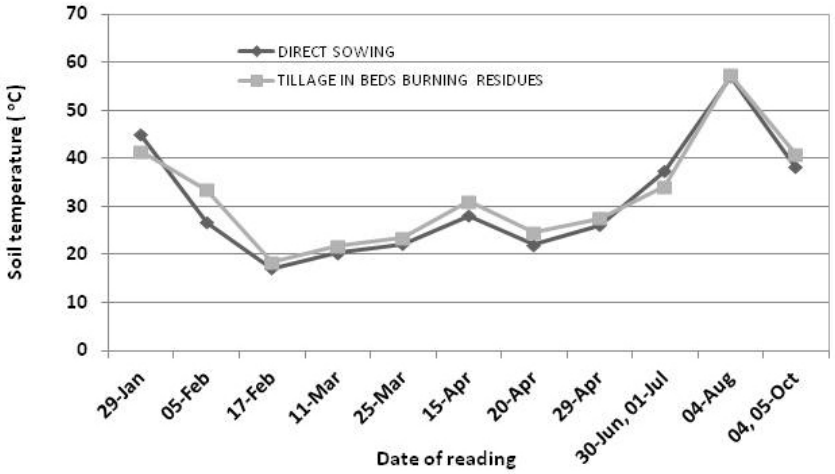

Fig. 11. Temporal variability of the soil moisture content under two tillage systems

Conclusions. No significant differences were observed in CO_2 flux between tillage treatments. Direct sowing or no till increases the soil moisture content, and this variable is correlated with CO_2 flux in the soil. Direct sowing or no till reduces the soil temperature, and this variable is negatively correlated with the CO_2 flux in the soil. It is necessary to consider other biotic and abiotic factors that influence the quantification of CO_2 flux, such as the microbial population and the content of organic and inorganic carbon in the soil, in order to understand in detail the functioning of agricultural soils as carbon reservoir, and the impact that tillage systems and crop production might have on the atmosphere and the global warming.

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Effect on seedling emergence of placing seed of bread wheat cultivar Rayón F89 in direct contact with three different fertilizers separately during sowing.

Juan Manuel Cortés-Jiménez, Alma Angélica Ortiz-Ávalos, Guillermo Fuentes-Dávila, and Teresa de Jesús Ruiz-Vega.

Summary. The effect on seedling emergence of placing seed of the bread wheat cultivar Rayón F89 in direct contact with three different fertilizers separately during sowing was studied through a factorial experiment with two seed densities (40 and 80 kg/ha) and fertilizer rates of 0, 40, and 80 kg/ha of urea, ammonium sulphate, and mono-ammonium phosphate. Significant differences were found for seedling emergence/m^2 among the fertilizers evaluated, between seed densities, and fertilizer rates. No negative effect on seedling emergence was observed when wheat seed was placed in contact with mono-ammonium phosphate, whereas urea and ammonium sulphate caused a significant reduction in seedling emergence that was not compensated for by using a greater seed density.

Introduction. Any natural or industrialized material that contains at least 5% of one or more of the primary nutrients (N, P_2O_5, and K_2O) can be called a fertilizer. The presentation of mineral fertilizers is highly variable. Based on the process of fabrication, particles of mineral fertilizers can be of very different size and shape, granules, pellets, powder of coarse grain/compacted, or fine. Most fertilizers are provided in a solid form (FAO 2002). The efficiency of a fertilizer var-

ies according to the nutrient and fertilizer source, and it depends on rates, method, timing, cultivar, management of the crop, and environmental conditions. Therefore, proper selection, timing, and way of application of a fertilizer will help to achieve greater agronomic efficiency and profitability by using this input (SAGARPA 2012). In order to increase the absorption efficiency by the plant roots, the most recommended method for application of a fertilizer is to place it in a band at the bottom of the furrow beside the seed. Although most fertilizers are soluble salts of various kinds, they vary greatly in their damage to seed germination and emergence of crops, which essentially depends upon the saline index. In wheat in the Yaqui Valley, México, an average of 250 kg/ha of nitrogen is applied. Fertilization costs in wheat represent 25–30% of the total production costs for the crop, depending on the rate and the nitrogen source used. Of the total rate, 75% is applied during presowing and the rest right before the first and second complementary irrigations. The efficiency of recovery of granulated fertilizers by the crop has been calculated to be 38%, which means that 62% of the fertilizer and resources that this represents are not being used by the wheat plant (Cortés 2008). At the commercial level, efficiencies of 20–48% have been observed (Cortés et al. 2011); however, there are no references about the use of fertilizers applied in direct contact with the seed in northwest Mexico. Our objective was to determine the effect of placing the seed in direct contact with three different fertilizers separately during sowing on seedling emergence of the bread wheat cultivar Rayón F89.

Materials and methods. The evaluation was carried out at the Norman E. Borlaug Experimental Station during the fall-winter 2000–01 agricultural season in a compacted clay soil with 101 kg/ha of N–NO_3 available in the topsoil layer, and 71 kg in the subsoil. The effect of three fertilizers on the emergence of bread wheat cultivar Rayón F89 was studied through a factorial experiment with two sowing densities (40 and 80 kg/ha of seed), and fertilizer rates of 0, 40, and 80 kg/ha of urea, ammonium sulphate, and mono-ammonium phosphate. Before sowing, land preparation was as follows: disking (2), planking, and bed formation. Sowing was done on moist soil on 15 December in beds with 80 cm separation and one row. The fertilizer was applied in a band at the bottom of the row on the bed, and the seed was placed over the fertilizer. A single, complementary irrigation was applied to the crop. An application of insecticide was necessary for aphid control, and weeds were eliminated manually. A randomized block experimental design with split plots and two replications was used. The experimental unit consisted of four 8-m rows; two linear meters from the central rows were used for seedling counts. The variables evaluated were seedling emergence and the percent of seedling emergence for each treatment. Data were analyzed with Statgraphics Plus 5.1. Mean comparison was done with Tukey´s test (0.01 and 0.05).

Results and discussion. Significant differences were found for seedling emergence/m^2, between the fertilizers evaluated, between seed densities, and fertilizer rates (Table 7), as well as in the interaction 'source x rate of fertilizer' and seed density and fertilizer rate. The average number of emerged seedlings/m^2 was 56, 77, and 112 for urea, ammonium sulphate, and mono-ammonium phosphate, respectively. The average number of emerged seedlings with seed densities of 40 and 80 kg/ha was 55 and 108, respectively, whereas with fertilizer rates of 0, 40, and 80 kg/ha, it was 119, 76, and 51, respectively. The interaction 'source x rate' showed an average number of emerged seedlings of 116, 39, and 15 for urea;

Table 7. Effect of placing the seed in direct contact with three different fertilizers separately during sowing, on seedling emergence of bread wheat cultivar Rayón F89 during the fall–winter of the 2000–01 agricultural season. Source of fertilizer, Tukey (0.01) = 54.24; rate of fertilizer, Tukey (0.05) = 41.03.

	Seedling emergence/m^2								
	40 kg/ha of seed rate of fertilizer (kg/ha)				80 kg/ha of seed rate of fertilizer (kg/ha)				
Fertilizer	0	40	80	Mean	0	40	80	Mean	Mean
Urea	75	24	15	38	156	53	16	75	56 a
Ammonium sulphate	90	43	24	52	146	120	39	101	77 a
Mono-ammonium phosphate	80	71	75	75	167	143	136	148	112 ab
Fertilizer rate mean	82 a	46 ab	38 b	55	156 a	105 b	64 b	108	

118, 81, and 31 for ammonium sulphate; and 124, 107, and 105 for mono-ammonium phosphate. The interaction 'seed density x rate of fertilizer' showed an average number of emerged seedlings of 82, 46, and 38 for 40 kg/ha of seed and 0, 40 and 80 kg/ha of fertilizer, and 156, 105, and 64 for 80 kg of seed with the corresponding fertilizer rates. Significant differences were observed for the percent of seedling emergence between the source of fertilizer, rate, and the interaction 'source x fertilizer rate'. The percent of seedling emergence was 46% for urea, 61% for ammonium sulphate, and 90% for mono-ammonium phosphate (Table 8, p. 111). Relative seedling emergence for seed densities of 40 and 80 kg/ha was 65.58 and 65.75%, respectively. Seedling emergence associated with the a fertilizer rate of 0 was 94.7%, 60.1% at 40 kg,

and 42.2% at 80 kg. The interaction 'source x rate of fertilizer' showed that each rate of urea and ammonium sulphate reduced significantly the percentage of seedling emergence, but that was not the case for mono-ammonium phosphate. The observed values were 93.1, 30.8, and 13.7% for 0, 40, and 80 kg of urea; 94.1, 62.7, and 26.2% for ammonium sulphate; and 97.0, 86.7, and 86.7% for mono-ammonium phosphate.

Table 8. Effect of placing the seed in direct contact with three different fertilizers separately during sowing on the percentage of seedling emergence of bread wheat cultivar Rayón F89 during the fall–winter 2000–01 agricultural season. Fertilizer, Tukey (0.01) = 22.46; rate of fertilizer, Tukey (0.01) = 14.2; and 'source x rate of fertilizer', Tukey (0.01) = 24.61.

	Seedling emergence (%)/m²			
Fertilizer (kg/ha)	Urea	Ammonium sulphate	Mono-ammonium phosphate	Mean
0	93.14 a	94.05 a	96.95 a	94.71 a
40	30.79 b	62.65 b	86.74 a	60.06 b
80	13.72 b	26.22 c	86.73 a	42.22 c
Mean	45.88 b	60.97 b	90.14 a	

Conclusions. It is feasible to physically place wheat seed with mono-ammonium phosphate in direct contact during sowing, because there was no significant reduction in the percentage of seedling emergence with the rates evaluated. We do not recommended the use urea or ammonium sulphate in contact with the wheat seed, because of significant reduction in seedling emergence. Increasing the seed rate partially compensates the reduction in the percentage of wheat seedling emergence, when seed and fertilizer are placed in direct contact in the soil.

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Effect of residual nitrogen applied to wheat, on maize grain yield as a summer crop in the rotation, in the Yaqui, Valley Sonora, México.

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Introduction. In the Yaqui Valley, Sonora, México, fertilization costs in wheat and maize represent from 25 to 30% of the total production costs for the crop, depending on the rate and the nitrogen source used. Wheat is fertilized with an average of 250 kg/ha, 75% of the rate is applied during presowing and the rest right before the first and second complementary irrigations. Wheat efficiency to recover nitrogen is 38%, whereas 25% is lost by lixiviation and volatilization, and 37% is retained in the soil as residual nitrogen (Cortés and Uvalle 1998). The residual nitrogen from 250 units applied to wheat represent 92.5 units/hectare. Rates of 0, 75, 150, 225, and 300 nitrogen units applied to wheat, were associated to yields of 3.817, 3.973, 4.573, 4.744, and 5.176 ton/ha of maize, as a follow up crop when it was not fertilized (Cortés and Uvalle 1996). For wheat fertilization, the maximum economic yield and quality required by the market are taking into consideration, primarily the protein content. Therefore, it is important to apply an additional quantity of nitrogen (N), although not necessary for yield, it is very important for quality. For farmers who practice wheat–maize rotation (fall–winter–summer), the best viable solution consists in assuring the quality standards required by the industry (a minimum of 12.5% protein content), which implies increasing the rate of N in wheat in order to comply with an appropriate supply of N in the soil during formation and grain filling, with the understanding that a great quantity of residual N will be available in the soil at harvest, which will be used by maize, and will allow a significant reduction in production costs of this second crop, primarily on the concept of fertilization. However, the increasing use of chemical fertilizers is of great

concern with respect to the effect on the environment. Nitrates lixiviated from the top soil layer are considered the main source of contamination, which are produced primarily by N-based fertilizers that were not taken up by the crop, and after harvest they remain as residual N in the soil. The amount of residual N increases based on the rate applied, method and time of application (Cortés et al. 1994; Isfan et al. 1995; Cortés and Uvalle 1998). Our objective was to determine the impact of residual N of wheat, on the maize response to N fertilization.

Materials and methods. The study was conducted at the Norman E. Borlaug Experimental Station, which belongs to the Mexican National Institute for Forestry, Agriculture and Livestock Research (INIFAP), during the fall–winter 1996–97 crop season. The station is located at 27°22' north latitude and 109°55' west longitude. Two experiments were established in order to determine the impact of residual N of wheat, on the maize response to N fertilization. Experiment 1: three N rates (150, 225, and 300 kg/ha) in the form of urea were applied to wheat, and four N rates (0, 75, 150, and 225 kg/ha) in the form of urea and ammonium sulphate were applied to summer maize. Experiment 2: three N rates (0, 150, and 300 kg/ha) in the form of urea were applied to wheat, and the four N rates used in experiment 1 in the form of urea were applied to maize. A split-split plot design was used with three replications; large plots corresponded to N sources, medium plots to N rates in wheat, and small plots to N rates in maize. For both experiments, the soil was prepared with minimum tillage based on the use of the same bed on which wheat was cultivated. Sowing was on 16 December with durum wheat cultivar Nacori C97 at the rate of 50 kg/ha on beds 80 cm apart with two rows. N was applied in band between the wheat rows and before the first complementary irrigation. Three complementary irrigations were applied, insecticide was used for aphid control, and weeds were eliminated mechanically and manually. Maize hybrid H-431 was sown on 20 June at a density of six seeds/m or 75,000 seeds/ha, which produced an average of 64,062 plants/ha at the end of the crop season. The total N rate was applied in band 25 days after sowing maize. The plot size was 38.4 m^2 and 6.4 m^2 was used for yield evaluation. Four complementary irrigations were applied and two insectide applications for pest control. Weed control was done mechanically and manually. The variable evaluated was grain yield of maize.

Results. ***Experiment 1.*** An increment of 938 kg/ha was observed in maize yield when N rates of 150 and 300 units applied to wheat, which is equivalent to a residual effect of 6.25 kg of maize per each kg of N applied to wheat. No significant differences were observed between urea and ammonium sulphate; maize yields were 4.555 and 4.614 ton/ha, respectively. When maize was not fertilized, the yield associated with the N rate of 150 kg/ha was 2.640 ton/ha, 3.389 tons/ha at an N rate of 225 kg, and 4.399 ton/ha at 300 kg N (Table 9). The application of N to maize caused a significant grain yield increase, however, only the difference between the treatments fertilized and the check was detected. The yields obtained with the N rates were 3.476 tons/ha at 0 kg, 4.641 at 75 kg, 4.967 at 150 kg, and 5.254 at 225 kg. The interaction between the maize response to N fertilization in function of the N applied to wheat as a previous crop indicated that when wheat is fertilized with 150 N units the average response of maize to fertilizer application is 15.98 kg of grain/kg N; the response to N is reduced to 10.58 kg of grain when wheat is fertilized with 225 kg/ha N, and when 300 kg/ha N are applied to wheat, the average response of maize to N application es 6.80 kg of grain/kg N applied.

Table 9. Nitrogen fertilization in wheat and its effect on nitrogen response in summer maize as a follow up crop in the Yaqui Valley, Sonora, México, during the fall–winter 1996–97 and summer 1997 agricultural seasons. Maize grain yield is the average of results obtained from applications of urea and ammonium sulphate, since there were no differences in maize response between these fertilizers.

Nitrogen rate in wheat (kg/ha)	Nitrogen rate in maize (kg/ha)	Maize grain yield (ton/ha)
150	0	2.640
150	75	4.642
150	150	4.310
150	225	4.922
Mean		4.128
225	0	3.389
225	75	4.158
225	150	5.242
225	225	5.450
Mean		4.560
300	0	4.399
300	75	5.124
300	150	5.349
300	225	5.389
Mean		5.066

Experiment 2. An increment of 2.437 ton/ha was observed in maize yield when N rates of 0 and 300 units applied to wheat were compared, which is equivalent to a residual effect of 8.12 kg of maize/each kg N applied to wheat. When maize was not fertilized, the yields associated with the N rates of 0, 150, and 300 kg of N applied to wheat were 2.020, 2.594, and 4.457 ton/ha, respectively. Significant differences between N rates in maize were observed; the yields obtained with the N rates of 0, 75, 150, and 225 kg/ha were 3.024c, 4.207b, 5.083ab, and 5.384a ton/ha, respectively (Tukey 0.01, 1.116 ton/ha). When wheat was not fertilized, the average response of maize to fertilizer application was 19.10 kg of grain/kg N. The response to N was reduced to 13.29 kg of grain when wheat was fertilized with 150 kg/ha, whereas for at 300 kg/ha N in wheat, the average response of maize to N application was 7.61 kg of grain/kg N applied (Table 10, p. 113). In terms

of the cost/benefit of wheat and summer maize, it is economically feasible to increase the N rates in wheat, which is not possible in maize, and reduce significantly the N rate in maize. This is based on the low response found to N fertilization at high levels of fertilization in wheat. When wheat was not fertilized, the economic threshold for N use by maize was a rate of 225 kg/ha, 150 kg/ha when wheat was fertilized with 150 kg of N, and the highest economic threshold was obtained with 75 kg/ha when wheat was fertilized with 300 kg of N.

Table 10. Nitrogen fertilization in wheat and its effect on nitrogen response in summer maize as a follow up crop in the Yaqui Valley, Sonora, México, during the fall–winter 1996–97 and summer 1997 agricultural seasons. Urea was used as the nitrogen source.

Nitrogen rate in wheat (kg/ha)	Nitrogen rate in maize (kg/ha)	Maize grain yield (ton/ha)
0	0	2.020
0	75	3.831
0	150	4.679
0	225	5.491
Mean		4.005
150	0	2.594
150	75	3.475
150	150	5.173
150	225	5.051
Mean		4.073
300	0	4.457
300	75	5.316
300	150	5.396
300	225	5.609
Mean		5.194

Conclusions.

1. There were no differences between fertilization with urea and ammonium sulphate under conditions of the Yaqui Valley, Sonora.
2. A significant residual effect of nitrogen applied to wheat was detected on maize yield and on the nitrogen rate needed for this crop.
3. Fertilization with 300 units of nitrogen in wheat, provides a physiological wheat yield with a protein content similar or superior to 12.5% and, also, maize as a follow up crop in the rotation can be cultivated with only 75 nitrogen units

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Nitrogen fertilization and its effect on quality and yield of durum wheat cultivar Nacori C97.

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Summary. We evaluated the interaction between nitrogen (N) fertilization, protein content, and yield of the durum wheat cultivar Nacori C97 at the Norman E. Borlaug Experimental Station during the 1998–99 agricultural season. The variables evaluated were percent grain protein at 12% humidity and grain yield. The sources of N, urea and ammonium sulphate, were used at the rates of 150, 225, and 300 kg/ha. A randomized block design with split plots and three replications was used; the large plots corresponded to the N source and the small plots to the rate. No significant differences were found between N sources for the two variables evaluated. The application of urea and ammonium sulphate produced yields and protein contents of 4.368 and 4.395 ton/ha and 12.57 and 13.28%, respectively. Rates of N increased grain yield with a significance of 0.055; yields with 150, 225, and 300 kg/ha were 4.074, 4.490, and 4.581 ton/ha, respectively; however, there were no statistical differences. The rate of N 225 kg/ha optimizes wheat yield and quality considering 12.5% of protein, however, in order to obtain greater grain yield, it should be considered to increase the rate to 300 units of this element.

Introduction. Protein concentration is one of the main parameters for wheat grain quality, and the availability of nitrogen (N) in the soil is the simple factor that increases its value (Ottman and Doerge 1994). N is an essential component of aminoacids, which constitute the primary structure of the protein molecule (Kent 1983). The effect of N availability

on wheat yield and quality has been a topic of numerous investigations. An increment in the accumulation of N may be obtained by the application of greater rates or for the same rate of N, which can be achieved by increasing the efficiency recovery of the fertilizer applied (Sowers et al. 1994; Ortiz et al. 1997). Cortés et al. (1994a) and Ortiz et al. (1997) have indicated that rates, source, timing, and application method have an important role on the efficient management of these inputs. In general, the highest rates are less efficient than the lowest ones (Uvalle et al. 1997; Sayre and Moreno 1997; Ortiz et al. 1997). Application in band is better than broadcast (Cortés et al. 1994a; Keeney 1982), fractionated fertilization is better than total application (Ruiz 1985; Mahler et al. 1994; Cortés et al. 1994a, 1994b), and no significant differences have been found between N sources if managed properly (Cortés et al. 1994a; Ortiz et al. 1997). For optimum use of N-based fertilizers, the fractionated fertilization should consider the demand curve of the crop, the N available in the soil, and the quality requirements for the product. For quality purposes, Kent (1983) indicates that the N aborbed after heading promotes an increment on the protein level of the grain, whereas the N taken up during the early growth phases has a primary impact on production. Therefore, our objective was to evaluate the interaction between N fertilization, protein content, and yield of durum wheat cultivar Nacori C97.

Materials and methods. The study was conducted at the Norman E. Borlaug Experimental Station, which belongs to the Mexican National Institute for Forestry, Agriculture and Livestock Research (INIFAP), during the 1998–99 agricultural season. The station is located at 27°22' N latitude and 109°55' W longitude. A factorial experiment was established to evaluated two sources of N (urea and ammonium sulphate), and three N rates (150, 225, and 300 kg/ha) in wheat. A randomized block design with split plots and three replications was used; the large plots corresponded to the N source and the small ones to the rate of N. The experimental plot consisted of six 8-m rows, and the experimental unit consisted of the two central 4-m rows. Soil preparation consisted of minimum tillage reusing the bed from the previous crop. Sowing was on 16 December 16 in beds 80 cm apart with two rows with durum wheat cultivar Nacori C97 at a density of 50 kg/ha. Three complementary irrigations were provided to the crop during the beginning of jointing, heading, and beginning of grain formation. Weeds were controlled mechanically and manually. N rates were applied in band between the bed rows before the first complementary irrigation. Based on soil analysis and its availability, phosphorus was not applied. The variables evaluated were percent grain protein at 12% humidity and grain yield. Data were analyzed with MSTAT (Russell D. Freed, MSTAT Director Crop and Soil Sciences Deparment Michigan State University). Tukey`s test (0.05) was used for mean comparison.

Results. No significant differences for N source or for the interaction 'N sources x rate' were observed (Table 11). The mean grain yield was practically the same whether urea or ammonium sulphate were applied, the difference between these sources was only 27 kg/ha. The differences in yield between the sources and rates were 5 kg at 150 units, 19 kg at 225 units, and 105 kg at 300 units. There were no statistical differences between N rates, although the maximum difference in grain yield was 507 kg between the 300 and 150 N unit rates, followed by a difference of 416 kg between the 225 and 150 N unit rates, and 91 kg between the 225 and 300 N unit rates (Table 12). Protein content was slightly higher when Nacori C97 was fertilized with ammonium sulphate, however, the difference was not statistically significant (Table 13). On average, each N rate applied promoted a statistically

Table 11. Grain yield of the durum wheat cultivar Nacori C97 obtained with two nitrogen sources and the interaction of 'source x nitrogen rate', during the 1998–99 agricultural season, in the Yaqui Valley, Sonora, México.

Nitrogen source	Nitrogen rate (kg/ha)	Grain yield (ton/ha)
Urea	150	4.076
	225	4.500
	300	4.528
Mean		4.368
Ammonium sulphate	150	4.071
	225	4.481
	300	4.633
Mean		4.395

Table 12. Grain yield of the durum wheat cultivar Nacori C97 obtained with three nitrogen rates during the 1998–99 agricultural season in the Yaqui Valley, Sonora, México.

Nitrogen rate (kg/ha)	Grain yield (ton/ha)
150	4.074
225	4.490
300	4.581

Table 13. Percent grain protein of the durum wheat cultivar Nacori C97 obtained with three nitrogen rates during the 1998–99 agricultural season in the Yaqui Valley, Sonora, México.

Nitrogen source	Protein content (%)
Urea	12.57
Ammonium sulphate	13.28

Table 14. Percent grain protein of the durum wheat cultivar Nacori C97 obtained with three nitrogen rates during the 1998–99 agricultural season in the Yaqui Valley, Sonora, México (Tukey 0.05 = 0.652).

Nitrogen rate (kg/ha)	Protein content (%)
150	11.90 a
225	13.06 b
300	13.80 c

significant increase in grain protein content. A N rate of 225 units was sufficient to obtain the quality standard of 12.5% of protein (Table 14, p. 114). As an average, 75 N units applied before the first complementary irrigation caused an increment of 0.95% in the grain protein (Table 15).

Conclusions.

1. No differences were found in grain yield of durum wheat cultivar Nacori C97 between the application of urea and ammonium sulphate.
2. A rate of 225 nitrogen units optimizes yield and quality of durum wheat cultivar Nacori C97.
3. The application of 300 nitrogen units increased quality, but not the grain yield of durum wheat cultivar Nacori C97.

Table 15. Percent wheat grain protein obtained from the interaction 'source x rate of nitrogen' in the durum wheat cultivar Nacori C97, during the 1998–99 agricultural season in the Yaqui Valley, Sonora, México.

Nitrogen source	Nitrogen rate (kg/ha)	Protein (%)
Urea	150	11.19
	225	12.79
	300	13.72
Ammonium sulphate	150	12.61
	225	13.33
	300	13.88

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Effect of foliar nitrogen on yield and protein content of the durum wheat cultivars Nacori C97 in 1998–99 and Rafi C97 in 1999–2000, in the Yaqui Valley, Sonora, México.

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Summary. These studies were conducted at the Norman E. Borlaug Experimental Station in Sonora, México, during the 1998–99 and 1999–2000 agricultural seasons. In 1998–99, urea, a 32% urea solution, 45% ammonium nitrate, and 7.5% Flussigg nitrogen (10% nitrogen solution) were applied to the foliage of thedurum wheat cultivar Nacori C97 during jointing initiation, boot, and grain filling, and were compared with an untreated check. The soil was not fertilized. In 1999–2000, foliar applications of urea (16%), urea Lobi, Nitrocel, and Blitzer at the rate of 2.0 kg/ha were applied dur-

ing the milk-dough stage and compared with an untreated check. Soil fertilization rate was 200–52–00 of NPK. Average grain yield increase in 1998–99 was 1.305 ton/ha with foliar nitrogen (N) applications. Average yield of treatments with foliar N was 2.491 ton/ha and 1.428 ton/ha for the check. The highest yield was obtained with Flussigg, because it did not cause foliar damage as the other sources of N. The protein value increased 0.77% with one or two applications and 1.51% with the third. The highest protein value was 12.7% with three applications of ammonium nitrate. The highest grain yield (7.213 ton/ha) in 1999–2000 was obtained with urea (16%), statistically superior to the check with a difference of 1.095 ton/ha. There were no significant statistical differences between treatments for protein content. Grain yield was 7.213 ton/ha for 16% urea (11.22% protein), 6.199 ton/ha for Lobi (11.27% protein), 6.834 ton/ha for Nitrocel (11.35% protein), 6.510 ton/ha for Blitzer (11.37% protein), and 6.118 ton/ha for the check (11.32% protein).

Introduction. The average annual area established with wheat in the state of Sonora during the period 2000–09 was 252,586 ha, with a maximum of 320,476 ha and a minimum of 104,268 ha. During the same period, the average grain yield was 5.80 ton/ha for a production of 1,464,408 ton with a value of approximately \$253 x 10^6 USD. For the 2010–11 agricultural season, the area established with wheat in Sonora was 287,574 ha, 172,422 ha corresponded to the District of Rural Development (DRD) 148-Cajeme (Yaqui Valley) and 80,134 ha for the DRD-149-Navojoa, for a total of 252,556 ha in southern Sonora. The average price/ton fluctuated from \$100 USD in 2002 to \$340 USD in 2008 (OEIDRUS 2010). In Sonora, durum wheat production is oriented predominantely for the international market, whereas production of bread wheat is for the national market. Farmers must select cultivars that comply with the market requirements but, at the same time, with regional strategies for control of leaf rust and Karnal bunt. In the case of durum wheat, it is important that wheat cultivars also comply with quality standards established by the buyers, such as 90% of vitreous grains and 12% grain protein content (Cortés et al. 2011). Protein concentration is one of the main quality parameters for the wheat grain, and the nitrogen (N) available in the soil is the simple factor that has more influence on increasing its content (Ottman and Doerge 1994). Kent (1983) reported that the N absorbed after heading promotes an increment on the protein level of the grain, but the N absorbed in the early growth stages has an impact mainly on production. Plants take up nutrients primarily by the roots, however, leaves also have the capacity for absorbing water and nutrients. In theory, a plant can be nourished completely through leaves, but in practice, foliar fertilization is used as a complementary way to supply N, magnesium, and micro-elements. Foliar nutrition is important because frequently in soils with a sufficient nutrient content, nutrients are not available to the plant, such as iron and phosphorus under alcaline soil conditions. In this case, foliar application has a greater efficiency than applied to the soil. For an optimum application of this method of fertilization, it is important to consider the relative humidity, light, temperature, leaf age, contact zone and wet surface, and the chemical characteristics of the solution. Urea solutions may be applied to a maximum concentration of 15%, however, the critical growth periods in which foliar fertilization should not be applied must be taken into consideration. In the case of cereals, these periods occur between sowing and the appearance of the third leaf, as well as right after heading (Fink 1988). Gros and Domínguez (1992) reported that 100 kg/ha of urea can be applied in 300 L of water to wheat and other cereals, with minimum risks of leaf damage or consequences, which can be attributed to the waxy cuticle. The objective of this study was to determine the effect of foliar application of N on yield and protein content of the durum wheat cultivar Nacori C97.

Materials and methods. The study was conducted at the Norman E. Borlaug Experimental Station, which belongs to the Mexican National Institute for Forestry, Agriculture and Livestock Research (INIFAP), during the 1998–99 and 1999–2000 agricultural seasons. The station is located at 27°22'N latitude and 109°55'W longitude, in a compacted clay soil. In 1998–99, urea, a 32% urea solution, 45% ammonium nitrate, and 7.5% Flussigg nitrogen (10% nitrogen solution) were applied to the foliage of the durum wheat cultivar Nacori C97 during jointing initiation, boot, and grain filling, and were compared with an untreated check. The soil was not fertilized and the beds from the previous crop was reutilized. Sowing was on 16 December after summer maize in beds with two rows, at the rate of 50 kg/ha. Two complementary irrigations were applied; one application of an insecticide for pest control, and weeds were eliminated mechanically and manually. A randomized block design with split plots and two replications was used, the large plots corresponded to sources of foliar N and the small ones to the number of applications. The experimental unit consisted of six beds 6-m long and 80 cm apart, and the evaluation was carried out on two beds 4-m long. In 1999–2000, urea (16%), urea Lobi, Nitrocel, and Blitzer were applied at the rate of 2.0 kg/ha during heading, and were compared with an untreated check. Wheat was established under a conservation tillage system with drip irrigation; the previous summer crop was maize. Durum wheat cultivar Rafi C97 was sown at the rate of 100 kg/ha on 17 December in beds 80 cm apart with two rows. The irrigation lamina was 4.3 cm and fertilization 200–100–00 of NPK. Weeds were eliminated manually and there was no need for insect control. A randomized block design with four replications was used. The experimental unit consisted of two beds, 20-m long, 80 cm apart, and the evaluation was carried out on two beds, 4-m long. N solutions were applied in 381 L of water. Data were analyzed with MSTAT (Russell D. Freed, MSTAT Director Crop and Soil Sciences Deparment Michigan State University). Tukey's test (0.01, 0.05) was used for mean comparison.

Results and discussion. No significant differences between the number of applications for grain yield and protein content were observed in 1998–99. However, significant differences were found between the N sources evaluated (Table 16). Basically, the difference was observed between whether or not to apply the source of N, because 1, 2, or 3 applications were statistically similar, but different from the untreated check. The average grain yield increment when N was applied in relation to the check was 1,063 ton/ha. The average grain yield of the treatments was 2.491 ton/ha, whereas the check showed a yield of 1.428 ton/ha. The highest yield recorded was obtained with Flussig g; this possibly is due to the low level of leaf damage that was observed on plants treated with the other sources of N. The protein value increased 0.77% with one or two applications and 1.51% with the third. The highest value (12.7%) was obtained with three applications of ammonium nitrate (Table 17). In 1999–2000, significant differences were detected between treatments for grain yield. The highest yield (7.213 ton/ha) was obtained with urea (16%), statistically superior to the check with a difference of 1.095 ton/ha. Grain yields of 6.834, 6.510, and 6.199 ton/ha were obtained with Nitrocel, Blitzer, and urea Lobi, respectively, and were statistically similar to urea (16%). There were no significant statistical differences between treatments for protein content (Table 18). From the experimentation during both crop seasons, we determined that the response of the wheat to foliar N applications depends upon the base soil fertilization; under low availability of N in the soil, such as in 1998–99, all the N sources evaluated increased grain yield and protein content, which was not observed when 200 N units were applied to the soil.

Table 16. Grain yield of the durum wheat cultivar Nacori C97 after foliar application of four different sources of nitrogen, during the 1998–99 agricultural season, in the Yaqui Valley, Sonora, México (Tukey 0.01= 0.703).

Fertilizer	Grain yield (ton/ha)				
	Number of applications				
	0	1	2	3	Mean
Urea	1.211	2.368	2.413	2.476	2.117
Ammonium nitrate	1.755	2.432	2.332	2.128	2.162
Urea + ammonium nitrate	1.561	2.650	2.128	2.104	2.111
Flussig g	1.185	2.704	2.959	3.190	2.510
Mean	1.428 a	2.539 b	2.458 b	2.475 b	

Table 17. Grain protien content of the durum wheat cultivar Nacori C97 after foliar application of four different sources of nitrogen, during the 1998–99 agricultural season, in the Yaqui Valley, Sonora, México (Tukey 0.01= 0.703).

Fertilizer	Grain protein (%)				
	Number of applications				
	0	1	2	3	Mean
Urea	10.20	10.15	11.75	11.20	10.83
Ammonium nitrate	10.60	11.85	10.95	12.70	11.53
Urea + ammonium nitrate	10.45	10.55	10.15	12.25	10.85
Flussig g	9.50	11.30	11.00	10.65	10.61
Mean	10.19 a	10.96 a	10.96 a	11.70 b	

Table 18. Grain yield and protein content of the durum wheat cultivar Rafi C97 after foliar application of four different sources of nitrogen during the 1999–2000 agricultural season, in the Yaqui Valley, Sonora, México (Tukey 0.05 = 1.039).

Foliar nitrogen	Grain yield (ton/ha)	Protein (%)
Untreated check	6.118 b	11.32
Urea (16%)	7.213 a	11.22
Urea Lobi 2.0 kg/ha	6.199 ab	11.27
Nitrocel 2.0 kg/ha	6.834 ab	11.35
Blitzer 2.0 kg/ha	6.510 ab	11.37

Conclusions. Foliar application of nitrogen increases grain yield and the protein content when the soil is not fertilized; however, nitrogen application to the soil or a highly availability of this element reduces the possibility for a significant response to the foliar application of this element.

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Interaction between accumulation of cold hours and sowing date for wheat in the Yaqui Valley, Sonora, México.

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Summary. To study the interaction between accumulation of cold hours and sowing date for wheat in the Yaqui Valley, Sonora, México, data from the weather station network (14 stations) reported in www.pieaes.org.mx were used. Data covered the wheat crop seasons from 2005–06 to 2009–10, on different wheat sowing dates: 15 November and 1, 15, and 30 December. We considered that each season lasted 120 days. Cold hour (CH) production was 88.9% from December to March; 10% during November, April, and May; and 1% during June to October. The accumulation of CH according to sowing date was 1 December > 15 December > 15 November > 30 December. The greatest CH accumulation occurred during the second half of December. Based on the positive correlation (r = 0.50) between production of cold hours and wheat yield, 1 December 1 can be considered as the optimum sowing date in the Yaqui Valley, Sonora.

Introduction. Temperature is the main factor that controls the response of plants to the environment, especially those that require the accumulation of a total of cold hours (CH) in order to change from a vegetative stage to a reproductive stage (Flood and Halloran 1984). A CH is defined as the quantity of hours within a time range, when the temperature is lower than a specific amount of degrees (Gil 1997), or the period of vernalization that occurs between 0 and 12°C (FAO 2001; Miralles 2004). The requirement of CH by crops is not constant and varys according to the vegetative stage, the species, and cultivar. Crops must complete their reproductive development within the available growth stage in order to achieve the maximum yield, so that stresses might be avoided during vulnerable stages (Loomis and Connor 2002). In the case of wheat, temperature is the most important factor that induces plant growth from emergence to flowering and maturity (Rawson and Macpherson 2001; Miralles 2004). The ideal temperature for growth and development of this crop ranges from 10 to 24°C; wheat stops growth at 0°C (Rawson and Macpherson 2001). The Yaqui Valley comprises 252,000 ha for irrigated agriculture and, for the last 30 years, wheat has been the most cultivated crop covering 90% of the area and, therefore, the most investigated. New scientific and technological information explains the behaviour of this crop under different weather conditions and soils, which allows farmers the proper management of wheat in this region (INIFAP 2009). The objective of this work was to study the interaction between accumulation of CH and sowing date for wheat in the Yaqui Valley, Sonora, México.

Materials and methods. The data used for this study was obtained from the weather station network, which is published at www.pieaes.org.mx. Accumulated CH from the 14 weather stations in the Yaqui Valley during the wheat crop seasons 2005–06 to 2009–10 were recorded and analyzed, considering the sowing dates of 15 November and 1, 15, and 30 December. Although the optimum sowing date for this cereal has a range from 15 November to 15 December (INIFAP 2009), there are farmers that delay the sowing date for various reasons, such as water and seed availability, therefore, the date 30 December was taken into consideration for this study. Each agricultural season was estimated to consist of 120 days. The number of CH was calculated according to sowing date, month, and crop season. The wheat yield reported by OEIDRUS (2010) for the state of Sonora was used to make a correlation with the value of CH. A database was created in Excel with the geoposition in UTM units of weather stations, and the average value of CH during the seasons of evaluation. The database was exported to the program Idrisi Kilimanjaro (Eastman 2003), and a spatial distribution map of CH in the Yaqui Valley was created.

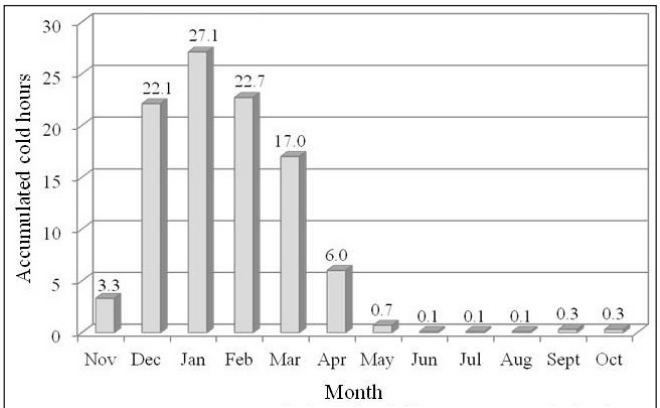

Fig. 12. Average accumulation of cold hours/month in the Yaqui Valley, Sonora, México, during the 2005–06 to 2009–10 crop seasons.

Results and discussion. The majority (89%) of accumulated CH during the year is produced during the months of December, January, February, and March. November,

April, and May added another 10%, and the rest (1%) is produced during the months of June to October (Fig. 12, p. 118). It is possible to find CH during the summer months in some years. Félix et al. (2009) reported CH in June and July in 2008. The weather stations of the Yaqui Valley show recordings of the climatic variables every 10 minutes. The generation of CH regularly take place between the first hours (0–8 h) and the last (22–24 h) during the day, which correlates with the temperature (Fig. 13). Hours with the highest temperature difference was 06:50 and 15:00 with 16.3°C on 25 January, 2010. Of the seasons evaluated, 2007–08 had the greatest accumulation of CH (868), followed by 2005–06 (763), 2006–07 (742), 2009–10 (689), and 2008–09 (572) (Fig. 14).

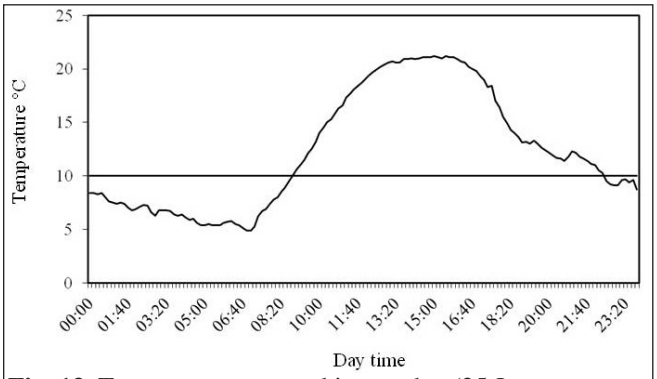

Fig. 13. Temperature reported in one day (25 January, 2010) with recording every 10 minutes in block 609, Yaqui Valley, Sonora, México.

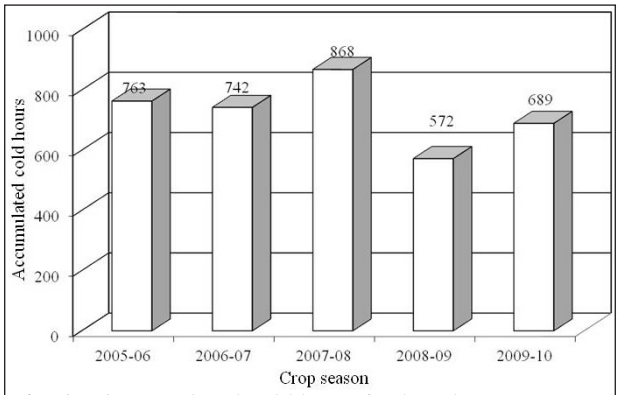

Fig. 14. Accumulated cold hours in the wheat crop seasons 2005–06 to 2009–10 in the Yaqui Valley, Sonora, México.

The accumulation of CH is related and has an effect on the wheat development depending on the sowing date. For the Yaqui Valley, INIFAP (2009) recommends an optimum sowing date 15 November to 15 December. This recommendation was based primarily on climatic conditions, water availability, and the probabilities of rust epidemics. The best sowing date is when a crop produces the highest yields within the local limitations. Once this date is determined, any delay will cause reductions in yield (Rawson and Macpherson 2001). The greatest accumulation of CH (658) in the Yaqui Valley was obtained with the sowing date of 1 December, followed by 15 December with 643 CH, 15 November with 600 CH, and 30 December with 573 CH (Fig. 15). The accumulation of CH every two weeks during 15 November to 15 April is shown (Table 19). For practical purposes, it should be considered that a proper tillering will take place with 150 CH (Félix et al. 2009). Average wheat yield in the Yaqui Valley in 2005–06 was 6.16 ton/ha, 6.25 in 2006-07, 6.07 in 2007-08, 5.68 in 2008-09, and 6.40 ton/ha in 2009-10 (OEIDRUS 2010). There was a positive correlation (r = 0.50) between CH and wheat yield (Fig. 16, p. 120). The spatial distribution of accumulated CH in the Yaqui Valley is shown (Fig. 17, p. 120). The areas with more accumulation of CH were the north-central, south, and southeastern parts of the valley. This figure can be a useful tool for farmers in order to plan crop management, and based on the location of their fields, select cultivars based on physiological maturity and the sowing date.

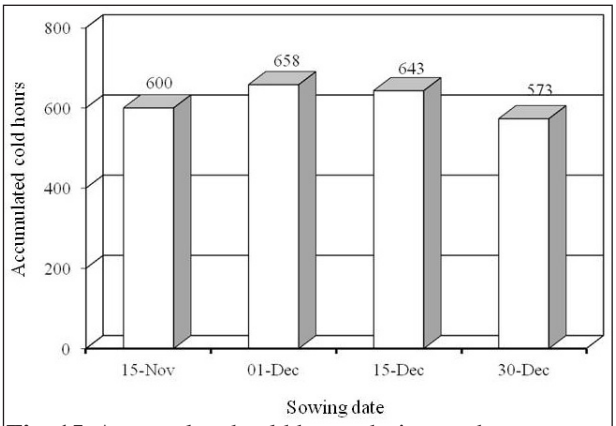

Fig. 15. Accumulated cold hours during a wheat season according to planting date and considering a season of 120 days (average of five crop seasons 2005–06 to 2009–10).

Table 19. Average accumulation of cold hours every two weeks during wheat crop seasons 2005–06 to 2009–10 in the Yaqui Valley, Sonora, México (SD = standard deviation).

Period	Accumulation of cold hours			
	Maximum	Minimum	Mean	SD
15–30 November	52	0	7	13.2
1–15 December	153	7	55	32.7
16–31 December	165	47	138	30.8
1–15 January	148	60	109	22.1
16–31 January	173	32	102	40.0
1–15 February	132	46	93	21.9
16–29 February	113	23	63	20.4
1–15 March	150	18	79	34.1
16–31 March	109	18	60	19.2
1–15 April	76	5	27	15.3

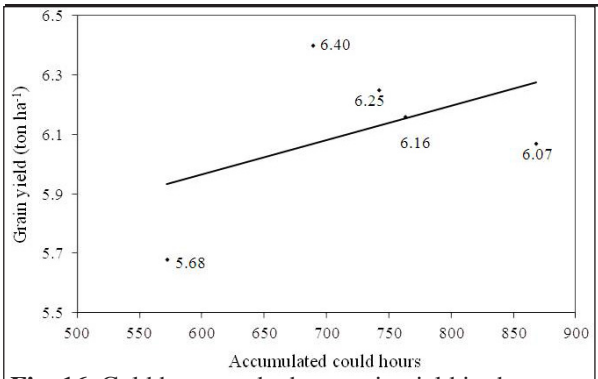

Fig. 16. Cold hours and wheat grain yield in the Yaqui Valley, Sonora, México, during the 2005–06 to 2009–10 crop seasons.

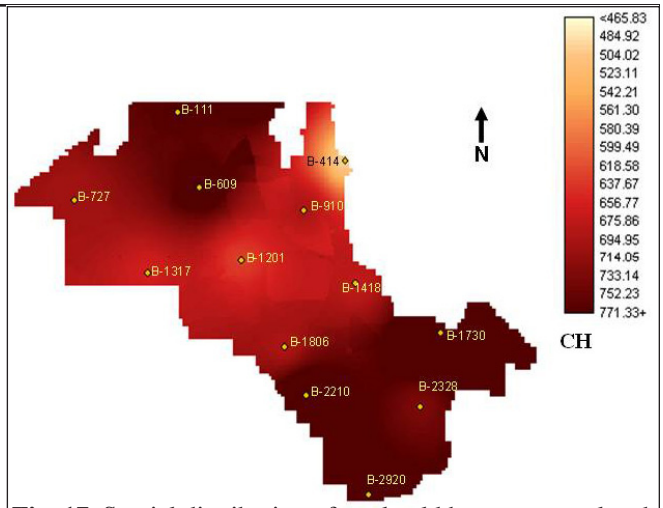

Fig. 17. Spatial distribution of total cold hours accumulated in the Yaqui Valley, Sonora, México, during the period from 15 November to 15 May (B = block).

Conclusions.

1. Accumulation of cold hours according to sowing date was 1 December > 15 December > 15 November > 30 December.
2. Based on the positive correlation (r = 0.50) between production of cold hours and wheat yield, 1 December can be considered as the optimum wheat sowing date in the Yaqui Valley.

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Effect of biofertilization on yield and quality of wheat in the Yaqui Valley, Sonora, México.

Alma Angélica Ortiz-Ávalos, Juan Manuel Cortés-Jiménez, Teresa de Jesús Ruiz-Vega, and Guillermo Fuentes-Dávila.

Summary. We evaluated the effect of biofertilizers on grain yield and quality of wheat. The study was carried out during the fall–winter 2010–11 wheat season in the Yaqui Valley, Sonora, México. Trials were established in a farmer's field where inoculations of wheat seed with *Azospirillum brasilense* (A), *Glomus intraradices* (G), and the combination

(A+G) were evaluated under the fermer's management scheme by the farmer and an untreated check. Wheat grain yield ranged from 6.46 to 10.81 ton/ha and a grain protein content from 9.78 to 12.92%. There was no response to the application of the fertilizers in relation to grain yield and quality, therefore, it is necessary to continue this type of study with native strains and analyze the soil content where trials will be established in order to assure proper conditions for the experiment.

Introduction. Wheat is the most important crop during the fall–winter in the Yaqui Valley, Sonora, and for the last 30 years it has covered more than 90% of the agricultural area. New scientific and technological information explains the behavior of this crop under different weather conditions and soils, which allows farmers proper management in this region (INIFAP 2009). However, the importance of this crop in the sustainability of agricultural systems has not been well studied, so there is interest in testing new inputs (biofertilizers) available in the market that offer similar or better grain yields at low cost and maintain a balance with nature. The first studies with biofertilizers were done in the central plateau of Mexico, which covers parts of the states of Guanajuato, Queretaro, Mexico, the Federal District, Tlaxcala, Puebla, and Morelos, with crops such as maize, wheat, barley, oats, sorghum, beans, and orange. Results indicated that biofertilizers provide 29% of the nitrogen that cereals require and almost 70% of the N requirement by grain legumes and allow the reduction of mineral fertilizers from 20 to 40%; they are cheap and easy to handle and apply (INIFAP-SAGARPA 2011). However, in the state of Morelos, maize grain yield was reduced when the microorganisms were used to inoculate the seed. In the state of Puebla, wheat yield increased 5% when seed was inoculated with *Glomus* (Gl) in relation to the fertilized check with 80-40-30 of NPK; however, yield was reduced when inoculation was carried out with *Azospirillum* (Az), and with a mixture of Gl+Az. In Amoloya, Hidalgo, in barley treatments inoculated with biofertilizers produced less yield than the fertilized check (46-23 of NP) (Irízar et al., 2003). Okon and Labandera (1994) reported that benefits from inoculation with *Azospirillum* depend on multiple factors, many of which can not be controlled under field conditions, such as temperature, rainfall, O_2 difusion, soil pH, poor soils, and the presence of competitive microorganisms, among others. Caballero et al. (1992) suggest that many more strains must be studied for each cereal, and Irízar el al. (2003) recommend to use strains from the region since a strain may not be effective in all locations and crops. We evaluated the effect of biofertilizers on grain yield and quality of wheat.

Materials and methods. The evaluation of biofertilizers was conducted during the fall–winter 2010–11 crop season in the Yaqui Valley, Sonora, México. Trials were stablished in nine modules with coöperating farmers, where the effects of the wheat seed inoculation with *Azospirillum brasilense* (A) and *Glomus intraradices* (G) from two different companies were evaluated, as well as the combination A+Z, under the management scheme of farmers (the average nitrogen units were 280, and 52 units of P2O5/ha), leaving an untreated check. Inoculation was carried out using 1 kg of biofertilizer for each 30 kg of seed. The durum wheat cultivar Patronato Oro C2008 was cultivated in module 1 and CIRNO C2008 in modules 2–7 and 9, and the bread wheat cultivar Kronstad F2004 in module 8. At harvest, grain yield and grain protein content at 12% humidity were recorded.

Results and discussion. Inoculation with *Azospirillum* and the combination *Azospirillum* + *Glomus* produced 8.76 ton/ha, 90 kg/ha less than the check, whereas *Glomus* 1 and *Glomus* 2 were 100 and 240 kg/ha, respectively, behind the check (Fig. 18). The yield range of the inoculation with *Azospirillum* was 6.46–10.81 ton/ha, 6.68–10.41 for *Glomus* 1, 6.84–10.28 for *Glomus* 2, 6.89–10.71 for the combination A+G, and 7.21–10.56 for the check (Table 19, p. 122). The yield range of bread wheat cultivar Kronstad F2004 in module 8 was 6.46–7.47 ton/ha with the lowest average of all modules with 6.89; module 3 showed the range 9.67–10.81 with an average of 10.33 and module 5 a range of 9.98-10.71 with an average of 10.30 ton/ha. Fallik et al. (1985) cited by Mendoza et al. (2004) indicate that the ability of *Azospirillum* to compete is significantly reduced in soils poor in organic matter, which is the case of the Yaqui Valley (Cortés et al. 2009), that could be the reason for the limited response of wheat to the inoculation with *Azospirillum*. Mendoza et al. (2004) also cites Caballero (personal communication) who reported an adequate symbiotic establishment when inoculations are carried out with native strains of

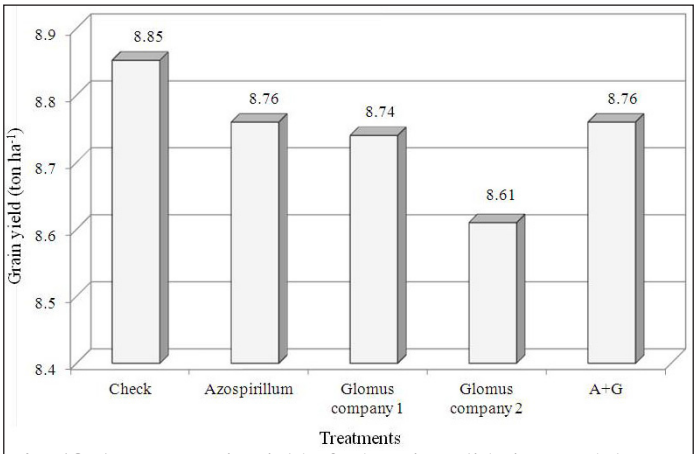

Fig. 18. Average grain yield of wheat in validation modules evaluated in the Yaqui Valley, Sonora, México, during the fall–winter 2010–11 crop season.

Azospirillum isolated from the same maize genotypes. Caballero et al. (1992) recommend as necessary to study the strains for cereals that can be used. Regarding grain protein, the highest percentage was obtained with *Glomus* (10.93), followed by the combination A+G, the check, and *Azospirillum* (Fig. 19). Protein content in module 2 was not possible to determine. The range of protein percent from inoculation with *Azospirillum* was 9.94–12.92, 9.78–12.87 for *Glomus* 1, 9.94–12.88 for *Glomus* 2, 9.78–12.87 for the combination A+G, and 9.98–12.81 for the check (Table 20). The range of protein percentage of bread wheat cultivar Kronstad F2004 in module 8 was 12.81–12.92 with the highest average (12.87). In general, there was no response of wheat in grain yield and percent of protein to the inoculation with biofertilizers. Mendoza et al. (2004) indicate that at least two factors are involved for the proper functioning of these fertilizers, the initial number of bacteria in the soil and the adaptation of bacteria to environmental conditions that prevail in arid regions. The introduction of a competitive strain of *Azospirillum* in completely different environment does not assure that such a strain will have the same behaviour as in its original niche.

Table 19. Effect of the inoculation with *Azospirillum brasilense*, *Glomus intraradices*, and their combination *Azospirillum+Glomus*, on percent grain yield of wheat in validation modules in the Yaqui Valley, Sonora, México, during the fall–winter 2010–11 crop season.

Module	Grain yield (ton/ha)				
	Check	*Azospirillum brasilense*	*G. intraradices* Company 1	*G. intraradices* Company 2	*Azospirillum + Glomus*
1	7.64	7.45	7.61	7.70	7.68
2	7.21	7.31	7.20	6.84	7.26
3	10.56	10.81	10.34	10.28	9.67
4	8.65	9.44	8.46	8.23	8.41
5	10.26	10.14	10.41	9.98	10.71
6	9.88	9.62	9.74	10.02	9.58
7	8.69	8.34	8.78	8.23	9.20
8 (module with bread wheat)	7.47	6.46	6.68	6.93	6.89
9	9.31	9.31	9.43	9.24	9.43

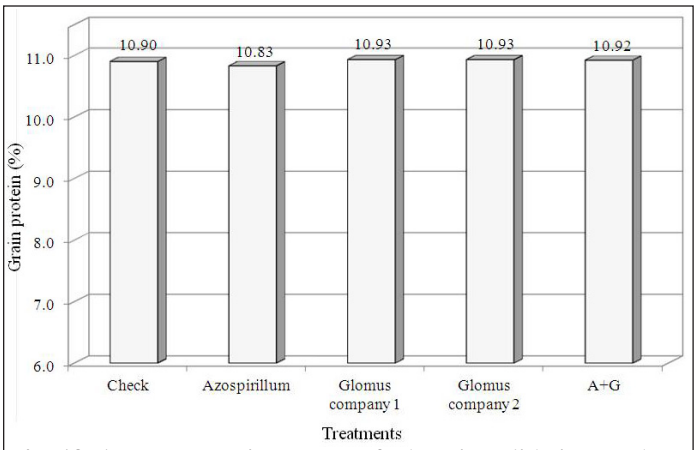

Fig. 19. Average protein percent of wheat in validation modules evaluated in the Yaqui Valley, Sonora, México, during the fall–winter 2010–11 crop season.

Table 20. Effect of the inoculation with *Azospirillum brasilense*, *Glomus intraradices*, and their combination *Azospirillum+Glomus*, on percent grain protein of wheat in validation modules in the Yaqui Valley, Sonora, México, during the fall–winter 2010–11 crop season.

Module	Grain yield (ton/ha)				
	Check	*Azospirillum brasilense*	*G. intraradices* Company 1	*G. intraradices* Company 2	*Azospirillum + Glomus*
1	9.98	10.07	9.94	9.94	9.99
2	10.53	10.35	10.71	10.51	10.77
3	10.13	10.22	9.78	10.01	9.82
4	10.03	10.22	10.56	10.41	10.66
5	10.10	9.94	9.80	9.87	9.78
6	11.98	11.96	12.08	12.08	11.88
7	12.81	12.92	12.87	12.88	12.87
8 (module with bread wheat)	11.64	10.97	11.67	11.68	11.63
9	9.31	9.31	9.43	9.24	9.43

Conclusions. There was no response to the application of biofertilizers (*Azospirillum brasilense* and *Glomus intraradices*) for wheat grain yield and quality. More experimental research should be carried out with the use of fertilizers in this crop, in relation to native strains, soil content, and the interaction chemical fertilization x organic fertilization.

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Two promising, elite, durum wheat lines for the Yaqui Valley, Sonora, México.

Pedro Figueroa-López, Gabriela Chávez-Villalba, Guillermo Fuentes-Dávila, José Luis Félix-Fuentes, Miguel Alfonso Camacho-Casas, Alberto Borbón-Gracia, and César Martín Armenta-Castro.

Introduction. Durum wheat cultivation in southern Sonora has been an excellent and convenient solution for the wheat producer in Sonora after the complex problems derived from epidemic levels of Karnal bunt present during the 1980s and the low competiveness of the bread wheat produced in the region, as shown by the manifest after the signing of the North American Free Trade Agreement (NAFTA) in the middle of the 1990s. On one hand, agronomic management of durum wheat is practically the same as that for bread wheat, so that the experience acquired by generations has not been wasted. On the other hand, durum wheat has shown greater yield potential than bread wheat and more tolerance to Karnal bunt, a disease that occurs endemically in the area. However, constraints for the crop have not disappeared completely, because leaf rust, a disease unknown in durum wheat in the region before the crop season 2000–01, currently represents a greater risk of yield loss. Grain quality, related to its pigmentation, also has become a trait to improve after the release of the cultivar Júpare C2001, which was resistant to the leaf rust but low in this characteristic.

Recently, several new durum wheat cultivars with different sources of resistance to leaf rust have been released in order to create a genetic mosaic that will diminish the risk of loss by epidemics. These cultivars also have been selected for their quality and grain yield, however, the Wheat Program of INIFAP in northwest Mexico faces a dissociation between quality and yield criteria, because the cultivar CIRNO C2008 was sown on more than 70% of the area cultivated with wheat in southern Sonora (152,838 out of 217,578 ha) due to its greatest yield potential, although it is one of the current cultivars with the lowest levels of grain pigmentation.

Other high-quality cultivars released during 2008, such as CEVY Oro C2008, Patronato Oro, and Sáwali Oro C2008, and Huatabampo Oro C2009 in 2009, have not attracted the attention of farmers, because they have a lower grain yield potential than CIRNO C2008. The collaborative project between INIFAP and CIMMYT on wheat breeding, aims at the release of new cultivars with better attributes, which will improve the phytosanitary status of the region and make a more profitable crop for the farmers domestically and internationally.

The identification of outstanding durum wheat experimental lines is one important component of the collaborative breeding project, where every year a set of 25 elite lines/cultivars are assembled and sown on several dates and managed under two irrigation regimes, using a randomized block design with three replications. Data recorded are grain yield, specific weight, 1,000-kernel weight, grain pigmentation, percentage of protein, days-to-flowering, days-to-maturity, height, percent lodging, percent black point, yellow berry, and Karnal bunt.

Progress report. The main characteristics of two outstanding durum wheat lines during the 2009–10 and 2010–11 agricultural seasons compared to the high-yielding cultivar CIRNO C2008 and the high-quality cultivar Sáwali Oro C2008 are given (Table 21). Line No. 1 (Fig. 20) GODRIN/GUTROS//DUKEM/3/THKNEE_11/4/DUKEM_1//PATKA_7/YAZI_1 /3/PATKA_7/YAZI_1/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/ADAMAR showed an average grain yield of 6,945 ton/ha. Line No. 2 (Fig. 21) ARMENT//2*SOOTY _9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN had 6,734 ton/ha, CIRNO C2008 was 6,648 ton/ha, and Sáwali Oro C2008 was 6,347 ton/ha. Both lines showed high levels of pigmentation, greater than that of CIRNO C2008, and line No. 2 was even greater than that of Sáwali Oro C2008. Both lines showed a lot of similarity to the

Fig. 20. Elite durum wheat line GODRIN/GUTROS//DUKEM/3/THKNEE_11/4/DUKEM_1//PATKA_7/YAZI_1 /3/PATKA_7/YAZI_1/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/ADAMAR.

Fig. 21. Elite durum wheat line ARMENT//2*SOOTY _9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN.

Table 21. Average grain yield, agronomic and quality characteristics, and reaction to diseases of two elite durum wheat lines, compared to the high-yielding cultivar CIRNO C2008 and the high-quality cultivar Sáwali Oro C2008, in four sowing dates during the 2009–10 and 2010–11 agricultural seasons, in the Yaqui Valley, Sonora, México.

Cultivar/line	Grain yield (ton/ha)	Specific weight	1,000-kernel weight (g)	Pigmentation	Protein (%)	Days-to-flowering	Days-to-maturity	Height (cm)	Lodging	Black point (%)	Karnal bunt (%)	Yellow berry (%)
CIRNO C2008	6,648	82	60.6	22.1	13.5	82	125	81	0	4.6	2.3	0.2
Sáwali Oro C2008	6,347	82	46.1	28.6	13.2	83	125	88	1	2.4	1.1	0.6
GODRIN/GUTROS//DUKEM/3/THKNEE_11/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/ADAMAR	6,945	82	51.8	28.2	12.7	82	124	83	0	1.8	4.5	0.6
ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN	6,734	83	50.8	30.1	12.6	81	123	93	0	2.9	0.6	0.6
Mean	6,668	82	52.3	27.3	13.0	82	124	86	0	2.9	2.1	0.5

cultivar checks in days-to-flowering and maturity, as well as in specific weight. Thousand-kernel weight was similar for both lines (51.8 and 50.8 g), greater than that of Sáwali Oro C2008 (46.1) but lower than that of CIRNO C2008 (60.6). Although acceptable, the protein percent was lower in both lines than in the cultivar checks. Grain protein percent, as well as yellow berry, can be managed by an adequate rate of nitrogen as well as a proper timing. Line No. 2 is considerably taller than line No. 1 and the cultivars, however, it did not show any lodging. The percent black point was lower than that of the cultivar checks. Line No. 1 showed the highest level of Karnal bunt infection, however, it is still considered a resistant reaction (Fuentes-Dávila and Rajaram 1994). Both lines carry the gene *Lr14a* as does Sáwali Oro C2008, which has remained resistant to leaf rust for several years. Their release would be justified and the yield gap between high-yielding and high-quality cultivars would be reduced considerably. A more balanced set of commercial wheat cultivars with different genetic basis for resistance to leaf rust in the Yaqui Valley, Sonora, México, will be available as the preference for CIRNO C2008 is expected to decrease.

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Tepahui F2009, new bread wheat cultivar with high grain protein content and resistant to leaf rust for northwest Mexico.

Gabriela Chávez-Villalba, Miguel Alfonso Camacho-Casas, Pedro Figueroa-López, Guillermo Fuentes-Dávila, Víctor Valenzuela-Herrera, José Luis Félix-Fuentes, and José Alberto Mendoza-Lugo.

Summary. As a strategy to broaden the spectrum of resistant cultivars to leaf rust and increase its durability, the Mexican National Institute of Forestry, Agriculture, and Livestock Research released a new bread wheat (*Triticum aestivum* L.) cultivar with the name **Tepahui F2009**. Evaluations of experimental elite wheat lines were carried out under irrigation during the 2007–08 to 2009–10 fall–winter wheat seasons. Tepahui F2009 is resistant to the common leaf rust races in Mexico and it showed similar grain yield to the check Tacupeto F2001 with an average of 6.4 ton/ha. Average test weight of this cultivar was 81.9 kg/hL and grain protein content 13.12%. Tepahui F2009 presented a gluten strength of 362 W x 10-4 J. The balance of tenacity and elasticity (P/G) was 4.1. Tepahui F2009 is considered an excellent option for farmers in northwest Mexico, which will partly meet the domestic market demand.

Introduction. Wheat cultivars in Mexico are classified into five groups based on its species, hardness, gluten type, and potential use by the food industry (Peña et al. 2007). Bread-making wheat from group 1 is the most demanded by the national industry (CANIMOLT 2008); however, in the state of Sonora, which is the main wheat-producer in the nation with 47%, the preference for durum wheat (*Triticum durum*) from group 5 has prevailed since the 1994–95 crop season. During the 2010–11 crop season, the area grown with bread wheat in southern Sonora was 87,648 ha, which represents 30% of the area grown with wheat (OEIDRUS 2011). The production of this type of wheat in this region is more competitive with imported bread wheat, which is reflected in less imports by the national milling industry. However, the most grown bread wheat cultivar by farmers and the most demanded by the industry Tacupeto F2001 has lost its resistance to races of leaf rust present in this region, and the low yield potential of high protein cultivar Kronstad F2004, have led to a new generation of bread wheat cultivars. The collaborative project between INIFAP and CIMMYT on wheat breeding, aims at the release of new cultivars with better attributes that will improve the phytosanitary status of the region and make a more profitable crop for the farmers domestically and internationally. The identification of outstanding bread and durum wheat experimental lines is one important component of this collaborative breeding project. This article deals with a bread wheat line released as commercial cultivar for northwest Mexico.

Materials and methods. The evaluations of elite experimental wheat lines were carried out at the Norman E. Borlaug Experimental Station which belongs to the Mexican National Institute for Forestry, Agriculture, and Livestock Research located in block 910 in the Yaqui Valley, Sonora (27º22´3.01" N and 109º55´40.22" W, at 38 masl), during the 2007–08 to 2009–10 wheat seasons. Sowing dates were 15 November, 1 and 15 December, and 1 January. In 2007–08, lines/cultivars were subjected to two and three complementary irrigations, and in 2008–09 and 2009–10 to three and four. A randomized block design with three replications was used for these evaluations. Plots consisted of a bed 5-m long with two rows and a density of 100 kg/ha. Strong gluten-type regional check bread wheat cultivars included Tacupeto F2001 and Kronstad F2004. Agronomic management followed INIFAP's technical recommendations (Figueroa-López et al. 2011). Data recorded were: grain yield, test weight, 1,000-kernel weight, grain protein (determined with a NIR-Perten 9100 analyzer), flour extraction (with an experimental grinder Brabender), gluten strength (with an alveograph Chopin), optimum

mixing time (with the Swanson mixer), and experimental bread making trials.

Results and discussion. Bread wheat experimental line 'Bettu/3/CHEN/*Ae. tauschii*//2*Opata' (later released as commercial cultivar Tepahui F2009) was identified as resistant to the new races of leaf rust (*Puccinia triticina*) present in southern Sonora during the 2007–08 wheat season and has maintained the resistance reaction during the last four seasons. This is one of the most important traits for the release of experimental lines as commercial cultivars in this region. The cross and selection history of this line is CMSW00WM00150S-040M-040Y-030M-030ZTM-3ZTY-0M-0SY-0CEVY-OCEVY. Grain yield is another important characteristic that a farmer will consider in order to adopt a new cultivar; as a result of the experimental trials during three crop seasons, Tepahui F2009 showed similar yield to the check Tacupeto F2001 with an average of 6.4 ton/ha, 3.1% higher than that of the other check Kronstad F2004 (Table 22). The range of the average yield during the three crop seasons was from 5.8 to 7.2 ton/ha. The highest yield was obtained when cultivars were sown from 15 November to 1 December (Table 23). The maximum yield difference between sowing dates (15 November–1 December and 1 January) for Tepahui F2009 was 700 kg, 1.2 ton between 1 December and 1 January for Tacupeto F2001, and 1.2 ton between 1 and 15 December for Kronstad F2004. Average test weight of Tepahui F2009 was 81.9 kg/hL, slightly superior to those of Tacupeto F2001 and Kronstad F2004 (80.5) (Table 24). These values are greater than those specified by the Mexican Norm NMX-FF-036-1996, which regulates wheat commercialization within the country (DGN, 1996), and establishes a minimum of 74 kg/hL for the quality grade Mexico 1. Test weight is partly positively related to flour yield (Finney et al. 1987), so Tepahui F2009 will likely produce high quantities of this subproduct.

Table 22. Experimental grain yield (ton/ha) of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. The results are the average of three wheat seasons (2007–08 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México.

	Cultivar		
Season	Tepahui F2009	Tacupeto F2001	Kronstad F2004
2007–08	5.8	5.7	6.0
2008–09	6.0	6.0	5.6
2009–10	7.2	7.5	6.9
Mean	6.4	6.4	6.2

Table 23. Experimental grain yield (ton/ha) by sowing date of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. Average of three wheat seasons (2007–08 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México.

	Cultivar		
Sowing date	Tepahui F2009	Tacupeto F2001	Kronstad F2004
15 November	6.7	6.1	6.2
1 December	6.7	7.2	6.8
15 December	6.1	6.1	5.6
1 January	6.0	6.0	6.0
Mean	6.4	6.4	6.2

Table 24. Test weight (kg/hL) of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. Average of three wheat seasons (2007–09 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México.

	Cultivar		
Season	Tepahui F2009	Tacupeto F2001	Kronstad F2004
2007–08	81.5	79.4	80.5
2008–09	82.7	81.8	81.0
2009–10	81.5	80.2	80.0
Mean	81.9	80.5	80.5

Table 25. Percent grain protein (at 12.5% humidity) of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. Average of three wheat seasons (2007–09 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México.

	Cultivar		
Season	Tepahui F2009	Tacupeto F2001	Kronstad F2004
2007–08	13.1	12.7	14.1
2008–09	13.5	13.0	14.1
2009–10	12.8	12.4	13.4
Mean	13.1	12.7	13.9

The average grain protein content of Tepahui F2009 was 13.1%, whereas the check cultivar Tacupeto F2001 showed 12.7 and Kronstad F2004 13.9% (Table 25). Tepahui F2009 presents a gluten strength of 362 W x 10-4 J, a characteristic of strong gluten wheats, and is greater than that of Tacupeto F2001 (315 W x 10-4 J), but lower than that of Kronstad F2004 (494 W x 10-4 J) (Table 26, p. 127). These three cultivars are appropriate for mechanized bread-making. The value of Tepahui F2009 with respect to the balance of tenacity and elasticity (P/G) was 4.1 (Table 27, p. 127). Tepahui F2009 represents a new option for farmers in northwest Mexico, but particularly for those in southern Sonora. This

cultivar has shown tolerance levels acceptable to Karnal bunt and resistance to the races of leaf rust present in the region, it has a high protein content, and strong gluten. Its origin partially comes from *Aegilops tauschii*, a wild wheat used as a parent that is resistant to drought and it is a source of new genes for resistance to diseases (Cox et al. 1992). Therefore, Tepahui F2009 was released with the objective to have a cultivar with better baking quality than Tacupeto F2001 the most grown bread wheat cultivar, and it also has better grain yield than Kronstad F2004. According to the law of seed production and commercialization, after complying with the requirement of the International Union for the Protection of New Varieties of Plants (UPOV 1994), Tepahui F2009 was described and protected in the Catalogue of cultivars feasible for registration with the number TRI-119-270510.

Table 26. Gluten strength of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. Average of three wheat seasons (2007–09 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México (*Kronstad F2004 was not evaluated in 2007–08).

Season	Cultivar		
	Tepahui F2009	Tacupeto F2001	Kronstad F2004*
2007–08	342	270	—
2008–09	328	253	545
2009–10	417	421	443
Mean	362	315	49

Table 27. Balance of tenacity and elasticity (P/G) of the bread wheat cultivar Tepahui F2009 and the checks Tacupeto F2001 and Kronstad F2004. Average of three wheat seasons (2007–09 to 2009–10) of evaluation at the Norman E. Borlaug Experimental Station, Sonora, México (*Kronstad F2004 was not evaluated in 2007–08).

Season	Cultivar		
	Tepahui F2009	Tacupeto F2001	Kronstad F2004*
2007–08	3.8	4.5	—
2008–09	4.5	3.0	3.9
2009–10	3.9	3.9	3.7
Mean	4.1	3.8	3.8

Conclusions.

1. The release of cultivar Tepahui F2009 for northwest Mexico will contribute to reduce the risk of leaf rust epidemics in the region.
2. Tepahui F2009 will extend the durability of cultivars with resistance to leaf rust.
3. Tepahui F2009 will provide the necessary time for the breeding program to increase the genetic diversity of the two types of wheat grown in the region.
4. Tepahui F2009 will make bread wheat to be more competitive in the regional market.

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Identification of rust resistance genes in experimental bread wheat lines and cultivars.

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Introduction. With the implementation of molecular marker-assisted selection in wheat breeding to incorporate new sources of resistance to pathogens, it has been posible to protect this crop against diseases. The accumulation of different sources of resistance to a disease in the same genotype is a process known as pyramiding, which constitutes a promising estrategy for achieving durable resistance through time. This idea is difficult to implement in traditional breeding, because once a resistance gene is incorporated into a line, it becomes difficult to monitor other resistance genes for the same pathogen as all plants show the same resistant phenotype, unless the selection process is assisted by molecular markers for each of the genes target of pyramiding (Johnson 1984). Our objective was to evaluate experimental bread wheat lines and cultivars in order to determine the presence of genes *Lr34*, *Sr2*, *Sr24*, *Sr25*, and *Sr26*.

Materials and methods. From the experimental trial established every wheat season at the Norman E. Borlaug Experimental Station, which belongs to the Mexican National Institute for Forestry, Agriculture, and Livestock Research (INIFAP) located in block 910 in the Yaqui Valley, Sonora (27º22´3.01" N and 109º55´40.22" W, at 38 masl), during the 2010–11, fall–winter, agricultural season, 20 experimental, elite lines from the International Maize and Wheat Improvement Center (CIMMYT) Bread Wheat Program were evaluated for the presence of genes *Lr34*, *Sr2*, *Sr24*, *Sr25*, and *Sr26*, as well as four cultivars released by INIFAP and a bread wheat commercial cultivar from a private company (Table 28, pp. 128-129). DNA was extracted from developing leaves of lines and cultivars during heading, based on the methodology by Saghai-Maroof et al. (1984). PCR reactions were carried out at the CIMMYT Biotechnology Laboratory in El Batan, state of Mexico. For gene *Lr34*, a final volume of 9.5 µl was used for the reaction (6.5 µl de RED taq sigma, 1.5 µl of primer CSLV34, and 1.5 µl of L34PLUS), and 5 µl of DNA. For the rest of the genes, the amount of primer changed in order to adjust to 9.0 µl per reaction: *Sr2* (2.5 µl of BSPH1), *Sr24* (2.5 µl of Sr24#12), *Sr25* (1.5 µl of WMC221), and *Sr26* (2.5 µl of Sr26#43). The PCR products obtained were separated by horizontal electrophoresis in agarose gels at 2.5% and 3.0 % depending on the gene. The separation was done in 1X TBE buffer, stained in ethidium bromide, visualized under UV light, and documented with digital photography.

Table 28. Commercial bread wheat cultivars and elite advanced lines evaluated for the presence of genes *Lr34*, *Sr22*, *Sr24*, *Sr26*, and *Sr36*, during the 2010–11 fall–winter agricultural season at the Norman E. Borlaug Experimental Station, Sonora, México.

Entry	Line	Selection history
1	Tacupeto F2001	
2	Kronstad F2004	
3	Navojoa M2007	
4	Roelfs F2007	
5	RSM-Norman F2008	
6	Thelin/2*WBLL1	CGSS02Y00079T-099B-099B-099Y-099M-6Y-0B
7	PBW343//CAR422/ANA/3/Elvira	CMSS02M00409S-030M-1Y-0M-040Y-10ZTB-0Y-02B-0Y
8	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/*Ae. tauschii* (213)//PGO/4/HUITES	CMSS06B00704T-099TOPY-099ZTM-099Y-099M-23WGY-0B
9	Tacupeto F2001/Brambling*2/5/KAUZ//Altar 84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS06B00707T-099TOPY-099ZTM-099Y-099M-2WGY-0B
10	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ	CMSS06Y00619T-099TOPM-099Y-099ZTM-099Y-099M-5WGY-0B
11	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CMSS06Y00859T-099TOPM-099Y-099ZTM-099Y-099M-35WGY-0B
12	Becard/5/PGO//CROC_1/*Ae. tauschii* (224)/3/ 2*BORL95 /4/ Circus	CMSS06B00411S-0Y-099ZTM-099Y-099M-12WGY-0B

Table 28. Commercial bread wheat cultivars and elite advanced lines evaluated for the presence of genes *Lr34*, *Sr22*, *Sr24*, *Sr26*, and *Sr36*, during the 2010–11 fall–winter agricultural season at the Norman E. Borlaug Experimental Station, Sonora, México.

Entry	Line	Selection history
13	KAUZ//Altar 84/AOS/3/MILAN/KAUZ/4/HUITES/5/ C80.1/3*Batavia//2*WBLL1/6/KAUZ//Altar 84/AOS/3/MILAN/ KAUZ/4/HUITES	CMSS06Y01283T-099TOPM-099Y-099ZTM-099Y-099M-14WGY-0B
14	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/ 2*KAUZ/6/ Pastor/7/WHEAR/8/CAL/NH//H567.71/3/SERI/4/CAL/NH// H567.71/5/2*KAUZ/6/Pastor	CMSS06Y01284T-099TOPM-099Y-099ZTM-099Y-099M-24WGY-0B
15	PBW343*2/Kukuna*2//FRTL/PIFED	CMSS06Y00831T-099TOPM-099Y-099ZTM-099NJ-099NJ-5WGY-0B
16	WBLL1*2/Kurufu//Kronstad F2004/3/WBLL1*2/Brambling	CMSS06B00720T-099TOPY-099ZTM-099Y-099M-5RGY-0B
17	YAV_3/SCO//JO69/CRA/3/YAV79/4/*Ae. tauschii* (498)/5/ LINE1073/6/KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ/7/Krpm-stad F2004/8/KAUZ/Pastor//PBW343	CMSS06B00762T-099TOPY-099ZTM-099Y-099M-11RGY-0B
18	MILAN/KAUZ//Prinia/3/BAV92/4/Pastor*2/BAV92/5/Tacupeto F2001*2/Kukuna	CMSA05Y01021T-040M-040ZTP0Y-040ZTM-040SY-14ZTM-01Y-0B
19	SOKOLL*2/TROST	CMSA05Y01186T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-03Y-0B
20	SOKOLL*2/3/BABAX/LR42//BABAX	CMSA05Y01225T-040M-040ZTP0Y-040ZTM-040SY-12ZTM-01Y-0B
21	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KAUZ// ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	CMSS05B00060S-099Y-099M-099Y-099ZTM-14WGY-0B
22	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES/7/CAL/NH// H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/Pastor	CMSS05B00581S-099Y-099M-099Y-099ZTM-2WGY-0B
23	TRCH/SRTU/5/KAUZ//Altar 84/AOS/3/MILAN/KAUZ/4/HU-ITES	CGSS05B00189T-099TOPY-099M-099NJ-099NJ-7WGY-0B
24	MONR/3/GAV/ROM//BAR	TC-060024-10R-0OAX-0R-6C
25	PAMDOLY-PABG	SRGD(1erCSR06.06)-55R-0OAX-0R-1C

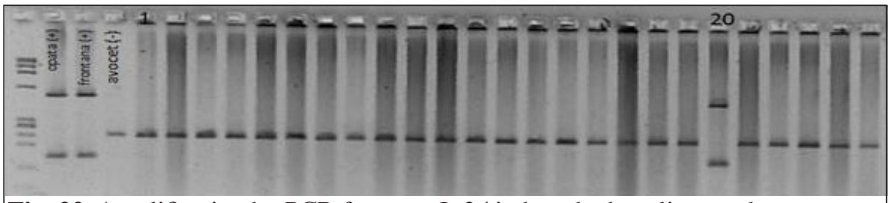

Fig. 22. Amplification by PCR for gene *Lr24* in bread wheat lines and commercial cultivars evaluated during the 2010–11 wheat season in the Yaqui Valley, Sonora, Mexico, using Red taq Sigma primer *Lr34* plus – SCLV24, with the positive parents Opata and Frontana and the negative Avocet (3% agarose gel).

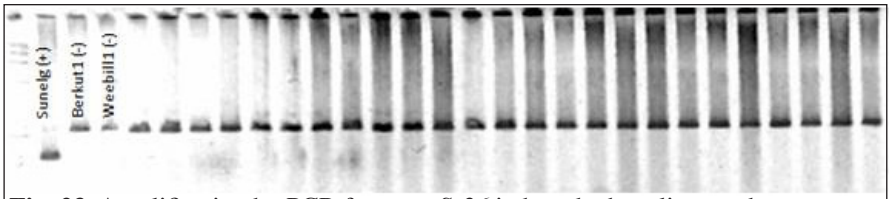

Fig. 23. Amplification by PCR for gene *Sr26* in bread wheat lines and commercial cultivars evaluated during the 2010–11 wheat season in the Yaqui Valley, Sonora, Mexico, using Red taq Sigma primer BES186379–Sr26#43, with the positive parents Sunelg and the negative Berkut 1 and WBLL1 (2.5% agarose gel).

Results. From the PCR products, only the line SOKOLL*2/3/BABAX/LR42//BABAX was positive for gene *Lr34* (Fig. 22). This line produced the highest grain yield in experimental trials, which differs from a report by Singh and Huerta-Espino (1997) who indicate that genes such as *Lr34* for nonspecific races, called durable resistance or low rusting, are expressed in adult plants and are related with a reduction in grain yield. For gene *Sr24*, a positive band was produced for line PBW343*2/KUKUNA*2//FRTL/PIFED, which confers resistance to most races of stem rust, including virulent race Ug99 (TTKSK). No positives for genes *Sr25* and *Sr26* were observed (Fig. 23). *Sr26* is effective against the family of TTKS races. The low frequency of this gene in modern cultivars make it ideal for use in breeding programs as a strategy for accumulation of genes. *Sr2* is a rust resistance gene that has been used for more than 60 years as a durable source and with a broad spectrum that includes resistance to Ug99. The incorporation of *Sr2* has been difficult, because its reccesive nature and to the fact that its phenotype is only evident in adult plant, which can be influenced by the genetic background and the environment (Spielmeyer et al. 2003). Gene *Sr2* was present in only the following lines: TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES and WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP //KAUZ*2/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ. With the incorporation of molecular marker-assisted selection within the traditional breeding system, it is

possible to solve specific demands and problems of susceptibility to certain pathogens in advanced lines, which would be discarded otherwise. Genomic-molecular information published about wheat in relation to characters of agronomic interest is increasing. The most simple and efficient way to incorporate this information for developing new wheat cultivars is through breeding with molecular marker-assisted selection.

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Effect of the irrigation lamina on grain yield, 1,000-kernel weight, protein content, and pigment in four durum wheat cultivars in the Yaqui Valley, Sonora, México.

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Summary. The effect of two, three, and four complementary irrigations (CI) applied in 4.8, 6.0, and 7.1 x 10^3 m^3/ha, respectively, on the industrial quality and grain yield of durum wheat cultivars Huatabampo Oro C2009, Movas C2009, CIRNO C2008, and Samayoa C2004 was evaluated during the 2010–11 wheat season. Sowing was carried out in moist soil, using 100 kg of seed/ha. Each cultivar occupied three strips 90-m long consisting of six beds 0.80 m apart, each with two rows, and with three replications. The highest grain yield was shown by Huatabampo Oro C2009 with 6.099 ton/ha when provided with four CI, followed by CIRNO C2008 with 6.067. The application of four and three CI or the increment on the lamina caused a reduction in grain protein content. The highest grain protein content was shown by CIRNO C2008 with 14.98% when provided with two CI, followed by Movas C2009 with 14.52. All cultivars showed a consistently greater 1,000-kernel weight when they were provided with a greater irrigation lamina. The highest 1,000-kernel weight was shown by Huatabampo Oro C2009 with 53 g when provided with four CI, followed by CIRNO C2008 with 51.20. The highest b value was shown by Samayoa C2004 with 28.27 when provided with two CI, followed by Huatabampo Oro C2009 with 27.43 when provided with four CI, CIRNO C2008 with 24.63 and Movas C2009 with 22.90 when provided with two CI.

Introduction. The many investigations about the effect of irrigation on wheat production all conclude that irrigation is necessary in order to achieve the grain yield potential. However, in nature, there is a negative physiological association between grain yield and its protein content. Wheat cultivars with the highest yield potential have the tendency to produce grain with lower protein levels, and as a consequence lower aptitude to produce pasta or bread of quality and vice versa. Protein content is of great industrial interest, because it is directly related to the bread-making, pasta-making, and nutritional values of the wheat (Caputo et al. 2009). In the irrigated areas of Sonora, Mexico, the percentage of grain protein content in general is moderate, because the agronomic management practiced is focused on obtaining the highest grain yield. However, recently more interest has been given to the industrial quality aspects or processes, primarily on durum wheat, since this type of wheat is grown in 80% of the area. There are few investigations related to the effects of irrigation on the characteristics of the industrial quality; however, Nasseri et al. (2009) demonstrated that nitrogen translocation is favored with a good irrigation, but limited with excess water; they also mentioned that during the grain-filling period, nitrogen assimilation by cereals is an important process which determines yield and quality, and is also associated with the genotype and the agronomic practices, among them, the availability of water. The nitrogen required for protein synthesis, which is accumulated in the developing wheat grain, comes primarily from the nitrogen previously assimilated and accumulated in the leaves (Feller and Fisher 1994). Kouchaki et al (1997) found that increasing the number of irrigations caused an increase in grain yield and a reduction in grain weight and protein content. There is the need to generate information about the effect of the factors involved in the main parameters of the industrial quality; therefore, our objective was to carry out a preliminary study to determine the effect of different water lamina on grain yield and on

some parameters of industrial quality, such as protein, pigment, and 1,000-kernel weight, in four durum wheat commercial cultivars grown in northwest Mexico.

Materials and methods. We evaluated three irrigation levels, 4.8, 6.0, and 7.1 x 10^3 m^3/ha, on grain yield and the quality of cultivars Huatabampo Oro C2009, Movas C2009, CIRNO C2008, and Samayoa C2004 at the Norman E. Borlaug Experimental Station which belongs to the Mexican National Institute for Forestry, Agriculture, and Livestock Research located in block 910 in the Yaqui Valley, Sonora (27º22´3.01" N and 109º55´40.22" W, at 38 masl), during the 2010–11 wheat season. Sowing was carried out on 8 December in moist soil irrigated on 16 November, 2010, using 100 kg of seed/ha; each cultivar occupied three strips 90-m long consisting of six beds 0.80 m apart, each with two rows, and with three replications. The first strip was irrigated with a total of 4.8 x 10^3 m^3/ha, the second with 6.0, and the third with 7.1. The first lamina was administered in two irrigations 64 and 91 days after sowing (das), the second in three 55, 79, and 104 das, and the third in four irrigations 44, 71, 96, and 114 das. From each strip, three samples from the two central 8-m beds were analyzed to determine grain yield. Fertilization was done during presowing using urea and monoammonium phosphate (MAP) to complete the formula 280–80–00 of NPK/ha for all combinations. The variables evaluated were grain yield (ton/ha), protein content (percentage at 12.5% of grain humidity), semolina pigment, and 1,000-kernel weight (g). The protein content was estimated using a NIR equipment model 1488 Perten, and the pigment with a reflectance colorimeter model CR 300 Minolta.

Results and discussion. ***Grain yield.*** In general, the results obtained agree with the reports by Kouchaki et al. (1997) and Nasseri et al. (2009), because all cultivars showed greater yield when they were provided four complementary irrigations (CI), and a consistent reduction with lower total lamina. The average yield of all cultivars with four CI was 5.59 ton/ha, 5.14 with three, and 3.86 with two (Table 29). The average difference for all cultivars between four and three CI was 458 kg, and 1,741.5 between four and two. CIRNO C2008 showed the highest differences, 1,467 (four CI) and 1,934 kg (two CI). The highest grain yield was shown by Huatabampo Oro C2009 with 6.099 ton/ha when provided with four CI, followed by CIRNO C2008 with 6.067, Movas C2009 with 5.433, and Samayoa C2004 with 4.800.

Table 29. Grain yield (ton/ha) of four durum wheat cultivars evaluated under different irrigation regimes at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, México, during the 2010–11 wheat season.

Cultivar	Complementary irrigations			
	Two	Three	Four	Average
Huatabampo Oro C2009	6.099	5.900	4.333	5.433
CIRNO C2008	6.067	4.600	4.133	4.933
Movas C2009	5.433	5.367	3.867	4.889
Samayoa C2004	4.800	4.700	3.100	4.200
Average	5.592	5.142	3.858	4.864

Grain protein. The wheat market in Mexico is almost 80% for human consumption and for the industry (CANIMOLT 2008), which requires wheat of high protein content for the regional and for the export market. Average grain protein content of cultivars with four CI was 13.66%, 14.09 with three, and 14.59 two (Table 30). The application of four and three CI or the increment on the lamina caused a strong reduction in protein content in the grain. These results corroborate the report by Kouchaki et al. (1997), who indicated that the increase in the number of irrigations caused a grain yield increase and a reduction in the protein content. The highest grain protein content was shown by CIRNO C2008 with 14.98% when provided with two CI, followed by Movas C2009 with 14.52, and Huatabampo Oro C2009 and Samayoa C2004 both with 14.42.

Table 30. Grain protein content (%) of four durum wheat cultivars evaluated under different irrigation regimes at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, México, during the 2010–11 wheat season.

Cultivar	Complementary irrigations			
	Two	Three	Four	Average
CIRNO C2008	13.82	14.35	14.98	14.38
Movas C2009	13.71	13.96	14.52	14.06
Samayoa C2004	13.40	14.30	14.42	14.04
Huatabampo Oro C2009	13.71	13.76	14.42	13.96
Average	13.66	14.09	14.59	14.11

1,000-kernel weight. all cultivars showed consistently greater weight when they were provided with a greater irrigation lamina (Table 31). The average 1,000-kernel weight of all cultivars with four CI was 50.80 g, 46.98 with three, and 42.62 with two. The highest 1,000-kernel weight was shown by Huatabampo Oro C2009 with 53 g when provided with four CI, followed by CIRNO C2008 with 51.20, Movas C2009 with 50.73, and Samayoa C2004 with 48.27. The highest difference in 1,000-kernel weight between four and two CI was shown by Movas C2009 with 14.33 g, followed by CIRNO C2008 with 11.53.

Table 31. 1,000-kernel weight (g) of four durum wheat cultivars evaluated under different irrigation regimes at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, México, during the 2010–11 wheat season.

Cultivar	Complementary irrigations			
	Two	Three	Four	Average
Huatabampo Oro C2009	53.00	50.27	48.93	50.73
Samayoa C2004	48.27	46.47	45.47	46.73
CIRNO C2008	51.20	48.07	39.67	46.31
Movas C2009	50.73	43.13	36.40	43.42
Average	50.80	46.98	42.62	46.80

Semolina pigment. The accumulation of carotenoids confers the yellow color to flour and semolina. This trait is negative for the bread-making industry because the consumer prefers white bread, but on the contrary, it is a very important characteristic for the pasta-making industry, because this feature is sought by the consumer. Genetic studies have demonstrated that the yellow color of the endosperm is highly heritable (65–90%) (Cenci et al. 2004). With the exception of Huatabampo Oro C2009, the other cultivars showed greater pigment b value when provided with two CI (Table 32). The b value remained at 27 for Huatabampo Oro C2009. The average b value of all cultivars with four CI was 24.59, 25.07 with three, and 25.80 with two. The highest b value was shown by Samayoa C2004 with 28.27 when provided with two CI, followed by Huatabampo Oro C2009 with 27.43 when provided with four CI, CIRNO C2008 with 24.63 and Movas C2009 with 22.90 when provided with two CI. In general, cultivars did not show great differences when managed with different irrigation lamina. Movas C2009 consistently showed lower pigment values with all irrigation lamina.

Table 32. Semonlina pigment (Minolta b value) of four durum wheat cultivars evaluated under different irrigation regimes at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, México, during the 2010–11 wheat season.

Cultivar	Complementary irrigations			
	Two	Three	Four	Average
Huatabampo Oro C2009	27.43	27.10	27.40	27.31
Samayoa C2004	26.23	27.20	28.27	27.23
CIRNO C2008	23.90	23.90	24.63	24.14
Movas C2009	22.40	22.07	22.90	22.46
Average	24.99	25.07	25.80	25.29

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ITEMS FROM PAKISTAN

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Diversity and distribution of rust diseases of wheat (Puccinia spp.) in southern Pakistan.

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Pakistan is an agricultural country. A large portion of the population is directly or indirectly dependent on agriculture. Presently, a burgeoning population is demanding more food, which only can be accomplished when the production of cereal crops in the country is increased. To achieve this, sustainable control of biotic and abiotic constraints is needed. Among the biotic constraints, rust diseases (stem, leaf, and stripe) caused by *Puccinia spp*. are important fungal diseases of the wheat crop attributed to substantial grain losses (Chen 2005; Bux et al. 2012).

Leaf, stem, and yellow rust prevail in all the wheat-growing areas of southern Sindh, spanning from Thatta to Sanghar and the Nawabshah area. Leaf rust is common in all the areas and occurs regularly. Yellow rust is sporadic and prevails when the climatic conditions are favorable for the pathogen. Stem rust is a regularly occurring and most dangerous rust occurring in the southern wheat-growing areas of Sindh. These diseases effect the wheat crop by damaging the respiratory system, killing foliage, stunting growth, and most importantly reducing grain yield by shriveling grain, reducing weight, and effecting quality (Chen 2005). Grain losses of 10–70% caused by these devastating pathogens have been reported. In severe disease epidemics, the grain damage may be up to 100% (Chen 2005).

These destructive fungal pathogens are controlled by fungicides and host-genetic resistance through developing resistant wheat cultivars. The pathogens are dynamic, changing their genetic make-up over time to become more aggressive and devastating. Therefore, for sustainable control, studies on the diversity of the pathogen and identifying genetic resources through molecular technology are needed. To achieve this, developing efficient molecular markers for the genes conferring resistance are indispensible (Bux et al. 2011).

We carried out a survey of wheat rust diseases across Sindh province, southern Pakistan. Occurrence of leaf, stem, and yellow rust is limited to particular areas or overlap each other. In 2012, leaf rust was common and observed across the wheat tract in the Sindh province. We observed a high incidence of the disease in Kunri, Umar kot, Petaro, Jamshoro, Tandom Adam, Mirpur Matrhelo, Kandiaro, and other areas. Stem rust was limited to a few areas. During our survey, we observed a few pustules of stem rust on wheat growing around Matiari and Sanhgar. Yellow rust was completely absent in the southern parts of Sindh province. However, a few diseased leaves were observed in Ghotki wheat-growing regions lying on the border of Sindh–Punjab provinces.

All three rust diseases are damaging the rural economy in Sindh. To control these diseases sustainably, monitoring the pathogen's prevalence is necessary before applying the appropriate control measures. However, sustainable control can be accomplished by increasing the genetic diversity of existing wheat cultivars. Furthermore, agricultural extension services will assist in combating the pathogen by creating awareness among the farmers. Research on modern lines, in parallel with those in developed countries, will help resolve the problem and devise new avenues for sustainable control.

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Relationship of pasting parameters with gluten and some other attributes of flour.

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Pearson's correlation coefficients were used to analyze the relationships between pasting parameters and quality attributes of hard white spring wheat flours. The relationships between pasting parameters and flour quality attributes are given in Table 1. A significant positive relationship was found between pasting temperature and gluten content (wet and dry). Addo et al. (2001) addressed the requirement for higher temperature by high-gluten flours over other components, such as prime starch, tailing starch, and their mixtures.

Table 1. Physio-chemical attributes and arabinoxylan contents of some wheat cultivars (SD = starch damage; FN = falling number; PC = protein content; WG = wet gluten; DG = dry gluten; GI = gluten index; MC = moisture content; TOAX = total arabinoxylan content; WEAX = water extractable arabinoxylan content; and WUAX = water unextractable arabinoxylan content).

Cultivar	SD (UCD)	FN (s)	Ash (%)	PC (%)	WG (%)	DG (%)	GI	MC (%)	TOAX (mg/g)	WEAX (mg/g)	WUAX (mg/g)	%WEAX in TOAX
TD-1	23.0	594	0.54	11.0	30.4	9.7	68	15.1	13.6	4.3	9.4	31.2
Imdad	23.1	566	0.72	11.2	29.5	9.6	95	15.1	16.5	6.3	10.3	37.8
Mehran	22.0	902	0.56	12.2	31.0	10.6	78	14.7	14.5	4.7	9.8	32.4
Abadgar	22.4	586	0.66	13.0	38.2	11.0	56	12.7	15.1	5.1	10.1	33.4
Moomal	22.9	582	0.69	11.1	27.8	9.1	96	14.7	16.6	6.1	10.5	36.6
Anmol	22.1	505	0.71	11.5	28.4	8.7	95	14.7	17.2	6.1	11.1	35.2
SKD-1	22.5	844	0.52	11.3	33.0	9.1	87	14.5	16.1	6.0	10.1	37.0
TJ-83	20.9	583	0.60	9.4	28.5	8.3	78	14.5	11.7	3.3	8.3	28.6

Regarding the soundness of wheat kernels, the cultivars used in this study were found to have very low α-amylase activity as interpreted by higher values for falling number (> 450s) and peak viscosity (> 960 BU), because falling number is inversely related with α-amylase activity (D'Appolonia et al. 1982; Moot and Every 1990) and peak viscosity (D'Appolonia et al. 1982). But when the α-amylase activity is too low, as in our study, the resultant falling number will exclusively be the function of flour viscosity instead of α-amylase. Therefore, the falling number was found to strongly corelate with amylograph peak viscosity. Vijayakumar et al. (2009) also found a positive relationship between falling number and PV in composite flours. Noda et al. (2003) includes sprouted wheat (high α-amylase activity) in their study and displayed the curvilinear relationship between peak viscosity and α-amylase activity.

The arabinoxylan (AX) content and percentage of water-extractable AX in total AX weakly related with peak viscosity of wheat flour. In heat-treated flour paste, the AX contents significantly correlated with apparent viscosity (Iriki et al. 2003).

A significantly positive relationship was found between time to reach peak viscosity and cold paste viscosity (r = 0.626**, Table 2, p. 135). The relationship revealed that the flours with long peak-paste time had a greater ability to form a paste or gel after cooling. Wiesenborn et al. (1994) suggested that flours exhibiting longer time to reach their maximum viscosity are more resistant to mechanical damage. Moreover, the flours having good cold thickening capacity are suitable for use in recipes for instant soup, cream, and sauces (Alves et al. 1999).

Hot paste viscosity was strongly and positively related with cold paste viscosity (r = 0.857**, Table 2, p. 135). Kaur et al. (2007) also showed a significantly positive relationship between hot paste viscosity and cold paste viscosity in potato cultivars.

Breakdown viscosities were moderately related with peak viscosity (r = 0.433, Table 2), suggesting that flours having a greater peak viscosity can be broken down rapidly resulting in weak gels or pastes. These flours are suitable in food applications where low thickening power is required such as in pastries. Tsakama et al. (2010) also reported a significant positive relationship between peak viscosity and the breakdown viscosity of sweet potato starches.

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Table 2. Correlation coefficients for wheat flour pasting parameters and quality attributes (PT, pasting temperature; PV, peak viscosity; TPV, time to reach peak viscosity; HPV, hot paste viscosity; CPV, cold paste viscosity; BD, breakdown viscosity; SB, setback viscosity; SD, damaged starch; FN, falling number; AC, ash content; PC, protein content; WG, wet gluten; DG, dry gluten; GI, gluten index; MC, moisture content; TOAX, total arabinoxylan; WEAX, water–extractable arabinoxylan; WUAX, water–unextractable arabinoxylan; and %WEAX, percent water–extractable arabinoxylan in total arabinoxylan (*0.05 and **0.01 level of significance.).

	PT	PV	TPV	HPV	CPV	BD	SB	SD	FN	ASH	PC	WG	DG	GI	MC	TOAX	WEAX	WUAX
PV	0.041																	
TPV	–0.542*	0.309																
HPV	0.164	0.66**	0.418															
CPV	–0.031	0.470	0.626**	0.857**														
BD	–0.156	0.433	–0.124	–0.390	–0.447													
SB	–0.097	0.36	0.192	0.289	0.502*	–0.30												
SD	–0.168	–0.354	–0.192	–0.005	–0.021	–0.427	0.116											
FN	–0.008	0.901**	0.365	0.568*	0.371	0.427	–0.014	–0.458										
AC	–0.187	0.171	0.291	0.343	0.516*	–0.187	0.614*	0.202	0.209									
PC	0.512*	–0.102	–0.110	0.029	0.198	–0.148	0.021	–0.154	–0.220	0.14								
WG	0.633**	0.124	–0.211	0.165	0.209	–0.40	–0.098	–0.254	–0.101	–0.12	0.88**							
DG	0.504**	–0.133	–0.13	–0.076	0.112	–0.068	–0.088	–0.108	–0.288	–0.035	0.947**	0.864**						
GI	–0.337	–0.086	0.223	0.008	0.10	–0.109	0.239	0.172	0.071	0.236	–0.265	–0.543*	–0.373					
MC	–0.60	–0.442	0.014	0.046	0.083	–0.612*	0.303	0.526*	–0.343	0.044	–0.213	–0.420	–0.178	0.149				
TOAX	–0.150	–0.262	0.038	0.172	0.200	–0.519*	0.531*	0.567*	–0.238	0.504*	–0.036	–0.273	–0.196	0.486	0.426			
WEAX	–0.102	–0.289	–0.037	0.166	0.171	–0.547*	0.566*	0.588*	–0.278	0.456	–0.069	–0.290	–0.226	0.542*	0.457	0.983**		
WUAX	–0.203	–0.203	0.132	0.189	0.244	–0.464	0.455	0.518*	–0.173	0.542*	0.02	–0.220	–0.137	0.380	0.356	0.971**	0.911**	
%WEAX	0.004	–0.369	–0.194	0.057	0.060	–0.517*	0.584*	0.573*	–0.388	0.310	–0.037	–0.242	–0.179	0.561*	0.461	0.891**	0.955**	0.764**

Effect of water-extractable pentosan on the cold paste viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

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Breakdown viscosity (BD) is the measure of fragility of starch granules. A high breakdown viscosity indicates fewer tendencies of starch granules to resist shear. The BD viscosities of flours varied insignificantly between 487 BU to 644 BU. Singh et al. (2010) relates climatic conditions with breakdown viscosity and found a decrease in breakdown viscosity under rain-fed conditions. Flour from the cultivar Imdad showed the maximum resistance to shear as interpreted by its least BD viscosity amongs cultivars. The higher breakdown viscosity found in the flour of cultivar TJ-83 was followed by those of Mehran, SKD-1, and Anmol. The addition of water-extractable pentosan (WEP) was found to increase the BD viscosity of the flour of all cultivars, suggesting that the presence of hydrophilic WEP exerts more stress on wheat starch granules resulting in a rapid decline in the viscosity of wheat flour suspensions. A statistically significant increase was found in the BD viscosity of cultivars TD-1, Imdad, Anmo, SKD-1, and TJ-83 (Fig. 1).

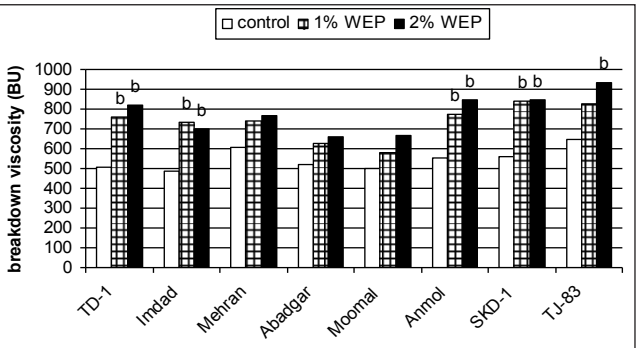

Fig. 1. The eEffect of water-extractable pentosan (WEP) on breakdown viscosity of flour from different wheat cultivars. Bars labeled 'b' indicate a significant difference from the control at $P < 0.05$.

The effect of WEP concentration. An increase in WEP concentration did increase the BD values of all flours, but the extent of the increase varied from cultivar to cultivar. The magnitude of increase (in terms of percentage) in BD viscosities of different cultivar flours with the substitution of WEP is presented in Fig. 2. At a 1% concentration, the increase in BD varied between 16% and 51% of the BD value of control flours. The BD values further increased and reached 63% (maximum) of the BD value of control flours when WEP was added up to the 2% level.

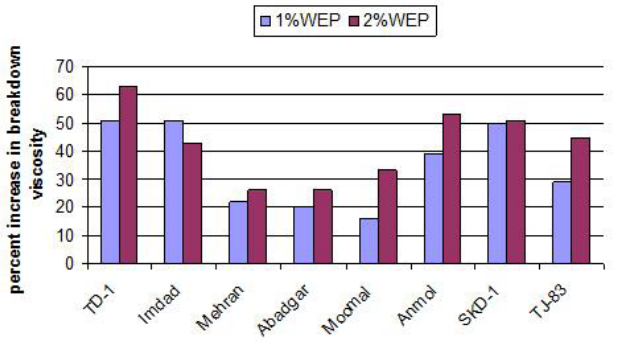

Fig. 2. Percent increase in breakdown viscosity values of different cultivar flours due to the addition of water-extractable pentosan (WEP).

Cultivar differences in the breakdown viscosities in WEP-substituted flours. Breakdown viscosities in 1% WEP-substituted flours of all cultivar (except SKD-1, TJ-83, and Moomal) varied between 624 and 774BU. Starch granules in the flour of SKD-1 and TJ-83 showed a low tendency against shearing in the presence of WEP (Fig. 3 (left)), whereas the highest tendency to resist shear was in the flour of Moomal. The lowest tendency was exhibited by the starch granules in flour of TJ-83 to resist shear in the presence of 2% WEP (Fig. 3 (right)). The breakdown viscosities of two-percent WEP substituted flours of all other varieties ranged between 660 and 850BU.

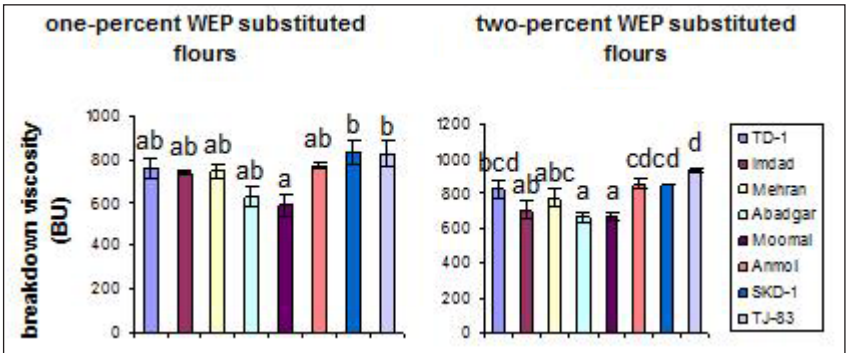

Fig. 3. Breakdown viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

The relationship between breakdown viscosity and other pasting parameters in presence of WEP. Correlation coefficients between BD and other pasting parameters in presence of WEP are given in Table 3. We found that WEP did not influence the relationship of BD with other pasting parameters. BD viscosity had a similar pattern of relationship with other pasting parameters in the presence or absence of WEP.

Table 3. Relationships between BD viscosity and other pasting parameters in presence of water-extractable pentosan (WEP).

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Setback
Breakdown viscosity	0.191	0.637**	–0.052	–0.335	–0.350*	–0.264

Reference.

Singh S, Gupta AK, Gupta SK, and Kaur N. 2010. Effect of sowing time on protein quality and starch pasting characteristics in wheat (*Triticum aestivum* L.) genotypes grown under irrigated and rain-fed conditions. Food Chem 122(3):559-565.

Effect of water-extractable pentosan on cold paste viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

Cold paste viscosity (CPV) is the viscosity measured after holding the slurry at 50°C for 10 minutes. The value of CPV serves as an indicator of paste stability after cooking. The paste or gel-forming ability of starch after cooling corresponds well through CPV values. Moreover, CPV is a significant attribute in some food-processing operations, such as canning, and predicts the starch property in the preparation of food items such as instant soup, creams, and sauces that require cold thickening capacity. All cultivar flours varied in their CPV ranging between 865 BU to 1187 BU. The differences among cultivars in CPV might be due to different flour composition.

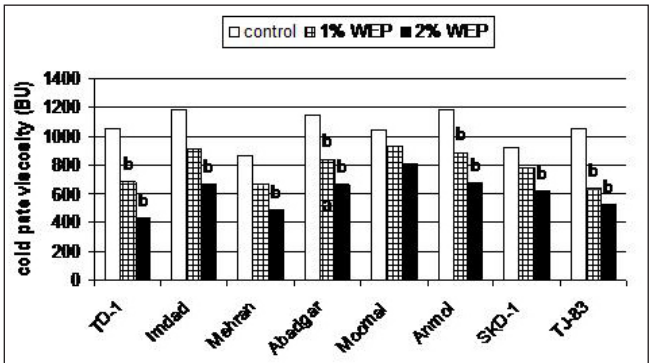

Fig. 4. Effect of water-extractable pentosan (WEP) on cold paste viscosity of flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

The addition of water-extractable pentosan (WEP) to flour induced significant reduction in CPV of all cultivar flours (Fig. 4), which may be a result of hydrophilic nature of WEP that weakens the gelling tendency of wheat flour possibly by hindering the reassociation of amylose molecules while they aggregate on cooling. Rao et al. (2007) reported an increase in CPV values when the water-soluble, nonstarch polysaccharides obtained from rice and ragi were added at 0.5% to a wheat flour sample. This is in contrast to present study in which pentosans were isolated from a hard-type wheat flour and were substituted at 1% and 2% into eight different genotypes of hard white spring wheats. The differences in the pentosan source, the supplementation level and the number and quality of wheat flour samples analyzed might be the possible reasons for contrary results.

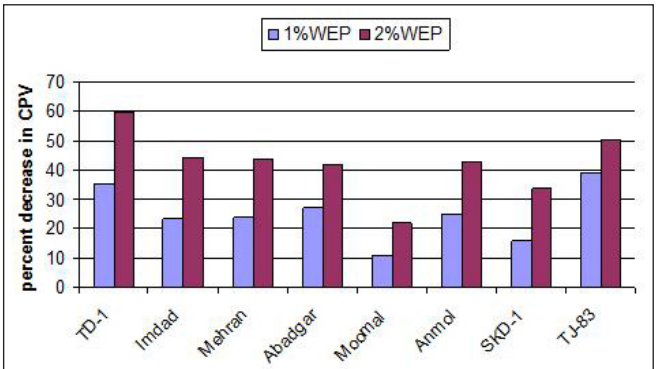

Fig. 5. Percent decrease in cold past viscosity of different cultivar flours due to the addition of water-extractable pentosan (WEP).

Effect of WEP concentration. The concentration of WEP did not influence the type of effect because CPV decreased with the substitution of WEP but the magnitude of decrease was found to be variable depending on the genotype of wheat flour. The decrease in CPV was estimated in terms of percent decrease from their control (Fig. 5). The CPV of all cultivar flours was nonlinearly

reduced with increasing concentration of WEP. The reduction varied from cultivar to cultivar at the same concentration level. At a concentration of 1%, the reduction varied between 11% and 39% with the highest reduction in CPV in the culivar TJ-83. A further decrease (22–60%) in CPV was found when the WEP concentration was increased upto 2%. At a concentration of 2%, the highest decrease was found in the CPV of cultivar TD-1, followed by TJ-83. The reduction with increasing WEP concentration of WEP showed further weakening in paste stability after cooking. A greater amount of WEP molecules could more strongly hinder the reassociation of amylose molecules upon cooling resulting in the further reduction in CPV.

Relationship between CPV and other pasting parameters in the presence of WEP. The Pearson's correlation coefficients between CPV and other pasting parameters in presence of WEP are given in Table 4. We found that CPV related in a similar fashion with other pasting parameters in the presence or absence of WEP.

Table 4. Relationship between cold pasting viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Breakdown	Setback
Cold paste viscosity	–0.234	0.426*	0.517**	0.910**	–0.350*	0.883**

Cultivar differences in CPV of WEP-substituted flours. A wide range (638-937BU) of cold paste viscosities was observed in one-percent WEP substituted flours (Fig. 6). The least and most viscous cold paste was formed with the flours of varieties TJ-83 and Moomal respectively. Amongst two-percent WEP substituted flours, all varieties had viscosities varied insignificantly between 426 and 816BU. The most viscous cold paste belonged to the variety of Moomal.

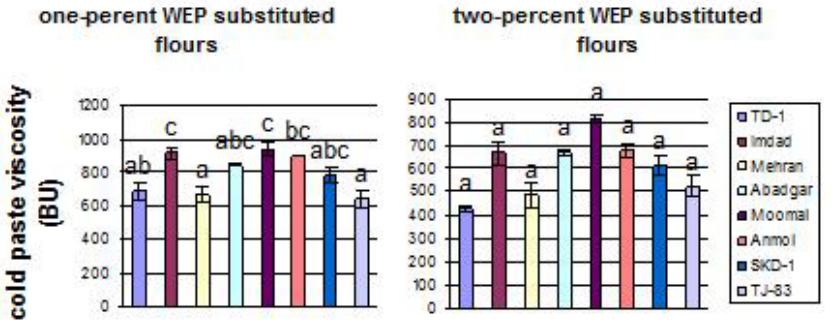

Fig. 6. Cold paste viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Reference.

Rao RSP, Manohar RS, and Muralikrishna G. 2007. Functional properties of water-soluble non-starch polysaccharides from rice and ragi: Effect on dough characteristics and baking quality. Food Sci Technol-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on hot paste viscosity of some wheat cultivars with with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, and Abdul Mujeeb-Kazi.

Hot paste viscosity (HPV) is defined as the viscosity measured after a holding period of 10 minutes at 95°C. Holding a wheat starch slurry at 95° C leads to a reduction in pasting viscosity, because it is subjected to mechanical stress during this isothermal phase that eventually results in the rupture of swollen starch granules that are responsible for viscosity development. Hot paste viscosity of flours of all cultivars (except Mehran) was found to range between 510BU to 671BU. The hot paste of flour of Mehran had an exceptionally low viscosity (351BU).

The addition of water-extractable pentosan (WEP) at both levels of supplementation induced a significant reduction in HPV of all cultivar flours (Fig. 7, p. 139), which may be a result of the hydrophilic nature of WEP that increases the rupturing tendency among starch granules during the isothermal phase or under induced mechanical stress while they are suspended in flour. Rao et al. (2007) reported an increase in HPV values when the water-soluble, nonstarch polysaccharides obtained from rice and ragi were added at 0.5% to a wheat flour sample. In our study, pentosans were isolated from a hard-type wheat flour and were substituted at 1% and 2% levels in to eight different hard white spring wheat

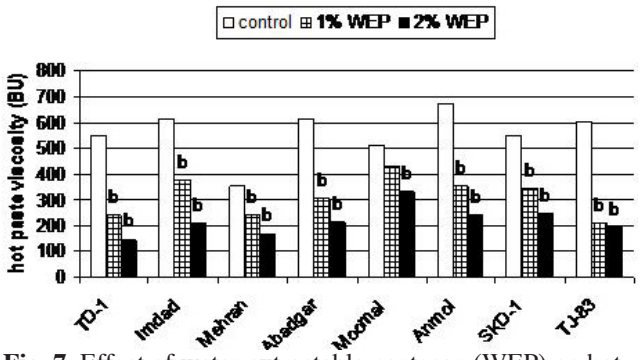

Fig. 7. Effect of water-extractable pentosan (WEP) on hot paste viscosity of flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

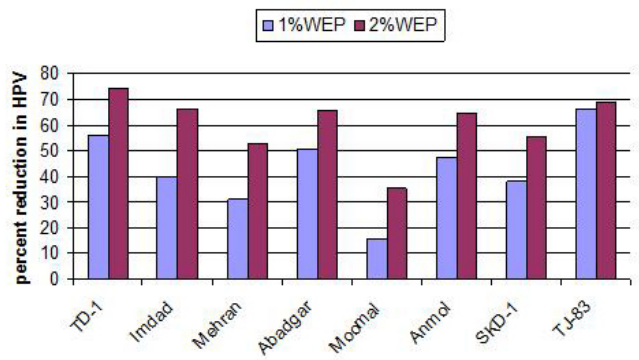

Fig. 8. Percent decrease in hot past viscosity of different cultivar flours due to the addition of water-extractable pentosan (WEP).

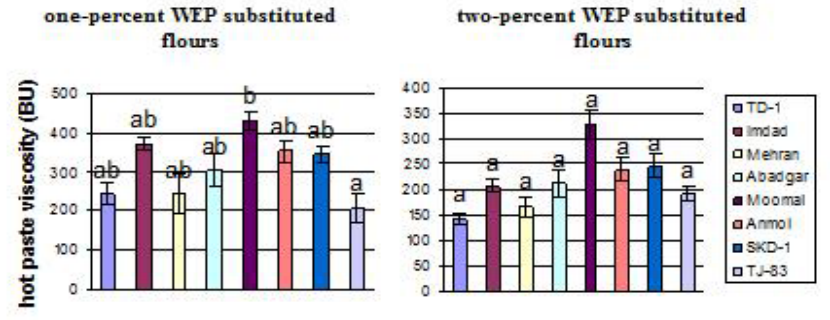

Fig. 9. Hot paste viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

genotypes. The differences in pentosan source, supplementation level, and the number and quality of wheat flour samples analyzed might be the possible reasons for different results.

The type of WEP effect, that is, decreasing the viscosity, on HPV was found to be same for all cultivars, but the magnitude of WEP varied from cultivar to cultivar.

Effect of WEP concentration. The HPV of all flours was found to be inversely related with the concentration of WEP. However, HPV of all cultivars reduced nonlinearly with increasing concentration of WEP. The decrease in HPV was larger from control to 1% WEP (79-398BU) than from 1% to 2% (16-165BU) in all cultivars except Moomal. Moomal flour was the least affected having the lowest reduction observed.

The magnitude of reduction in terms of percent decrease upon the addition of 1% and 2% of WEP to flours of each cultivar is in Fig. 8. In terms of percentage, the reduction in HPV ranged between 15% and 66% at 1% concentration, and a further reduction noticed when WEP concentration increased to 2%. At a concentration of 1%, the highest reduction was observed in TJ-83 flour, followed by TD-1 and Abadgar, with the percent decrease of 66%, 56%, and 51%, respectively. Further reductions (35–74%) were found with 2% WEP; the maximum reduction in the HPV of TD-1 flour.

Cultivar differences in the HPV of WEP-substituted flours. The hot paste of 1% WEP-substituted flours of all cultivars exhibited viscosities ranging between 206 and 431 BU (Fig. 9). The least and most viscous hot pastes belonged to cultivars TJ-83 and Moomal, respectively. Hot paste viscosities of 2% WEP-substituted flours varied between 142 and 330BU, however, the differences were not statistically significant.

Relationship between HPV and other pasting parameters in presence of WEP. Similar to the control flours, HPV strongly and positively corelated with CPV upon the addition of WEP in flour (Table 5). However, HPV was moderately related with setback, peak viscosity, and time to reach peak viscosity and weakly related with pasting temperature and breakdown viscosity.

Table 5. Relationship between hot pasting viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Cold paste viscosity	Breakdown	Setback
Hot paste viscosity	–0.370*	0.512**	0.453**	0.910**	–0.335	0.698**

Reference.
Rao RSP, Manohar RS, and Muralikrishna G. 2007. Functional properties of water-soluble non-starch polysaccharides from rice and ragi: Effect on dough characteristics and baking quality. Food Sci Technol-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on the pasting temperature of some wheat cultivars with with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, and Abdul Mujeeb-Kazi.

Pasting temperature (PT) is defined as the temperature when the first rise in viscosity is recorded by a viscoamylograph. The granules of starch undergo swelling and amylose leaching when the suspension is heated to more than a specific temperature in the presence of excess water. The pasting temperatures of flours from eight different wheat cultivars (TD-1, Imdad, Mehran, Abadgar, Moomal, Anmol, SKD-1, and TJ-83) were determined using a viscoamylograph. Pasting temperatures of these cultivars were found to be narrow, ranging from 56.8–59.9°C. The highest temperature to gelatinize the starch was in flour of Abadgar, followed by those of cultivars SKD-1 and TJ-83.

Water-extractable pentosan (WEP) was found to marginally increase the pasting temperature of all cultivar flours except those of Abadgar and SKD-1 (Fig. 10). The shift in PT was found not statistically significant in any cultivar. In the presence of WEP, the onset of the pasting process took place earlier (at lower temperatures) in Abadgar and SKD-1 flour and was delayed (took place at higher temperatures) in all the other cultivar flours. WEP facilitates the process of granule swelling–amylose leaching in the flours of Abadgar and SKD-1, but also hindered the same process, resulting in a delay in pasting because they required higher temperature for onset.

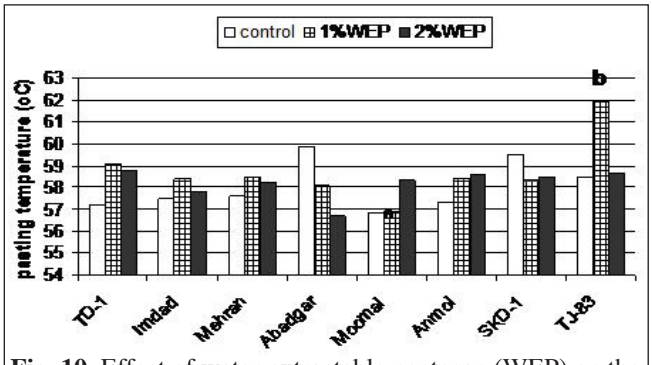

Fig. 10. Effect of water-extractable pentosan (WEP) on the pasting temperature of different hard white spring wheat flours from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

Effect of WEP concentration. WEP, substituted at 1% and 2% were studied to reveal their effect on PT in the different cultivar flours. Results showed that the concentration of WEP did not influence the type of effect (whether increasing or decreasing) but induced variable changes in the magnitude of the effect on PT on the cultivar.

The PT of all cultivars (except two) was delayed in the presence of WEP up to the 2% level (Fig. 11). The magnitude of increase (0.2–0.6%) or decrease (1.7–5.3%) varied from cultivar to cultivar at the same WEP concentration. The maximum increase at 1% was in TJ-83 flour and maximum decrease at 2% was in Abadgar flour.

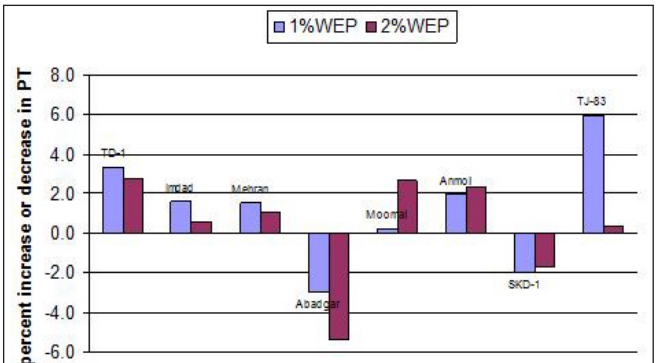

Fig. 11. Magnitude (% increase or decrease) of the effect of water-extractable pentosan (WEP) on the pasting temperature of different hard white spring wheat flours.

Relationship between PT and other pasting parameters in presence of WEP. Pasting temperature was found to relate differently with other pasting parameters upon the addition of WEP to flour (Table 6). Pasting properties of flour are different in the presence of WEP. A moderately negative correlation was found between PT and hot paste viscosity in the presence

Table 6. Relationship between pasting temperature and other pasting parameters in presence of water-extractable pentosan.

	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown	Setback
Pasting temperature	–0.129	0.088	–0.370*	–0.234	0.191	0.061

of WEP. With other pasting parameters, there was no linear correlation.

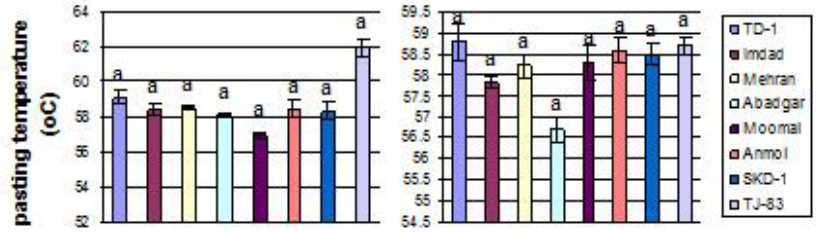

Fig. 12. Pasting temperatures of water-extractable pentosan (WEP)-substituted, hard white spring wheat flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Cultivar differences in the PT of WEP-substituted flours. The pasting temperature of WEP-substitute flours from different cultivars at the same supplementation level was not found to be statistically different (Fig. 12). The pasting temperatures of 1% WEP-substituted flours ranged between 56.9° and 62°C, whereas 2% WEP varied from 56.7° to 58.8C. The greatest temperature needed to trigger the starch swelling–amylose leaching process was in TJ-83 flour in the presence of 1% WEP. In the 2% WEP-substituted flours, the lowest temperature needed to gelatinize starch granules was in the flour of Abadgar and the highest in TD-1 flour. The pasting temperature of flour of Anmol, SKD-1, and TJ-83 were only 2°C, 3°C, and 1°C, respectively, lower than that of TD-1.

Effect of water-extractable pentosan on peak viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, and Abdul Mujeeb-Kazi.

Peak viscosity (PV) is one of the most important pasting attributes that is useful for distinguishing starch properties. Peak viscosity is the equilibrium point between swelling and rupture of starch granules (Newport Scientific 1995). The viscosity of a wheat flour slurry reaches peak when there is the maximum number of swollen intact granules present. The value of PV corresponds to the extent of granule swelling. The PV value of the flour from different cultivars varied between 962 and 1,252 BU. The highest PV was in the flour of TJ-83, followed by Anmol and Abadgar. The flour of these cultivars could be a good thickening agent. The least viscous flour was found in the cultivar Mehran.

Water-extractable pentosan (WEP) appears to be one of the significant sources of variation in PV of hard white spring wheat flours. Yin and Walker (1992) found a significant affect of pentosan on RVA peak viscosity of starch and reported that the PV of starch decreased with the addition of WEP. They used only one commercial starch sample, whereas our study used flour samples from several cultivars. Moreover, the pasting properties of starch may not necessarily correspond to that of flour because differences have been found in the PV pattern of starch and flour from same wheat (Lin and Czuchajowska et al. 1997). In another study, the increase in the PV of wheat flour was reported with the addition of water-soluble, nonstarch polysaccharide from other sources, such as rice and ragi (Rao et al. 2007). In our study, the pentosans were isolated from wheat flour. Hence, slight differences in results would be due to different analytical techniques for pasting properties, the nature of the sample, the pentosan source and isolation procedure, and the number of samples analyzed. We found that WEP, at two levels of supplementation, made insignificant changes in the PV of each cultivar, except Abadgar at the 2% level (Fig. 13).

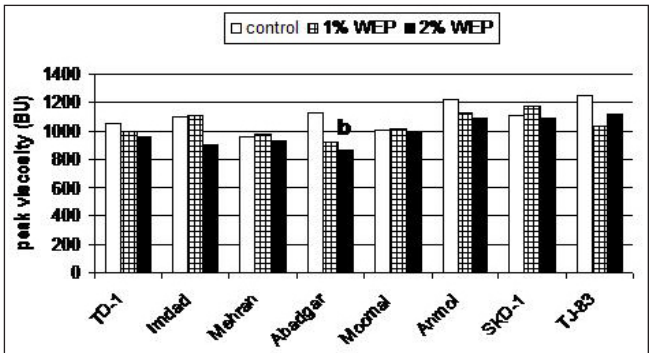

Fig. 13. Effect of water-extractable pentosan (WEP) on the peak viscosity of the flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

Effect of WEP concentration. An increasing concentration of WEP did not impart a uniform influence on the PV of all flours. WEP concentration did affect the PV, varied in type and magnitude, and largely depended on the genotype of the wheat flour. However, the addition of WEP at a concentration of 2% reduced the PV of all cultivar flours between 1.6%

and 23.3% (Fig. 14). The maximum decrease in PV was found in the flour of Abadgar.

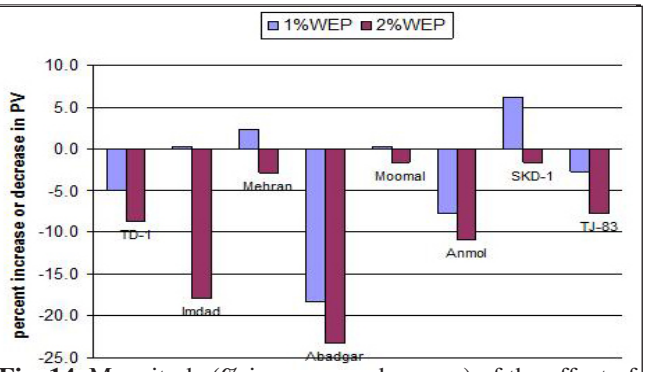

Fig. 14. Magnitude (% increase or decrease) of the effect of water-extractable pentosan (WEP) on the peak viscosity of different hard white spring wheat flours.

Varietal differences in PV of WEP-substituted flours. The peak viscosity exhibited by 1% WEP-substituted flours varied between 926 and 1,179 BU (Fig. 15). However, differences in PV among cultivars were not found to be statistically significant. With 2% WEP-substituted flours, the highest PV, 1,123 BU, was in TJ-83 flour and the lowest, 870 BU, was in Abadgar flour.

Relationship between PV and other pasting parameters in presence of WEP. A moderate relationship was found between PV and other pasting parameters with the addition of WEP in flour. Peak viscosity was positively correlated with hot paste viscosity (r = 0.512**), cold past viscosity (r = 0.426**), breakdown (r = 0.637**), and setback (r = 0.331).

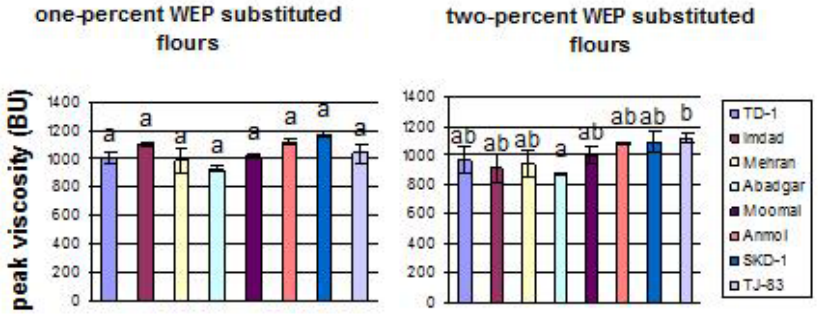

Fig. 15. Peak viscosity of water-extractable pentosan (WEP)-substituted, hard white spring wheat flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

References.

Lin P-Y, and Czuchajowska Z. 1997. Starch properties and stability of club and soft white winter wheats from the pacific northwest of the United States. Cereal Chem 74(5):639-646.

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Rao RSP, Manohar RS, and Muralikrishna G. 2007. Functional properties of water-soluble non-starch polysaccharides from rice and ragi: Effect on dough characteristics and baking quality. Food Sci Technol-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on setback viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Setback viscosity (SB) measures the retrogradation tendency of starch granules (Abd Karim et al. 2000). After gelatinization, the leached out linear amylose chains start reassociating with each other on cooling, which subsequently results in increased viscosity of flour pastes. Retrogradation of starch is a good indicator of bread staling. Other flour components such as gluten, lipids, and pentosans also may be involved in the process of staling (Martin et al. 1991). The setback value of all cultivar flours (except Imdad) varied between 459 and 571 BU. Flour of Imdad had an SB viscosity of 641 BU, exceptionally higher than that of all other cultivars.

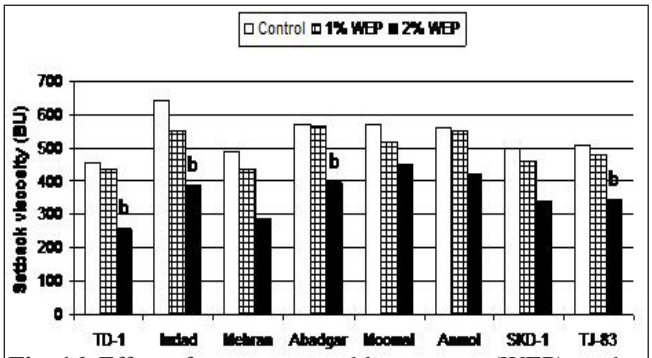

Fig. 16. Effect of water-extractable pentosan (WEP) on the setback viscosity of the flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

The addition of water-extractable pentosan (WEP) reduced the SB viscosity of all cultivar flours (Fig. 16). Because WEP is hydrophilic in nature, bound water molecules may interact with solubilized amylose chains

and curtail the tendency of the amylose chains to reassociate. Based on low firmness values, previous reports indicate that pentosan reduces starch retrogradation and bread staling (Kim & D'Appolonia 1997 a, b; Jankiewicz and Michniewicz 1987). The addition of 1% WEP did not significantly reduce SB values; a significant reduction was found in TD-1, Imdad, Abadgar, and TJ-83 at 2% WEP.

Effect of WEP concentration. The concentration of WEP did influence the magnitude of the reduction in SB values. The extent of reduction was higher at 2% WEP compared to 1% (Fig. 17). The reduction in SB values was found to vary from cultivar to cultivar at same level of WEP supplementation. At a 1% concentration level, the reduction in setback value varied between 2% and 14%. The reduction degree increased with increasing pentosan concentration (2% level) and the setback values reduced up to 44%. Further decreases in setback viscosity were possibly because WEP reduces the amount of starch components available for crystallization. The maximum reduction in setback value was found in the flour of TD-1, followed by Mehran and Imdad. Differences in flour composition may have causede this, because flour components, including gluten, pentosans, and lipids, are involved in bread staling (Martin et al. 1991). The flours used in this study were found to have different compositions and differences in setback values were found among the cultivars. Moreover, setback viscosity largely depends on the amylose content of starch. Variation in the amylose-to-amylopectin ratio also could be responsible for the differences in setback viscosities of flour and their subsequent interaction with WEP.

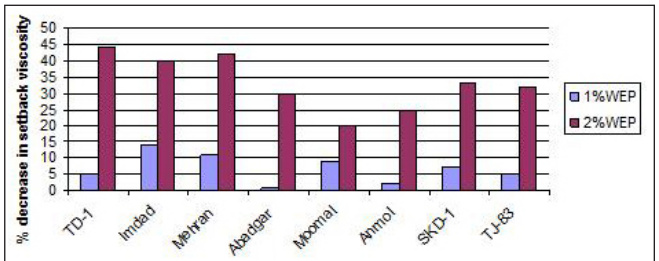

Fig. 17. Percent decrease in setback viscosity of the flour from different wheat cultivars after the addition of water-extractable pentosan (WEP).

Varietal differences in setback viscosity of WEP-substituted flours. No statistically significant differences were found among the 1% and 2% WEP-substituted flours. We found a narrow range in the setback viscosity (438–567) in 1% WEP-substituted flours (Fig. 18). The least retrograded flours were those of TD-1 and Mehran and the highest setback viscosities were found in the flour of Abadgar. All setback viscosities of the 2% WEP-substituted flours were between 258 and 454 BU. The least and most retrograded flours were in the flour of TD-1 and Moomal, respectively.

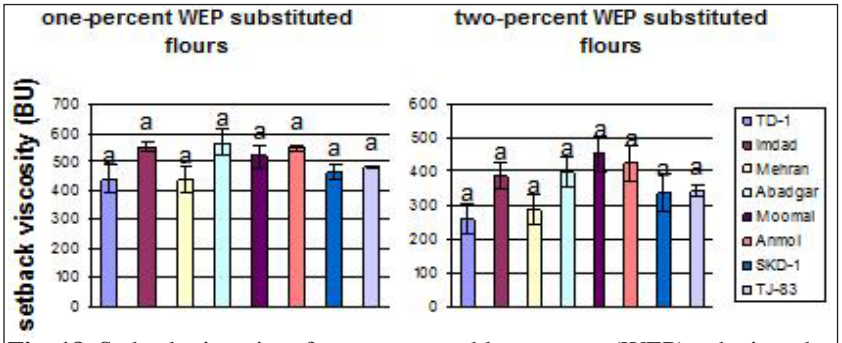

Fig. 18. Setback viscosity of water-extractable pentosan (WEP)-substituted flour from different wheat cultivars. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Relationship between setback viscosity and other pasting parameters in presence of WEP. The relationship between SB viscosity and other pasting parameters in WEP-substituted flours are given (Table 7). Setback value was positively related with cold past viscosity, hot paste viscosity, time to reach peak viscosity, and peak viscosity, but negatively correlated with breakdown viscosity. Setback did not relate with PT; SB viscosity related more with other pasting parameters in the presence of WEP.

Table 7. Relationship between setback viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown viscosity
Setback viscosity	0.061	0.331	0.530*	0.698**	0.883**	–0.264

References.

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Martin ML and Hoseney RC. 1991. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem 68:503-508.

Effect of water-extractable pentosan on time to reach peak viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Time to reach peak viscosity (TPV) is an indication of the time required for cooking. Flours of all the cultivars, except that of SKD-1, were found to require more than 15 min to reach their maximum viscosity. Flour of SKD-1 took 13:50 min to reach its maximum viscosity. The less time required to reach peak viscosity indicates that more energy is required to gelatinize starch granules, which ultimately decreases energy costs. Thus, SKD-1 flour may give a lower energy cost compared to the other flours.

Water-extractable pentosan (WEP) was found to be one of the significant sources of variation in the TPV of wheat flour. However, WEP did not induce a significant change in the TPV of all the cultivars tested (Fig. 19). The insignificant influence of WEP on peak viscosity confirmed the weak interference of WEP in the process of granule swelling, which may be the reason that the time needed by flour paste to gain the maximum number of swollen granules did not shift drastically with the addition of WEP.

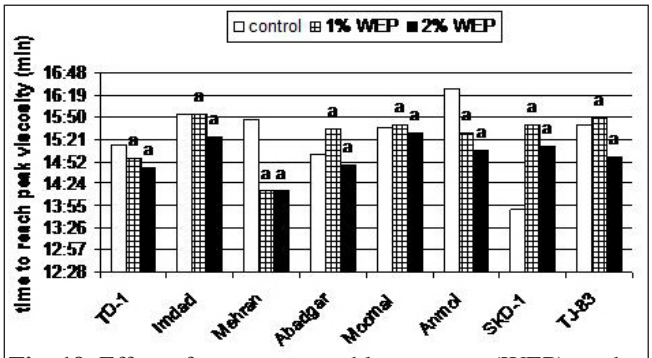

Fig. 19. Effect of water-extractable pentosan (WEP) on the time to reach peak viscosity of the flour from different hard white spring wheat cultivars. Bars labeled with an 'a' are not significantly different from the control at $P < 0.05$.

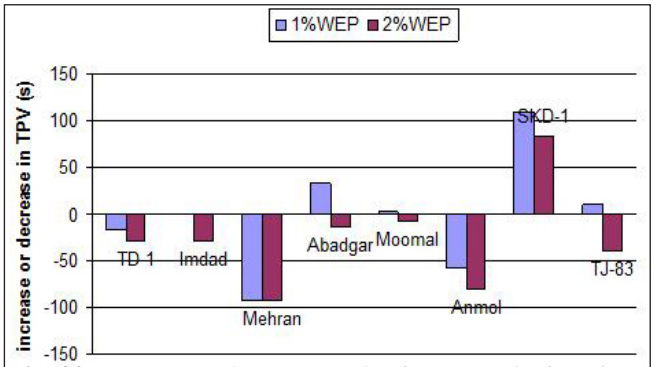

Fig. 20. Increase or decrease on the time to peak viscosity of the effect of water-extractable pentosan (WEP) on different hard white spring wheat flours.

Effect of WEP concentration. The difference between TPV (in seconds) in WEP-substituted flours (1% and 2%) and control flours is shown in Fig. 20. The addition of 1% and 2% WEP to flour induced similar effects, whether an increase or decrease) on the TPV of TD-1, Mehran, Anmol, and SKD-1. However, the difference in magnitude was not similar among all the cultivars except Mehran. At a 2% supplementation level, the TPV of all cultivars, except SKD-1, decreased from the control; the maximum reduction was found in the flour of Mehran.

Varietal differences in TPV of WEP substituted flours. The time for 1% WEP-substituted flours to reach their maximum viscosities ranged between 14:15 and 15:55 min (Fig. 21). The shortest time was in the flour of Mehran

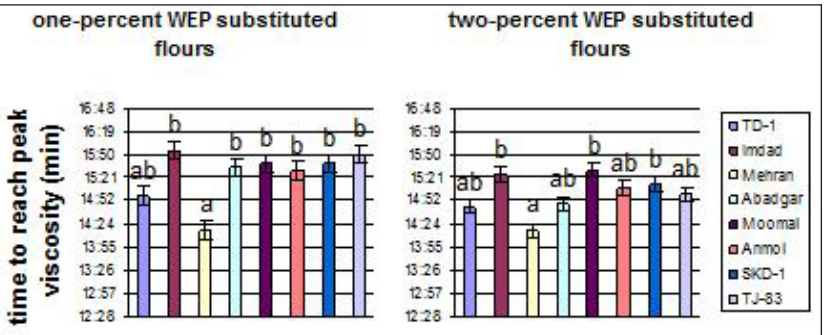

Fig. 21. Time to reach peak viscosity of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

and the longest in the flour of Imdad. At 2% WEP-substituted flours, the TPV for all cultivars was 14:15–15:30 min. The shortest time was in the flour of Mehran and the longest in the flour of Moomal.

Relationship between TPV and other pasting parameters in presence of WEP. WhenWEP is added to flour, the TPV was found to have a positive effect on all pasting parameters except breakdown (r = –0.052). The relationship between TPV with hot paste viscosity (r = 0.453**), cold paste viscosity (r = 0.517**), and setback (r = 0.532**) were statistically significant.

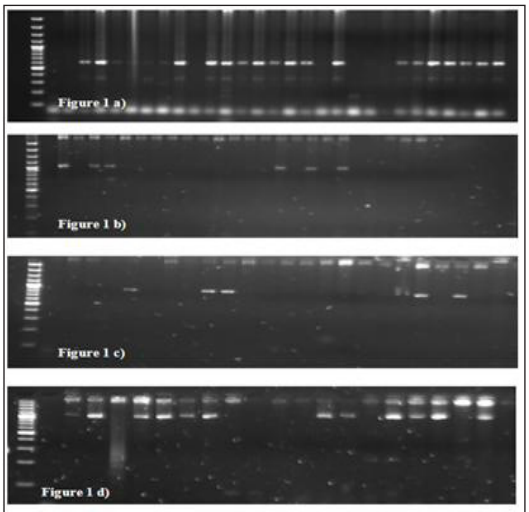

Fig. 22. Amplicons based on STS markers for low-molecular-weight glutenin subunit alleles, a) *Glu-A3c*, 573bp; b) *Glu-B3h*, 1022 bp; c) *Glu-B3g*, 853 bp; and d) *Glu-A3d*, 967bp.

Identification of allelic variation at the Glu-A3 and Glu-B3 loci using allele-specific PCR markers.

Mah-Jabeen Tariq, Kausar Nawaz Shah, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Glutenin proteins are the major factors responsible for the unique viscoelastic characteristics of wheat dough. They determine rheological properties and bread-making performance. The polymeric glutenin proteins, with molecular weights ranging from less than 300 to more than 1,000 kDa, are composed of two groups of subunits. These subunits include the low-molecular-weight glutenin subunits (LMW-GS), which are similar in size and structure to the γ- gliadins (30–40 kDa), and the high-molecular-weight glutenin subunits (HMW-GS), which range in molecular mass from ~65 to 90 kDa. The LMW-GS represent about one-third of the total seed protein and ~60% of the total glutenins, and are essential in determining dough properties, such as dough extensibility and gluten strength. Hence, characterizing allelic variation among cultivars and investigating their relationship with end-use quality has been a key area of research on quality improvement during the last 15 years and is the basis for the success of using specific LMW-GS alleles in breeding programs. Various methods are available for detecting LMW-GS in wheat, including SDS-PAGE, 2-D gel electrophoresis, MALDI-TOF-MS, and PCR-based identification of alleles. In this study, allele-specific, PCR markers developed by Wang et al. (2009; 2010) were used to characterize the LMW-GS composition in 27 wheat cultivars (Fig. 22). The presence of various alleles of *Glu-A3* and *Glu-B3* is given in Table 8.

Table 8. *Glu-A3* and *Glu-B3* alleles detected in wheat genotypes using STS markers.

Genotype	*Glu-A3*	*Glu-B3*
1x2-46	d	h
1x2-99	c	h
1x2-121	d	h
12x2-11	c	h
12x2-29	d	h
12x2-163	c	h
LLR-30	c	i
LLR-32	c	d
LLR-33	c	d
LLR-36	c	d
LLR-38	c	i
Bhakkar-2000	d	g
Chakwal-50	c	i
Miraj-2008	c	g
Abadghar-93	c	–
Seher-2006	c	–
Parvaz-94	d	–
Bhittai	d	–
Inqilab-91	g	–
Jauhar-78	g	–
Kirman	d	–
Zindad-2000	g	–
Shafaq	c	–
Faislabad-2008	c	g
Zamindar-80	d	i
Lasani	b	g
Pak-81	b	h

References.

Wang LH, Li GY, Peña RJ, Xia XC, and He ZH. 2010. Development of STS markers and establishment of multiplex PCR for *Glu-A3* alleles in common wheat (*Triticum aestivum* L.). J Cereal Sci 51:305-312.

Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, and Xia XC. 2009. Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theor Appl Genet 118:525-539.

Tolerance to boron toxicity in synthetic hexaploid wheats.

Mohammad Ilyas, Tariq Mahmood, Awais Rasheed, Alvina Gul-Kazi, and Abdul Mujeeb-Kazi.

Wheat is one of the most important food crops of the world, supporting 35% of the world population, but production is limited by several abiotic stress factors. Boron toxicity can cause serious loss in wheat production in different parts of the world. In synthetic hexaploid wheats (SH), the D-genome donor is one source for bringing in genetic variability in to common, hexaploid wheat. Forty-five SH wheats were screened for their tolerance to boron toxicity at seedling and adult stages. At the seedling stage, genotypes were screened in two different boron treatments; BO1 at 0.01M and BO2 at 0.025M along with a control using filter paper. Root growth suppression was expressed as a percent of the control was used as the selection criterion for tolerance to boron toxicity. The RGS % varied between 15.61% for SH-113 and 87.45% for SH-447. In addition to the RGS %, shoot growth suppression (SGS), expressed as a percent of the control, also was measured and varied between 1.84% for SH-117 and 94.74% for SH-385. The SGS % data further confirmed the results from the RGS % data for tolerant genotypes. At the adult-plant stage, genotypes were screened in a soil-assay experiment. Two different boron treatments, BO1 (25 mg/kg) and BO2 (50 mg/kg), along with control (no boron), were used and symptom data were taken 45 days after sowing. Using RGS %, SGS %, and symptom data, the plants were divided into four groups, tolerant, moderately tolerant, moderately susceptible, and susceptible. Genetic diversity for the respective genotypes was calculated using 10 SSR primers specific to 7D chromosome. The data was further subjected to cluster analysis using the NTSYS program. Simple sequence repeat primers amplified a total of 38 alleles with an average of 3.8 allele/primer. The polymorphism information content value was calculated and ranged from 0.34 (BARC214) to 0.69 (BARC53) with an average of 0.56. The nutritional quality of the respective genotypes was assessed by calculating iron (Fe) and Zinc (Zn) content for tolerant genotypes using an atomic absorption spectrophotometer, in order to use tolerant genotypes for breeding purposes without compromising on nutritional quality. A high level of variability was found among genotypes in response to different boron treatments at the seedling stage, adult-plant stage, and the molecular level. We concluded that genotypes SH-505, SH-380, SH-117, SH-131, SH-361, and SH-618 have the best potential for tolerance to boron toxicity that can be used in breeding program.

High-molecular-weight glutenin subunit composition of synthetic hexaploids derived from the durum wheat cultivar Decoy.

Madiha Khalid, Tariq Mahmood, Awais Rasheed, Alvina Gul-Kazi, and Abdul Mujeeb-Kazi.

Characterizing high-molecular-weight glutenin subunits is a fundamental approach for categorizing genotypes with good bread-making quality. Allelic variation at the *Glu-D^{1}1* locus is major determinant of end-use quality of bread wheat. In synthetic hexaploids (SHs), the D genome encodes numerous allelic variants of HMW-GS that require appropriate identification prior to their exploitation for bread wheat improvement. This study was conducted to identify allelic variation at *Glu-D^{1}1* loci of 47 accessions of D-genome SHs derived from crossing durum wheat Decoy with different accessions of *Ae. tauschii*. Biochemical (SDS-PAGE) and molecular-marker techniques were used to identify allelic variation at *Glu-D^{1}1* locus (Table 9, p. 147). Ten different alleles at *Glu-D^{1}1* were observed, which formed 16 different subunit combinations. The frequency of inferior quality encoding allele, 1Dx2+1Dy12, is low (19.14%) compared to the frequency of superior quality encoding allele, 1Dx5+1Dy10, (21.27%). A large allelic diversity was observed at *Glu-D^{1}1* with improved frequency of occurrence. This attribute makes the SHs able to be utilized for improving bread-making quality. Codominant molecular markers were used to validate the *Glu-A1c* (null), *Glu-D1d* (1Dx5+1Dy10), *Glu-D1a* (1Dx2+1Dy12), and *Glu-D1-1g* (1Dx2.1) alleles. The high number of glutenin subunits observed in the SHs indicated narrow genetic base for the D-genome-encoding glutenin subunits in bread wheat, which can be broaden by using synthetic hexaploids and inferior alleles in the D genome can be replaced with other better allelic variants from *Glu-D1* locus of SHs that are inherited from *Ae. tauschii*.

Table 9. Allelic identification and nomenclature of *Glu-D1* alleles in 47 sunthetic hexaploid (SH) wheats derived from Decoy durum wheat.

SH line	*Glu-D1*	Alleles
SH-6	2.1 + 10.5	*Glu-D1ai*
SH-43	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-115	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-117	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-123	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-128	1.5 + 10	*Glu-D1ah*
SH-129	1.5 + 12/10	*Glu-D1ah/Glu-D1aj*
SH-131	5 + 10	*Glu-D1d*
SH-302	3/4 + 10	*Glu-D1z/Glu-D1ac*
SH-323	3 + 12.2	*Glu-D1y*
SH-326	2 + 12.2	*Glu-D1x*
SH-330	2 + 12.2	*Glu-D1x*
SH-341	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-349	5 + 10	*Glu-D1d*
SH-361	3 + 10	*Glu-D1z*
SH-363	2 + 10	*Glu-D1e*
SH-373	2 + 12	*Glu-D1a*
SH-377	2 + 12	*Glu-D1a*
SH-380	2.1 + 10.5	*Glu-D1ai*
SH-381	5 + 10	*Glu-D1d*
SH-385	5 + 10	*Glu-D1d*
SH-395	5 + 10	*Glu-D1d*
SH-396	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-398	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-401	2/2.1 + 12.2	*Glu-D1x/Glu-D1ae*
SH-403	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-409	2.1 + 10.5	*Glu-D1-ai*
SH-422	5 + 10	*Glu-D1d*
SH-424	2 + 12	*Glu-D1a*
SH-426	5 + 10	*Glu-D1d*
SH-434	2 + 12	*Glu-D1a*
SH-441	5 + 10	*Glu-D1d*
SH-444	2 + 12	*Glu-D1a*
SH-447	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-500	1.5 + 10	*Glu-D1ah*
SH-505	3 + 12.2	*Glu-D1y*
SH-509	3 + 12.2	*Glu-D1y*
SH-618	1.5 + 12.2	*Glu-D1ag*
SH-638	2 + 12	*Glu-D1a*
SH-648	2 + 12	*Glu-D1a*
SH-649	1.5 + 12	*Glu-D1aj*
SH-672	2 + 12	*Glu-D1a*
SH-675	1.5 + 12	*Glu-D1aj*
SH-677	2 + 12	*Glu-D1a*
SH-678	2/2.1 + 12	*Glu-D1a/Glu-D1-1g + Glu-D1-2a*
SH-852	5 + 10	*Glu-D1d*
SH-907	5 + 10	*Glu-D1d*

Optimizing a PCR marker-based technique to identify low-molecular-wheat s in wheat.

Awais Rasheed, Tariq Mahmood, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Characterizing allelic variation among cultivars and investigating their relationships with end-use quality has been a key area of research for quality improvement during the last 15 years and is the basis for the success of using specific low-molecular-weight glutenin subunit (LMW-GS) alleles in breeding programs. The genes coding for LMW-GS are located on the short arms of homoeologous group-1 chromosomes at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci. Identification of LMW glutenins through SDS-PAGE does not allow differentiating several of alleles with certainty. On the other hand, 2-D gel electrophoresis is not generally recommended for use in breeding programs, due to its time consuming procedure, high costs, and skill requirements. Recently, a simple, rapid, and sensitive PCR approach has proven to be a very useful tool for identifying LMW-GS composition in common wheat. The PCR markers developed by Wang et al. (2010) for the *Glu-A3* alleles and by Wang et al. (2009) for the *Glu-B3* alleles were optimized in Wheat Wide Crosses and Cytogenetics Lab for their further utilization in identification of alleles in breeding material (Fig. 23, p. 148). The list of cultivars optimized as standards based on earlier literature is given in Table 10.

Table 10. Cultivars used as standards to optimize a marker-assisted selection strategy for *Glu-A3* and *Glu-B3* alleles.

Locus	Allele	Cultivar
Glu-A3	*a*	Chinese Spring
	b	Pavon, King Bird
	c	Waxwing
	d	Pastor
	e	Kiriati
	f	—
	g	Inquilab-91
Glu-B3	*a*	Chinese Spring
	b	Pitic
	c	—
	d	—
	e	—
	f	—
	g	PRL*2/Paston
	h	Pavon, King Bird
	i	Kiriati

References.

Wang LH, Li GY, Peña RJ, Xia XC, and He ZH. 2010. Development of STS markers and establishment of multiplex PCR for *Glu-A3* alleles in common wheat (*Triticum aestivum* L.). J Cereal Sci 51:305-312.

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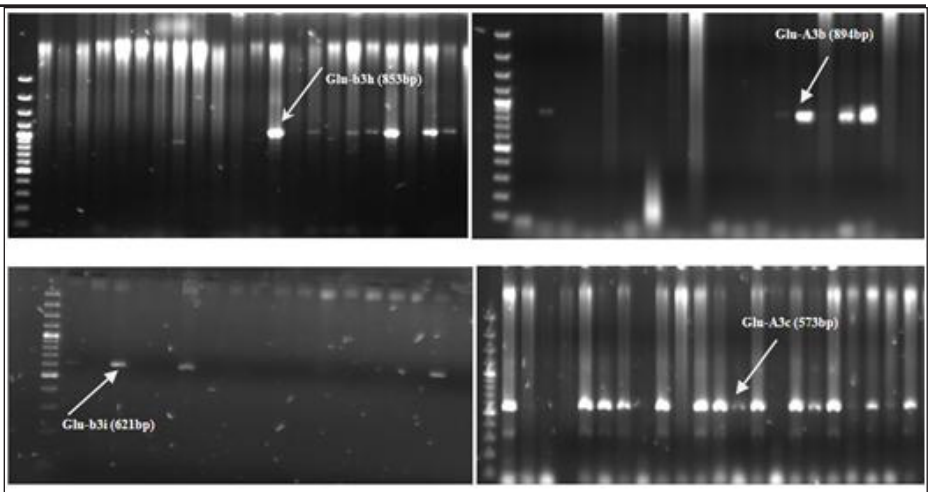

Fig. 23. Identification *Glu-3* alleles in wheat genotypes using allele specific markers for *Glu-B3h*, *Glu-A3b*, *Glu-B3i*, and *Glu-A3c*.

Drought tolerance studies in diverse wheats involving land races, local cultivars, and novel exotic germ plasm.

Misbah Sehar, Ghulam Shabbir, Sami Ullah, Jalal-Ud-Din, Alvina Gul Kazi, Awais Rasheed, Hadi Bux, and Abdul Mujeeb-Kazi.

Breeding for drought tolerance has been recognized as an important target in Pakistan's wheat improvement because, out of a total cultivated area of 20.9 mha, 4.8 mha (24.4%) is rainfed. To utilize this area efficiently and get high yields, the main emphasis is to develop drought-tolerant wheat cultivars that are able to survive under conditions of limited water. To complement the conventional variability, other options have been identified globally and also within Pakistan, including land races of wheat and species within the Triticeae gene pools. Around this material, conventional wheats, land races, and the progenitor species, we tried to identify sources of for drought, a key abiotic stress drought that leads to improving our wheat cultivars through recombination breeding. The evaluation parameters involved *in vitro* and *in vivo* studies conducted under laboratory, controlled screen houses, and field conditions, and the best drought-tolerant cultivars were identified.

The research material was comprised of 51 entries in three categories; local land races (29), local cultivars (12), and exotic germ plasm (10 genotypes) (Table 11, p. 148-149). This germ plasm was studied and investigated for drought tolerance under *in vivo* and *in vitro* conditions at the National Agriculture Research Centre (NARC), Islamabad, during the November 2009–May 2010 crop cycle. *In vivo* parameters were days-to-heading, days-to-maturity, plant height, spike length, number of grains/spike, and 1,000-kernel weight. *In vitro* parameters on wheat seedlings were proline content, chlorophyll content, protein content, sugar content and superoxide dismutase (SOD) content. For statistical analysis of all field and laboratory data, analysis of variance (ANOVA) was computed by using MINITAB software, and the treatment means were compared by Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) test at a probability level of 0.05 by using MSTATC software.

Table 11. List of selected local Landraces and cultivars of Pakistan and exotic germ plasm (synthetic derivatives) used in the drought studies.

ID	Parent/pedigree	ID	Parent/pedigree
Landraces		**Local cultivars**	
Landrace 1	T1 (*T. durum* subsp. *durum*)	Inqilab 91	
Landrace 2	T2 (*T. durum* subsp. *durum*)	Baviacora	
Landrace 3	T3 (*T. durum* subsp. *durum*)	Opata M85	
Landrace 5	T5 (*T. aestivum* subsp. *sphaeorcoccum*)	Suleman 96	
Landrace 7	T7 (*T. aestivum* subsp. *sphaeorcoccum*)	Sitta	
Landrace 8	T8 (*T. aestivum* subsp. *aestivum*)	Weebill	

Table 11. List of selected local Landraces and cultivars of Pakistan and exotic germ plasm (synthetic derivatives) used in the drought studies.

ID	Parent/pedigree	ID	Parent/pedigree
Landraces		**Local cultivars**	
Landrace 9	T9 (*T. aestivum* subsp. *aestivum*)	Nesser	
Landrace 12	T12 (*T. aestivum* subsp. *aestivum*)	Dharwar	
Landrace 14	T14 (*T. aestivum* subsp. *aestivum*)	Zarghoon	
Landrace 15	T15 (*T. aestivum* subsp. *aestivum*)	Chakwal 86	
Landrace 16	T16 (*T. aestivum* subsp. *aestivum*)	Margalla 99	
Landrace 17	T17 (*T. aestivum* subsp. *aestivum*)	Marwat	
Landrace 18	T18 (*T. aestivum* subsp. *aestivum*)	**Exotic germ plasm**	
Landrace 20	T20 (*T. aestivum* subsp. *aestivum*)	F4 719	SH DR#45/Sehar
Landrace 24	T24 (*T. aestivum* subsp. *aestivum*)	F4 786	S.RIC-62/NR-26
Landrace 26	8A (Selection)	F4 826	DR.MP.1-95/NN(L)R1-4
Landrace 27	D-9 (Barani Selection)	F4 834	L.SEQ.15/N(N)17R1
Landrace 28	C-217 (C-516/C-591)	F4 841	S.RIC-10/NN(L)R2-48
Landrace 29	C-288 (Hard Federation/9D)	F4 883	DR.MP.2-26/NNR1-2
Landrace 30	C-245	F4 922	S.RIC-75/Wafaq
Landrace 31	C-247	F4 925	S.RIC-51/Pastor 68
Landrace 32	C-248 (Lr28, 14A)	F4 1992	F1460 Seq.3/Seq.4-36//Wafaq
Landrace 33	C-250 (Hard Federation/9D) (Lr30, 14A)	F4 2011	F1484 Seq.4-78/IBWSN152//NN(L) R1-8
Landrace 34	C-256 (Lr10, 23, 30)		
Landrace 35	C-258		
Landrace 36	C-269 (Lr2a, 18)		
Landrace 37	C-271(C-220/IP165)		
Landrace 39	C-288		
Landrace 40	C-518 (T9/8A)		

Significant differences were obtained between all field parameters, days-to-heading, days-to-maturity, plant height, spike length, number of grains/spike, and 1,000-kernel weight at $P < 0.05$ for interaction between the control and drought stress of different wheat genotypes along with the genotype, treatment, and replication.

Days to heading significantly decreased in most wheat genotypes under stress conditions compared to the control but increased significantly in other genotypes. In drought conditions, the percent reduction in late-heading genotypes was 0.8% in landrace 15, 5.6% in Chakwal 86, 7.0% in Inqilab-91, 11.0% in landrace 11, and 5.0% in landrace 3 compared to the control (Table 12, p. 150). In the early-heading genotypes, the percent reduction was 1.8% in F4 834, 3.5% in landrace 37, 6.0% in landrace 35, 6.8% in landrace 34, 8.0% in F4 826, and 10.0% in F4 786, compared to the control (Table 12, p. 150). Various studies have already revealed that drought stress decreases the number of days to heading, as we also observed in most wheat genotypes, and this decrease is due to water stress. Heading days increased in F4 841, F4 883, F4 925, F4 2011, and landrace 27 compared to the control. Early heading genotypes also may give higher yields. In our study, genotype F4 2011 had increased heading days and high yield compared to other genotypes.

Days to maturity significantly decreased under stress conditions compared to the control in most wheat genotypes except a few in which it significantly increased. Maximum days to maturity were reduced in landrace 15 (4.8%), Inqilab-91 (9.5%), Baviacora (11.3%), and Opata M 85 (10.2%) under drought condition compared to the control. The minimum number of days to maturity were reductions of 12.0% in Nesser, 12.3% in landrace 33, 16.0% in landrace 39, and 13.8% in landrace 20 compared to control (Table 13, p. 150). Studies on drought stress revealed that it reducs the days to maturity in different wheat cultivars, in sunflower, and in common bean cultivars. Some genotypes had reduction in maturity days were F4 826, F4 1992, and F4 719, whereas late maturity or increase days compared to control were F4 2011, F4 925, F4 922, F4 883, F4 841, and F4 834. These genotypes differed in their response for days to maturity may be because of the different genetic makeup in different wheat cultivars.

The maximum plant height reduction was observed in line F4 2011 (3.9%), F4 841 (7.9%), and landrace 34 (11.4%) under drought condition in comparison to control. Minimum plant height reductions were observed in Opata M 85 (10.3%), Baviacora (16.0%), and Chakwal 86 (38.6%) compared to the control (Table 14, p. 151). These results are supported by other studies indicating that plant height significantly decreased under water stress and is significantly effects wheat cultivars differently.

Table 12. Means for days-to-heading of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 1.610, LSD (0.05) of genotypes (G) = 1.138, and LSD (0.05) of treatments (T) = 0.224, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	112.0 PQ	111.0 QR	111.5 TU	Landrace 7	119.0 IJ	115.0 MN	117.0 JKL
F4 786	119.0 IJ	107.0 UV	113.0 RS	Landrace 8	118.0 JK	116.0 LM	117.0 JKL
F4 826	114.0 NO	104.0 W	109.0 WX	Landrace 9	123.0 F	115.0 MN	119.0 FGH
F4 834	111.0 QR	109.0 ST	110.0 VX	Landrace 12	118.0 JK	113.0 OP	115.5 MNO
F4 841	107.0 UV	110.0 RS	108.5 XY	Landrace 14	120.0 HI	116.0 LM	118.0 HIJ
F4 883	104.0 W	111.0 QR	107.5 Y	Landrace 15	122.0 FG	121.0 GH	121.5 DE
F4 922	115.0 MN	113.0 OP	114.0 PQR	Landrace 16	117.0 KL	112.0 PQ	114.5 OPQ
F4 925	106.0 V	111.0 QR	108.5 XY	Landrace 17	123.0 F	116.0 LM	119.5 FG
F4 1992	115.0 MN	110.0 RS	112.5 ST	Landrace 18	119.0 IJ	117.0 KL	118.0 HIJ
F4 2011	111.0 QR	114.0 NO	112.5 ST	Landrace 20	119.0 IJ	112.0 PQ	115.5 MNO
Inqilab-91	127.0 D	118.0 JK	122.5 CD	Landrace 24	121.0 GH	115.0 MN	118.0 HIJ
Baviacora	120.0 HI	110.0 RS	115.0 NOP	Landrace 26	120.0 HI	117.0 KL	118.5 GHI
Opata M 85	125.0 E	115.0 MN	120.0 F	Landrace 27	115.0 MN	117.0 KL	116.0 LMN
Sitta	122.0 FG	111.0 QR	116.5 KLM	Landrace 28	115.0 MN	112.0 PQ	113.5 QRS
Suleman 96	123.0 F	113.0 OP	118.0 HIJ	Landrace 29	118.0 JK	111.0 QR	114.5 OPQ
Weebill	125.0 E	114.0 NO	119.5 FG	Landrace 30	120.0 HI	116.0 LM	118.0 HIJ
Nesser	122.0 FG	112.0 PQ	117.0 J-L	Landrace 31	118.0 JK	117.0 KL	117.5 IJK
Dharwar	125.0 E	115.0 MN	120.0 F	Landrace 32	122.0 FG	115.0 MN	118.5 GHI
Zarghoon	128.0 D	117.0 KL	122.5 CD	Landrace 33	117.0 KL	111.0 QR	114.0 PQR
Chakwal 86	125.0 E	118.0 JK	121.5 DE	Landrace 34	117.0 KL	109.0 ST	113.0 RS
Margalla 99	128.0 D	110.0 RS	119.0 FGH	Landrace 35	115.0 MN	108.0 TU	111.5 TU
Marwat	127.0 D	116.0 LM	121.2 E	Landrace 36	119.00 IJ	113.00 OP	116.0 LMN
Landrace 1	134.0 B	118.0 JK	126.0 B	Landrace 37	113.0 OP	109.0 ST	111.0 UV
Landrace 2	130.0 C	117.0 KL	123.5 C	Landrace 39	117.0 KL	110.0 RS	113.5 QRS
Landrace 3	143.0 A	121.0 GH	132.0 A	Landrace 40	121.0 GH	112.0 PQ	116.5 KLM
Landrace 5	134.0 B	116.0 LM	125.0 B	Mean	119.9 A	113.4 B	

Table 13. Means for days-to-maturity of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 2.540, LSD (0.05) of genotypes (G) = 1.796, and LSD (0.05) of treatments (T) = 0.353, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	143.6 S-X	143.0 T-Z	143.3 RS	Landrace 7	164.0 B-D	140.3 Y-f	152.1 F-K
F4 786	146.0 Q-T	141.6 W-c	143.8 RS	Landrace 8	164.6 BC	143.6 S-X	154.1 B-F
F4 826	141.3 W-d	141.3 W-d	141.3 TU	Landrace 9	163.6 B-E	144.0 S-X	153.8 B-G
F4 834	140.3 Y-f	141.0 X-e	140.6 U	Landrace 12	158.0 I-M	140.0 Z-g	149.0 M-P
F4 841	139.0 b-h	143.0 T-Z	141.0 U	Landrace 14	160.0 G-K	142.0 N-b	151.0 I-M
F4 883	141.0 X-e	145.3 R-V	143.1 R-T	Landrace 15	161.3 D-H	148.3 PQ	154.8 B-D
F4 922	143.3 S-Y	150.3 P	146.8 Q	Landrace 16	162.3 B-H	138.0 e-h	150.1 K-N
F4 925	138.6 c-h	145.6 Q-U	142.1 S-U	Landrace 17	157.3 J-N	138.6 c-h	148.0 O-Q
F4 1992	150.3 P	150.3 P	150.3 J-N	Landrace 18	163.6 B-E	144.0 S-X	153.8 B-G
F4 2011	142.6 U-Z	153.3 O	148.0 O-Q	Landrace 20	159.3 H-L	137.3 f-i	148.3 N-Q
Inqilab-91	163.3 B-F	147.6 P-R	155.5 AB	Landrace 24	162.3 B-H	141.6 W-c	152.0 G-K
Baviacora	165.0 B	146.3 Q-S	155.6 AB	Landrace 26	160.3 F-J	143.0 T-Z	151.6 H-L
Opata M 85	162.6 B-G	146.0 Q-T	154.3 B-E	Landrace 27	157.0 K-N	143.3 S-Y	150.1 K-N
Sitta	160.6 E-I	145.3 R-V	153.0 D-I	Landrace 28	158.3 I-M	140.0 Z-g	149.1 M-P
Suleman 96	163.3 B-F	142.0 W-b	152.6 E-I	Landrace 29	159.3 H-L	141.3 W-d	150.3 J-N
Weebill	163.6 B-E	141.0 X-e	152.3 E-J	Landrace 30	158.3 I-M	142.3 V-a	150.3 J-N
Nesser	154.6 NO	135.0 i	144.8 R	Landrace 31	155.3 M-O	140.3 Y-f	147.8 O-Q
Dharwar	161.6 C-H	139.0 b-h	150.3 J-N	Landrace 32	156.0 M-O	140.0 Z-g	148.0 O-Q
Zarghoon	164.0 B-D	142.6 U-Z	153.3 C-H	Landrace 33	156.3 L-N	137.0 ghi	146.6 Q
Chakwal 86	169.0 A	141.6 W-c	155.3 A-C	Landrace 34	159.3 H-L	139.3 a-h	149.3 M-P
Margalla 99	167.6 A	142.6 U-Z	155.1 A-C	Landrace 35	158.0 I-M	138.3 d-h	148.1 N-Q
Marwat	160.6 E-I	142.6 U-Z	151.6 H-L	Landrace 36	157.0 K-N	142.6 U-Z	149.8 L-O
Landrace 1	169.0 A	142.6 U-Z	155.8 AB	Landrace 37	155.6 M-O	139.3 a-h	147.5 PQ
Landrace 2	163.6 B-E	144.3 S-W	154.0 B-G	Landrace 39	162.3 B-H	136.3 hi	149.3 M-P
Landrace 3	169.6 A	144.3 S-W	157.0 A	Landrace 40	158.3 I-M	138.3 d-h	148.3 N-Q

Table 14. Means for plant height of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 3.720, LSD (0.05) of genotypes (G) = 2.631, and LSD (0.05) of treatments (T) = 0.517, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	90.21 K-N	81.35 R-U	85.78 E-I	Landrace 7	82.75 Q-T	61.77 jkl	72.26 U
F4 786	80.51 S-V	80.48 S-V	80.49 M-Q	Landrace 8	92.36 I-M	70.12 a-e	81.24 K-O
F4 826	84.95 P-S	73.59 X-b	79.27 O-R	Landrace 9	96.53 E-I	62.13 i-l	79.33 O-R
F4 834	91.46 J-M	80.31 S-V	85.89 E-I	Landrace 12	93.56 H-L	72.80 Y-c	83.18 I-N
F4 841	91.57 J-M	84.25 P-S	87.91 B-E	Landrace 14	93.19 H-L	70.61 a-e	81.90 J-O
F4 883	81.30 R-U	80.92 S-V	81.11 L-P	Landrace 15	97.53 D-H	71.22 Z-d	84.38 F-K
F4 922	79.43 T-N	74.46 X-a	76.95 RS	Landrace 16	100.93 B-E	80.89 S-V	90.92 AB
F4 925	98.62 C-G	81.49 R-U	90.06 A-C	Landrace 17	101.29 B-D	75.03 N-Z	88.16 B-E
F4 1992	85.79 O-R	77.88 U-X	81.83 J-O	Landrace 18	106.02 A	77.98 U-X	92.00 A
F4 2011	90.21 K-N	86.61 N-Q	88.41 B-E	Landrace 20	97.12 D-H	75.91 W	86.51 D-H
Inqilab-91	89.45 L-O	63.75 h-l	76.59 RS	Landrace 24	86.30 N-Q	65.34 f-j	75.82 ST
Baviacora	70.85 a-e	59.45 lm	65.15 VW	Landrace 26	94.78 G-K	71.47 Z-d	83.13 I-N
Opata M 85	66.53 e-i	59.66 lm	63.09 W	Landrace 27	93.06 H-L	70.18 a-e	81.62 J-O
Sitta	91.77 J-M	72.19 Y-c	81.98 J-O	Landrace 28	102.33 BC	64.38 g-k	83.35 I-M
Suleman 96	70.47 a-e	60.62 kl	65.55 VW	Landrace 29	104.63 AB	74.35 X-a	89.49 A-D
Weebill	69.50 b-f	61.21 jkl	65.35 VW	Landrace 30	101.79 B-D	73.03 X-b	87.41 C-F
Nesser	71.38 Z-d	59.86 lm	65.62 VW	Landrace 31	100.32 B-E	70.83 a-e	85.57 E-I
Dharwar	71.98 Z-d	62.81 i-l	67.39 V	Landrace 32	99.11 C-F	66.51 e-i	82.81 I-N
Zarghoon	69.84 b-f	60.48 kl	65.16 VW	Landrace 33	94.72 G-K	73.37 X-b	84.05 G-L
Chakwal 86	90.43 K-N	55.44 m	72.94 U	Landrace 34	95.73 F-J	84.73 P-S	90.23 A-C
Margalla 99	99.25 C-F	68.25 c-g	83.75 H-L	Landrace 35	92.93 I-M	63.39 h-l	78.16 P-S
Marwat	84.47 P-S	70.73 a-e	77.60 Q-S	Landrace 36	83.72 Q-T	80.71 S-V	82.23 J-O
Landrace 1	76.27 V-Y	70.66 a-e	73.47 TU	Landrace 37	92.52 I-M	76.75 V-Y	84.64 F-J
Landrace 2	88.19 M-P	72.14 Y-c	80.16 N-Q	Landrace 39	93.95 H-L	79.85 T-W	86.91 D-G
Landrace 3	72.86 Y-c	72.80 Y-c	72.83 U	Landrace 40	101.35 B-D	79.24 T-W	90.30 A-C
Landrace 5	77.28 U-X	67.17 d-h	72.23 U	Mean	88.88 A	71.60 B	

Table 15. Means for spike length of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 0.826, LSD (0.05) of genotypes (G) = 0.584, and LSD (0.05) of treatments (T) = 0.114, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	12.23 B-D	11.22 E-J	11.72 C	Landrace 7	7.73 i-l	5.63 m	6.68 X
F4 786	10.28 I-Q	10.03 L-T	10.16 J-M	Landrace 8	10.13 K-S	8.28 a-j	9.20 O-T
F4 826	10.40 I-P	8.87 W-g	9.63 M-R	Landrace 9	10.28 I-Q	7.23 kl	8.76 S-V
F4 834	12.20 B-E	9.50 P-Y	10.85 F-I	Landrace 12	10.39 I-P	7.95 f-l	9.17 O-T
F4 841	11.06 G-L	9.88 N-W	10.47 G-K	Landrace 14	9.19 R-b	8.42 a-j	8.81 S-V
F4 883	10.69 G-O	9.03 T-e	9.85 K-P	Landrace 15	11.03 G-L	9.79 O-X	10.41 H-L
F4 922	10.14 K-S	9.55 P-Y	9.85 K-P	Landrace 16	10.21 I-R	7.93 g-l	9.07 Q-T
F4 925	11.51 D-H	8.00 e-l	9.76 L-Q	Landrace 17	12.14 B-E	11.24 E-I	11.69 CD
F4 1992	12.72 A-C	8.58 Y-i	10.65 F-J	Landrace 18	10.90 G-N	9.59 P-Y	10.25 I-M
F4 2011	13.48 A	11.59 D-H	12.54 B	Landrace 20	12.09 B-E	10.11 E-I	11.11 C-G
Inqilab-91	13.42 A	11.68 D-G	12.55 B	Landrace 24	10.98 G-N	8.21 b-k	9.59 M-R
Baviacora	9.53 P-Y	7.81 h-l	8.67 T-V	Landrace 26	8.39 a-j	8.10 d-l	8.23 V
Opata M 85	10.20 J-R	9.54 P-Y	9.87 K-O	Landrace 27	11.04 G-L	7.73 i-l	9.38 N-S
Sitta	13.00 A-C	9.05 T-d	11.03 D-H	Landrace 28	10.09 K-S	7.21 kl	8.65 T-V
Suleman 96	9.60 P-Y	8.97 U-f	9.28 N-T	Landrace 29	10.64 H-O	9.21 R-b	9.93 K-N
Weebill	12.16 B-E	10.30 I-Q	11.23 C-F	Landrace 30	10.35 I-P	7.60 i-l	8.98 R-U
Nesser	12.02 C-F	9.98 M-U	11.01 E-H	Landrace 31	10.68 H-O	8.94 V-g	9.81 K-P
Dharwar	10.86 G-N	10.35 I-P	10.61 F-J	Landrace 32	7.58 i-l	7.42 jkl	7.50 W
Zarghoon	12.30 B-D	9.95 M-V	11.13 C-G	Landrace 33	9.21 R-b	8.34 a-j	8.78 S-V
Chakwal 86	13.06 AB	10.18 K-R	11.62 C-E	Landrace 34	9.28 Q-a	9.00 T-e	9.14 P-T
Margalla 99	11.06 G-L	10.23 I-R	10.65 F-J	Landrace 35	11.08 F-K	8.14 b-l	9.61 M-R
Marwat	13.48 A	13.36 A	13.42 A	Landrace 36	9.13 S-c	8.34 a-j	8.74 S-V
Landrace 1	9.47 P-Z	8.93 V-g	9.20 O-T	Landrace 37	9.91 N-V	8.78 X-h	9.35 N-T
Landrace 2	8.37 a-j	8.36 a-j	8.37 UV	Landrace 39	9.59 P-Y	9.21 R-b	9.41 N-S
Landrace 3	9.87 N-W	9.51 P-Y	9.69 M-Q	Landrace 40	8.47 Z-i	8.07 d-l	8.27 V
Landrace 5	7.14 l	5.37 m	6.25 X	Mean	10.56 A	9.07 B	

Table 16. Means for grains/spike of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 1.308, LSD (0.05) of genotypes (G) = 0.925, and LSD (0.05) of treatments (T) = 0.182, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	52.33 MN	50.67 O	51.50 E-G	Landrace 7	51.33 NO	50.67 O	51.00 FG
F4 786	51.00 NO	36.67 V	43.83 K	Landrace 8	51.33 NO	36.67 V	44.00 K
F4 826	46.33 Q	40.33 T	43.33 K	Landrace 9	52.33 MN	24.67 ef	38.50 O
F4 834	53.33 LM	42.33 S	47.83 I	Landrace 12	62.33 F	11.67 k	37.00 P
F4 841	57.67 HI	48.67 P	53.17 D	Landrace 14	47.33 PQ	25.67 de	36.50 PQ
F4 883	39.67 TU	29.67 Za	34.67 R	Landrace 15	57.33 HI	24.67 ef	41.00 L
F4 922	52.67 MN	43.33 RS	48.00 I	Landrace 16	52.00 MN	28.33 ab	40.17 LM
F4 925	58.33 GH	36.67 V	47.50 IJ	Landrace 17	69.67 B	23.67 f	46.67 J
F4 1992	56.33 IJ	44.67 R	50.50 G	Landrace 18	70.33 A	32.33 Y	51.33 FG
F4 2011	67.67 C	56.33 IJ	62.00 A	Landrace 20	57.67 HI	16.67 ij	37.17 P
Inqilab-91	51.33 NO	35.67 VW	43.50 K	Landrace 24	51.67 NO	30.67 Z	41.17 L
Baviacora	38.67 U	26.67 cd	32.67 S	Landrace 26	61.33 F	36.67 V	49.00 H
Opata M 85	51.33 NO	34.67 WX	43.00 K	Landrace 27	59.67 G	27.67 bc	43.67 K
Sitta	58.33 GH	42.67 S	50.50 G	Landrace 28	57.33 HI	21.67 g	39.50 MN
Suleman 96	65.33 DE	54.33 KL	59.83 B	Landrace 29	64.67 E	38.33 U	51.50 EFG
Weebill	56.33 IJ	47.67 PQ	52.00 EF	Landrace 30	52.33 MN	28.67 ab	40.50 LM
Nesser	40.33 P	15.67 j	28.00 U	Landrace 31	52.33 MN	33.67 XY	43.00 K
Dharwar	40.33 P	19.67 h	30.00 T	Landrace 32	52.33 MN	28.67 ab	40.50 LM
Zarghoon	55.33 JK	48.67 P	52.00 EF	Landrace 33	53.33 LM	32.67 Y	43.00 K
Chakwal 86	61.00 F	51.67 NO	56.33 C	Landrace 34	50.33 O	35.67 VW	43.00 K
Margalla 99	65.00 DE	52.67 N	58.83 B	Landrace 35	47.67 PQ	15.67 j	31.67 T
Marwat	61.00 F	50.67 O	55.83 C	Landrace 36	61.67 F	33.67 XY	47.67 I
Landrace 1	29.33 Za	8.67 l	19.00 W	Landrace 37	66.33 CD	38.67 U	52.50 DE
Landrace 2	54.33 KL	17.67 i	36.00 Q	Landrace 39	47.67 PQ	26.67 cd	37.17 P
Landrace 3	38.67 U	15.00 j	26.83 V	Landrace 40	51.67 NO	26.67 cd	39.17 NO
Landrace 5	43.33 RS	26.67 cd	35.00 R	Mean	54.34 A	33.71 B	

Table 17. Means for 1,000-kernel weight of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 0.455, LSD (0.05) of genotypes (G) = 0.322, and LSD (0.05) of treatments (T) = 0.063, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	30.72 R	29.24 S	29.98 TU	Landrace 7	33.02 O	20.11 a	26.56 b
F4 786	30.58 R	29.81 S	30.19 ST	Landrace 8	31.28 Q	27.24 U	29.31 WX
F4 826	34.24 N	30.30 R	32.27 O	Landrace 9	32.01 P	27.72 U	29.87 TU
F4 834	45.99 D	24.19 X	35.09 J	Landrace 12	25.72 W	24.84 X	25.28 e
F4 841	50.18 B	28.11 T	39.15 C	Landrace 14	31.18 Q	24.91 X	28.05 Z
F4 883	51.83 A	31.63 Q	41.74 A	Landrace 15	26.00 V	24.19 X	25.09 e
F4 922	35.22 M	30.41 R	32.81 MN	Landrace 16	38.16 J	26.97 V	32.56 NO
F4 925	43.40 E	31.08 Q	37.24 F	Landrace 17	30.05 R	19.24 b	24.65 f
F4 1992	41.00 G	33.20 O	37.10 F	Landrace 18	31.54 Q	22.47 Z	27.00 a
F4 2011	39.61 I	36.98 L	38.29 D	Landrace 20	32.43 P	19.64 b	26.04 d
Inqilab-91	34.16 N	25.25 W	29.70 UV	Landrace 24	30.01 R	27.52 U	28.77 Y
Baviacora	41.00 G	26.24 V	33.62 K	Landrace 26	30.34 R	27.69 U	29.02 XY
Opata M 85	33.36 O	28.31 T	30.84 R	Landrace 27	26.43 V	26.01 V	26.22 cd
Sitta	36.39 L	31.05 Q	33.72 K	Landrace 28	31.11 Q	25.54 W	28.32 Z
Suleman 96	32.87 P	25.19 W	29.03 XY	Landrace 29	37.45 K	26.25 V	31.85 P
Weebill	32.02 P	24.41 X	28.22 Z	Landrace 30	36.16 L	35.26 M	35.71 I
Nesser	41.09 G	31.51 Q	36.29 GH	Landrace 31	30.58 R	30.26 R	30.42 S
Dharwar	40.15 H	35.60 M	37.88 E	Landrace 32	30.41 R	26.99 V	28.69 Y
Zarghoon	41.44 G	23.11 Y	32.28 O	Landrace 33	40.13 H	25.74 W	32.93 LM
Chakwal 86	48.03 C	24.23 X	36.13 H	Landrace 34	31.23 Q	27.76 U	29.49 VW
Margalla 99	48.52 C	33.45 O	36.48 G	Landrace 35	35.37 M	19.26 b	27.31 a
Marwat	40.01 H	32.08 P	36.05 H	Landrace 36	31.13 Q	25.36 W	28.25 Z
Landrace 1	41.11 G	38.53 J	39.82 B	Landrace 37	35.01 M	31.30 Q	33.16 L
Landrace 2	32.33 P	30.03 R	31.18 Q	Landrace 39	42.16 F	27.67 U	34.92 J
Landrace 3	40.71 H	37.69 K	39.19 C	Landrace 40	28.43 T	24.62 X	26.52 bc
Landrace 5	35.46 M	28.43 T	31.94 P	Mean	35.63 A	27.99 B	

The maximum spike length was found in genotypes Marwat (13/36 cm, 0.8% reduction compared to the control), Inqilab-91 (11.68 cm, 12.9% reduction), and F4 2011 (11.59 cm, 14.0% reduction) under drought conditions. The minimum spike length was observed in landraces 5 (24.0% reduction) and 7 (27.0% reduction) compared to the control. No significant difference in the spike length of Marwat and landrace 2 compared to the control (Table 15, p. 151).

The maximum number of grains/spike was observed in landrace 7 (1.3% reduction from the control) and F4 719 (3.1% reduction). Lines Chakwal 86 (15.2% reduction), F4 2011 (16.7% reduction), Suleman 96 (16.8% reduction), Margalla 99 (18.9% reduction), Marwat (16.9% reduction) under drought conditions. The greatest reduction was in landraces 1, 3, 12, 20, and 35 and in the cultivar Nesser (Table 16, p. 152).

Under drought conditions, the maximum 1000-kernel weight was observed in landrace 30 (2.4% reduction from the control) followed by landrace 1 (6.2% reduction), F4 2011 (6.6% reduction), landrace 3 (7.4% reduction), Dharwar99, Margalla 99 (15.3% reduction), F4 1992 (19.0% reduction) and Marwat (19.8% reduction) . The minimum grain weights were observed in landraces 7, 15, 17, 18, 20, and 35 (Table 17, p. 152).

The *in vitro* parameters proline, chlorophyll, protein, sugar, and SOD content revealed significantly different results at $P < 0.05$ for genotypes, the treatments, and the interaction between genotype and treatment. The amount of proline, protein, sugar, and SOD content significantly increased under drought conditions in all wheat genotypes compared to the control; chlorophyll content significantly decreased under drought stress.

The highest proline content in drought conditions was in genotype F4 922 (a 96.1% over the control), followed by F4 1992 (91.6% increase), F4 719 (89.8% increase), F4 826 (86.1% increase), F4 2011 (78.9% increase), Marwat (61.3% increase), Margalla 99 (59.8% increase), and Suleman 96 (59.3% increase). The lowest proline content was observed in landraces 15, 20, and 35 (Table 18). Plants accumulate different amounts of proline in response to abiotic stress, which protect the plant by reducing the oxidative damage created by osmotic stress. This proline accumulation may create tolerance and protect the plant from oxidative stress.

Table 18. Means for proline content of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 640.3, LSD (0.05) of genotypes (G) = 452.8, and LSD (0.05) of treatments (T) = 78.9, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	297.1 Z-i	2,925.0 AB	1,611.0 A-C	Landrace 7	88.8 ghi	655.5 S-i	372.1 OP
F4 786	288.4 Z-i	1,847.0 E-L	1,067.7 E-J	Landrace 8	431.1 X-i	686.0 S-i	558.6 L-P
F4 826	323.4 Y-i	2,330.6 B-F	1,327.0 B-E	Landrace 9	85.9 ghi	1,213.4 K-V	649.6 J-P
F4 834	227.2 c-i	1,360.5 J-T	793.8 F-O	Landrace 12	1,009.4 M-Z	1,423.1 I-R	1,216.3 C-G
F4 841	171.9 d-i	1,876.1 E-K	1,024.0 E-L	Landrace 14	1,053.1 M-X	1,335.7 J-T	1,194.4 C-G
F4 883	263.6 a-i	1,669.3 F-N	966.5 E-M	Landrace 15	256.3 b-i	419.5 X-i	337.9 OP
F4 922	118.0 f-i	3,054.6 A	1,586.3 A-D	Landrace 16	552.0 V-i	1,491.6 H-Q	1,021.8 E-L
F4 925	486.5 W-i	2,053.9 D-I	1,270.2 C-F	Landrace 17	103.4 ghi	419.5 X-i	289.8 P
F4 1992	183.5 d-i	2,206.8 C-G	1,195.1 C-G	Landrace 18	471.9 W-i	1,360.5 J-T	916.2 E-N
F4 2011	640.9 T-i	3,041.5 A	1,841.2 A	Landrace 20	224.3 c-i	418.0 X-i	321.1 OP
Inqilab-91	938.1 O-c	1,194.4 K-W	1,066.2 E-J	Landrace 24	756.0 R-i	1,707.2 F-M	1,231.6 C-F
Baviacora	834.6 P-f	1,535.3 G-P	1,185.0 C-H	Landrace 26	186.4 d-i	954.1 O-b	570.3 K-P
Opata M 85	719.6 R-i	1,621.2 G-O	1,170.4 C-I	Landrace 27	131.1 f-i	747.2 R-i	439.1 N-P
Sitta	145.6 e-i	5,95.7 U-i	370.7 OP	Landrace 28	74.3 hi	866.7 P-e	470.5 N-P
Suleman 96	1,009.4 M-Z	2,482.1 A-E	1,745.8 AB	Landrace 29	69.9 hi	598.6 U-i	334.3 OP
Weebill	303.0 Y-i	782.2 Q-h	542.6 L-P	Landrace 30	809.9 Q-g	1,136.2 M-X	973.0 E-M
Nesser	225.7 c-i	1,997.0 D-J	1,111.4 D-J	Landrace 31	151.5 e-i	1,166.7 L-W	659.1 J-P
Dharwar	100.5 ghi	678.8 S-i	389.6 OP	Landrace 32	578.3 U-i	1,691.1 F-M	1,134.7 C-J
Zarghoon	948.2 O-b	1,159.5 L-W	1,053.8 E-K	Landrace 33	604.5 U-i	1,698.4 F-M	1,151.4 C-I
Chakwal 86	212.6 d-i	1,171.1 L-W	691.9 I-P	Landrace 34	37.8 i	1,331.3 J-T	684.6 I-P
Margalla 99	1,089.6 M-X	2,713.7 A-C	1,901.6 A	Landrace 35	160.2 e-i	419.5 X-i	289.8 P
Marwat	1021.1 M-Y	2,639.4 A-D	1830.3 A	Landrace 36	319.0 Y-i	1076.4 M-X	697.7 H-P
Landrace 1	174.8 d-i	895.8 P-d	535.3 M-P	Landrace 37	568.1 U-i	2166.0 C-H	1367.0 B-E
Landrace 2	1286.2 K-U	1871.8 E-K	1579.0 A-D	Landrace 39	474.9 N-i	980.3 N-a	727.6 G-P
Landrace 3	85.9 ghi	642.3 T-i	364.1 OP	Landrace 40	677.3 S-i	1369.2 J-S	1023.3 E-L
Landrace 5	1160.9 L-W	1187.1 K-W	1174.0 C-I	Mean	453.6 B	1431.1 A	

Chlorophyll content significantly decreased in all wheat genotypes under drought conditions compared to the control. The maximum chlorophyll content under drought was observed in landrace 40 (a 35.7% reduction from the control) followed by Baviacora (18.1% reduction), landrace 39 (14.2% reduction), landrace 37 (14.1% reduction), and Marwat (0.9% reduction). The minimum chlorophyll content was observed in lines F4 834, F4 719, and F4 786 (Table 19).

Table 19. Means for chlorophyll content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 0.3387, LSD (0.05) of genotypes (G) = 0.2395, and LSD (0.05) of treatments (T) = 0.0471, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	0.62 O-b	0.19 c	0.41 U-X	Landrace 7	0.42 V-c	0.41 W-c	0.41 U-X
F4 786	0.43 U-c	0.19 c	0.31 X	Landrace 8	0.55 P-c	0.36 Y-c	0.45 S-X
F4 826	0.52 R-c	0.26 a-c	0.39 V-X	Landrace 9	0.41 W-c	0.32 Z-c	0.36 WX
F4 834	0.43 U-c	0.18 d	0.31 X	Landrace 12	1.17 B-M	0.81 I-W	0.99 C-L
F4 841	0.61 O-c	0.31 Z-c	0.46 S-X	Landrace 14	1.09 C-N	0.96 F-R	1.03 B-J
F4 883	0.55 P-c	0.31 Z-c	0.43 T-X	Landrace 15	0.97 E-R	0.59 P-c	0.78 I-Q
F4 922	0.57 P-c	0.37 X-c	0.47 R-X	Landrace 16	0.93 F-S	0.87 G-T	0.90 D-O
F4 925	0.54 Q-c	0.20 bc	0.37 WX	Landrace 17	1.08 C-N	0.85 H-V	0.97 C-M
F4 1992	0.82 I-W	0.57 P-c	0.70 L-T	Landrace 18	1.22 A-I	0.77 K-Y	0.99 C-L
F4 2011	1.06 C-N	0.83 I-W	0.95 C-N	Landrace 20	1.39 A-E	0.86 G-U	1.12 A-F
Inqilab-91	0.89 G-T	0.67 N-a	0.78 I-Q	Landrace 24	1.28 A-H	0.51 S-c	0.90 D-O
Baviacora	1.21 A-J	0.99 D-P	1.10 A-G	Landrace 26	0.66 N-a	0.58 P-c	0.62 O-W
Opata M 85	1.29 A-G	0.82 I-W	1.06 B-I	Landrace 27	0.93 F-S	0.76 L-Y	0.84 F-P
Sitta	0.90 G-T	0.74 M-Z	0.82 G-P	Landrace 28	1.03 D-O	0.71 N-Z	0.87 E-P
Suleman 96	1.22 A-I	0.77 K-Y	0.99 C-L	Landrace 29	1.19 B-L	0.72 N-Z	0.95 C-N
Weebill	1.06 C-N	0.76 L-Y	0.91 D-O	Landrace 30	1.61 A	0.68 N-a	1.14 A-E
Nesser	1.35 A-F	0.85 H-V	1.10 A-G	Landrace 31	0.74 M-Z	0.72 N-Z	0.73 K-S
Dharwar	0.89 G-T	0.56 P-c	0.73 K-S	Landrace 32	0.93 F-S	0.65 N-a	0.79 H-Q
Zarghoon	0.69 N-a	0.66 N-a	0.67 N-V	Landrace 33	1.51 AB	0.82 I-W	1.17 A-D
Chakwal 86	1.09 C-N	0.92 G-S	1.01 C-K	Landrace 34	0.99 D-P	0.72 N-Z	0.86 E-P
Margalla 99	0.73 N-Z	0.66 N-a	0.70 L-T	Landrace 35	1.35 A-F	0.71 N-Z	1.03 B-J
Marwat	1.08 C-N	1.07 C-N	1.08 B-H	Landrace 36	0.89 G-T	0.62 O-b	0.75 J-R
Landrace 1	0.97 E-R	0.78 J-Y	0.87 E-P	Landrace 37	1.48 A-C	1.27 A-H	1.37 A
Landrace 2	0.98 D-Q	0.66 N-a	0.82 G-P	Landrace 39	1.40 A-D	1.20 A-K	1.30 AB
Landrace 3	0.55 P-c	0.47 T-c	0.51 Q-X	Landrace 40	1.51 AB	0.97 E-R	1.24 A-C
Landrace 5	0.85 H-V	0.35 Y-c	0.60 P-W	Mean	0.95 A	0.65 B	

Highest protein content was observed in Landrace 31 followed by landrace 27 (68.7% increase), F4 2011 (48.0% increase), F4 922 (22% increase), and F4 719 (7.5% increase) under drought condition compared to the control. The lowest content was observed in landrace 26, followed by landraces 34 and 28 (Table 20, p. 155). Different studies have revealed that drought-resistant cultivars of wheat a specific type of protein, along with heat-shock protein, increased in a stress environment, which we observed also. Proteins probably function by protecting cells from dehydration, such as the enzymes required for the biosynthesis of various osmoprotectants and detoxification enzymes.

Under drought conditions, the highest sugar content was observed in F4 922 (51.6% increase over the control) followed by F4 1992 (48.2 % increase), F4 925 (47.6% increase), Marwat (41.1% increase), Margalla 99 (45.7% increase), F4 826 (41.7% increase), and F4 719 (38.4% increase). The lowest sugar contents were observed in landraces 20 and 40 (Table 21, p. 155). The complex, essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, and energy production. Under drought stress conditions, even sugar flux may be a signal for metabolic regulation.

The maximum SOD content was observed in Marwat followed by Margalla 99, F4 2011, F4 719, F4 922, F4 826 and F4 1992 with 24, 11.6, 2.4, 24, 21.7, 4.4 and 10.9 percent increase respectively under drought condition in comparison to control while minimum SOD content was observed in Landrace 20, Landrace 35 (Table 22, p. 156). Antioxidative enzymes (e.g., superoxide dismutase, catalase and ascorbic peroxidases) have been found to be related with water deficiency and considered as the main components of anti-oxidative machinery for drought resistance in higher plants. These antioxidants efficiently scavenge active oxygen species and prevent damaging effects of free radicals. Thus an antioxidant system was induced in drought stress in different wheat varieties as observed in this study.

Table 20. Means for protein content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 258.5, LSD (0.05) of genotypes (G) = 182.8, and LSD (0.05) of treatments (T) = 35.9, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	1,143.1 D-P	1,473.8 A-D	1,308.5 A-F	Landrace 7	1,203.3 B-P	1,229.5 B-P	1,216.4 A-G
F4 786	1,222.0 B-P	1,379.4 B-L	1,300.7 A-F	Landrace 8	1,153.0 C-P	1,160.2 C-P	1,156.6 C-H
F4 826	1,303.5 B-M	1,424.9 B-I	1,364.2 A-D	Landrace 9	1,207.8 B-P	1,326.9 B-L	1,267.4 A-F
F4 834	1,104.4 H-Q	1,157.0 C-P	1,130.7 D-I	Landrace 12	1,210.0 B-P	1,270.9 B-O	1,240.4 A-G
F4 841	1,050.4 L-Q	1,102.2 H-Q	1,076.3 F-I	Landrace 14	921.1 P-S	1,395.3 B-K	1,158.2 C-H
F4 883	1,072.5 J-Q	1,335.6 B-L	1,204.1 A-G	Landrace 15	1,114.8 G-P	1,163.7 C-P	1,139.2 D-I
F4 922	1,302.7 B-M	1,461.2 B-E	1,381.9 A-C	Landrace 16	1,191.2 C-P	1,358.9 B-L	1,275.1 A-F
F4 925	1,051.4 L-Q	1,224.1 B-P	1,137.7 D-I	Landrace 17	1,200.3 B-P	1,372.5 B-L	1,286.4 A-F
F4 1992	1,213.4 B-P	1,416.1 B-I	1,314.8 A-E	Landrace 18	1,240.4 B-P	1,359.7 B-L	1,300.0 A-F
F4 2011	1,374.9 B-L	1,487.4 A-C	1,431.2 A	Landrace 20	1,145.0 D-P	1,305.7 B-M	1,225.3 A-G
Inqilab-91	1,265.8 B-O	1,342.0 B-L	1,303.9 A-F	Landrace 24	1,064.0 K-Q	1,234.0 B-P	1,149.0 C-H
Baviacora	1,224.1 B-P	1,275.7 B-N	1,249.9 A-F	Landrace 26	355.7 V	668.8 R-U	512.2 L
Opata M 85	1,251.9 B-P	1,358.1 B-L	1,304.9 A-F	Landrace 27	790.4 Q-T	1,535.1 AB	1,162.7 B-H
Sitta	568.4 T-V	942.3 N-S	755.4 JK	Landrace 28	459.0 UV	729.6 R-U	594.3 KL
Suleman 96	1,327.8 B-L	1,454.3 B-F	1,391.1 AB	Landrace 29	714.4 R-U	1,133.9 E-P	924.1 IJ
Weebill	1,307.0 B-M	1,378.1 B-L	1,342.5 A-E	Landrace 30	1,351.4 B-L	1,405.1 B-J	1,378.2 A-C
Nesser	1,279.2 B-M	1,336.1 B-L	1,307.7 A-F	Landrace 31	547.2 T-V	1,750.9 A	1,149.1 C-H
Dharwar	1,243.1 B-P	1,301.4 B-M	1,272.2 A-F	Landrace 32	1,257.8 B-O	1,261.3 B-O	1,259.5 A-F
Zarghoon	1,155.1 C-P	1,321.4 B-L	1,238.3 A-G	Landrace 33	1,230.8 B-P	1,330.8 B-L	1,280.8 A-F
Chakwal 86	938.9 O-S	975.2 M-R	957.1 HI	Landrace 34	662.7 S-U	717.4 R-U	690.0 KL
Margalla 99	1,349.8 B-L	1,450.8 B-G	1,400.3 A	Landrace 35	1,046.9 L-Q	1,404.8 B-J	1,225.9 A-G
Marwat	1,296.3 B-M	1,428.1 B-H	1,362.2 A-D	Landrace 36	1,138.6 D-P	1,179.2 C-P	1,158.9 B-H
Landrace 1	1,128.1 E-P	1,205.1 B-P	1,166.7 B-H	Landrace 37	1,269.8 B-O	1,452.7 B-F	1,361.3 A-D
Landrace 2	1,216.4 B-P	1,297.9 B-M	1,257.1 A-F	Landrace 39	1,136.2 D-P	1,166.4 C-P	1,151.3 C-H
Landrace 3	975.8 M-R	1,057.9 K-Q	1,016.8 G-I	Landrace 40	1,104.6 H-Q	1,120.1 F-P	1,112.4 E-I
Landrace 5	939.7 O-S	1,088.8 I-Q	1,014.3 G-I	Mean	1,098.4 B	1,268.7 A	

Table 21. Means for sugar content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 290.6, LSD (0.05) of genotypes (G) = 205.5, and LSD (0.05) of treatments (T) = 40.4, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	1,367.6 B-F	2,222.0 A	1,794.8 A	Landrace 7	614.3 O-f	640.8 N-f	627.6 K-R
F4 786	627.8 N-f	774.0 I-b	700.9 J-P	Landrace 8	657.8 N-f	742.5 J-e	700.2 J-P
F4 826	830.4 I-Y	1,424.8 B-D	1,127.6 C-E	Landrace 9	338.0 e-f	681.6 L-e	509.8 N-R
F4 834	726.2 J-d	745.0 J-d	735.6 I-O	Landrace 12	488.7 V-f	604.1 P-f	546.4 M-R
F4 841	712.0 K-e	1,350.8 B-F	1,031.4 D-G	Landrace 14	555.7 R-f	617.3 O-f	586.5 L-R
F4 883	641.1 N-f	765.5 I-c	703.3 J-P	Landrace 15	855.9 I-V	907.4 H-T	881.7 F-K
F4 922	999.3 G-N	2,065.6 A	1,532 5 B	Landrace 16	449.0 Z-f	548.9 R-f	499.0 O-R
F4 925	836.4 I-Y	1,597.4 B	1,216.9 CD	Landrace 17	1,023.1 F-M	1,039.1 F-L	1,031.1 D-G
F4 1992	824.7 I-Z	1,594.9 B	1,209.8 CD	Landrace 18	415.7 b-f	522.9 U-f	469.3 P-R
F4 2011	835.7 I-Y	1,396.6 B-E	1,116.1 C-F	Landrace 20	368.3 d-f	470.2 X-f	419.2 R
Inqilab-91	660.3 M-f	673.8 L-f	667.1 J-R	Landrace 24	672.0 L-f	693.5 L-e	682.8 J-Q
Baviacora	492.9 V-f	786.7 I-b	639.8 J-R	Landrace 26	666.8 L-f	717.3 J-d	692.0 J-Q
Opata M 85	450.7 Z-f	961.1 G-Q	705.9 J-P	Landrace 27	474.9 W-f	596.4 P-f	535.6 M-R
Sitta	534.9 T-f	594.4 P-f	564.6 L-R	Landrace 28	568.9 R-f	777.5 I-b	673.2 J-R
Suleman 96	873.2 I-U	1,402.3 B-E	1,137.8 CD	Landrace 29	618.3 O-f	689.6 L-e	653.9 J-R
Weebill	688.3 L-e	826.4 I-Z	757.4 H-N	Landrace 30	459.4 Y-f	632.8 N-f	5,46.1 M-R
Nesser	773.2 I-b	849.4 I-W	811.3 G-L	Landrace 31	589.3 Q-f	798.2 I-a	693.8 J-Q
Dharwar	861.9 I-U	923.9 H-R	892.9 E-J	Landrace 32	1,031.3 F-M	1,074.8 E-K	1,053.0 C-F
Zarghoon	707.3 K-e	915.6 H-S	811.5 G-L	Landrace 33	605.3 O-f	690.3 L-e	647.8 J-R
Chakwal 86	856.2 I-V	1,089.5 D-J	972.9 D-I	Landrace 34	725.3 J-d	809.9 I-Z	767.6 H-M
Margalla 99	810.0 I-Z	1,493.5 B-C	1,151.8 CD	Landrace 35	555.1 R-f	635.3 N-f	595.2 L-R
Marwat	954.6 H-R	1,621.2 B	1,287.9 C	Landrace 36	982.3 G-O	1,236.9 C-H	1,109.6 C-F
Landrace 1	838.0 I-X	1,120.0 D-I	979.0 D-H	Landrace 37	970.9 G-P	1,307.1 B-G	1,139.0 CD
Landrace 2	370.5 d-f	547.4 R-f	459.0 P-R	Landrace 39	430.2 a-f	534.4 T-f	482.3 O-R
Landrace 3	388.2 c-f	537.1 T-f	462.7 P-R	Landrace 40	409.2 b-f	472.7 a-f	441.0 QR
Landrace 5	302.5 f	539.6 S-f	421.1 R	Mean	676.3 B	926.7 A	

Table 22. Means for SOD contentof different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 8.624, LSD (0.05) of genotypes (G) = 6.098, and LSD (0.05) of treatments (T) = 1.200, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	30.13 C-T	39.69 B-D	34.91 B-D	Landrace 7	17.65 X-b	24.69 K-a	21.17 L-Q
F4 786	28.70 D-X	32.18 C-P	30.44 D-J	Landrace 8	18.87 U-b	20.80 Q-b	19.84 M-R
F4 826	37.24 B-H	38.96 B-F	38.10 A-C	Landrace 9	30.39 C-T	32.20 C-P	31.29 C-I
F4 834	21.81 N-b	26.10 I-Z	23.96 I-Q	Landrace 12	33.82 B-M	34.50 B-L	34.16 B-E
F4 841	26.94 H-Y	34.44 B-L	30.69 D-J	Landrace 14	21.25 O-b	29.43 D-V	25.34 G-P
F4 883	20.14 Q-b	25.30 J-a	22.72 K-Q	Landrace 15	20.83 Q-b	21.75 N-b	21.29 L-Q
F4 922	30.59 C-S	39.07 B-E	34.83 B-D	Landrace 16	27.57 G-Y	32.57 C-N	30.07 D-K
F4 925	20.85 Q-b	21.12 P-b	20.98 L-R	Landrace 17	19.75 S-b	19.83 R-b	19.79 M-R
F4 1992	32.47 C-N	36.48 B-I	34.48 B-E	Landrace 18	32.91 B-N	35.12 B-K	34.01 B-F
F4 2011	39.54 B-D	40.53 A-C	40.03 AB	Landrace 20	13.39 b	14.31 ab	13.85 R
Inqilab-91	25.41 I-a	27.87 G-Y	26.64 F-N	Landrace 24	28.41 E-X	36.44 B-J	32.43 C-G
Baviacora	32.11 C-P	32.59 C-N	32.35 C-G	Landrace 26	24.75 K-a	26.70 H-Y	25.72 G-O
Opata M 85	23.78 L-b	26.54 H-Y	25.16 G-P	Landrace 27	25.33 I-a	26.61 H-Y	25.97 G-O
Sitta	19.57 S-b	25.67 I-Z	22.62 K-Q	Landrace 28	16.74 Y-b	20.67 Q-b	18.70 O-R
Suleman 96	19.19 T-b	22.47 N-b	20.83 L-R	Landrace 29	27.31 G-Y	29.50 D-U	28.41 D-L
Weebill	18.26 V-b	20.22 Q-b	19.24 N-R	Landrace 30	23.86 L-b	25.33 I-a	24.60 H-P
Nesser	25.04 K-a	25.82 I-Z	25.43 G-P	Landrace 31	19.51 S-b	28.17 E-X	23.84 I-Q
Dharwar	28.98 D-W	29.12 D-V	29.05 D-K	Landrace 32	22.98 M-b	23.76 L-b	23.37 J-Q
Zarghoon	32.67 C-N	34.78 B-L	33.73 B-F	Landrace 33	17.89 W-b	18.80 U-b	18.35 O-R
Chakwal 86	31.08 C-Q	32.33 C-O	31.71 C-H	Landrace 34	24.76 K-a	29.28 D-V	27.02 E-M
Margalla 99	38.33 B-G	43.37 AB	40.85 AB	Landrace 35	14.98 Z-b	18.78 U-b	16.88 QR
Marwat	37.48 B-H	49.37 A	43.43 A	Landrace 36	25.06 K-a	30.98 C-R	28.02 D-L
Landrace 1	23.67 L-b	31.18 C-Q	27.42 D-M	Landrace 37	27.92 F-Y	34.82 B-L	31.37 C-I
Landrace 2	15.24 Z-b	20.55 Q-b	17.89 P-R	Landrace 39	24.55 K-a	26.83 H-Y	25.69 G-O
Landrace 3	17.49 X-b	27.94 F-Y	22.72 K-Q	Landrace 40	19.43 S-b	30.98 C-R	25.20 G-P
Landrace 5	22.02 N-b	29.37 D-V	25.70 G-O	Mean	25.07 B	29.13 A	

Genotypes that performed best under drought (rainout shelter), field, and laboratory conditions were F4 2011, F4 719, F4 922, Margalla 99, Marwat, F4 826, F4 1992, landrace 37, Suleman 96, F4 841, and F4 786. These lines gave high yield in both field and laboratory tests and exhibited a high amount of proline, protein, sugar, and SOD content. Thus, having better osmoregulation mechanisms to tolerate water-deficit conditions. These genotypes also provided wide genetic diversity, because they have diverse alleles compared to the local cultivars.

In vitro studies evaluating elite D-genome synthetic hexaploid wheat for drough tolerance.

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Abstract. Pakistan falls into arid and semi-arid regions of climatic conditions. Areas receiving a rainfall less than 100 to 250 mm to less than 500 mm are called semi-arid lands. These lands constitute about 88% of the country's total geographic area of 79.6 x 10^6 ha and are prone to occasional drought resulting in low or no production. Drought is one key constraint that influences our national wheat yield. For breeding targets to be achieved, unique diversity is paramount. Synthetic hexaploid (SH) wheats may provide this diversity. Earlier, international screening under reduced irrigation conditions led to the identification of 53 SH wheats. Twenty-seven of these SH wheats have been tested now under Pakistani conditions for the first time along with three standard, global, drought-tolerant check wheat cultivars. The germ plasm evaluation was done under *in vitro* conditions by giving a drought stress of 30% PEG to the SH wheats and checks. Their proline, protein, sugar, chlorophyll, and SOD content were assessed by comparing with the control lines. We observed that the degree of drought tolerance varied in all 27 SH wheats and thr checks, because of their genetic variability. SHDR 17, SHDR 18, SHDR 45, SHDR 46, SHDR 49 SHDR 50, SHDR 51, SHDR 52, and SHDR 53 were drought tolerant, whereas three lines, SHDR 29, SHDR 43, and SHDR 21, were sensitive.

Introduction. Drought stress remains an ever increasing problem that severely limits crop production by preventing crop plants from expressing their full genetic potential (Rampino et al. 2006; Karmer 1980) and can cause yield losses greater

than any other environmental factor, particularly in arid and semi-arid areas. Of the total area of Pakistan, 75% is arid and semi-arid with average temperature ranging between 2°C–50°C. An extensive, canal irrigation system was introduced for use in the vast agricultural area. Nevertheless, a large part of the country is still rain fed (Ashraf and McNeilly 1988).

Depending on the duration and extent of drought stress, a range of plant processes occurring at molecular, biochemical, cellular, and whole-plant levels may be altered. Plant adaptation to drought stress can involve avoidance mechanisms such as morphological changes in the roots and shoots. In addition to avoidance mechanisms, plant response to water shortage can involve changes in biochemical pathways and the expression of genes encoding proteins that contribute to drought adaptation. Such changes can bring about drought tolerance, whereby plants continue to function at the low water potentials caused by water deficit. Osmotic adjustment is one of the most effective physiological mechanisms underlying plant resistance to water deficit (Turner and Jones 1980; Morgan 1984; Blum 1988). With 50% of the world's arable land being arid or sensitive to drought, developing drought-tolerant wheat cultivars is an important aim of plant scientists (Ruivenkamp and Richards 1994) with better adaptation to water deficits and augmenting the productivity of rain fed wheat (Rajaram 2002).

Because of land limitations, enhanced wheat production must come from higher absolute yields, which can only be met by the concerted efforts in plant breeding (Braun et al. 1998). Synthetic hexaploid wheat is a relatively new germ plasm obtained by artificially crossing durum wheat, *Triticum turgidum* subsp. *durum* (2n = 4x = 28, AABB) and *Aegilops tauschii* (2n = 14, DD). This germ plasm has proven to be very useful as a source of resistance to diseases and pests, as well as for tolerance to environmental stresses (Gorham 1990; Limin and Fowler 1993). Synthetic hexaploids are routinely crossed and backcrossed with common wheat (2n = 6x = 42, AABBDD) to achieve acceptable agronomic types. In defining a strategy for wheat breeding under drought stress, Rajaram et al. (1996) suggested that simultaneous evaluation of the germ plasm should be carried out under both near-optimum conditions (to utilize high heritabilities and identify genotypes with high yield potential) and stress conditions (to preserve alleles for drought tolerance).

Table 23. Mean chlorophyll a (mmol/g fresh weight) and chlorophyll b (mmol/g fresh weight) content of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. Means with same letters in each column do not differ significantly.

Population	Chlorophyll a		Chlorophyll b	
	T_0	T_1	T_0	T_1
SHDR 5	3.183 A-G	2.546 A-M	0.977 B-J	0.778 C-K
SHDR 6	3.105 A-H	2.564 A-L	1.312 A-C	0.762 D-K
SHDR 7	3.382 AB	3.352 AB	1.342 AB	1.255 A-D
SHDR 9	2.611 A-L	3.354 AB	0.757 D-K	1.117 B-F
SHDR 11	2.297 D-N	2.992 A-J	0.632 E-K	0.939 B-K
SHDR 13	2.320 D-N	2.268 E-N	1.016 B-I	0.763 D-K
SHDR 14	3.266 A-E	2.220 F-N	1.338 AB	0.684 E-K
SHDR 16	3.227 A-F	2.432 B-N	1.036 B-H	0.753 D-K
SHDR 17	2.888 A-K	1.228 O-Q	0.921 B-K	0.374 K
SHDR 18	3.121 A-H	2.374 B-N	1.143 B-F	0.676 E-K
SHDR 19	3.384 AB	1.751 L-Q	1.086 B-G	0.643 E-K
SHDR 21	3.297 A-D	1.471 N-Q	1.177 B-E	0.521 G-K
SHDR 23	3.095 A-H	1.031 Q	0.934 B-K	0.452 I-K
SHDR 24	3.226 A-F	1.991 I-Q	0.905 B-K	0.682 E-K
SHDR 29	3.005 A-I	2.247 E-N	0.709 D-K	0.781 C-K
SHDR 32	2.946 A-J	1.823 L-Q	0.596 F-K	0.649 E-K
SH DR 33	3.210 A-G	1.984 J-Q	1.168 B-E	0.689 E-K
SHDR 37	3.113 A-H	2.049 I-O	0.959 B-J	0.705 D-K
SHDR 38	3.332 A-C	1.810 L-Q	0.765 D-K	0.661 E-K
SHDR 43	3.442 A	1.094 PQ	1.743 A	0.506 H-K
SHDR 45	1.897 K-Q	1.533 M-Q	0.534 G-K	0.440 JK
SHDR 46	2.374 B-N	2.259 E-N	0.688 E-K	0.661 E-K
SHDR 49	2.408 B-N	2.263 E-N	0.702 D-K	0.596 F-K
SHDR 50	2.562 A-L	1.977 J-Q	0.808 B-K	0.574 F-K
SHDR 51	2.203 G-O	2.407 B-N	0.618 E-K	0.761 D-K
SHDR 52	2.542 A-M	2.063 I-P	0.746 D-K	0.589 F-K
SHDR 53	2.340 C-N	2.079 I-O	0.703 D-K	0.609 E-K
Baviacora	2.324 C-N	2.270 E-N	0.676 E-K	0.728 D-K
Nesser	2.301 D-N	1.897 K-Q	0.673 E-K	0.529 G-K
Marwat	2.159 H-O	2.158 H-O	0.616 E-K	0.630 E-K
LSD 1%	0.822		0.453	

Results and discussion. According to Saadalla (2001), drought-tolerant genotypes of wheat showed higher chlorophyll a/b contents and dry weight than drought-susceptible genotypes. We found this assumption to be true as SHDR 9, SHDR 11, SHDR 51, SHDR 53, SHDR 52, SHDR 46, SHDR 45, SHDR 50, and

SHDR 9 showed a higher amount of chlorophyll a than the susceptible lines, and SHDR 9, SHDR 11, SHDR 29, SHDR 32, and Baviacora showed a higher chlorophyll b content after drought stress (Table 23, p. 157). Similarly, Nikolaeva et al. (2010) found that the chlorophyll content in wheat leaves at the beginning of a drought treatment (3 days) increased insignificantly with respect to that in untreated plants. As water limitation becomes more pronounced (5 days), the chlorophyll content decreased but the chlorophyll a/b ratio remained unchanged. According to Garg et al. (1998), chlorophyll a and b and chlorophyll a/b ratios are not affected by drought stress; we found similar results (Table 23, p. 157). In some lines, SHDR 5 (2.546 mmol/g fresh weight), SHDR 6 (2.564), SHDR 7 (3.352), SHDR 9 (3.354), SHDR 13 (2.268), SHDR 29 (2.247), SHDR 37 (2.049), SHDR 45 (1.533), SHDR 53 (2.079), and SHDR 52 (2.063), no significant change in chlorophyll content was found. The check lines, Baviacora, Marwat, and Nesser, also showed no significant change in chlorophyll content after drought stress. All these results contrast the assumption that photosynthesis is sensitive to heat and drought stresses and it is often the first process affected by stress. Photosynthetic activity was reduced by water stress and SHDR 14 (2.220 mmol/g fresh weight), SHDR 17 (1.228), SHDR 19 (1.751), SHDR 21 (1.471), SHDR 23 (1.031), SHDR 24 (1.991), SHDR 32 (1.823), SHDR 33 (1.984), SHDR 38 (1.810), and SHDR 43 (1.094) showed significantly low chlorophyll a contents under drought stress (Table 23). These results agree with those of Balouchi (2010), who found a marked reduction in chlorophyll and carotenoid content under water stress in all cultivars. Sairam et al. (1998) also found that total chlorophyll and carotenoid contents showed a decreasing trend with age, under both control and stress conditions.

The limitation of photosynthesis by water stress, especially when it is combined with conditions of high temperature and light, may cause photo-oxidative damage to the photosynthetic apparatus if the plant does not avoid or utilize the excess excitation energy (Asada 1999). However, if the photoprotective mechanisms are insufficient, the leaves are protected from stress-induced oxidative damage by several antioxidant systems (Foyar et al. 1994). Carotenoids, such as β-carotene, are key scavengers of reactive oxygen species, such as singlet oxygen, and so protect thylakoid membranes from oxidative damage (Young 1991). The carotenoids serve at least two important functions in photosynthesis, namely light harvesting and photoprotection and, hence, their comparative levels in a genotype will determine its relative tolerance. Lines SHDR 51 (1.152 mmol/g fresh weight), SHDR 6 (0.203), SHDR 7(0.2631), SHDR 11 (0.196), and SHDR 14 (0.175) showed an increase in carotenoid content after drought stress compared to control lines (Table 24). These results agree with those of Rahman et al. (2003), Kraus et al. (1995), and Sairam et al. (1998), who reported higher carotenoid levels in tolerant genotypes. The remaining 25 lines did not have any significant change in their carotenoid content. Price and Hendry (1991) also reported unchanged carotenoid concentration in wheat leaves under drought. Lines SHDR 24 (0.063 mmol/g fresh weight) and SHDR 29 (0.072 mmol/g fresh weight) were significantly lowest in their carotenoid content of all germ plasm tested, as confirmed by Balouchi (2010) and Tas and Tas (2007).

Table 24. Mean carotenoid (mmol/g fresh weight) content of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. LSD 1% = 0.09557 Means with same letters in each column do not differ significantly.

Population	Carotenoid content		Population	Carotenoid content	
	T_0	T_1		T_0	T_1
SHDR 5	0.181 A-K	0.167 A-K	SHDR 32	0.197 A-H	0.089 F-K
SHDR 6	0.142 B-K	0.203 A-G	SHDR 33	0.168 A-K	0.080 H-K
SHDR 7	0.152 A-K	0.263 A	SHDR 37	0.146 A-K	0.066 JK
SHDR 9	0.237 A-C	0.231 A-E	SHDR 38	0.144 A-K	0.101 F-K
SHDR 11	0.155 A-K	0.196 A-H	SHDR 43	0.075 I-K	0.064 JK
SHDR 13	0.113 D-K	0.112 E-K	SHDR 45	0.090 F-K	0.084 G-K
SHDR 14	0.102 F-K	0.175 A-K	SHDR 46	0.107 F-K	0.095 F-K
SHDR 16	0.184 A-J	0.179 A-K	SHDR 49	0.174 A-K	0.147 A-K
SHDR 17	0.172 A-K	0.123 C-K	SHDR 50	0.116 D-K	0.101 F-K
SHDR 18	0.232 A-D	0.205 A-F	SHDR 51	0.159 A-K	0.152 A-K
SHDR 19	0.203 A-G	0.140 B-K	SHDR 52	0.170 A-K	0.147 A-K
SHDR 21	0.155 A-K	0.070 JK	SHDR 53	0.173 A-K	0.124 C-K
SHDR 23	0.155 A-K	0.104 F-K	Baviacora	0.195 A-I	0.155 A-I
SHDR 24	0.248 AB	0.063 K	Nesser	0.164 A-K	0.125 C-K
SHDR 29	0.240 AC	0.072 JK	Marwat	0.174 A-K	0.110 F-K

One biochemical response to dehydrative stress is the accumulation of a family of proteins called dehydrins, which are believed to protect membranes and macromolecules against denaturation. Previous studies demonstrated the

Table 25. Mean protein content (mg/g fresh weight) of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. LSD 1% = 0.1352; means with the same letters in each column do not differ significantly.

Population	Protein content		Population	Protein content	
	T_0	T_1		T_0	T_1
SHDR 5	1.058 H-L	1.060 H-L	SHDR 32	1.067 H-L	1.063 H-L
SHDR 6	1.030 KL	1.053 I-L	SHDR 33	1.060 H-L	1.035 J-L
SHDR 7	1.061 H-L	1.069 H-L	SHDR 37	1.042 J-L	1.046 J-L
SHDR 9	1.048 J-L	1.067 H-L	SHDR 38	1.008 L	1.046 J-L
SHDR 11	1.063 H-L	1.059 H-L	SHDR 43	1.103 H-L	1.074 H-L
SHDR 13	1.078 H-L	1.079 H-L	SHDR 45	1.107 G-L	1.331 A-E
SHDR 14	1.093 H-L	1.101 H-L	SHDR 46	1.219 C-I	1.369 A-C
SHDR 16	0.999 L	1.042 J-L	SHDR 49	1.200 D-J	1.343 A-D
SHDR 17	0.992 L	1.065 H-L	SHDR 50	1.288 A-F	1.382 AB
SHDR 18	0.992 L	1.030 KL	SHDR 51	1.280 A-F	1.368 A-C
SHDR 19	1.076 H-L	1.069 H-L	SHDR 52	1.256 A-G	1.362 A-C
SHDR 21	1.063 H-L	1.061 H-L	SHDR 53	1.151 F-L	1.362 A-C
SHDR 23	1.051 J-L	1.078 H-L	Baviacora	1.292 A-F	1.397 A
SHDR 24	1.035 J-L	1.086 H-L	Nesser	1.225 B-H	1.308 A-E
SHDR 29	1.065 H-L	1.050 J-L	Marwat	1.178 E-K	1.376 A-C

accumulation of dehydrins in drought-stressed wheat (Lopez et al. 2003). These proteins have been assumed to function not as enzymes but to protect the plant cell during dehydration. Under drought conditions, all wheat lines accumulated soluble proteins except for SHDR 45 (1.331 mg/g fresh weight) and SHDR 53 (1.362), where the accumulation was highly significant (Table 25). Marwat (1.376), SHDR 46 (1.369), SHDR 49 (1.343), SHDR 52 (1.362), Baviacora (1.397), and SHDR 50 (1.382) had protein accumulation that was better than the control lines, and so can be considered as drought tolerant. Bensen et al. (1988) reported that drought stress resulted in an increase of some soluble proteins and a decrease of others. Lines SHDR 43, SHDR 29, SHDR 33, and SHDR 32 accumulated the lowest amount of soluble proteins and can be considered as sensitive.

Compatible solutes are overproduced under osmotic stress aiming to facilitate osmotic adjustment (Hasegawa et al. 2000; Shao et al. 2005; Zhu 2000). In this study, all germ plasm accumulated a significant amount of proline after stress (Fig. 24). The beneficial roles of proline in conferring osmotolerance have been widely reported (Bajji et al. 2000). Proline has been shown to have a key role in stabilizing cellular proteins and membranes in presence of high concentrations of osmoticum (Errabii et al. 2006). Tatar and Gevrek (2008) showed that wheat dry matter production, relative water content decreased and proline content increased under drought stress. Higher proline content in wheat plants after water stress was reported by Vendruscolo et al. (2007). Our results are similar; proline content in all wheat lines increased significantly (Fig. 24). Line SHDR 53 accumulated the maximum amount of proline (91%) during drought exceeding the check lines, whereas SHDR 18 (88%), Baviacora (85%), Nesser (64%), SHDR 32 (80%) SHDR 38 (88%), SHDR 45 (78%), SHDR 49 (83%), SHDR 50 (79%), SHDR 17 (72%), SHDR 9 (68%), SHDR 52 (64%), and SHDR 51 (67%) accumulated the highest proline content and can be considered as tolerant. However, Johari-Pireivatlou et al. (2010) also advocated that proline content increases significantly with stress compared to the control. The lowest proline accumulation was in SHDR 29 (23%) and SHDR 43 (38%); these lines can be considered as sensitive.

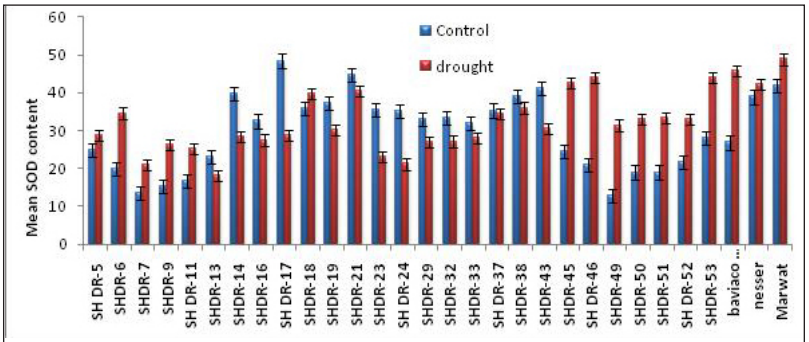

Fig. 24. Proline content (mg/g fresh weight) of synthetic hexaploid wheat lines grown at control (T_0) and drought (30% PEG (T_1)) conditions.

The increase in soluble sugars under water stress has been reported (Johari Pireivatlou et al. 2010). A higher amount of soluble sugars and a lower amount of starch were found under stress by Mohammad Khani and Heidari (2008). In both studies, sugars are suggested to play a role in osmotic adjustment. Therefore, the accumulation of glucose and fructose in drought may be involved in a signal transduction pathway or an increase in osmotic pressure leading

to drought stress tolerance. We observed a similar increase (Fig. 25) in lines SHDR 16 (30%), SHDR 17 (38%), SHDR 18 (39%), SHDR 49 (52%), SHDR 45 (57%), SHDR 50 (56%), SHDR 52 (38%), SHDR 53 (37%), and Baviacora (59%) after drought. These lines can be considered drought tolerant; they performed well under drought stress by accumulating soluble sugars. Lines SHDR 21 (7%), SHDR 32 (2%), SHDR 29 (15%), and SHDR 43 (9%) accumulated a minimum amount of soluble sugars (Fig. 25) and can be considered sensitive. Soluble sugar content proved to be a better marker for selecting improvement to drought tolerance in durum wheat than proline content (Al Hakimi et al. 1995).

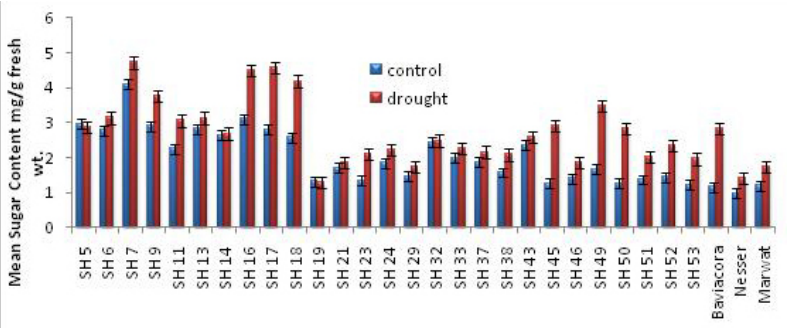

Fig. 25. Soluble sugar content (mg/g fresh weight) of synthetic hexaploid wheat population grown at control (T_0) and drought (30% PEG (T_1)) conditions.

Drought stress induced oxidative stress in plants that exhibited high H_2O_2 and oxidized ascorbate levels in relation to the prolonged drought period. A weak antioxidant enzymes response leading to enhanced membrane damage during severe drought stress was indicated by the accumulation of malondialdehyde. Suproxide dismutase, catalase, and andascorbic peroxidises have been related with water deficiency and are considered the main components of anti-oxidative machinery for drought resistance in higher plants (Bergmann et al. 1999). Tian and Lei (2007) advocated that the activities of superoxide dismutase increased under drought. Lines SHDR 6 (42%), SHDR 7 (36%), SHDR 9 (41%), Baviacora (41%), SHDR 46 (52%), SHDR 53 (36%), SHDR 45 (42%), SHDR 49 (60%), SHDR 50 (42%), SHDR 51 (43%), and SHDR 52 (34%) showed a significant increase in SOD after drought stress (Fig. 26). These lines have the good, anti-oxidative machinery for drought resistance and can be considered tolerant, which was confirmed in Al-Ghamdi (2009).

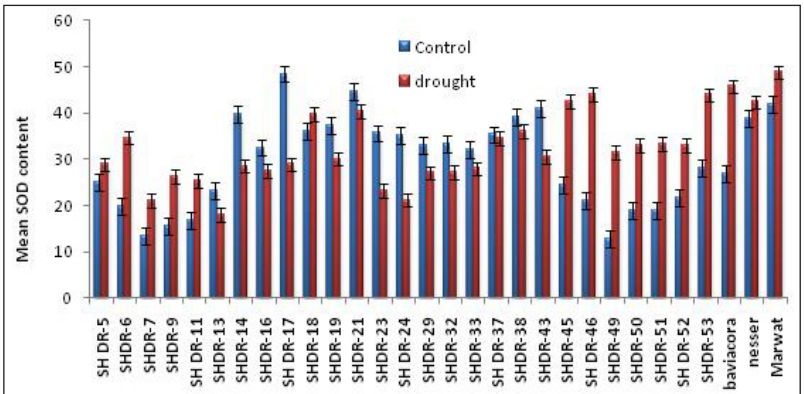

Fig. 26. Superoxide dismutase content (μ units/mg fresh weight) of synthetic hexaploid wheat population grown at control (T_0) and drought (30% PEG (T_1)) conditions.

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Evaluation of molecular mapping population from a 'wheat/synthetic hexaploid' cross for drought tolerance.

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In Pakistan, wheat production is very low in the rain-fed areas because of an uneven and unpredictable rainfall. Therefore, the production of high-yielding and drought tolerant cultivars is the main challenge for the plant breeders. QTL mapping, identifying groups of genes by mapping regions of the genome responsive to a particular stress, is used to enhance the efficiency of plant breeding through the use of molecular markers. Genotyping and phenotyping are the two categories of QTL mapping. The combined use of molecular, morphological, and physiological markers is proven to be one of the best approaches for coping with major abiotic stresses such as drought. This study is a morphological and physiological characterization of a double-haploid-based, molecular mapping population (bread wheat/synthetic hexaploid (SH) wheat) for various phenotypic parameters for drought tolerance. Our main objectives were to (a) evaluate and phenotypically characterize a double-haploid mapping population under *in vivo* conditions for drought tolerance and (b) evaluate and phenotype the mapping population under *in vitro* conditions.

The germ plasm studied was a molecular mapping population for drought tolerance produced by the cross 'Opata (susceptible bread wheat cultivar)/SH (tolerant D67.2/P66.270//*Ae. tauschii* 257)'. The F_1s were crossed with maize to produce haploids, which after a colchicine application, produced double haploids (DH). Of the original 142 individuals, 128 entries were evaluated under *in vivo* and *in vitro* conditions. The data recorded were subjected to an analysis of variance technique at probability level of 0.05 for all the parameters recorded in the field, tunnel, and for PEG-induced stress studies to observe the level of variation in all accessions.

In vivo* evaluation. *Plant height (cm). Plant height under controlled conditions ranged from 54.5 to 117.2 cm with an average height of 95.1 cm. Under drought conditions, height ranged from 46.9 to 115.0 cm with an average plant height of 85.0 cm; a decreasing trend with an average decrease of 10.6% compared to the average plant height of the control. Mapping population entries 126, 80, 125, 42, 89, and 145 had a decreased height by 2.0, 5.3, 3.7, 14.0, 30.0, and 33.1% respectively, when compared with control (Table 26, continued on p. 163).

Table 26. Mean values for plant height (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	107.3	82.7	45	95.6	79.8	85	96.6	92.4	119	99.9	83.1
4	106.3	96.1	46	93.4	64.2	86	92.57	78.4	120	101.5	94.2
5	102.3	90.6	47	92.4	63.1	87	111.7	105.1	121	103.1	81.4
7	101.3	92.4	48	95.7	90.3	88	97.6	87.4	122	106.3	105.0
8	104.1	84.5	49	96.7	90.2	89	69.1	48.7	123	106.3	99.2
9	92.6	80.0	51	80.7	76.9	90	100.6	82.6	124	106.6	103.2
12	98.8	88.5	53	66.1	57.2	91	97.4	80.4	125	110.4	106.3
13	91.3	70.5	55	97.9	94.5	92	92.1	76.2	126	117.2	115.7
14	94.5	83.7	57	96.7	90.1	93	81.5	77.2	127	100.5	95.3
15	85.4	72.2	58	63.3	57.8	94	85.6	85.1	128	107.3	97.4
16	80.4	74.6	59	102.5	94.3	95	103.9	102.4	130	80.7	71.7
17	90.2	82.5	60	98.9	87.7	97	77.8	73.1	132	87.5	77.3
18	87.3	83.1	61	90.1	88.8	98	96.5	83.9	133	104.7	100.2
19	82.6	78.5	62	93.5	93.3	99	95.6	92.2	134	108.1	101.3
21	96.4	78.3	63	112.9	99.9	100	98.8	92.2	135	100.4	93.6
22	95.6	89.7	64	81.8	62.4	101	95.4	90.5	136	112.2	81.4
23	100.6	93.6	67	86.2	76.5	102	97.0	86.2	137	101.1	92.3
24	97.8	96.4	68	96.2	95.3	103	96.6	80.6	138	95.7	86.4
26	96.3	98.6	69	105.3	101.2	104	86.2	68.6	139	102.9	87.4

Table 26. Mean values for plant height (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
27	100.9	96.8	71	91.2	86.5	105	104.1	91.1	140	102.9	76.3
28	100.4	90.7	72	89.6	76.6	106	75.60	57.6	141	75.0	73.4
30	61.6	55.4	73	100.3	98.6	108	95.83	87.5	142	103.1	81.2
31	78.3	69.9	74	100.2	98.1	109	103.0	91.4	143	81.5	55.9
32	87.3	65.3	75	94.3	84.7	110	102.3	94.4	144	93.7	68.6
33	95.4	89.2	76	103.3	81.1	111	103.3	92.0	145	74.0	49.5
35	83.4	65.5	77	101.5	92.5	112	99.2	88.2	146	99.5	93.1
37	96.5	89.2	78	94.5	88.5	113	98.6	94.1	147	94.5	88.5
39	109.7	100.5	79	104.4	94.6	114	93.3	74.5	148	94.5	84.5
40	105.6	99.1	80	113.7	107.7	115	85.6	71.2	149	92.4	83.6
41	97.5	59.2	81	117.2	103.0	116	81.5	72.3	150	100.7	97.4
42	54.5	46.9	82	110.2	104.4	117	102.3	99.1	151	101.0	97.1
43	61.4	50.3	84	88.4	82.4	118	99.7	98.9	152	103.6	95.0

Days-to-flowering. The data regarding mean values for days-to-flowering showed a range of 107 to 149 days with an average of 127 days under field conditions (Table 27). Entry 31 took 107 days to flower and was the earliest of all the genotypes studied in the field, followed by entries 26 and 151 with 109 and 111 days.

Table 27. Mean values for days-to-flowering under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	122.0	105.7	45	144.0	121.7	85	121.0	115.0	119	111.0	109.7
4	114.7	109.0	46	137.0	134.7	86	125.0	121.0	120	138.0	126.0
5	132.7	110.0	47	131.0	103.3	87	123.0	112.0	121	130.0	114.0
7	132.0	130.0	48	132.0	127.3	88	129.0	114.0	122	140.0	129.0
8	123.0	128.7	49	119.0	112.7	89	130.0	110.0	123	124.0	121.0
9	125.7	115.7	51	119.0	118.3	90	126.0	112.0	124	129.0	117.7
12	138.7	130.3	53	125.0	113.3	91	126.0	117.0	125	135.0	117.7
13	127.3	125.3	55	121.0	105.7	92	124.0	121.0	126	134.0	120.7
14	113.3	111.7	57	119.0	107.0	93	130.0	128.3	127	137.3	122.3
15	121.0	109.7	58	135.7	124.7	94	132.0	129.0	128	121.0	109.0
16	122.3	121.0	59	116.0	113.0	95	135.0	130.3	130	139.0	126.0
17	121.0	120.7	60	118.0	113.7	97	123.0	120.7	132	122.0	115.7
18	119.7	118.3	61	115.0	113.3	98	130.0	120.7	133	134.0	117.0
19	130.3	121.0	62	120.0	112.3	99	133.0	120.7	134	137.0	118.0
21	116.7	112.3	63	112.0	105.0	100	134.0	124.0	135	140.0	121.0
22	125.3	112.7	64	131.0	113.7	101	129.0	125.3	136	143.0	122.0
23	125.3	107.3	67	131.0	129.7	102	136.0	131.0	137	130.0	127.0
24	127.0	117.0	68	115.0	109.3	103	135.0	128.3	138	142.0	123.3
26	109.0	118.3	69	121.0	110.0	104	133.0	129.7	139	118.0	105.0
27	120.0	110.3	71	129.0	121.3	105	132.0	127.3	140	120.0	117.0
28	132.0	121.0	72	139.0	121.7	106	135.0	131.0	141	121.0	113.0
30	127.0	122.7	73	149.0	117.3	108	138.0	125.0	142	132.0	106.3
31	107.0	106.7	74	120.0	117.7	109	140.0	131.0	143	118.0	117.0
32	114.0	107.0	75	121.0	106.0	110	131.0	108.0	144	112.0	107.3
33	134.0	123.3	76	116.0	105.0	111	126.0	110.0	145	131.0	109.7
35	129.3	111.0	77	140.0	122.0	112	112.0	119.0	146	112.0	107.3
37	145.0	134.3	78	136.0	115.0	113	123.0	116.0	147	115.0	113.0
39	131.0	129.0	79	124.0	123.0	114	122.0	113.0	148	112.0	107.0
40	124.0	107.7	80	115.0	109.0	115	141.0	130.0	149	116.0	106.0
41	130.0	120.0	81	133.0	115.0	116	139.0	126.0	150	128.0	109.0
42	132.0	117.7	82	140.0	123.3	117	120.0	113.7	151	111.0	107.3
43	133.0	122.0	84	135.0	134.3	118	120.0	101.7	152	134.0	119.0

In the tunnel, under drought conditions, entry 118 was the earliest of all the genotypes studied with mean value of 101.7 days, followed by entry 47 (103.3 days), and 2, 55, 63, 76, and 139 all with 105 days-to-flowering. Under drought conditions, days-to-flowering ranged from 101.7 to 134.5 days with an average of 118 days.

Days-to-physiological maturity. The average number of days to physiological maturity was 154 for the genotypes sown under control conditions, the range was 139.0–168.7 days. The minimum number of days to plant maturity under field conditions were recorded in genotypes 23 and 26 (138.7 days), followed by 149 (139.7) and 73 (139.0). Entry 118 was the earliest maturing genotype, taking 124 days to mature under drought conditions. The mean value of days-to-physiological maturity ranged from 124 to 155 days, with an average of 138 days under drought stress. A decrease in days to physiological maturity was observed in genotypes 67 (11 days earlier); 12 (12 days earlier than the control); 46, 93 and 109, (13 days earlier), 68 (14 days earlier); 95 (15 days earlier); 76 and 118 (19 days earlier); 151 (22 days earlier), and 90 (23 days earlier) compared to entries grown under control conditions (Table 28).

Table 28. Mean values for days-to-flowering under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Days-to-physiological maturity			Days-to-physiological maturity			Days-to-physiological maturity			Days-to-physiological maturity	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	147.7	127.3	45	164.0	140.0	85	153.0	134.3	119	140.7	129.0
4	145.7	128.3	46	168.7	155.0	86	156.7	140.0	120	164.3	146.3
5	152.3	129.0	47	168.0	142.3	87	145.7	132.0	121	155.7	134.7
7	161.0	148.3	48	162.7	148.7	88	156.3	132.3	122	163.7	150.3
8	156.3	141.3	49	148.0	132.7	89	155.0	129.3	123	155.0	141.7
9	153.0	131.3	51	147.3	135.7	90	149.7	126.7	124	152.7	137.0
12	163.7	151.0	53	153.0	133.3	91	152.7	134.0	125	152.7	135.0
13	160.3	144.3	55	146.7	127.7	92	150.3	142.7	126	151.3	138.3
14	146.7	132.0	57	149.0	129.7	93	164.0	151.0	127	155.7	138.7
15	152.0	137.7	58	161.0	141.0	94	159.3	149.3	128	150.0	136.3
16	145.3	137.0	59	147.3	130.3	95	166.0	151.0	130	160.7	143.0
17	149.7	137.7	60	141.3	133.0	97	148.3	131.3	132	151.0	137.0
18	149.3	142.7	61	143.7	131.0	98	159.0	141.7	133	149.3	135.7
19	161.0	148.7	62	147.3	133.3	99	163.7	145.7	134	157.3	140.7
21	145.0	139.7	63	147.3	128.0	100	161.0	146.0	135	159.0	139.7
22	144.7	138.3	64	154.0	131.0	101	165.0	147.3	136	168.0	144.7
23	138.7	126.0	67	162.3	151.0	102	167.0	150.3	137	157.7	147.3
24	149.3	135.3	68	140.0	126.7	103	164.0	147.7	138	157.0	145.7
26	138.7	129.3	69	146.0	129.3	104	164.7	149.0	139	146.3	127.7
27	151.7	136.7	71	160.3	144.0	105	161.7	146.7	140	152.0	141.3
28	156.0	147.0	72	160.7	139.0	106	163.3	152.3	141	150.0	138.7
30	152.7	143.7	73	139.0	130.3	108	161.0	146.0	142	154.0	128.0
31	141.3	131.7	74	151.0	136.3	109	164.3	151.0	143	145.7	132.3
32	141.7	127.7	75	151.3	129.3	110	152.0	132.3	144	142.3	130.0
33	157.7	142.3	76	144.7	125.7	111	147.3	131.0	145	149.0	129.0
35	154.7	127.3	77	162.3	145.3	112	151.0	138.7	146	142.3	127.3
37	167.3	150.3	78	156.3	132.7	113	149.0	131.0	147	152.3	132.7
39	154.7	141.0	79	153.7	136.7	114	146.0	127.3	148	146.7	127.7
40	152.3	133.7	80	144.7	129.3	115	157.7	148.7	149	139.7	127.0
41	155.3	135.3	81	156.0	135.3	116	159.7	145.3	150	150.7	129.7
42	160.7	138.0	82	163.0	144.0	117	146.0	132.0	151	147.7	125.3
43	156.3	137.0	84	166.3	155.0	118	143.0	124.0	152	150.3	144.7

Spike length. The maximum spike length of 13.30 cm was observed in 99 and 63 followed by entries 103 and 144 with spike length of 12.83 and 12.73 cm respectively. The data recorded for spike length under control condition ranged from 6.20 to 13.30 cm with an average spike length of 9.83 cm. Spike length under drought conditions ranged from 5.55 to 11.20 cm with an average value of 8.55 cm. Maximum spike length of 11.20 cm was recorded in entry 87 followed by genotypes 80, 78 with common value of 10.97 cm and 108 with 10.87 cm of spike length (Table 29, p. 165).

Table 29. Mean values for spike length (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Spike length			Spike length			Spike length			Spike length	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	11.20	10.47	45	10.43	9.30	85	10.27	8.40	119	9.60	8.60
4	10.10	10.13	46	9.43	8.60	86	9.13	8.73	120	10.50	9.73
5	8.80	6.66	47	10.63	9.13	87	12.27	11.20	121	8.83	6.86
7	8.73	6.93	48	11.60	10.57	88	9.53	8.83	122	11.37	10.43
8	9.40	7.76	49	11.67	9.30	89	7.13	5.66	123	10.37	7.53
9	9.76	8.50	51	12.27	10.50	90	8.60	7.76	124	9.26	8.63
12	10.20	9.36	53	9.90	7.80	91	8.30	8.46	125	8.80	8.50
13	8.50	7.60	55	11.40	10.07	92	9.83	8.10	126	9.10	8.30
14	11.20	10.53	57	10.33	10.13	93	9.66	7.66	127	11.10	10.50
15	8.56	7.86	58	6.20	5.55	94	7.56	7.80	128	10.47	9.26
16	9.73	7.60	59	10.30	7.76	95	9.30	8.30	130	8.60	7.76
17	11.13	10.13	60	8.50	7.06	97	9.60	8.63	132	9.40	8.60
18	10.43	8.50	61	8.10	8.10	98	9.66	8.40	133	8.23	7.73
19	7.60	5.66	62	10.70	8.20	99	13.30	9.50	134	10.43	9.80
21	10.50	9.86	63	13.30	10.63	100	8.73	6.70	135	10.37	9.66
22	10.30	8.30	64	12.13	10.07	101	9.16	8.70	136	11.37	8.80
23	10.87	10.57	67	8.73	7.50	102	9.90	9.03	137	10.57	8.60
24	8.63	7.03	68	9.66	8.70	103	12.83	10.53	138	9.50	8.63
26	8.76	7.56	69	8.33	7.63	104	10.50	9.23	139	9.36	7.16
27	7.20	5.60	71	12.63	9.50	105	10.73	10.57	140	8.86	7.43
28	7.73	7.43	72	8.20	7.36	106	8.20	5.83	141	9.03	7.23
30	8.76	7.23	73	9.83	9.20	108	11.67	10.87	142	9.33	8.50
31	9.36	6.76	74	9.03	8.20	109	9.53	6.66	143	8.90	7.13
32	8.86	8.20	75	8.26	7.50	110	11.30	7.40	144	12.73	9.73
33	10.20	9.36	76	10.53	9.83	111	10.53	9.73	145	9.13	6.06
35	8.73	7.60	77	9.53	8.53	112	10.40	10.20	146	10.70	10.10
37	8.33	7.50	78	11.43	10.97	113	9.73	8.43	147	11.47	9.43
39	10.30	8.56	79	9.36	8.63	114	10.13	9.13	148	9.40	9.26
40	11.17	10.17	80	12.13	10.97	115	8.50	7.73	149	9.73	8.60
41	9.70	7.30	81	10.43	9.43	116	8.76	7.66	150	11.57	10.40
42	8.86	6.26	82	11.57	9.70	117	10.73	9.00	151	9.50	8.20
43	7.30	5.93	84	7.93	7.00	118	11.80	10.70	152	9.30	9.46

Number of grains/spike. The range of values of the drought mapping population for the number of grains/spike under control and stress conditions were 21.86–64.03 and 17.63–51.93, respectively (Table 30, p. 166). The average number of grains/spike was 42.87 under control conditions and 34.13 under drought stress. The highest mean value was recorded for entry 144, with mean value of 51.93, followed by genotypes 57 (51.40) and 150 (50.50).

1,000-kernel weight. Mean values for 1,000-kernel weight ranged from 25.56 to 60.66 g under control conditions and 15.60 to 50.53 g under drought. The maximum 1,000-kernel weight under control conditions of 60.66 g was for entry 63, followed by entries 108 (59.06 g) and 146 (58.90 g). Entry 148 had the highest 1,000-kernel weight (50.53 g) among all the genotypes under stress conditions (Table 31, pp. 166-167).

Pubescence. Only 14 entries, 4, 12, 16, 17, 18, 62, 84, 93, 99, 121, 123, 142, 147, and 151, were pubescent (Table 32, p. 167).

Waxiness. Of the drought mapping population, lines 4, 12, 17, 21, 37, 45, 46, 49, 99, 102, 121, 137, 142, 143, and 147 were waxy on their leaves. Genotypes 4, 12, 21, 37, 99, 121, 137, 142, and 147 were both waxy and pubescent (Table 32, p. 167).

***In vitro* evaluation.** The *in vitro* parameters included coleoptile length, proline content, chlorophyll content, and superoxide dismutase.

Coleoptile length. We had significant results for the mean value of the coleoptile length at four different treatment levels, control, 7.5% PEG, 15.0% PEG, and 22.5% PEG. Coleoptile length was 2.53–4.50 cm (3.18 cm average) in the control

Table 30. Mean values for number of grains/spike under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Grains/spike			Grains/spike			Grains/spike			Grains/spike	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	46.13	34.43	45	53.96	47.26	85	41.60	39.50	119	39.26	36.30
4	38.23	35.23	46	32.26	17.66	86	49.30	27.56	120	37.60	26.13
5	32.96	28.06	47	27.46	18.56	87	50.73	44.26	121	41.00	21.66
7	31.40	22.53	48	64.03	40.36	88	44.46	26.96	122	56.50	50.03
8	35.36	28.06	49	38.73	26.36	89	30.73	18.33	123	45.83	29.13
9	36.46	29.26	51	49.16	44.16	90	45.20	29.40	124	40.13	29.13
12	31.16	26.13	53	32.46	27.43	91	44.53	28.56	125	38.73	30.83
13	40.33	33.20	55	51.10	48.33	92	54.30	24.23	126	42.06	31.46
14	53.26	49.40	57	56.23	51.40	93	45.73	44.46	127	47.36	29.30
15	39.03	33.40	58	36.40	19.60	94	42.43	40.36	128	48.93	35.73
16	55.13	35.13	59	33.40	31.56	95	33.26	17.63	130	49.60	42.10
17	60.90	49.26	60	42.36	36.23	97	44.00	42.50	132	45.50	32.46
18	53.76	49.40	61	41.90	36.03	98	40.20	33.40	133	46.40	42.16
19	41.06	20.46	62	43.30	29.40	99	53.73	28.43	134	39.26	34.13
21	40.73	35.66	63	48.26	43.50	100	40.56	31.56	135	41.16	33.76
22	40.00	33.23	64	40.90	32.26	101	39.36	28.76	136	43.43	34.63
23	54.20	48.30	67	36.30	32.00	102	45.56	30.76	137	37.43	41.70
24	38.23	26.33	68	42.33	41.93	103	45.40	36.23	138	36.46	29.00
26	39.00	32.46	69	40.10	38.50	104	53.66	35.23	139	46.43	41.86
27	36.53	20.60	71	48.06	41.23	105	37.70	29.66	140	43.40	36.13
28	49.13	48.30	72	43.40	33.73	106	35.13	19.56	141	40.53	39.06
30	31.20	20.30	73	49.23	42.86	108	51.86	45.96	142	38.93	25.60
31	46.16	35.63	74	41.33	37.53	109	25.26	18.66	143	41.20	38.16
32	38.23	31.63	75	37.56	33.36	110	47.06	31.20	144	53.46	51.93
33	40.10	29.16	76	43.13	35.66	111	21.86	18.43	145	30.00	18.63
35	39.06	30.53	77	41.36	34.66	112	44.63	40.36	146	52.76	41.43
37	36.36	22.96	78	48.30	32.30	113	38.36	29.40	147	42.23	39.06
39	37.36	31.60	79	45.50	41.50	114	43.63	38.23	148	49.36	45.56
40	52.20	47.50	80	53.36	42.13	115	51.53	38.60	149	48.73	37.83
41	41.50	31.10	81	50.43	41.20	116	50.50	44.56	150	53.13	50.50
42	29.26	19.43	82	45.43	36.16	117	40.30	40.26	151	28.46	26.96
43	27.56	21.50	84	43.43	35.06	118	52.20	49.40	152	44.50	23.63

Table 31. Mean values for 1,000-kernel weight (g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	1,000-kernel weight			1,000-kernel weight			1,000-kernel weight			1,000-kernel weight	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	51.23	23.33	45	57.03	46.70	85	40.13	33.10	119	41.26	31.96
4	49.56	25.46	46	41.10	29.86	86	36.70	30.20	120	43.30	25.50
5	43.53	22.53	47	33.10	16.93	87	44.43	28.56	121	42.30	30.96
7	38.43	27.86	48	42.50	19.70	88	38.50	25.46	122	53.16	47.03
8	38.66	22.43	49	34.36	34.36	89	33.96	19.26	123	43.16	31.80
9	42.20	35.46	51	51.73	44.70	90	44.46	39.40	124	43.23	23.23
12	41.86	21.46	53	35.43	24.30	91	42.13	30.00	125	44.33	35.46
13	32.46	29.50	55	57.36	49.50	92	44.56	26.53	126	37.43	30.70
14	51.00	48.03	57	35.56	32.60	93	30.40	25.13	127	39.30	29.43
15	39.63	31.23	58	32.16	20.20	94	31.83	15.60	128	47.70	39.60
16	37.53	33.56	59	42.23	40.86	95	42.33	22.00	130	41.43	23.53
17	51.76	45.46	60	41.66	35.43	97	39.63	28.26	132	39.13	20.16
18	38.56	29.73	61	40.36	30.23	98	33.60	26.36	133	43.53	32.40
19	37.33	28.93	62	42.33	31.40	99	33.63	31.26	134	36.56	26.50
21	39.00	33.40	63	60.66	47.60	100	28.46	25.30	135	33.76	26.56
22	34.70	32.33	64	38.80	28.06	101	28.63	20.70	136	39.16	34.53
23	55.26	47.83	67	32.46	31.06	102	39.43	21.56	137	48.63	28.66
24	38.53	33.43	68	37.40	23.23	103	28.66	19.66	138	36.56	29.33

Table 31. Mean values for 1,000-kernel weight (g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	1,000-kernel weight			1,000-kernel weight			1,000-kernel weight			1,000-kernel weight	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
26	42.76	34.56	69	39.60	34.43	104	25.56	18.20	139	50.60	28.76
27	36.83	17.36	71	43.06	24.30	105	30.76	28.43	140	34.50	21.60
28	28.63	21.73	72	40.13	25.00	106	32.53	16.63	141	41.56	25.93
30	40.43	18.93	73	40.46	34.26	108	59.06	46.03	142	46.70	32.46
31	41.70	28.00	74	39.76	27.36	109	32.83	16.16	143	30.30	19.56
32	41.36	31.43	75	39.50	34.40	110	45.23	37.16	144	39.63	30.76
33	35.70	28.36	76	41.33	34.16	111	32.16	21.00	145	33.56	17.46
35	36.20	28.40	77	39.46	33.56	112	51.76	33.16	146	58.90	48.23
37	37.30	23.53	78	51.26	29.43	113	42.50	33.43	147	41.16	38.36
39	37.10	33.56	79	31.80	23.56	114	39.50	29.16	148	54.43	50.53
40	56.26	46.70	80	46.30	45.50	115	38.50	24.43	149	41.73	32.36
41	29.26	27.70	81	44.06	31.43	116	36.26	26.70	150	50.60	47.63
42	31.36	18.40	82	53.13	47.43	117	44.16	43.60	151	55.76	41.43
43	29.90	16.50	84	34.66	22.26	118	54.13	46.13	152	49.53	40.10

Table 32. Observations (+ = presence and – = absence) for leaf pubescence (PUB) and waxiness (WAX) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

Entry	PUB	WAX	Entry	PUB	WAX	Entry	PUB	WAX	Entry	PUB	WAX
2	–	–	45	–	+	85	–	–	119	–	–
4	+	+	46	–	+	86	–	–	120	–	–
5	–	–	47	–	–	87	–	–	121	+	+
7	–	–	48	–	–	88	–	–	122	–	–
8	–	–	49	–	+	89	–	–	123	+	–
9	–	–	51	–	–	90	–	–	124	–	–
12	+	+	53	–	–	91	–	–	125	–	–
13	–	–	55	–	–	92	–	–	126	–	–
14	–	–	57	–	–	93	+	–	127	–	–
15	–	–	58	–	–	94	–	–	128	–	–
16	+	–	59	–	–	95	–	–	130	–	–
17	+	+	60	–	–	97	–	–	132	–	–
18	+	–	61	–	–	98	–	–	133	–	–
19	–	–	62	+	–	99	+	+	134	–	–
21	–	+	63	–	–	100	–	–	135	–	–
22	–	–	64	–	–	101	–	–	136	–	–
23	–	–	67	–	–	102	–	+	137	–	+
24	–	–	68	–	–	103	–	–	138	–	–
26	–	–	69	–	–	104	–	–	139	–	–
27	–	–	71	–	–	105	–	–	140	–	–
28	–	–	72	–	–	106	–	–	141	–	–
30	–	–	73	–	–	108	–	–	142	+	+
31	–	–	74	–	–	109	–	–	143	–	+
32	–	–	75	–	–	110	–	–	144	–	–
33	–	–	76	–	–	111	–	–	145	–	–
35	–	–	77	–	–	112	–	–	146	–	–
37	–	+	78	–	–	113	–	–	147	+	+
39	–	–	79	–	–	114	–	–	148	–	–
40	–	–	80	–	–	115	–	–	149	–	–
41	–	–	81	–	–	116	–	–	150	–	–
42	–	–	82	–	–	117	–	–	151	+	–
43	–	–	84	+	–	118	–	–	152	–	–

plants, 1.36–3.16 cm (2.42 cm average) in the 7.5% PEG treatment, 0.83–2.50 (1.70 cm average) in the 15.0% PEG treatment, and 0.0–1.83 cm (0.57 cm average) in the 22.5% PEG treatment. Entry 77 had the best performance, the highest among the genotypic means with a coleoptile length of 2.97 cm, followed by genotypes 14 (2.76 cm) and 122 (2.71 cm). The data for all four levels of treatment showed that coleoptile length decreased under artificially induced stress (Table 33, continued on p. 169). The minimum reduction was observed at 7.5% PEG with an average decrease of 28.89% in coleoptile length compared to the average mean of the control; the maximum reduction was at 22.5% PEG, where the average decrease was 82.07% compared to the treatment mean of the control. At 15.0% PEG, a decrease of 46.54% was inbetween that of 7.5% and 22.5%, showing a continued decrease from lower to higher PEG concentrations. The genotypes showing a response under the maximum stress (22.5%) are more tolerant to drought and those higher mean values for coleoptiles length under all three PEG treatments are good for drought conditions.

Table 33. Mean values for coleoptile length (cm) at 0.0%, 7.5%, 15.0%, and 22.5% polyethylene glycol for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Coleoptile length (cm)						Coleoptile length (cm)				
Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean	Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean
2	3.16	2.50	1.43	0.86	1.99	86	3.03	2.70	1.83	0.00	1.89
4	3.56	2.66	1.70	1.23	2.29	87	2.86	2.23	1.63	0.00	1.68
5	3.06	2.40	1.70	1.16	2.08	88	3.16	2.50	1.66	1.76	2.27
7	2.96	2.40	1.26	0.00	1.65	89	3.56	2.73	1.96	0.00	2.06
8	2.96	2.60	1.73	0.00	1.82	90	3.06	2.60	1.80	0.00	1.86
9	3.73	2.56	1.76	0.00	2.01	91	3.16	2.43	1.80	0.00	1.85
12	3.00	2.16	1.43	0.83	1.85	92	3.43	2.66	1.60	0.00	1.92
13	3.00	2.20	1.63	1.00	1.95	93	3.16	2.40	1.70	0.00	1.81
14	4.06	3.06	2.23	1.70	2.76	94	3.33	2.53	1.80	0.00	1.91
15	2.90	2.03	1.36	0.00	1.57	95	3.60	2.80	1.83	0.00	2.05
16	3.00	2.26	1.53	0.00	1.70	97	2.86	2.23	1.60	0.00	1.67
17	3.80	2.96	2.36	1.40	2.63	98	3.33	2.33	1.73	0.00	1.85
18	2.96	2.43	1.73	1.20	2.08	99	3.70	3.03	2.10	0.00	2.20
19	3.13	2.43	1.63	1.23	2.10	100	3.50	2.90	1.93	0.00	2.08
21	2.96	2.50	1.73	1.33	2.13	101	3.43	2.83	1.96	0.00	2.05
22	3.06	2.40	1.53	0.96	1.99	102	3.06	2.33	1.76	0.00	1.79
23	3.33	2.76	2.13	1.16	2.35	103	3.33	2.60	1.80	0.00	1.93
24	3.03	2.33	1.80	1.33	2.12	104	2.86	1.96	1.36	0.00	1.55
26	3.43	2.46	1.73	1.23	2.21	105	3.00	2.43	1.60	0.00	1.75
27	2.53	1.83	1.13	0.00	1.37	106	3.00	2.43	1.73	0.00	1.79
28	3.53	2.60	1.83	0.00	1.99	108	3.26	2.30	1.93	0.00	1.87
30	3.03	2.40	1.66	1.33	2.10	109	3.70	2.93	1.80	0.00	2.10
31	3.03	2.53	1.73	1.23	2.13	110	2.96	2.30	1.93	0.00	1.80
32	3.16	2.53	1.80	1.20	2.17	111	3.00	1.73	0.90	0.00	1.40
33	3.16	2.50	1.73	1.40	2.20	112	3.03	2.13	1.93	0.00	1.77
35	3.36	2.76	2.13	1.46	2.43	113	2.63	1.80	1.03	0.00	1.36
37	2.96	2.10	1.63	1.20	1.97	114	3.06	2.13	1.63	0.00	1.70
39	3.56	2.56	1.83	1.43	2.35	115	3.40	2.33	1.56	0.00	1.82
40	3.13	2.26	1.80	1.13	2.08	116	3.03	2.46	1.70	0.00	1.80
41	2.90	2.26	1.50	1.03	1.92	117	3.23	2.46	1.86	0.00	1.89
42	2.90	1.80	1.10	0.00	1.45	118	3.50	2.76	2.00	0.00	2.06
43	2.93	1.73	1.03	0.00	1.42	119	3.36	2.83	2.00	0.00	2.05
45	2.86	2.33	1.66	1.23	2.02	120	3.26	2.50	2.03	0.00	1.95
46	3.33	2.63	2.00	1.56	2.38	121	3.40	2.50	1.66	0.00	1.89
47	2.56	1.60	1.03	0.00	1.30	122	3.76	3.00	2.50	1.60	2.71
48	3.00	2.60	1.73	1.43	2.19	123	3.06	2.46	2.03	1.43	2.25
49	3.50	2.80	1.93	1.16	2.35	124	2.96	1.36	0.83	0.00	1.29
51	3.43	2.60	1.83	1.36	2.30	125	3.13	2.26	2.00	1.53	2.23
53	3.46	2.63	1.90	1.33	2.33	126	3.00	1.96	1.43	1.00	1.85
55	3.06	2.50	1.66	0.00	1.80	127	3.13	2.33	1.66	0.00	1.78
57	2.96	2.63	1.86	0.00	1.86	128	2.86	1.90	1.30	0.00	1.51
58	2.86	2.40	1.83	1.00	2.02	130	3.30	2.33	1.73	1.30	2.16
59	2.86	2.30	1.60	0.00	1.69	132	3.26	2.40	1.86	1.30	2.20
60	3.06	2.53	1.80	1.40	2.20	133	3.70	2.90	2.06	1.63	2.57

Table 33. Mean values for coleoptile length (cm) at 0.0%, 7.5%, 15.0%, and 22.5% polyethylene glycol for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Coleoptile length (cm)						Coleoptile length (cm)				
Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean	Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean
61	3.16	2.76	2.03	1.46	2.35	134	3.56	2.96	2.16	1.60	2.57
62	4.10	3.16	2.43	0.00	2.42	135	3.10	2.26	1.60	1.13	2.02
63	3.20	2.50	1.86	0.00	1.89	136	2.90	1.76	1.10	1.03	1.70
64	3.00	2.53	1.83	1.23	2.15	137	3.23	2.43	1.80	1.33	2.20
67	3.03	2.53	1.90	0.96	2.10	138	3.16	2.26	1.70	0.00	1.78
68	3.13	2.50	1.83	1.13	2.15	139	3.23	2.36	1.70	1.20	2.12
69	3.43	2.60	1.86	0.00	1.97	140	3.16	2.36	1.70	0.00	1.80
71	2.86	1.70	1.03	0.00	1.40	141	2.96	2.23	1.06	0.00	1.56
72	3.06	2.40	1.66	0.00	1.78	142	3.00	2.23	1.33	0.00	1.64
73	2.96	2.46	1.53	0.00	1.74	143	2.90	2.20	1.50	0.96	1.89
74	2.80	1.76	0.90	0.00	1.36	144	3.03	2.10	1.56	1.40	2.02
75	3.23	2.53	1.73	0.00	1.87	145	3.30	2.40	1.73	0.00	1.85
76	2.96	2.20	1.60	1.06	1.95	146	3.06	2.40	1.53	1.00	2.00
77	4.50	3.06	2.50	1.83	2.97	147	2.86	1.90	1.13	0.56	1.61
78	3.20	2.33	1.76	1.46	2.19	148	3.40	2.36	1.36	1.00	2.03
79	3.00	2.43	1.80	0.00	1.80	149	3.23	2.43	1.90	0.00	1.89
80	3.70	3.00	2.00	0.00	2.17	150	3.53	2.93	1.83	1.33	2.40
81	3.33	2.53	1.93	0.00	1.95	151	3.10	2.26	1.50	1.20	2.01
82	3.20	2.90	2.13	0.00	2.05	152	3.16	2.46	1.50	0.56	1.92
84	3.00	2.40	1.80	0.00	1.80	Mean	3.19	2.42	1.70	0.57	—
85	3.33	2.50	1.86	0.00	1.92						

Physiological laboratory tests. ***Analysis of proline content.*** Proline content ranged from 84.10 to 5324.00 μg/g with an average value of 949.22 μg/g under control conditions. The maximum proline content accumulation under controled conditions was in entry 128 (5,324.00 μg/g), followed by entries 14 (5,016 μg/g) and 17 (4,958 μg/g). The highest proline accumulation under drought conditions was 9,277.30 μg/g in genotype 14, followed by entries 17 (8,837.90 μg/g) and 152 (8,199.80 μg/g). Proline content ranged from 399.00 to 9,277.30 μg/g with an average of 3,626.79 μg/g under water-stress (Table 34, p. 170).

Analysis of chlorophyll content. Examination of photosynthetic activity, including various other physiological parameters, is a useful approach. The germ plasm under study was measured for chlorophyll a, chlorophyll b, and total chlorophyll content.

Chlorophyll a content. The chlorophyll a content 0.293–1.396 mg/g under control and 0.115–1.190 mg/g under drought conditions with average values of 0.697 mg/g and 0.472 mg/g, respectively (Table 35, p. 171). The maximum chlorophyll a content was in entry 152 (1.396 mg/g), followed by entries 151 (1,180 mg/g) and 148 (1.168 mg/g). Under drought conditions, the maximum mean value was 1.190 mg/g for entry 152, followed by genotypes 89 (0.909 mg/g) and 49 (0.893 mg/g). When the entries of the mapping population with the highest and lowest means (drought tolerant and drought susceptible) under drought were compared to the control means, a decreasing trend in chlorophyll a content was observed for most of the genotypes. A decrease in chlorophyll a content compared to their mean values under controled conditions were noted in entries 152 (14.75%), 89 (18.83%), 149 (16.22%), 99 (61.66%), 100 (74.67%), and 75 (67.99%). Entries 152, 89, and 149 were drought tolerant; the percent reduction was less. A significant decrease in chlorophyll a content was observed in genotypes 99, 100, and 75, showed their poor performance under drought because lower chlorophyll content directly imposes negative effects on photosynthetic activity.

Chlorophyll b content. The maximum value for chlorophyll b content under control conditions was observed in entry 152 (0.665 mg/g), followed by entries 89 (0.562 mg/g) and 151 (0.545 mg/g). Chlorophyll b means were 0.158–0.655 mg/g with an average mean value of 0.331 mg/g for the control conditions (Table 36, p. 170). Under drought conditions, the maximum mean value for chlorophyll b content was in genotype 82 (0.656 mg/g), followed by genotypes 86 (0.623 mg/g) and 152 (0.561 mg/g). Comparing the mapping population entries with the high and low mean values under drought conditions to the control conditions revealed that the chlorophyll b content decreased under stress, except in entries 82 (65.00% increase) and 86 (70.46% increase). This increasing mean value from the general decreasing trend in other genotypes for mean chlorophyll b content may suggest that these genotypes will perform better under drought con-

Table 34. Mean values for proline content (μg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Proline content			Proline content			Proline content			Proline content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	4,492.0	5,894.8	45	1,578.0	4,913.6	85	1,715.0	3,613.2	119	761.6	3,572.7
4	1,062.0	2,839.2	46	3,134.0	7,006.8	86	1,090.0	3,683.3	120	1,086.0	3,401.4
5	1,532.0	4,127.2	47	286.8	444.7	87	1,037.0	4,539.9	121	637.0	2,312.8
7	756.9	4,268.3	48	1,216.0	4,857.6	88	1,070.0	455.5	122	1,780.0	6,091.5
8	485.9	6,959.3	49	658.8	3,680.2	89	3,576.0	5,827.6	123	574.7	453.8
9	543.5	4,529.0	51	1,019.0	3,614.7	90	593.4	5,517.9	124	345.4	4,398.2
12	1,803.0	3,272.1	53	870.6	1,932.7	91	1,715.0	4,186.3	125	903.3	2,926.4
13	638.5	3,751.8	55	753.8	3,258.1	92	725.8	3,141.3	126	565.3	1,384.5
14	5,016.0	9,277.3	57	467.2	3,251.8	93	517.1	3,624.1	127	845.7	5,510.1
15	1,059.0	7,017.7	58	531.1	3,991.7	94	552.9	3,730.0	128	5,324.0	7,248.9
16	191.6	640.1	59	467.2	3,991.6	95	448.5	4,222.2	130	652.6	1,370.5
17	4,958.0	8,837.9	60	352.0	1,389.2	97	496.8	4,667.6	132	487.5	1,537.2
18	394.0	1,348.7	61	426.7	2,766.0	98	1,109.0	3,289.3	133	610.5	5,765.5
19	250.7	1,168.1	62	576.2	2,404.6	99	704.0	3,547.8	134	465.7	4,381.0
21	319.3	974.9	63	3,926.0	4,309.0	100	825.4	2,817.4	135	336.4	2,818.9
22	422.1	1,585.4	64	465.7	2,963.8	101	443.9	2,230.2	136	411.2	4,315.6
23	4,900.0	6,044.1	67	380.0	2,382.8	102	118.4	819.2	137	529.5	5,740.6
24	542.0	411.8	68	856.6	2,748.8	103	119.9	1,002.9	138	456.3	6,200.1
26	183.8	730.4	69	345.7	2,431.1	104	84.1	543.5	139	342.6	6,234.3
27	239.2	971.8	71	359.8	594.0	105	95.0	942.2	140	894.0	7,153.2
28	311.5	669.7	72	291.2	2,794.0	106	154.2	1,264.6	141	404.9	6,033.4
30	241.4	399.0	73	451.7	4,402.8	108	87.2	6,450.2	142	478.1	8,020.7
31	232.1	1,094.9	74	570.0	4,810.8	109	95.0	3,198.9	143	372.2	5,405.8
32	292.8	769.4	75	462.6	3,111.7	110	193.1	4,578.8	144	403.4	4,486.9
33	574.7	1,060.6	76	352.0	3,063.4	111	767.8	1,784.8	145	580.9	6,039.7
35	1,726.0	4,130.3	77	383.1	3,236.3	112	922.0	1,350.3	146	325.5	6,402.5
37	2,402.0	3,754.9	78	319.3	2,912.4	113	341.3	411.4	147	344.2	6,742.0
39	1,880.0	4,963.5	79	640.1	1,094.9	114	1,104.0	1,348.7	148	447.0	6,888.5
40	4,306.0	7,575.6	80	390.9	2,426.5	115	1,036.0	642.4	149	363.8	439.8
41	3,221.0	4,893.4	81	602.7	3,554.0	116	1,335.0	3,026.0	150	417.4	6,924.3
42	369.8	3,645.9	82	627.6	5,991.5	117	996.7	4,102.2	151	587.1	7,416.4
43	4,224.0	481.4	84	146.4	3,142.9	118	881.5	6,290.4	152	336.4	8,199.8

Table 35. Mean values for chlorophyll a content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll a content			Chlorophyll a content			Chlorophyll a content			Chlorophyll a content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.380	0.289	45	0.624	0.418	85	0.582	0.551	119	0.863	0.831
4	0.365	0.225	46	0.868	0.407	86	1.000	0.486	120	0.505	0.433
5	0.312	0.247	47	0.730	0.519	87	0.642	0.309	121	0.898	0.790
7	0.362	0.306	48	1.067	0.476	88	0.804	0.490	122	0.964	0.498
8	0.357	0.270	49	0.785	0.622	89	1.120	0.909	123	0.479	0.314
9	0.424	0.274	51	0.754	0.281	90	0.695	0.194	124	0.730	0.542
12	0.374	0.293	53	0.942	0.654	91	0.794	0.614	125	0.796	0.587
13	0.353	0.237	55	0.791	0.581	92	0.517	0.419	126	0.596	0.481
14	0.381	0.285	57	0.764	0.406	93	0.664	0.459	127	0.745	0.529
15	0.595	0.336	58	0.619	0.424	94	0.661	0.568	128	0.908	0.753
16	0.658	0.497	59	0.519	0.303	95	0.631	0.413	130	0.802	0.657
17	0.751	0.639	60	0.659	0.466	97	0.934	0.634	132	0.919	0.669
18	0.861	0.675	61	0.473	0.268	98	0.293	0.169	133	0.970	0.520
19	0.961	0.712	62	0.757	0.324	99	0.300	0.115	134	0.852	0.731
21	0.719	0.393	63	0.661	0.233	100	0.538	0.135	135	1.100	0.724
22	0.794	0.518	64	0.660	0.263	101	0.438	0.228	136	1.090	0.768
23	0.628	0.290	67	0.521	0.217	102	0.574	0.348	137	0.846	0.696

Table 35. Mean values for chlorophyll a content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll a content			Chlorophyll a content			Chlorophyll a content			Chlorophyll a content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
24	0.716	0.578	68	0.366	0.154	103	0.371	0.306	138	0.843	0.728
26	0.592	0.473	69	0.821	0.187	104	0.585	0.331	139	0.598	0.428
27	0.613	0.424	71	0.613	0.369	105	0.901	0.370	140	0.604	0.518
28	0.673	0.643	72	0.782	0.232	106	0.873	0.479	141	0.809	0.646
30	0.744	0.468	73	0.663	0.399	108	0.760	0.455	142	0.798	0.530
31	0.763	0.418	74	0.300	0.476	109	0.586	0.516	143	0.855	0.819
32	0.591	0.272	75	0.453	0.145	110	0.640	0.515	144	0.670	0.425
33	0.691	0.452	76	0.360	0.175	111	0.759	0.447	145	1.046	0.875
35	0.476	0.328	77	0.509	0.438	112	0.638	0.516	146	0.992	0.596
37	0.514	0.338	78	0.435	0.367	113	0.580	0.366	147	1.038	0.867
39	0.582	0.410	79	0.324	0.219	114	0.678	0.401	148	1.168	0.686
40	0.898	0.856	80	0.653	0.469	115	0.655	0.451	149	1.066	0.893
41	0.823	0.592	81	0.637	0.458	116	0.405	0.178	150	1.101	0.862
42	0.749	0.622	82	0.835	0.491	117	0.612	0.316	151	1.180	0.866
43	0.704	0.648	84	0.773	0.520	118	0.673	0.375	152	1.396	1.190

Table 36. Mean values for chlorophyll b content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll b content			Chlorophyll b content			Chlorophyll b content			Chlorophyll b content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.307	0.216	45	0.296	0.217	85	0.330	0.285	119	0.422	0.227
4	0.265	0.205	46	0.421	0.239	86	0.184	0.623	120	0.272	0.235
5	0.226	0.219	47	0.344	0.241	87	0.168	0.447	121	0.420	0.377
7	0.290	0.231	48	0.454	0.222	88	0.199	0.491	122	0.496	0.280
8	0.262	0.217	49	0.331	0.269	89	0.562	0.380	123	0.268	0.214
9	0.317	0.239	51	0.336	0.123	90	0.383	0.152	124	0.389	0.261
12	0.271	0.273	53	0.303	0.244	91	0.430	0.341	125	0.448	0.262
13	0.332	0.305	55	0.338	0.235	92	0.293	0.236	126	0.280	0.237
14	0.195	0.163	57	0.326	0.209	93	0.335	0.255	127	0.398	0.238
15	0.239	0.242	58	0.267	0.165	94	0.342	0.311	128	0.459	0.299
16	0.283	0.228	59	0.204	0.149	95	0.307	0.224	130	0.385	0.328
17	0.335	0.291	60	0.287	0.218	97	0.412	0.307	132	0.518	0.363
18	0.372	0.303	61	0.207	0.189	98	0.198	0.108	133	0.441	0.311
19	0.435	0.303	62	0.334	0.203	99	0.219	0.104	134	0.442	0.291
21	0.315	0.173	63	0.283	0.125	100	0.317	0.111	135	0.454	0.384
22	0.294	0.238	64	0.275	0.164	101	0.279	0.163	136	0.465	0.207
23	0.337	0.150	67	0.227	0.107	102	0.284	0.187	137	0.402	0.357
24	0.347	0.267	68	0.167	0.069	103	0.203	0.150	138	0.476	0.344
26	0.239	0.208	69	0.356	0.122	104	0.193	0.179	139	0.308	0.265
27	0.266	0.175	71	0.270	0.191	105	0.431	0.204	140	0.293	0.275
28	0.336	0.208	72	0.335	0.096	106	0.332	0.166	141	0.437	0.341
30	0.309	0.179	73	0.271	0.169	108	0.346	0.219	142	0.395	0.259
31	0.265	0.199	74	0.275	0.084	109	0.298	0.255	143	0.455	0.429
32	0.236	0.100	75	0.196	0.090	110	0.318	0.270	144	0.384	0.199
33	0.283	0.166	76	0.191	0.104	111	0.383	0.254	145	0.497	0.352
35	0.241	0.173	77	0.272	0.249	112	0.338	0.264	146	0.417	0.364
37	0.228	0.154	78	0.214	0.177	113	0.295	0.204	147	0.475	0.392
39	0.268	0.156	79	0.161	0.126	114	0.357	0.186	148	0.500	0.307
40	0.417	0.247	80	0.371	0.289	115	0.344	0.234	149	0.509	0.400
41	0.367	0.276	81	0.222	0.406	116	0.158	0.113	150	0.515	0.376
42	0.318	0.261	82	0.224	0.656	117	0.312	0.197	151	0.545	0.355
43	0.303	0.264	84	0.406	0.299	118	0.365	0.226	152	0.655	0.561

ditions. Chlorophyll content decreases with water deprivation, such as under drought conditions, and dehydration occurs in the plant cell.

Total chlorophyll content. Entry 152 had the maximum total chlorophyll content with a mean value of 2.043 mg/g, followed by genotypes 151 (1.717 mg/g) and 89 (1.675 mg/g). Total chlorophyll content under control conditions was 0.483–2.043 mg/g with an average value of 1.023 mg/g. Total chlorophyll content values under drought were 0.219–1.751 mg/g with an average of 0.719 mg/g. Entry 152 had the highest mean value, 1.751 mg/g, under drought conditions, entry 149 had 1.293 mg/g and 89 had 1.289 mg/g. Chlorophyll content under drought conditions in most of the mapping population entries decreased with the exception of very few genotypes (Table 35–37, pp. 170-172). Drought affects photosynthesis negatively and, thus, lowers the yield of the cultivars in drought-prone environments. In this mapping population, lines 152, 89, 149, 99, and 68 showed better performance with a low percent decrease in chlorophyll content and can be used in breeding wheat for improved photosynthetic activity.

Superoxide dismutase activity. The maximum superoxide dismutase activity under controled conditions was observed in entry 74 (49.97 units/g), followed by entries 109 (49.91 u/g) and 103 (49.59 u/g) Mean values show that SOD activity under normal conditions was 12.86–49.97 u/g with an average of 32.44 u/g (Table 38, p. 173). SOD mean values were high under the stress condition in most lines, ranging from 18.85 to 51.88 u/g with an average of 38.00 u/g. The maximum SOD activity under drought was in entry 103, with mean value of 51.88 u/g, followed by entries 106 (49.92 u/g) and 68 (49.71 u/g). When genotypes with highest and lowest mean values under stress were compared with the control, both increasing (4.41%, 1.24%, and 25.48 % increases in genotypes 103, 106, and 68, respectively) and decreasing (15.81%, 53.75%, and 55.62% decreased in entries 149, 111, and 124, respectively) trends were observed. The degree

Table 37. Mean values for total chlorophyll content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Total chlorophyll content			Total chlorophyll content			Total chlorophyll content			Total chlorophyll content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.684	0.505	45	0.916	0.635	85	0.908	0.836	119	1.280	1.058
4	0.627	0.430	46	1.284	0.646	86	1.028	1.108	120	0.774	0.669
5	0.535	0.467	47	1.070	0.760	87	0.786	0.756	121	1.314	1.167
7	0.649	0.537	48	1.516	0.699	88	1.001	0.981	122	1.454	0.778
8	0.616	0.487	49	1.112	0.892	89	1.675	1.289	123	0.744	0.528
9	0.737	0.513	51	1.086	0.404	90	1.074	0.346	124	1.115	0.803
12	0.642	0.566	53	1.241	0.898	91	1.219	0.955	125	1.239	0.849
13	0.682	0.542	55	1.125	0.816	92	0.807	0.655	126	0.873	0.718
14	0.574	0.448	57	1.086	0.615	93	0.995	0.715	127	1.138	0.767
15	0.831	0.578	58	0.882	0.589	94	0.998	0.879	128	1.362	1.052
16	0.938	0.725	59	0.690	0.452	95	0.935	0.638	130	1.182	0.985
17	1.082	0.930	60	0.942	0.684	97	1.341	0.940	132	1.430	1.032
18	1.229	0.978	61	0.677	0.457	98	0.490	0.278	133	1.405	0.831
19	1.391	1.016	62	1.088	0.527	99	0.517	0.219	134	1.290	1.022
21	1.031	0.566	63	0.940	0.358	100	0.851	0.246	135	1.548	1.109
22	1.084	0.756	64	0.932	0.427	101	0.714	0.391	136	1.558	0.976
23	0.961	0.440	67	0.746	0.324	102	0.854	0.535	137	1.243	1.054
24	1.059	0.845	68	0.531	0.223	103	0.572	0.456	138	1.314	1.072
26	0.828	0.681	69	1.172	0.309	104	0.823	0.510	139	0.902	0.694
27	0.875	0.599	71	0.879	0.560	105	1.328	0.573	140	0.894	0.794
28	1.006	0.852	72	1.113	0.328	106	1.201	0.645	141	1.241	0.987
30	1.049	0.647	73	0.930	0.568	108	1.101	0.674	142	1.189	0.788
31	1.025	0.617	74	0.572	0.561	109	0.880	0.772	143	1.304	1.248
32	0.824	0.373	75	0.646	0.235	110	0.953	0.784	144	1.050	0.624
33	0.971	0.618	76	0.548	0.279	111	1.138	0.702	145	1.537	1.227
35	0.714	0.500	77	0.778	0.680	112	0.972	0.780	146	1.404	0.960
37	0.740	0.492	78	0.646	0.544	113	0.872	0.571	147	1.506	1.259
39	0.847	0.567	79	0.483	0.344	114	1.031	0.587	148	1.661	0.993
40	1.311	1.104	80	1.019	0.758	115	0.995	0.686	149	1.569	1.293
41	1.186	1.104	81	0.864	0.857	116	0.561	0.291	150	1.609	1.239
42	1.063	0.883	82	1.147	1.055	117	0.918	0.514	151	1.717	1.221
43	1.003	0.912	84	0.819	1.175	118	1.034	0.601	152	2.043	1.751

of oxidative stress and antioxidant activity seems to be closely associated with the tolerance/susceptibility of a genotype to water stress, therefore, the reduced SOD activity in genotypes 140, 111, and 124 indicates their high susceptibility to drought.

Table 38. Mean values for superoxide dismutase (SOD) activity (units/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	SOD activity			SOD activity			SOD activity			SOD activity	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	26.09	41.43	45	35.67	35.89	85	25.03	26.48	119	39.88	42.94
4	32.27	48.15	46	40.85	36.69	86	30.30	28.66	120	48.55	48.30
5	27.21	45.07	47	41.49	21.44	87	33.88	37.28	121	39.97	40.92
7	17.55	31.02	48	28.18	32.02	88	35.88	36.10	122	37.15	47.90
8	21.70	45.28	49	42.39	42.48	89	24.03	31.18	123	35.73	47.76
9	19.39	47.89	51	27.11	46.14	90	34.24	34.82	124	43.88	19.47
12	24.24	49.25	53	38.18	39.76	91	34.36	43.07	125	38.39	35.89
13	26.42	49.48	55	36.21	44.25	92	23.91	28.41	126	33.18	35.07
14	28.00	45.22	57	30.33	43.15	93	27.39	27.66	127	33.09	40.48
15	38.94	35.23	58	32.76	45.48	94	22.82	23.46	128	32.73	33.69
16	45.94	44.30	59	24.91	45.25	95	31.21	30.07	130	37.33	38.79
17	37.09	48.41	60	32.67	40.76	97	24.82	27.17	132	38.70	37.46
18	38.52	38.87	61	31.46	40.82	98	24.85	39.00	133	24.03	30.46
19	32.97	32.74	62	31.64	40.53	99	44.82	46.59	134	24.18	25.56
21	27.97	30.12	63	40.94	49.56	100	33.09	41.02	135	28.85	31.20
22	32.97	38.10	64	34.52	40.71	101	28.94	38.05	136	21.21	30.41
23	35.64	46.48	67	26.49	35.51	102	49.27	48.64	137	27.03	26.48
24	46.45	47.21	68	37.03	49.71	103	49.59	51.88	138	26.24	29.94
26	35.55	40.10	69	33.70	38.71	104	44.76	42.74	139	32.73	36.07
27	12.86	21.50	71	15.99	21.56	105	43.49	49.10	140	29.15	32.82
28	26.33	29.12	72	29.12	41.46	106	49.30	49.92	141	33.39	37.74
30	15.20	20.50	73	30.21	46.77	108	48.85	46.00	142	37.94	41.00
31	27.15	30.94	74	49.98	39.94	109	49.91	49.46	143	36.51	39.00
32	38.36	36.97	75	40.73	47.20	110	48.30	49.61	144	32.70	29.20
33	42.36	42.64	76	32.73	43.95	111	41.39	19.14	145	28.12	31.43
35	35.70	35.05	77	34.39	36.41	112	15.44	48.07	146	31.82	48.07
37	39.97	39.17	78	29.39	43.89	113	49.27	49.64	147	25.42	23.58
39	32.67	36.23	79	34.76	44.20	114	23.76	48.35	148	26.15	25.71
40	24.55	45.15	80	23.36	31.30	115	16.38	21.04	149	22.39	18.85
41	25.03	29.28	81	27.94	39.64	116	32.09	42.79	150	21.79	24.61
42	32.61	34.51	82	26.73	38.87	117	31.27	49.30	151	30.21	47.01
43	18.31	21.08	84	34.58	33.18	118	35.91	48.10	152	24.12	29.35

Conclusion. To enhance the breeding efficiency in stress-prone environments, several molecular mapping populations have been generated globally. Our focus was to phenotype a mapping population derived from an 'Opata/Synthetic hexaploid' cross combination for various phenological and physiological parameters to evaluate for drought tolerance under *in vivo* and *in vitro* conditions. The combined use of morphological and physiological markers is one of the best approaches to evaluate genotypes for their potential tolerance to abiotic and biotic stresses.

The data from the phenological parameters showed that genotypes 87, 80, 78, 108, 118, 63, 105, 23, 48, 103, 14, 51, 127, and 122 had a very good spike length under drought conditions, which is directly related to higher yield performance of the plants; entries 144, 57, 150, 122, 14, 18, 118, 28, 55, 23, 40, 17, 45, and 108 were found to be good for number of grains/spike. Genotypes with excellent performance for the most important and key parameter, 1,000-kernel weight, which is a direct measure of the yield, include 148, 55, 146, 14, 23, 150, 63, 82, 118, 45, 41, and 17. These genotypes can be used in wheat breeding programs of Pakistan.

Genotypes under *in vitro* conditions also showed better defense mechanisms against drought. Entries 14, 17, 152, 142, 40, 151, 128, 118, 15, 46, 103, 106, 68, 12, 4, 9, 23, 105, 102, 75, 73, 63, 109, 110, 117, 149, 89, 147, 150,

145, 151, 121, and 82 had a high accumulation of proline and had more antioxidant activity under stress conditions. These lines can be used in any breeding program targeted to improve wheat against drought stress.

The best lines of mapping population, based on both morphological and physiological evaluation, are 14, 17, 23, 55, 108, 118, 122, 150, and 152. These genotypes performed the best equally under both *in vivo* and *in vitro* testing. This research study based on morphological and physiological evaluation suggests that unique genetic diversity from *Ae. tauschii* can be harnessed for increased yield by improving the existing cultivars against biotic and abiotic stresses. Such a targeted approach to incorporate novel genes for tolerance against drought can revolutionize wheat production in water-stressed environments.

Molecular validation and utilization of T1BL·1RS wheat and its isogenic lines in wheat breeding.

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Wheat occupies 220 million hectare or 17% of the total cultivated land in the world and supports nearly 35% of the world's population. One of the biggest adaptabilities of wheat is that it can grow in a variety of environments, ranging from fully irrigated to high rainfall and drought regions; it also faces a wide range of biotic and abiotic stresses. The wheat crop needs more focus on improvement in each area for higher production and to fulfill consumer demand.

Wheat, being an ancient crop of Asia, is the most important crop of Pakistan and occupies a central position in agriculture and economy. Pakistan is the 6th largest wheat producer, contributing about 3.2% of world's wheat production. As the leading crop in Pakistan, wheat contributes 14.4% to the value added in agriculture and 3.1% to the GDP. Wheat was cultivated on area of 9.042 x 10^6 ha and production was 23.864 x 10^6 tons during 2010 (Government of Pakistan, 2009–10).

In wheat improvement programs, two major problems are the targets regarding quantity and quality that are biotic and abiotic constraints. Of the biotic stresses, rusts are a major problem; abiotic stresses include drought, salinity, and heat. In order to meet the rapidly growing demand for food, genetic resistance or tolerance of these stresses has become important for increasing production.

Wheat, the world's leading food crop, is mainly grown on rainfed lands, so low moisture is a primary constraint on wheat production in the semiarid areas of developing countries. Worldwide, 37% of the semiarid, wheat-growing areas are effected by drought. Pakistan falls in the arid and semiarid climatic conditions, 14% of the rainfed wheat growing area is under drought stress and almost 15 x 10^6 ha of cultivated land is affected Government of Pakistan, 2009–10). Therefore, drought is a major factor in Pakistan that is detrimental to wheat production significantly depressing the wheat yield.

In order to increase grain yield/ha, wheat genotypes with high yield potential and resistance to biotic and abiotic stresses have been developed. Wheat genotypes having alien chromosomes are highly adapted with higher yield potential. Among them, the T1BL·1RS wheat genotype is a successful example. This translocation was first found in the winter wheat cultivar Kavkaz and was transferred to spring bread wheat at CIMMYT from rye sources. Other sources of T1BL·1RS are Buho, Kalyansona, and Blue Bird. From Kalyansona, Veery S, an advanced line of bread wheat, was derived at CIMMYT and, from this line, various sister lines were released as Seri 82, Ures 81, Genaro 81, Glennson 81, and V 10. By selection from Seri 82, the cultivar Pak 81 was released in Pakistan.

The homozygous chromosome T1BL·1RS involves short arm of *S. cereale* chromosome 1R and the long arm of chromosome 1B of *T. aestivum*. Genes for leaf rust (*Lr26*), yellow rust (*Yr9*), stem rust (*Sr31*), and powdery mildew (*Pm8*) have been associated with the rye chromosome arm 1RS. The T1BL·1RS lines have more vigorous root growth, but generally have inferior dough and breadmaking quality, mainly dough stickiness, and poor mixing tolerance. The T1BL·1RS wheats exhibit a yield advantage over normal wheat, and this positive yield advantage is expressed more under a water deficit. Worldwide, wheat cultivars with T1BL·1RS occupy 5 x 10^6 ha of cultivated area. Keeping in view the effect of T1BL·1RS on yield components and other parameters, this study was designed to focus on the following:

– check for the presence of T1BL·1RS in an experimental set of Seri 82 lines,
– find the importance of T1BL·1RS isogenic lines for wheat production in Pakistan under drought conditions via evaluation tests conducted under a rain shelter, and,

– determine the yield advantage of T1BL·1RS and test the response of isogenic lines under varying drought conditions through some standard *in vitro* tests.

Germplasm. The experimental material was comprised of bread wheat isogenic lines of Seri 82, which is a T1BL·1RS cultivar. By crossing with a 1B cultivar, Pavon 76, and several backcrosses to Seri 82, isogenic lines were obtained. Twenty isogenic lines of Seri 82 were studied, ten were T1BL·1RS and ten were normal chromosomes from Pavon 76. This set of 20 isogenic lines were validated and stringently used to define the contribution of the translocated chromosome under simulated drought conditions at National Agriculture Research Centre, Islamabad. Genetic material was obtained from Wheat Wide Crosses and Cytogenetics Program, National Agriculture Research Centre, Islamabad. Details of the germ plasm are given in Table 39.

Table 39. List of derived wheat isogenic lines from Seri 82.

Pedigree	Origin
SERI/PVN//8*SERI	02-515
SERI/PVN//8*SERI	02-516
SERI/PVN//8*SERI	02-517
SERI/PVN//8*SERI	02-518
SERI/PVN//8*SERI	02-519
SERI/PVN//8*SERI	02-520
SERI/PVN//8*SERI	02-521
SERI/PVN//8*SERI	02-522
SERI/PVN//8*SERI	02-523
SERI/PVN//8*SERI	02-524
SERI/PVN//8*SERI	02-525
SERI/PVN//8*SERI	02-526
SERI/PVN//8*SERI	02-527
SERI/PVN//8*SERI	02-528
SERI/PVN//8*SERI	02-529
SERI/PVN//8*SERI	02-530
SERI/PVN//8*SERI	02-531
SERI/PVN//8*SERI	02-532
SERI/PVN//9*SERI	02-533
SERI/PVN//9*SERI	02-534

Molecular validation. A rye-specific, SSR marker was used to validate the presence or absence of the 1RS arm in all the 20 isogenic lines. The protocol involved standard DNA analysis using a RYE–NOR marker that was rye chromosome specific. The oligonucleotide sequence of the RYE–NOR forward primer was GCATGTAGCGACTAACTCATC and reverse primer was CCCAGTTTTCCATGTCGC.

***In vitro* screening for drought tolerance.** Drought tolerance was investigated under *in vitro* conditions conducted in a moisture-controlled (rain shelter) tunnel; the control was grown under field conditions in the research area of Wheat Wide Crosses and Cytogenetics Program, NARC, Islamabad. Test parameters included plant growth development culminating in a final measure of standard yield.

PEG treatment. The *in vitro* tests were for root-growth parameters involving coleoptile emergence. To identify drought-tolerant, Seri 82 isogenic lines at the seedling stage, the treatments were control (0% PEG), treatment 1 (20% PEG, 200 g/800 mL dH_2O), treatment 2 (30% PEG, 300 g/700 mL dH_2O), and treatment 3 (40% PEG, 400 g/600 mL dH_2O).

Seeds of all isogenic lines were initially treated with solution of sodium hypochlorite for ten minutes. Residual chlorine was eliminated by washing seeds with distilled water. Five seeds of each line were placed on the moist filter papers under osmotic potentials of 0, –4, –10, and –17 bars induced by a polyethylene glycol solution (PEG-6000). Distilled water (2 mL) was added to each petri dish in the control treatment and 2 mL of each PEG solution (20%, 30%, and 40%) was added to the each petri plate under osmotic stress conditions every two days. All the petri plates were placed in a growth chamber for ten days at an average temperature of 22°C±2°C and 50% relative humidity. Data were recorded when the radicals reached at least 2 mm in length up to the tenth day after sowing. Data for coleoptile length, shoot length, and root length were recorded at four different moisture stress levels from five seedlings of each line.

The maximum germination and seedling emergence was observed at 0% PEG but markedly decreased at 20%, 30%, and 40% PEG concentrations due to PEG-induced drought stress. PEG concentration and time of application affect growth, and these concentrations also had lethal effects. At 0% and 20% PEG, all 20 lines germinated and had seedling emergence, but at no seedling emergence was shown at 30% and 40% PEG. The mean values of coleoptile, shoot, and root length of the 20 isogenic lines under four different levels of PEG-6000 are given (Table 40, p. 176).

Considerable variation was observed for coleoptile, shoot, and root length in the 0% and 20% PEG treatments. The highest coleoptile, shoot, and root lengths were observed at 0% PEG. Data recorded for various parameters were subjected to analysis of variance, which indicated a considerable amount of variability for all the parameters at 0% and 20% PEG. Analysis of variance indicated that the difference among the treatments for seedling traits was highly significant and that the genotypes were significantly different from each other for all seedling traits except root length, which was not significantly different (Table 41, p. 176).

Coleoptile length. The osmoregulation capability of the 20 Seri 82 isogenic lines was examined for coleoptile length under four different levels of PEG. Considerable variation for coleoptile length was observed under 0% and 20% PEG,

Table 40. Mean values for coleoptile length (CL), shoot length (SL), and root length (RL) of Seri 82 isogenic lines under different treatments of polyethylene glycol (PEG-6000).

Line	0% PEG (control)			20% PEG			30% PEG			40% PEG		
	CL	SL	RL	CL	SL	RL	CL	SL	RL	CL	SL	RL
02-515	1.70	8.33	15.50	1.10	2.23	4.23	0	0	0	0	0	0
02-516	1.83	10.00	14.83	0.90	0.87	1.83	0	0	0	0	0	0
02-517	2.00	9.17	13.00	0.00	0.00	1.33	0	0	0	0	0	0
02-518	2.13	9.07	8.57	1.83	2.23	6.20	0	0	0	0	0	0
02-519	2.50	12.73	13.27	1.33	2.03	3.57	0	0	0	0	0	0
02-520	1.67	7.33	9.60	1.40	3.03	4.90	0	0	0	0	0	0
02-521	1.90	9.97	16.30	1.33	2.87	4.40	0	0	0	0	0	0
02-522	1.87	7.70	10.10	0.93	1.07	2.23	0	0	0	0	0	0
02-523	1.87	9.33	12.57	0.83	0.73	2.50	0	0	0	0	0	0
02-524	1.93	9.73	13.23	1.17	1.80	3.20	0	0	0	0	0	0
02-525	1.73	9.60	8.83	1.07	1.17	3.63	0	0	0	0	0	0
02-526	2.60	10.47	17.53	2.23	7.30	7.97	0	0	0	0	0	0
02-527	1.80	8.03	10.37	1.03	2.10	2.00	0	0	0	0	0	0
02-528	1.87	10.07	11.57	1.20	2.10	3.20	0	0	0	0	0	0
02-529	1.97	9.50	14.30	1.40	1.10	3.67	0	0	0	0	0	0
02-530	2.33	11.77	12.00	1.83	2.77	5.33	0	0	0	0	0	0
02-531	2.37	10.73	15.10	1.37	1.30	1.70	0	0	0	0	0	0
02-532	2.10	10.57	11.33	1.53	2.57	4.87	0	0	0	0	0	0
02-533	2.03	9.90	14.07	1.57	2.07	3.87	0	0	0	0	0	0
02-534	2.17	9.93	15.23	2.03	1.77	3.07	0	0	0	0	0	0

and both treatments were significantly different from each other. The maximum coleoptile emergence was at 0%, least at 20%, and no emergence in the 30% and 40% PEG treatments (Table 40).

At 0% PEG (control), the coleoptile length was 1.67–2.60 cm (Table 40). The greatest coleoptile length was in isogenic line 02-526 and the shortest in line 02-520. Coleoptile length was 0.00–2.23 cm at 20% PEG (Table 40); the greatest length was in line 02-526 and the shortest in 02-517. At 30% and 40% PEG, coleoptile emergence was not observed in any isogenic line. The greatest reduction was noted for coleoptile length under osmotic stress.

Table 41. Mean square values of Seri isogenic lines for coleoptile, shoot, and root length under control and drought-stress conditions (* significant at the 5% level of probability; ** significant at the 1% level of probability).

Parameter	df	Coleoptile length	Shoot length	Root length
Genotype	19	0.726*	6.670*	14.603
Treatment	1	14.560**	1,759.502**	2,528.172**
G x E	19	0.288	4.715	13.900
Error	80	0.398	3.592	9.224

An analysis of variance revealed that there was significant difference among all lines for coleoptile length under 0% and 20% PEG (Table 41). The LSD determines the level of significance among all isogenic lines. On the basis of significance level, all of the 20 isogenic lines were divided in different ranges. Line 02-526 was significantly different from all the lines except 02-518, 02-519, 02-530, 02-531, 02-532, and 02-533, and 02-534. The highest mean coleoptile length was 2.146 cm at 0% and 20% PEG. the lowest mean coleoptile lenght of 1 cm was in line 02-517 under both 0% and 20% treatments, and it was significantly different from all other lines except 02-515, 02-516, 02-520, 02-521, 02-522, 02-523, 02-524, 02-525, 02-527, 02-528, and 02-529.

Shoot length. Shoot emergence was observed only in the 0% (control) and 20% PEG treatments. Emergence did not take place in 30% and 40% PEG treatments. The maximum shoot length was in the 0% treatment, ranged from 7.33 to 12.73 cm and the minimum was at 20% PEG, ranging from 0.00 to 7.30 cm (Table 40). At 0% PEG, the greatest shoot length was in line 02-519 and the shortest in line 02-520. At 20% PEG, isogenic line 02-526 had the greatest shoot length and line 02-517 the shortest.

The analysis of variance of shoot length showed that both treatments were significantly different from each other and all lines were found to be significantly different under both treatments with minute variation (Table 41). The LSD of the Seri 82 isolines for shoot length showed a significant difference among lines. Isogenic line 02-526 was not significantly different from 02-519 and 02-530 but was significantly different from all other lines, with the highest mean

value of 8.883 cm at 0% and 20% PEG. Isogenic line 02-522 was significantly different for shoot length from lines 02-519, 02-526, 02-530, and 02-532, with the lowest mean value of 4.383 cm under both PEG treatments.

Root length. Root emergence was observed in the isogenic lines subjected only to 0% and 20% PEG. Root length of the lines at 0% PEG were 8.57–17.53 cm (Table 40, p. 176). The longest root length was in line 02-526 and the shortest in 02-518. At 20% PEG, root length was 1.33–7.97 cm (Table 40, p. 176), with the longest shown in line 02-526 and the shortest in 02-517. Root emergence was not observed in any isogenic line of the 30% and 40% PEG treatments.

The analysis of variance showed that all lines were not significantly different from each other for root length, but both treatments were significantly different from each other (Table 41, p. 176). The level of significance between the lines was determined by an LSD test, and all lines showed almost same root length with minute variations. Isogenic line 02-526 had the highest mean value of 12.75 cm for root length at 0% and 20% PEG, which was significantly different from all other lines except 02-515 and 02-521. Line 02-522 was significantly different from isogenic lines 02-515, 02-521, and 02-526, with the lowest mean value of 6.166 cm. All other lines were not significantly different from each other at 0% and 20% PEG.

Correlation coefficients computed between the coleoptile, shoot, and root lengths which revealed that the three traits were strongly, positively correlated with each other with the highest correlation values in the 0% (control) and 20% PEG treatments. A strong correlation of 0.902 was shown by shoot length with root length at 0% and 20% PEG. Lines with a greater coleoptile length also showed greater shoot and root lengths and, therefore, performed better under drought conditions.

These results indicate that Seri 82 isogenic line 02-526 performed best under conditions of moisture availability and moisture stress with the longest coleoptile length (2.416 cm), shoot length (8.883 cm), and root length (12.75 cm). Other best performing lines were 02-519, 02-521, 02-524, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534. These lines have a good genotypic potential for drought tolerance.

Morphological evaluation. Data on eight morphological parameters were recorded for the 20 isogenic lines under both field (control) and rain-shelter conditions (moisture-stress conditions). Five plants of each entry were used for data and the arithmetic mean calculated. The correlation of Seri 82 isogenic lines data set was computed with the objective to determine the effect of various morphological and phenological traits (pubescence, waxiness, days-to-heading, days-to-physiological maturity, plant height, and spike length) on yield parameters (grains/spike and 1,000-kernel weight) under irrigated and drought-stressed conditions and the inherent association among the parameters. (Table 42, p. 178).

Seri 82 isogenic lines 02-522, 02-524, 02-525, 02-527, 02-528, 02-529, 02-530, 02-533, and 02-+534 performed well for all the morphological parameters and yield attributes under controlled and moisture-stress conditions. These lines are agronomically good, with high yield potential. The morphological analysis showed that there was a little diversity among the entries under controlled and stressed environments. Lines 02-519, 02-522, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 had the 1RS translocation and were drought tolerant. These lines were validated for 1RS through molecular and cytological analysis and were selected on the basis of their root growth and morphological parameters, having the longest coleoptile, shoot, and root lengths under *in vitro* conditions and performed well for days-to-heading, days-to-physiological maturity, plant height, spike length, grains/spike, and 1,000-kernel weight under moisture stressed conditions.

Molecular diagnostics. Characterization and validation of chromosome arm 1RS in the Seri 82 isogenic lines at the DNA level used microsatellite markers or SSRs that were chromosome specific. The SSR-marker technique helped to characterize 1RS in wheat genotypes. SSRs are valuable and widely used molecular markers in plant species, especially wheat, for marker-assisted selection, because they are variable, co-dominant, genome specific, and need less DNA.

Chromosome arm 1RS of rye is intensively used in wheat breeding because of presence of many useful genes. The sequence-specific, SSR marker RYE–NOR, known to be specific for 1RS, was used for the molecular validation of the 1RS translocation in the isogenic lines. This primer amplified polymorphic bands in sizes ranged from 400 bp to 800 bp. The maximum number of scorable bands was three and the minimum was two.

The RYE–NOR primer amplification profile of the 20 Seri 82 isogenic lines detected scorable bands lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 531, 02-532, 02-533, and 02-534, which ranged from 400 bp to 800 bp. Two had 1RS translocations. A maximum of three scorable bands were detected in lines 02-519, 02-526, 02-528,

Table 42. Mean values for morphological parameters of the Seri 82 isogenic lines under control (field) and stress (rain shelter) conditions.

Line	Pubescence	Waxiness	Days–to–heading	Days–to–physiological maturity	Plant height (cm)	Spike length (cm)	Grains/spike	1,000–kernel weight (g)
Control								
02-515	–	+	123	159	50.00	9.22	51	33.1
02-516	–	–	122	157	50.96	10.38	47	37.4
02-517	–	–	120	156	56.24	11.02	63	36.2
02-518	–	–	120	156	64.62	12.40	67	43.6
02-519	–	–	120	155	67.82	9.96	52	31.0
02-520	–	–	128	159	64.50	11.78	75	37.6
02-521	–	–	126	160	72.96	11.38	69	29.3
02-522	–	–	125	160	75.84	11.40	66	34.5
02-523	–	–	122	154	71.56	9.90	39	16.5
02-524	–	–	121	154	75.02	10.00	53	34.9
02-525	–	+	120	151	70.02	10.86	53	37.4
02-526	–	–	112	135	60.30	9.70	47	29.3
02-527	–	+	114	150	71.32	10.94	50	28.9
02-528	+	–	118	152	67.28	10.90	54	30.1
02-529	–	–	120	151	65.42	10.52	55	25.8
02-530	–	–	120	151	63.40	9.98	56	29.9
02-531	–	–	110	149	74.82	9.70	43	27.8
02-532	–	+	120	151	71.64	10.48	47	29.5
02-533	–	–	118	149	59.92	9.84	59	30.8
02-534	–	–	118	151	61.54	10.98	67	23.6
Drought stress								
02-515	–	+	118	151	60.06	8.62	23	21.8
02-516	–	–	111	151	71.00	9.66	29	20.0
02-517	–	–	116	147	69.56	9.84	29	22.7
02-518	–	–	117	152	68.92	11.06	39	25.6
02-519	–	–	116	147	61.68	11.24	44	13.5
02-520	–	–	118	148	68.30	10.60	38	19.5
02-521	–	–	118	152	71.50	11.36	37	11.9
02-522	–	–	116	151	72.56	11.54	49	23.5
02-523	–	–	113	143	68.02	12.06	48	22.8
02-524	–	–	113	145	69.48	12.38	48	26.8
02-525	–	+	111	147	63.28	11.68	41	23.2
02-526	–	–	115	138	62.50	9.00	43	28.5
02-527	–	+	118	144	63.84	11.74	49	32.6
02-528	+	–	111	145	58.90	12.22	48	31.2
02-529	–	–	118	147	59.26	12.00	40	26.5
02-530	–	–	118	145	60.20	11.82	43	23.0
02-531	–	–	121	149	49.06	11.44	46	30.0
02-532	–	+	111	149	59.20	12.52	57	30.5
02-533	–	–	111	149	57.24	10.98	43	26.8
02-534	–	–	111	146	70.48	11.06	48	28.6

02-529, 02-530, 02-531, 02-532, 02-533, and 02-534. Two bands were detected in lines 02-522 and 02-523 with band sizes ranging from 400 bp to 800 bp. Only one band was detected in isogenic line 02-527. No amplification was reported in 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, and 02-525.

The data of RYE–NOR was recorded in terms of the presence or absence of 2–3 bands within the range of 400–800 bp. The presence of bands within this range was recorded as positive (+), indicating the presence of rye chromatin (1RS), and the absence negative (–), no rye chromatin (Table 43, p. 179). Primer RYE–NOR showed the highest polymorphism in translocated lines.

Cytological diagnostics. Cytology of selected germ plasm was through validation by mitotic analysis and C-banding, which permit confirmation of T1BL·1RS. Routine mitotic analysis was done by staining the root tips with a 2% solution of aceto-orcein and visualizing the chromosomes under a microscope. The C-banding patterns were used to visualize the 1RS rye chromosome segment in the isogenic lines.

Mitotic analysis. For further validation of selected germ plasm, somatic cells of 20 isogenic lines were analyzed mitotically. In normal bread wheat, the short arms of chromosomes 1B, 6B, and 5D, have secondary constrictions, or satellites, but the resolutioj of the 5D satellites was inconsistent. In translocated wheat, T1BL1RS, a prominent satellite is associated with 6BS and 1RS replaces 1BS. Initial identification of 1B·1B and T1BL·1RS was by observing the satellites in somatic cells and through chromosome counts at metaphase. In all 20 lines, 42 chromosomes were counted. Four prominent satellites of 1B and 6B were observed in lines 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, 02-525, and 02-527, and two satellites of 6B were observed in isogenic lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 at metaphase from several root tips, which confirmed the presence of T1BL·1RS. In various preparations, 5D satellites also were observed. The translocation status of 20 isogenic lines is given in Table 43.

Table 43. Molecular and cytological validation of Seri 82 isogenic lines for T1BL·1RS (For molecular marker and C-banding results, a + indicates presence and a – absence of the translocation. For cytology, the number in parentheses indicates the presence of the translocation, 2 indicates presence and 4 indicates absence).

Isoline	Molecular marker diagnostic for 1RS	Cytology diagnostic for 1RS	C-banding diagnostic for 1RS
02-515	–	1B·1B, 6B·6B (4)	–
02-516	–	1B·1B, 6B·6B (4)	–
02-517	–	1B·1B, 6B·6B (4)	–
02-518	–	1B·1B, 6B·6B (4)	–
02-519	+	6B·6B (2)	+
02-520	–	1B·1B, 6B·6B (4)	–
02-521	–	1B·1B, 6B·6B (4)	–
02-522	+	6B·6B (2)	+
02-523	+	6B·6B (2)	+
02-523	+	6B·6B (2)	+
02-524	–	1B·1B, 6B·6B (4)	–
02-525	–	1B·1B, 6B·6B (4)	–
02-526	+	6B·6B (2)	+
02-527	–	1B·1B, 6B·6B (4)	–
02-528	+	6B·6B (2)	+
02-529	+	6B·6B (2)	+
02-530	+	6B·6B (2)	+
02-531	+	6B·6B (2)	+
02-532	+	6B·6B (2)	+
02-533	+	6B·6B (2)	+
02-534	+	6B·6B (2)	+

C-banding analysis. The C-banding technique characterized the T1BL·1RS and 1B·1B chromosomes through prominent bands on terminal and subterminal regions. The 1BL·1RS C-banded chromosomes are characterized by prominent bands on the centromeric, terminal, and subterminal sites of the short arm indicating the presence of 1RS and the terminal band on the end of the long arm of chromosome 1B. The C-banded homozygous 1B chromosome is characterized by a banding pattern on both the short (1BS) and long (1BL) arms.

We used C-banding for additional validation of T1BL·1RS in the Seri 82 isogenic lines. The C-banding pattern of lines 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, 02-525, and 02-527 showed prominent bands on the short and long arms of chromosome 1B, confirming the absence of T1BL·1RS. In lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534, a positive banding pattern was observed at the centromeric, terminal, and subterminal regions of 1B short arm and terminal bands on 1B long arm confirmed the presence of 1RS in these lines (Table 43).

The relationship of T1BL·1RS wheat and rye have been validated more clearly through conventional cytological techniques, because these techniques helped to understand the homoeologous relationship among the chromosomes of cultivated wheat and rye. Using the conventional cytological technique for root tips not only reduces the possibilities of misinterpretation but allows larger numbers of plants to be cytologically examined. The C-banded 1RS arm of rye is totally different from that of 1BS arm of wheat. Good metaphase chromosome preparations from root tip cells result in clear resolution of chromosomes 1B and 6B, which carry secondary constrictions on their short arms. The 1B and 6B satellites were observed in the present study and found to be more conspicuous in good preparations; less conspicuous secondary constrictions were observed for chromosome 5D in only a few preparations.

Rye chromosomes were first time identified through C-banding. Through preferential staining, wheat and rye chromosomes stain differently and are distinguished easily. Using the landmark bands on the short and long arms of chromosomes, the Seri 82 isogenic lines were characterized as T1BL·1RS or nontranslocated 1B·1B chromosomes (Table 43). Molecular and cytological techniques enable the characterization and validation of 1RS in Seri 82 isogenic lines of wheat. Through these techniques, Seri 82 isogenic lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 have T1BL·1RS, giving the same results with both techniques, and can be recommended for transferring 1RS to other wheat genotypes.

Cytological studies of genetic stocks from a Triticum aestivum/Thinopyrum bessarabicum intergeneric hybrid for wheat improvement.

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Wild species in the genus *Thinopyrum* constituting the tertiary gene pool represent a vast reservoir of useful agronomic traits for wheat and forage improvement. Chromosome engineering from *Thinopyrum* species into wheat has been practiced for 70 years. Approximately 30 economically important traits have been transferred from *Thinopyrum* species into wheat. Genetic transfer is usually realized through chromosome engineering, using *Triticum–Thinopyrum* partial amphiploids or chromosome addition, substitution, or translocation lines obtained from wide hybridization. The transfer and identification of desirable chromosome or chromosomal segments from *Thinopyrum* in wheat has benefited greatly from new developments in cytogenetics and molecular diagnostic techniques. Traditionally, the introduction of alien chromatin segments into wheat was confirmed by chromosome counting and pairing studies and/or with banding techniques. Although the conventional means are avenue to access the variability, newer technologies for the extraction of variability are needed to ameliorate the current cultivars. The study was conducted on a *T. aestivum* cv. Chinese Spring/*Th. bessarabicum* intergeneric hybrid, their disomic addition lines, and derived translocation stocks with their phenological characterization.

Our objectives were the cytological validation of the genetic stocks derived from bread wheat/*Th. bessarabicum* crosss as alien disomic addition (2x=6x=44) and translocation lines using conventional mitotic/meiotic analyses and giemsa C-banding and the development of fully characerized germ plasm with selections made for 56-chromosome amphiploid; 44-chromosome, disomic, addition lines; and full documentation of the translocation lines.

Experimental material. Germ plasm used included an intergeneric amphiploid of Chinese Spring bread wheat with *Th. bessarabiccum* that is an octaploid (2n=8x=56, AABBDDE^{b}E^b), seven disomic chromosome addition lines (2n=6x=42 + 1E^{b}E^b to 7E^{b}E^b) produced through backcrossing the amphiploid with wheat and cytologically identifying the complete set of seven additions, and wheat/alien chromosome translocation lines produced from backcrossing the amphiploid with a *phph* gene stock to facilitate alien introgressions leading to translocations through *ph*-based manipulation (Table 44). Seeds were germinated in petri dishes; 4,000 seeds of addition lines were planted. After germination, root tips were collected from individual seedling across all categories. Twenty-five seedlings of each amphiploid, addition, and translocation line were used for cytological studies. Individual seedlings were transplanted into Jiffy-7 peat pots. After 10 days growth, the seedlings of the amphiploid and *phph* stock were transplanted into pots filled with a soil:sand:leaf manure (2:1:1) mix and grown in a screen house. Chinese Spring and the seven addition lines were planted in the field.

Table 44. Chromosom status of wheat germ plasm with parentage.

Germ plasm	Parentage	Chromosome constitution
CS	Chinese Spring	42 (ABD)
Amphiploid	CS *Th. bessarabiccum*	56 (ABDE^b)
Addition line 1E^b	CS/ *Th. bessarabiccum*//Gen81	42+1E^{b}1E^b
Addition line 2E^b	CS/ *Th. bessarabiccum*//CS	42+2E^{b}2E^b
Addition line 3E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+3E^{b}3E^b
Addition line 4E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+4E^{b}4E^b
Addition line 5E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+5E^{b}5E^b
Addition line 6E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+6E^{b}6E^b
Addition line 7E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+7E^{b}7E^b
phph stock	CS/ *Th. bessarabiccum*//*ph*	49 (ABDE^b)

Cytological characterization. ***Mitotic analysis.*** Mitotic analysis was conducted to purify the genetic stocks. Somatic counts of mitotic chromosomes of the amphiploid, the seven addition lines, and the translocation stock focused on selecting plants with 56, 44, and 49 chromosomes, respectively, to establish seed purity. Cytology confirmed the status of amphiploid, addition lines, and translocation stock.

In Chinese Spring, the secondary constrictions and satellites were observed at metaphase. The secondary constrictions are present on the short arms of chromosomes 1B, 6B, and 5D (Fig. 27, p. 181). The secondary constriction of chromosome 5D of wheat was observed in some preparations. The secondary constriction of 5D frequently can be observed by shortening the pretreatment time.

The somatic cytology of the seven addition lines focused on selecting plants with 44 chromosomes (Fig. 28). In the addition lines, a variable number of chromosomes was observed, i.e., 41, 42, 43, and 44. Secondary constrictions, satellites, and telocentrics were observed. Telocentrics were detected only in some preparations. The presence of telocentrics indicates a whole-arm translocation and a chromosome number up to 45. For intergeneric hybrid, plants with 56 chromosomes, and in *phph* stock, plants with 49 chromosomes, were selected. Variable somatic counts of 49, 50, 51, and 53 were found in the translocation stock.

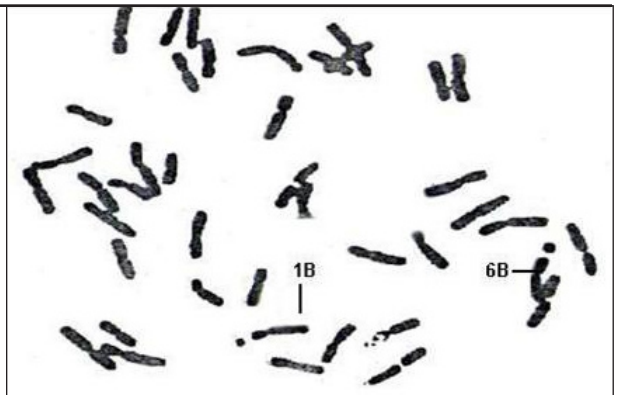

Fig. 27. Somatic chromosomes of wheat cultivar Chinese Spring (2n=6x=42).

Aceto-orcein binds with chromatin and stains chromosome nicely for optical microscopy. With traditional dyes, individual chromosomes in a complement can be identified only by chromosome size, the position of the centromere, and location of secondary constrictions. The pretreatment time for all samples was three hours, which seemed to be the optimum as inferred by the size of chromosomes at metaphase. The heat, pressure, and amount of the acetic acid determine the chromosomal spread, which aids in precise counting of the chromosomes and in observing the position of the centromere and secondary constrictions.

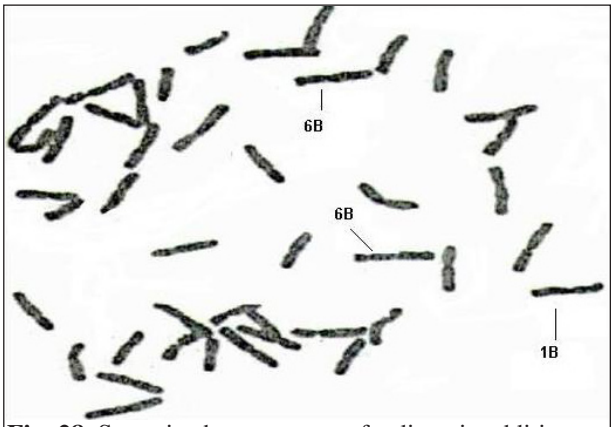

Fig. 28. Somatic chromosomes of a disomic addition line (2n=6x=44).

Cytological observation of each seed of the germ plasm was essential in order to maintain a normal euploid status, which aids in discerning hypo/hyperploidy. Cytological screening enabled the purification of the genetic stocks to give a wide array of plants with 56 (amphiploid), 44 (addition lines), and 49 (*phph* stock) chromosomes. Hybrid confirmation was obtained by a root-tip counts carried out either before or after the transplanting to peat pots or soil. Mitotic counts are essential for maintaining the normal euploid status of the seed stocks.

Quality, cytological procedures are essential for applied, alien transfers. Cytogenetic tools also are instrumental for studying the taxonomic relationships based upon karyotypic analysis. Metaphase chromosome spreads with optimum chromosome contraction and a high mitotic index are instrumental in the preparation of cytological preparations for use in chromosome banding.

Constitutive heterochromatin banding (C-banding). C-banding patterns of wheat/*Th. bessarabicum* amphiploid, the seven addition lines, and the translocation stock were determined by Giemsa C-banding. C-banding detects the presence of alien chromosomes to confirm *Th. bessarabicum* chromosome in the Chinese Spring background. *Thinopyrum bessarabicum* chromosomes exhibit prominent ,terminal heterochromatic regions and can be easily identified (Fig. 29). Distinct bands for each of the seven chromosomes of *Th. bessarabicum* were detected in the germ plasm. The C-banded karyotype distinctly identifies individual *Th. bessarabicum* chromosomes from those of *T. aestivum*.

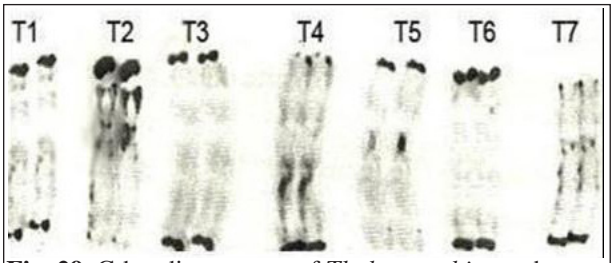

Fig. 29. C-banding pattern of *Th. bessarabicum* chromosomes.

C-banding of the CS–*Th. bessarabicum* showed that 14 of the 56 chromosomes were from *Th. bessarabicum* and the remaining 42 were wheat. In each of the seven addition lines, two of the 44 chromosomes were of *Th. bessarabicum* in $1E^b$ to $7E^b$ addition lines. In the *phph* translocation stock, seven of the 49 chromosome were *Th. bessarabicum* and the remaining were wheat. Due to an inactive *PhPh* locus, translocations were observed in this stock that need to be characterized further.

The bands present in the *Th. bessarabicum* chromosomes were medium to large and terminal (Fig. 29, p. 181). Chromosomes $1E^b$ (T1) and $2E^b$ (T2) had satellites on its short arms. Chromosomes $1E^b$, $3E^b$, (T3) and $6E^b$ (T6) had terminal bands on both the long and short arms. Terminal banding sites on the short arms were observed in chromosome $2E^b$ and $5E^b$ (T5). Chromosomes $4E^b$ (T4) and $7E^b$ (T7) had terminal bands on the long arms and variable terminal sites on the short arms. The banding details of *Th. bessarabicum* chromosomes confirm those reported earlier. C-banding is used to identify individual alien chromosomes in wheat background. The unique C-banding patterns of *Th. bessarabicum* chromosomes served as diagnostic markers for determining the chromosomes involved in translocations.

Meiotic analysis. Although the somatic evidence was adequate to validate the hybrid status, meiotic data reconfirms hybrids and provides a practical basis for advancing the F_1 hybrids. Chromosome pairing analysis in hybrids is based on the scoring of meiotic configurations i.e., univalents, rod and ring bivalents, trivalents, on metaphase I cells. The level of meiotic recombination between two chromosomes depends on the extent of chromosome pairing and chiasmata formation. Rod bivalents have one chiasmata, and ring bivalents have minimum two and maximum four chiasmata. Five chiasmata also are possible in ring bivalents, but it depends on personal observation. The extent of intergenomic recombination between the two parental species is determined by counting chromosomal arm associations. Meiotic studies of those plants were conducted, which showed a somatic count of 56 chromosomes (amphiploid), 44 chromosomes (addition lines), and 49 chromosomes (tanslocation stock).

Meiotic analysis of amphiploid showed a regular meiotic behavior. No multivalent or unpaired chromosomes were observed (Table 45). The amphilploid had the expected 28 bivalents. The amphiploid had a 2n=8x=56 chromosome composition with a mean association of 6.12 II rod bivalents, 21.89 II ring bivalents, and a mean chiasmata frequency of 64.12/cell. The high chiasmata frequency per cell supports the inactive 5B locus.

Table 45. Mean values for metaphase I chromosome associations in genetic stocks derived from *Th. bessarabicum*.

Line	Univalents	Bivalents		Trivalents	Chiasmata frequency per cell
		Rod	Ring		
Chinese Spring	–	18.20	2.80	–	56.60
Amphiploid	–	21.89	6.12	–	64.12
$1E^b$	–	18.84	3.15	–	56.92
$2E^b$	–	17.30	4.70	–	51.27
$3E^b$	–	18.26	3.74	–	49.70
$4E^b$	–	20.18	1.89	–	53.93
$5E^b$	–	19.67	2.34	–	52.50
$6E^b$	–	18.84	3.17	–	51.17
$7E^b$	–	19.11	2.88	–	55.55
phph stock	2.80	12.60	4.40	4.20	71.17

Cytological analysis of disomic addition lines provided evidence for normal meiotic metaphase I chromosome associations with 22 bivalents, as expected, and no multivalents or unpaired chromosomes (Fig. 30). The chromosome $1E^b$ addition line was 2n=6x=42 + $1E^b$ $1E^b$ or 44 chromosomes with a mean chromosome pairing of 18.84 II ring bivalents and 3.15 II rod bivalents and a chiasmata frequency of 56.92/cell. The chromosome $2E^b$ addition line was 2n=6x=42 + $2E^b$ $2E^b$ or 44 chromosomes with a mean chromosome pairing association of 17.30 II ring bivlalents, 4.70 II rod bivalents, abd a chiasmata frequency of 51.27/cell. The chromosome $3E^b$ disomic addition line was 2n=6x=42 + $3E^b$ $3E^b$ or 44 chromosomes with a mean chromosome pairing relationship of 18.26 II ring bivalents, 3.74 II rod bivalents, abd a chiasmata frequency of 49.70/cell. The $3E^b$ addition line showed a good meiotic index. The chromosome $4E^b$ addition line was 2n=6x=42 + $4E^b$ $4E^b$ or 44 chromosomes with a mean chromosome association of 20.18 II ring bivalents, 1.89 II rod bivalents, and a chiasmata frequency of 53.93/cell. At booting stage, eight florets/spikelet were observed in the $4E^b$ addition line whereas normally five florets are present in a spikelet. This addition line also had a high meiotic index.

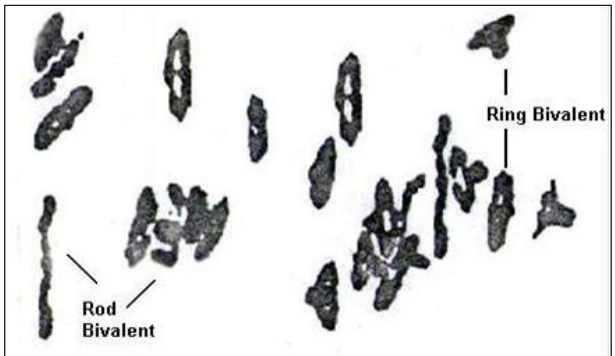

Fig. 30. Meiotic metaphase I associations in disomic addition lines 2n=6x=44 showing 22 ring and rod bivalents.

The chromosome $5E^b$ disomic addition line was 2n=6x=42 + $5E^b$ $5E^b$ or 44 chromosomes with a mean chromosome pairing of 19.67 II ring bivalents, 2.34 II rod bivalents, and a chiasmata frequency of 52.50/cell. The chromosome $6E^b$ disomic addition line had 2n=6x=42 + $6E^b$ $6E^b$ or 44 chromosomes with a mean chromosome pairing of 18.84 II ring

bivalents, 3.17 II rod bivalents, and a chiasmata frequency of 51.17/cell. The chromosome $7E^b$ disomic addition line was 2n=6x=42 + $7E^b$ $7E^b$ or 44 chromosomes with a mean chromosome pairing of 19.11 II ring bivalents, 2.88 II rod bivalents, and a chiasmata frequency of 55.55/cell.

The *phph* translocation stock was 2n=7x=49, AABBDDE^{b}E^b. This BC_1 progeny will become the source of BC_2 seed, which will have derivatives with 42 wheat chromosomes and zero to seven *Th. bessarabicum* chromosomes. This translocation stock showed 2.80 I univalents, 12.60 II ring bivalents, 4.40 II rod bivalents, and 4.20 III trivalents, with a chiasmata frequency of 71.17/cell.

Table 46. Phenotype of the genetic stocks derived from *Thinopyrum bessarabicum*. For grain color, BB = blackish brown, LB = light brown, and B = brown; for awn color, CW = creamy white.

Line	Days-to-heading	Days-to-physiological maturity	Plant height (cm)	Tillers/plant	Spike length (cm)	Grains/spike	Grains/plant	1,000-kernel weight (g)	Grain color	Awn color	Awns
Chinese Spring	123	174	120.00	–	10.20	67	315	24.40	B	–	Awnless
Amphiploid	134	163	70.70	6	13.20	19	32	–	LB	CW	Awnlets
Translocation stock	136	170	62.60	4	8.80	15	65	–	LB	CW	Awnlets
$1E^b$ addition line	79	110	67.00	12	11.20	41	384	19.50	B	CW	Awnlets
$2E^b$ addition line	78	110	70.20	14	10.60	35	316	17.30	LB	CW	Awnlets
$3E^b$ addition line	79	113	81.50	7	13.00	35	304	24.80	LB	-	Awnless
$4E^b$ addition line	80	113	77.70	10	12.70	26	305	22.80	BB	CW	Awned
$5E^b$ addition line	78	109	57.50	11	9.90	33	427	23.40	LB	CW	Awnlets
$6E^b$ addition line	80	104	55.00	2	6.90	17	35	–	LB	CW	Awnlets
$7E^b$ addition line	80	116	57.70	10	11.20	37	100	33.40	B	CW	Awned

The *phph* stock eliminates or suppresses the activity of the *Ph* gene to promote pairing between alien and wheat chromosomes to accelerate the process of gene transfer. The *phph*-based manipulation strategy offers great potential for obtaining alien transfers with minimum disturbance to the recipient genome. Pairing between wheat and alien chromosomes is a prerequisite for successful gene transfer. The *phph* stock showed extensive homoeologous pairing because of the lack of a dominant *Ph* gene. Such homoeologous pairing leads to the development of translocations, substitutions, and addition lines.

Regular meiotic behavior deciphers the transmission of the alien chromosomes to the progenies, and high fertility is beneficial for meeting the applied agricultural gains. Traditionally, alien introgressions in wheat have been characterized through meiotic metaphase I pairing. Germ plasm stability is of prime importance towards alleviating the constraints with biotic/abiotic screening for esoteric traits. Cytogenetics is valuable for a quick and accurate diagnosis of expected gene transfer and establishing stable gene introgressions. Cytogenetic manipulations can help in engineering desirable alien chromatin into wheat genome. Although high variability for biotic and abiotic stresses resides in the tertiary gene pool, very little has found its way to modern cultivars due to complex inheritance, unstable meiotic transmissions, and deleterious linked effects. Elucidating the compensating translocations, substitutions, and additions is facilitated by the newer technologies.

That most of the genetic stocks developed by the cytogeneticists over the past few decades are mostly maintained as oddities in germ plasm collections is unfortunate. These genetic stocks need to be characterized for response to biotic and abiotic stresses so that they can be used in applied breeding for stress tolerance.

Phenological characterization. Data on 11 morphological parameters was recorded for Chinese Spring wheat, the intergenric wheat/*Th. bessarabicum* hybrid, the seven addition lines, and the *phph* translocation stock. Three plants of each entry were used to record the data and the arithmetic mean was calculate (Table 46).

Days-to-heading. The amphiploid and *phph* translocation stock were very late heading compared to CS, the amphiploid, the *phph* translocation stock, and the seven addition lines, which were early heading. The addition lines had similar heading days.

Days-to-physiological maturity. The amphiploid had a rather early maturity compared to CS and the *phph* stock. Days-to-maturity in the addition lines was 104–116 days. Addition line $6E^b$ had early maturity and $7E^b$ was late.

Plant height. Plant height of the addition lines was 55–82 cm. Line $3E^b$ ws the tallest. Additions $3E^b$ and $4E^b$ were taller than the amphilploid and *phph* stock. The maximum plant height was in CS.

Tillers/plant. The number of productive tillers determines yield in terms of the number of spikes and grain produced. The amphiploid, *phph* stock, and $6E^b$ addition line showed poor growth. Tillering in the addition lines ranged from 7 to 14. The maximum number of tillers was in the $2E^b$ addition and the minimum in $3E^b$.

Spike length. Spike length contributes to yield determining the number of spikelets/spike and grain/plant. In CS, the amphiploid, and the *phph* stock, spike length ranged from 9 to 13 cm; in the addition lines, it ranged from 6 to 13 cm. The maximum spike length was in in addition line $3E^b$.

Grains/spike. Seed set in the addition lines was 17–41 grains/spike and 67 in CS. Addition $6E^b$ had poor seed set and poor growth in the screen house. The maximum seed set was in the $1E^b$ addition line. The minimum grains/spike was in addition $4E^b$ but it produced the maximum number of grains/spikelet.

Grains/plant. The *phph* stock, amphiploid, and the $6E^b$ addition line had poor reproductive growth. these lines had the least seed set. Grains/plant in the other addition lines ranged from 100 to 427. The maximum number of grains/plant was produced by $5E^b$ and the minimum by the $7E^b$ addition lines. A heavy pest and disease infestation on the $5E^b$ addition line did not preclude it from producing the maximum number of grains.

1,000-kernel weight. 1-000-kernel weight is an important yield parameter. Genotype were scored as either low-yielding or high-yielding, ranging from 17 to 34 g. The maximum grain weight was in addition line $7E^b$ and the least in the $2E^b$ addition line. Although the $7E^b$ addition produced the least number of grains, it had the maximum kernel weight, even greater than the Chinese Spring parent.

Grain color. The color of grain is an important parameter for characterizing and identifying a genotype and studying gene action. In these lines. grain color was brown, blackish-brown, or light brown.

Awn color. All the genotypes except Chinese Spring had a creamy white awn color.

Presence of awns. Chinese Spring is awnless. The $3E^b$ addition also was awnless. The amphiploid, its *phph* stock, and the $1E^b$, $2E^b$, $5E^b$, and $6E^b$ addition lines were awnletted. Additions $4E^b$ and $7E^b$ had long awns resembling normal wheat.

The vegetative morphology of the amphiploid resembled that of Chinese Spring, with spikes intermediate between the wheat parent and wheatgrass, a common observation for most intergeneric hybrids within the Triticeae and a valid morphological indicator of alien genetic expression in a wheat background. Seed set in the amphiploid and translocation stock was poor. A small number of seeds were produced but only sufficient for cytogenetic investigations in future. In the disomic addition lines, the phenotype of the wheat parent dominated. The addition lines showed good vegetative and reproductive growth, except for the $6E^b$ addition. This line produced an enormous amount of seed, which can be further utilized for cytogenetic manipulations and breeding. Cytological procedures helped in the purification of the genetic stocks.

Statistical analysis. Based on the statistical analysis, the genetic stocks derived from *Th. bessarabicum* showed considerable diversity (Table 47). Considerable variation was observed for days-to-heading and maturity, plant height, and number of grains/plant. These traits can be exploited further by breeders.

Table 47. Basic statistics for eight quantitative traits under study in *Thinopyrum bessarabicum*-derived addition lines.

Trait	Mean	Range	Standard deviation
Days-to-heading	94.70	78–136	±25.276
Days-to-physiological maturity	128.20	104–174	±28.448
Plant height (cm)	71.99	55–120	±18.999
Tillers/plant	8.45	2–14	±3.941
Spike length (cm)	10.78	6.9–13.2	±1.961
Grains/spike	32.50	17–67	±15.152
Grains/plant	228.30	32–427	±152.596
1,000-kernel weight (g)	23.65	17.31–33.38	±5.074

Currently, bread wheat improvement priorities worldwide are associated with the genetic security against the three rusts, Fusarium head blight and heat, drought, and salinity tolerance. Crop improvement is dependent on a continued supply of genetic variability. Alien grass species of tertiary gene pool can enrich

cultivated wheat. Considerable yield benefits can be gained from alien chromosome segments or through synthetic wheats using the genetic diversity residing in wild wheat relatives. *Thinopyrum* species have been extensively hybridized with wheat and have played an important role in wheat improvement. Many wheat–*Thinopyrum* amphiploids have been produced with varied chromosome constitutions and can be used as efficient bridges to transfer genes to wheat. Translocations between alien and wheat chromosomes are of worth because they carry a smaller number of alien genes into the crop. They may prove to be a potent source of variation and durable resistance under field conditions because of thier diverse origin. For this to happen, plant breeding, cytogenetics, and biotechnology should go hand-in-hand to accelerate germ plasm improvement programs.

Response of wheat germ plasm derived from the primary and tertiary Triticeae gene pools for Karnal bunt resistance.

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Wheat production is influenced by susceptibility to biotic and abiotic stresses. Karnal bunt (*Neovossia indica*) is a key biotic stress of significant concern to wheat production output; the pathogen is a serious quarantine matter. Fungicide can be used to control the disease, but this is highly uneconomical, hence, resistant cultivars are desirable. For resistance breeding, genetic diversity is required, and this diversity is present in all three gene pools of the wheat family. We exploited the primary and tertiary gene pools. The evaluation of synthetic hexaploids (SH) based on *Ae. tauschii* from the primary gene pool and on *S. cereale* from the tertiary gene pool.

Karnal bunt, or partial bunt, has been described as a smut disease of wheat. First reported in 1931 in experimental wheats at the Botanical Station at Karnal, India, for many years, it was known only in the plains of India. However, since 1974, Karnal bunt has been noted in many locations across northern India, and later Pakistan. Karnal bunt is known to occur in Mexico. Although it is reported from Iraq, this report has not been yet confirmed in the field. A similar situation occurred in Afghanistan and Lebanon, where the pathogen was found in wheat samples imported from the U.S.A. and India, respectively. Karnal bunt occurs naturally on bread wheat, durum wheat, and triticale. The Karnal bunt fungus, originally classified as *Tillitia indica*, was later placed in *Neovossia* on the basis of a long promycelium with a whorl of 32–128, nonfusing conidia at the apex. Karnal bunt is difficult to control because it is seed and soil borne. The focus of quarantine has been to restrict movement of the pathogen from country to country and within the countries where it has been detected. Chemical control of Karnal bunt is not feasible, thus, resistant cultivars are the best option. Derived SH wheat from durum/*Ae. tauschii* crosses plays a major role in the incorporation of Karnal bunt resistance in commercial cultivars. Karnal bunt can survive long periods of time outside its host.

Our focus was to (a) identify agronomically suitable, primary SH wheats based upon phenotype, (b) evaluate the response of advanced wheat germ plasm derived from crosses between breadwheat cultivars and Karnal bunt resistant, SH wheats for resistance, and (c) screen germ plasm of the tertiary gene pool for Karnal bunt resistance.

Forty-seven entries, including KB-12, 57, 171, 203, 245, 248, 268, 269, 272, 290, 301, 310, 369, 376, 380, 391, 405, 419, 520, 551, 640, 648, 706, 739, 789, 940, 1030, 1035, 1036, 1087, 1088, 1114, 1127, 1159, 1200, 1266, 1267, 1280, 1288, 1290, 1321, 1431, 1452, 1454, 1455, 1469, and 1475, were sown in four replications in a randomized complete block design. The infested grain ratio was calculated by the formula: % infestation = total number of infested grains / total number of grains x 100.

Phenotypic evaluation. Data were recorded for several characters (Table 48, p. 186).

Pubescence – pubescence grading was negative (–) or positive (+). Thirty-six genotypes were pubescent and 11 lines were not. The pubescent lines are resistant to insects/pests.

Plant height – Plant height in the genotypes under study was 88–97 cm.

Number of tillers/plant – ranged from 9.25 to 17.50 cm.

Number of grains/spike – ranged from 20.75 to 59.00 cm.

1,000-kernel weight – ranged from 39.25 to 57.50 g.

Spike length – ranged from 7 to 13 cm.

Days-to-flowering – was 89–105 days.

Table 48. Phenotypic characterization of 47 synthetic hexaploid wheat lines. For pubescence, + = present and – = absent; for waxiness, + = present and – = absent.

Entry	Pubescence	Waxiness	Plant height (cm)	Tillers/plant	Days–to–physical maturity	Spike length (cm)	Grains/spike	Days–to–flowering	Spikelets/spike	1,000–kernel weight (g)
KB–12	+	+	88	12	143	5.0	37	96	18	39
KB-57	+	–	87	18	129	11.0	57	96	18	58
KB-171	–	–	84	15	143	13.0	49	89	16	53
KB-203	+	+	87	12	127	9.0	25	95	18	37
KB-245	+	–	81	9	179	8.5	39	105	16	40
KB-428	+	+	85	12	132	10.0	34	99	18	45
KB-268	+	–	91	14	143	11.5	24	93	16	41
KB-269	–	+	82	12	136	8.0	49	100	18	49
KB-272	+	–	85	11	157	11.5	51	97	17	55
KB-290	+	+	81	10	162	10.0	24	98	15	44
KB-301	+	–	95	15	165	7.5	52	86	13	57
KB-310	–	–	96	13	179	8.0	47	102	13	52
KB-369	+	+	85	11	143	5.5	27	95	21	41
KB-376	+	–	94	10	151	12.0	32	89	15	38
KB-380	+	–	71	11	149	14.0	38	96	13	55
KB-391	+	+	89	12	143	10.0	27	94	13	56
KB-405	–	–	97	10	156	12.0	52	96	21	41
KB-419	+	+	85	11	179	12.0	29	100	17	41
KB-520	+	+	89	9	143	7.6	23	95	20	46
KB-551	+	–	85	10	172	8.3	34	95	19	45
KB-640	+	–	89	10	154	13.0	34	95	12	31
KB-648	–	+	84	14	149	9.0	47	92	19	47
KB-706	+	+	91	10	169	10.0	45	98	21	54
KB-739	–	–	85	12	127	11.5	43	92	17	58
KB-789	+	–	83	9	143	9.0	29	91	19	53
KB-940	+	+	85	11	127	9.0	38	97	16	47
KB-1030	+	–	94	10	158	11.0	36	84	18	40
KB-1035	+	+	89	15	143	9.0	52	98	20	55
KB-1036	–	–	89	10	159	11.0	51	94	19	55
KB-1087	+	–	71	10	168	11.0	30	95	17	49
KB-1088	–	+	89	10	162	13.0	33	96	18	45
KB-1114	+	–	97	12	143	9.0	29	95	19	44
KB-1127	+	–	85	10	179	11.0	38	92	15	47
KB-1159	+	+	89	7	163	11.0	26	100	20	42
KB-1200	–	+	85	12	169	13.0	49	95	19	52
KB-1266	+	–	89	14	127	8.0	59	97	17	48
KB-1267	+	–	84	14	179	7.0	51	105	18	57
KB-1280	+	–	84	10	179	10.0	34	98	16	45
KB-1288	+	+	89	9	161	8.0	39	93	15	48
KB-1290	–	+	85	12	173	6.8	45	100	20	45
KB-1321	+	–	89	9	143	8.5	42	97	19	56
KB-1431	+	+	87	9	158	12.0	21	98	17	48
KB-1452	+	–	95	13	149	8.0	21	86	18	48
KB-1454	+	–	94	10	152	12.0	33	102	19	48
KB-1455	–	–	82	12	143	8.0	24	97	15	45
KB-1469	+	–	94	12	140	11.0	22	98	14	41
KB-1475	+	+	85	16	139	10.0	46	92	16	48

Karnal bunt, disease-resistant genotypes. Of the 47 SH wheat genotypes, 15 were resistant to Karnal bunt (Table 49). These lines are recommended for further use in breeding programs in order to improve wheat yield and quality.

Table 49. Karnal bunt resistant genotypes selected for the breeding program of the Wheat Wide Crosses and Cytogenetics Program, National Agriculture Research Centre, Islamabad.

Line	Total grain	Infested grain	Karnal bunt score					
			0	1	2	3	4	5
KB-203	23	0	23	0	0	0	0	0
KB-245	22	0	22	0	0	0	0	0
KB-272	16	0	16	0	0	0	0	0
KB-290	10	0	10	0	0	0	0	0
KB-380	32	0	32	0	0	0	0	0
KB-391	25	0	25	0	0	0	0	0
KB-520	37	0	37	0	0	0	0	0
KB-1127	31	0	31	0	0	0	0	0
KB-1280	21	0	21	0	0	0	0	
KB-1321	29	0	29	0	0	0	0	0
KB-1452	26	0	26	0	0	0	0	0
KB-1454	38	0	38	0	0	0	0	0
KB-1455	12	0	12	0	0	0	0	0
KB-1469	21	0	21	0	0	0	0	0
KB-1475	31	0	31	0	0	0	0	0

Screening of wheat, a Thinopyrum bessarabicum amphiploid, and its disomic chromosome addition lines for salt tolerance.

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Salinity of arable land is a global problem that has restricted the productivity of 955 x 10^6 ha of land. Salinity has developed due to the accumulation of water soluble salts in the soil and its interactions with ground water to a level significantly affecting agricultural production, environmental health, and the economic welfare of many countries, especially those in the arid regions. Pakistan is located in an arid to semiarid climate zone, so high evapotranspiration is the basic cause of salt accumulation on the soil surface. The annual rainfall varies between 100–700 mm throughout the country. The evaporation rate exceeds the precipitation rate, rendering soil salinity and sodicity problems as common production constraints in arid and semiarid regions. In Pakistan, about 6.30 x 10^6 ha are salt affected, out of which 1.89 x 10^6 ha are saline, 1.85 x 10^6 ha are permeable saline-sodic, 1.02 x 10^6 ha impermeable saline-sodic, and 0.028 x 10^6 ha are sodic in nature. Of the 1.89 x 10^6 ha of saline patches, 0.45 x 10^6 ha are present in Punjab, 0.94 x 10^6 ha in Sindh, and 0.5 x 10^6 ha in KPK. The magnitude of the saline problem can be gauged from the fact that the area of productive land is being damaged at the rate of about 40,000 ha annually.

Thinopyrum bessarabicum (2n=2x=14, E^bE^b = JJ) is a wild, rhizomatous, maritime, sand couch grass distributed in Crimea recognized for its high tolerance to salinity and capable of withstanding up to 350 mol/mL^3 of NaCl. Understanding the organization of E^b genome and its phylogenetic relationship with other related genomes greatly facilitates the utilization of these grasses for the introgression of useful genes into wheat. *Th. bessarabicum* has been preferred for genetic transfers due to its diploid status, which theoretically permits swift genetic exchanges to occur as compared with an equally tolerant decaploid (2n=10x=70) *Th. ponticum*.

Alien chromosome addition lines are usually generated to detect agronomically important gene(s) of wild relatives expressed in a wheat background. Alien chromosome addition lines are useful for localizing genes for valuable traits on specific chromosomes, construction of DNA libraries for specific chromosomes following microdisjunction, and research on genome composition and chromosome structure.

Our objectives were the (a) *in vitro* screening for salt tolerance using K^+:Na^+ discrimination as an evaluation parameter under hydroponic testing conditions coupled with additional tests comprising of protein, proline, total chlorophyll content, sugars, super-oxide dismutase (SOD), and root and shoot fresh/dry weight, and (b) identification of the addition line/s that bestow salt tolerance.

The experimental lines were nine entries, including the wheat cultivar Chinese Spring, a CS/*Th. bessarabicum* amphiploid (2n=8x=56; $AABBDDE^bE^b$), and the seven, $1E^b$ to $7E^b$, disomic, chromosome addition lines with 44 chromosomes (2n=6x=42 + $1E^b1E^b$ to $7E^bE^b$). All addition lines were subjected to cytological validation for determining the presence of disomic addition status (42 + 1J1J–7J7J or $1E^b1E^b$ to $7E^bE^b$) through routine mitotic analysis and giemsa C-banding with a focus on detecting the added pair of *Th. bessarabicum* chromosomes. Standard wheat cultivars recognized as salt tolerant (Kharchia 65, Shorawaki, Lu26S, and S-24) and susceptible (PDW34 and PBW343) also were included in the experimental evaluation (Table 50, p. 187).

K^+:Na^+ discrimination. Salt-tolerant species have the ability to maintain low Na^+ and high K^+ concentration in leaves, therefore, a high K^+:Na^+ value indicates a high level of salt tolerance and a greater ability of a plant to exclude Na^+ and accumulate K^+ at high NaCl concentrations. The accumulation of more K^+ compared to Na^+ under saline conditions is a character that determines salinity tolerance at the seedling stage.

Table 50. Pedigree of the disomic, *Th. bessarabicum* addition lines (2n = 6x = 42 + 2 E^b) used to screen for salinity tolerance.

Pedigree	Addition chromosome	Status
CS/*Th. bessarabicum*//Gen81	1J	42 + 1J1J ($1E^b$)
CS/*Th. bessarabicum*//CS	2J	42 + 2J2J ($2E^b$)
CS/*Th. bessarabicum*//2*Gen81	3J	42 + 3J3J ($3E^b$)
CS/*Th. bessarabicum*//2*Gen81	4J	42 + 4J4J ($4E^b$)
CS/*Th. bessarabicum*//2*Gen81	5J	42 + 5J5J ($5E^b$)
CS/*Th. bessarabicum*//2*Gen81	6J	42 + 6J6J ($6E^b$)
CS/*Th. bessarabicum*//2*Gen81	7J	42 + 7J7J ($7E^b$)

The amphiploid, Chinese Spring, Kharchia-65, Shorawaki, and S-24 were found to be the most tolerant to salinity at 75 mol/m^3 with K^+:Na^+ values 6.00, 5.13, 4.81, 4.88, and 5.10, respectively (Table 51). Genotypes PBW-343 and PDW-34 were salt susceptible at 75 mol/m^3 with K^+:Na^+ values of 1.50 and 1.20, respectively. Among the addition lines, the K^+:Na^+ value ranged from 2.60–5.76. Addition lines 1J, 2J, 3J (translocated), and 6J were found to be semitolerant. Addition lines 3J, 4J, 5J, and 7J were the most salt tolerant at 75 mol/m^3 with K^+:Na^+ values of 5.21, 5.76, 4.98, and 4.78, respectively.

Agronomic characteristics under salt stress. Shoot length, root length, shoot and root fresh weight, and shoot and root dry weight were recorded on 35-day-old plants under 75 mol/m^3 NaCl salt stress (Table 52). The greatest shoot length of 38.0 cm was found in the cultivar S-24, and the greatest root length of 9.9 cm was found in Kharchia-65. The shortest shoot length of 30.0 cm was found in PDW-34, and the shortest root length of 4.5 cm was found in PBW-343. Both of these cultivars are susceptible checks and were found to be agronomically poor, with low biomass production. Overall, Chinese Spring, Kharchia-65, Shorawaki ,and S-24 showed better agronomic performance along with high K^+:Na^+ values, were regarded as tolerant genotypes (Table 52), and are of top priority for use in breeding for salt tolerance.

Addition line 4J showed good agronomic performance with high shoot (3.87 cm) and root (10.60 cm) lengths. Line 6J had the lowest shoot length (26 cm) and the lowest root length was 3.3 cm in addition 1J. Overall, addition lines 3J, 4J, 5J, and 7J were better than the others.

Table 51. Mean values for leaf fresh weight, leaf dry weight, and K^+:Na^+ for disomic *Thinopyrum bessarabicum* addition lines and standard wheat cultivars recognized as tolerant (Kharchia-65, Shorawaki, and S-24) and susceptible (PBW-343 and PDW-34) for salt tolerance.

Entry	Leaf fresh weight (gm)	Leaf dry weight (gm)	Na^+%	K^+%	K^+:Na^+
1J	0.106	0.0049	2.80	10.255	3.60
2J	0.920	0.0035	7.60	19.843	2.60
3J	0.159	0.0089	2.77	14.451	5.21
4J	0.132	0.0088	2.45	14.121	5.76
5J	0.108	0.0073	3.12	15.532	4.98
7J	0.167	0.0078	2.98	14.143	4.75
3J (translocation)	0.884	0.0056	6.30	17.566	2.78
6J	0.102	0.0086	5.90	17.465	2.96
Amphiploid	0.141	0.0094	1.80	10.772	6.00
PBW-343	0.031	0.0033	13.6	20.220	1.50
Chinese Spring	0.118	0.0088	2.73	14.000	5.13
PDW-34	0.084	0.0049	12.4	15.111	1.20
Kharchia-65	0.114	0.0075	3.21	15.440	4.81
Shorawaki	0.106	0.0073	2.99	14.581	4.88
S-24	0.109	0.0069	2.80	14.231	5.10

Table 52. Mean values for the agronomic parameters shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight for disomic *Thinopyrum bessarabicum* addition lines and standard wheat cultivars recognized as tolerant (Kharchia-65, Shorawaki, and S-24) and susceptible (PBW-343 and PDW-34) for salt tolerance.

Entry	Shoot length (cm)	Root length (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
1J	33.7	3.30	0.3173	0.0384	0.0268	0.0048
2J	30.8	7.40	0.0400	0.0624	0.0301	0.0058
3J	32.8	6.80	0.4449	0.0718	0.0349	0.0060
4J	37.8	10.6	0.3963	0.0951	0.0391	0.0081
5J	34.3	4.00	0.4999	0.0578	0.0389	0.0035
7J	34.7	3.60	0.4018	0.0877	0.0365	0.0076
3J (translocation)	30.0	3.90	0.3564	0.0605	0.0325	0.0062
6J	26.0	5.20	0.3349	0.0572	0.0309	0.0060
PBW-343	30.2	4.50	0.3673	0.0670	0.0355	0.0065
Chinese Spring	35.4	7.90	0.4995	0.1090	0.0428	0.0101
PDW-34	28.0	4.80	0.2600	0.0387	0.0279	0.0044
Kharchia-65	33.0	9.90	0.4150	0.0792	0.0407	0.0123
Shorawaki	35.5	6.80	0.4760	0.0895	0.0410	0.0085
S-24	38.8	9.80	0.5340	0.1193	0.0556	0.0899

Addition lines with high K^+:Na^+ values also showed high shoot dry weight, indicating sn association between K+/Na+ value and plant performance under stress. Lines 3J, 4J, 5J, and 7J showed high values for both parameters, whereas 1J, 2J, 3J (Tr), and 6J had average performance. Chinese Spring, Kharchia-65, Shorawaki, and S-24 exhibited high values for both parameters, whereas genotypes PBW-343 and PDW-34 were the lowest for both. These data are consistent with germ plasm tolerance catergorization.

Chlorophyll content. We found that the chlorophyll content significantly decreased under salt stress. The genotypes with tolerance to salinity had a low chlorophyll content compared to susceptibile lines. The tolerant genotypes Chinese Spring, Kharchia-65, Shorawaki, and S-24 had total chlorophyll content values of 1.1, 1.2, 1.0, and 1.3 mg/g, respectively, with high K^+:Na^+ values. Addition lines 3J, 4J, 5J, and 7J showed total chlorophyll values of 1.1, 0.9, 1.2, and 1.1 mg/g, respectively, with higher K^+:Na^+ values and more biomass production.

Proline content. Plants accumulated proline in response to abiotic stresses thereby protecting the plant by reducing oxidative damage created due to osmotic stress. The genotypes had increased proline contents in stress conditions. The maximum increase in proline content was observed in Kharchia-65 (4.111 mg/g) followed by Chinese Spring (4.068 mg/g), Shorawaki (3.998 mg/g), and S-24 (3.010 mg/g). The susceptible genotypes had proline content values of 1.061 and 1.010 mg/g. In the addition lines, the maximum proline content was 5.131 mg/g in line 3J, followed by 4J (4.666 mg/g), 5J (3.918 mg/g), and 7J (3.777 mg/g). Addition lines 3J, 4J, 5J, and 7J also showed a higher K^+:Na^+ value.

Protein content. The amount of soluble protein content increased during stress. Genotypes Chinese Spring, Kharchia-65, Shorawaki, and S-24 showed protein content values in the range of 2.001, 1.118, 1.669, and 1.703 mg/g, respectively, whereas genotypes PBW-343 and PDW-34 showed values as low as 0.968 and 0.898 mg/g, respectively. In the tolerant addition lines, high proteing content values were in 3J (2.101 mg/g), 4J (1.998 mg/g), 5J (1.896 mg/g), and 7J (1.999 mg/g). whereas the susceptible addition lines 1J, 2J, 3J(Tr), and 6J had values of 0.911, 1.018, 1.211, and 1.023 mg/g, respectively. Because 3J, 4J, 5J, and 7J showed higher K^+:Na^+ values, they were the most tolerant.

Sugar content. Under stress conditions, tolerant genotypes with high K^+:Na^+ showed high sugar content values, including Chinese Spring (22.9 mg/g), Kharchia-65 (24.6 mg/g), Shorawaki (19.5 mg/g) and S-24, whereas the susceptible genotypes PBW-343 (11.8 mg/g) and PDW-34 (13.6 mg/g) were low. Addition lines with high sugar content values were 3J (17.4 mg/g), 4J (20.2 mg/g), 5J (24.7 mg/g), and 7J (27.3 mg/g), were found to be the most salt-tolerant with high K^+:Na^+ values.

Superoxide dismutase (SOD) content. The maximum superoxide dismutase content was found in Shorawaki at 30.000 units/g fresh weight (u/gfw). Other genotypes with high SOD content incuded Chinese Spring (28.611 u/gfw), Khar-

chia-65 (27.000 u/gfw), and S-24 (21.671 u/gfw). PDW-34 had an SOD content 13.556 u/gfw, whereas the least value was 10.444 u/gfw in PBW-343. In the addition lines, the maximum SOD value was 28.056 u/gfw in line 5J.

Conclusion. The Chinese Spring, Kharchia-65, Shorawaki, and S-24 genotypes were found to be most tolerant to salinity at 75 mol/m^3; PBW-343 and PDW-34 were susceptible. Among the addition lines, K^+:Na^+ values were 2.6–6.0 in 3J, 4J, 5J, and 7J, the most tolerant to salinity at 75 mol/m^3. The tolerance of conventional wheats Chinese Spring, Kharchia, Shorawaki, S-24, and the susceptibility of PBW-343 and PDW 34, supports earlier observations by de Leon et al. (2010). The role of chromosomes 3J, 4J, 5J, and 7J suggests multiple influences, which is consistent with reports of Dvorak et al. (1988) for 3J, 4J, and 7J. The inclusion of 5J in this group is surprising, but it agrees with Forster et al. (1988). Future progress appears to be with more stringent testing with more replications and, if the tolerance comes from multiple alien chromosomes, exploit the amphiploid in a shot-gun manner to effect homoeologous exchanges around the *ph1b* system (Mujeeb-Kazi 2006).

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Characterization of wheat genotypes for salinity tolerance based on physiological attributes.

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Salinity is one of the major environmental stresses that cause significant reduction in grain yield productivity worldwide, especially in irrigated lands (Zhang et al. 2010). Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions and ion transport and homeostasis are issues of special significance (Pardo et al. 2006). Salt effected soils can be brought under cultivation by producing germ plasm tolerant to salinity, which involves identifying genotypes or cultivars on the basis of physiological/biochemical traits that are tolerant to salinity or the use of new genetic resources to introduce new genes for salt tolerance in existing cultivars (Farshadfar et al. 2008). The trait of salinity tolerance is present in D-genome ancestor of wheat *Ae. tauschii* and transferred to synthetics when crossed with susceptible durum parents (Schachtman et al. 1992). Material (landraces) developed in Pakistan before the Green Revolution possess very useful genes for tolerance to abiotic stresses, such as to salt and drought that prevail in the arid and semiarid regions. Our aim is to exploit the variation for salt tolerance within wheat to produce new salt-tolerant wheat cultivars. Many mechanisms, including growth response, selective uptake and transport of Na^+, maintaining high K^+:Na^+ in their cytoplasm, chlorophyll, antioxidative enzymes, compatible solute productio,n and osmotic adjustment have been associated with genetic variation in salt tolerance.

Thirty-two wheat genotypes (advanced lines, landraces, synthetic hexaploids, and checks (LU 26S and Kharchia 65) were obtained from Wheat Wide Crosses, Wheat Program and National Gene Bank, Plant Genetics Resources Program, IABGR, National Agricultural Research Centre, Islamabad, Pakistan. Wheat plants were grown in Jiffy-7 peat pots. Ten-day-old seedlings were transferred to black painted boxes (3 dm^3), containing 3 L full-strength, Hoagland's culture solution (Hoagland and Arnon 1950) and grown in a growth chamber at Plant Physiology Program, CSI, NARC, Islamabad. Thirteen-day-old wheat plants were exposed to 100 mM NaCl. Six days after salinization, recently matured fresh leaves were collected and Na^+ and K^+ concentrations were measured using the method of Yeo and Flower (1983). Chlorophyll content was calculated according to Arnon (1949). Twenty-day-old plants were harvested and shoot and root biomass were recorded.

Based on physiological parameters such as K^+:Na^+ ratio and chlorophyll content, seven genotypes, V-05066, NR-372, PK-01, V07194, Elite-10, Elte-9, and Elite-8, were found to be the most salt tolerant; PR-102, PK-04, V-05082, Elite-4, and Elite-3 were semi-tolerant, and rest of the genotypes were sensitive (Fig. 31, p. 191). The K^+:Na^+ ratio in tolerant genotypes was 2.0–3.5, 1.6–2.2 in the semi-tolerant genotypes, and 0.4–1.5 in the sensitive genotypes. Chlorophyll content in tolerant genotypes was 12.5–14.5 mg/g dry wt, 10.5–12.4 mg/g dry wt in the semi-tolerant genotypes,

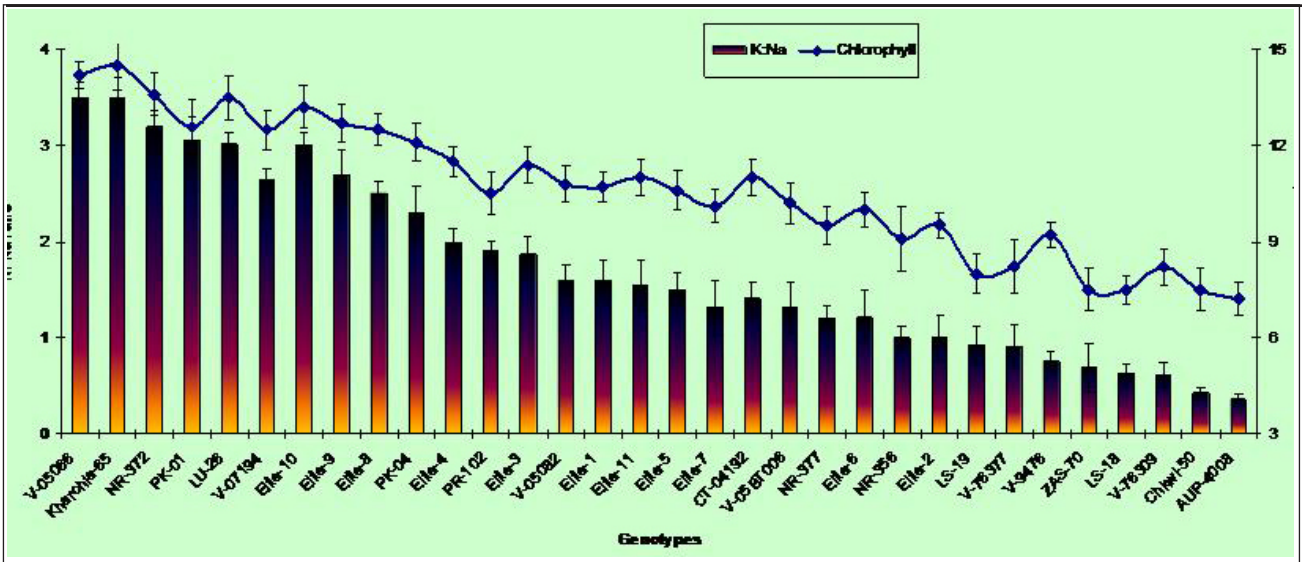

Fig. 31. The K^+:Na^+ ratio and chlorophyll content (mg/g dry weight) of the wheat genotypes is at 100 mM NaCl stress. Each bar represents the mean value of ten plants with standard error of means.

and 7.5–10.0 mg/g dry wt in the sensitive genotypes (Fig. 31). Shoot fresh and dry weight of the wheat genotypes were in the following order: tolerant genotypes > semi-tolerant > sensitive genotypes. The root dry weight of tolerant and semi tolerant genotypes were nearly equal, however the sensitive genotypes had lower root dry weight than tolerant and semi-tolerant genotypes (Table 53, p. 192).

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Genotypic differences in drought tolerance of wheat (Triticum aestivum L.) genotypes.

Jalal-ud-Din, Sami Ullah Khan, Ali Raza Gurmani, Yaqoob Mujahid, Alvina Gul Kazi, Awais Rasheed, Hadi Bux, and Abdul Mujeeb-Kazi.

Introduction. Drought is one of the most important factors, limiting plant growth and productivity more than any other environmental factor. In Pakistan about 15 x 10^3 ha of cultivated land is affected by this syndrome. Wheat is the most important grain crop grown, comprising about one-third of the total annual cereal production. In wheat, the reproductive stage is considered to be the most sensitive to drought. The best option to improve crop yield under moisture stress is to develop drought-tolerant crop cultivars. One important component is the evaluation of genetic variability in the cultivars to identify a tolerant cultivar that may sustain a reasonable yield under moisture stress. An increase in drought tolerance may be more successful if selection is based directly on the physiological characters conferring tolerance. Proline accumulation is considered to be associated with drought tolerance in wheat. Grain yield is a product of an organized interplay of several components that are highly susceptible to drought. This study assessed the drought tolerance of some commonly grown cultivars, advanced lines, and landraces collected from drought-prone areas.

Table 53. Shoot fresh weight, shoot dry weight, and root dry weight of wheat genotypes at 100 mM NaCl stress. The data represents the mean value of ten plants with standard error of means.

Genotype	Shoot fresh weight (g)	Shoot dry weight (g)	Rood dry weight (g)
V-05066	0.43±0.038	0.045±0.051	0.017±0.002
Kharchi-65	0.38±0.029	0.036±0.034	0.017±0.002
NR-372	0.40±0.040	0.042±0.049	0.016±0.001
PK-01	0.39±0.032	0.04±0.0470	0.022±0.002
LU-26	0.45±0.045	0.046±0.052	0.018±0.001
V-07194	0.37±0.034	0.037±0.039	0.015±0.002
Elite-10	0.35±0.045	0.032±0.021	0.018±0.003
Elite-9	0.34±0.060	0.033±0.036	0.016±0.004
Elite-8	0.31±0.050	0.029±0.032	0.015±0.003
PK-04	0.34±0.031	0.035±0.034	0.019±0.003
Elite-4	0.27±0.026	0.025±0.040	0.016±0.001
PR-102	0.38±0.029	0.039±0.044	0.015±0.004
Elite-3	0.24±0.022	0.022±0.026	0.015±0.002
V-05082	0.32±0.045	0.036±0.038	0.015±0.003
Elite-1	0.21±0.042	0.02±0.0250	0.014±0.001
Elite-11	0.22±0.037	0.019±0.022	0.013±0.003
Elite-5	0.23±0.025	0.02±0.0180	0.012±0.004
Elite-7	0.21±0.033	0.019±0.024	0.012±0.002
CT-04192	0.29±0.060	0.03±0.0280	0.016±0.001
V-05BT006	0.30±0.050	0.031±0.030	0.014±0.002
NR-377	0.31±0.026	0.033±0.035	0.015±0.001
Elite-6	0.20±0.020	0.018±0.017	0.011±0.001
NR-356	0.28±0.022	0.032±.0320	0.014±0.003
Elite-2	0.19±.0190	0.015±0.019	0.011±0.003
LS-19	0.17±0.037	0.015±0.033	0.010±0.004
V-76377	0.28±0.042	0.029±.0330	0.013±0.004
V-9476	0.31±0.037	0.032±.0370	0.012±0.002
ZAS-70	0.28±0.025	0.027±.0310	0.011±0.001
LS-18	0.15±0.025	0.013±0.027	0.009±0.002
V-76309	0.25±0.033	0.026±.0280	0.014±0.003
Chakwal-50	0.27±0.020	0.029±.0210	0.016±0.002
AUP-4008	0.25±.0190	0.024±.0250	0.012±0.001

Forty-five wheat genotypes, ZAS-70 NR-366, Inqilab-91, Zarlashta-99, NR-367, Wafaq-2001, Margalla-99, AUP-4008, NR-268, NR-375, ESH-9525, V-9476, Chakwal-97, NR-372, NR-377, Chakwal-50, NR-234, NR-368, NR-267, NARC-2009, QS-III, NR-360, HB-10, NR-358, NR-371, NR-356, GA-2002, V-05066, Sehar-2006, V-76309, V-10, Sariab-92, SD-4085/3, PR-102, EBWYT-514, V-07194, Punjab-96, V-76377, V-05082, V15, NR-356, DN-62, CT-04192, V-05BT006, and V-05BT006, were used in the study. Plants were grown in pots containing 10 kg sandy loam soil in a glasshouse at the National Agricultural Research Centre, Islamabad, during the winter/spring of 2005 and 2006 with average day/night temperatures 30±8ºC and 13±5ºC, respectively. A fertilizer mixture of 500 mg N, 300 mg P, 200 mg K, and 50 mg K per pot as urea, di-ammonium sulphate, potassium phosphate, and zinc sulphate was mixed in the soil before sowing. The pots were arranged in factorial, randomized, complete block design. Plants were subjected to three consecutive drought cycles at pre-anthesis (80 days after sowing) growth stages by withholding irrigation for 5–7 days, or until the signs of temporary wilting/leaf rolling started. After the drought stress treatment, fully emerged young leaves from control and stressed plants were sampled for quantification of proline and chlorophyll content. Control pots were irrigated as frequently as needed. After the drought treatment and sample collection, plants were regularly irrigated with water. Plants were harvested at physiological maturity and yield data recorded. Coleoptile emergence was recorded by germinating seeds in a 30% PEG (6000) for one week.

Results. Drought imposed at pre-anthesis significantly reduced grain yield (19–45%) in all the tested genotypes (Fig. 32). Relative reduction in grain yield due to drought was less in ZAS-70 (16%), NR-366 (19%), Inqalab-91 (19%), Zarlashta-99 (21%), NR-367(21%), Wafaq-2001(22%), Margalla-991(23%), AUP-4008 (26%), and NR-268 (26%). Under drought stress, a few additional genotypes, such Chakwal-97, NR-377, NR-360, and NR-356, also had high yields. However, based on some physiological traits such as coleoptile emergence, proline accumulation, chlorophyll content, and the yield component seeds/plant, the overall performance of wheat genotypes ZAS-70, NR-366, Inqalab-91, Zarlashta-99, NR-367, and Wafaq-2001, and the advance lines developed by NARC, NR-267 and NR-268, were found to be better than the other test cultivars. They produced a higher number of caryopses and a fewer number of sterile florets per spike under water stress conditions.

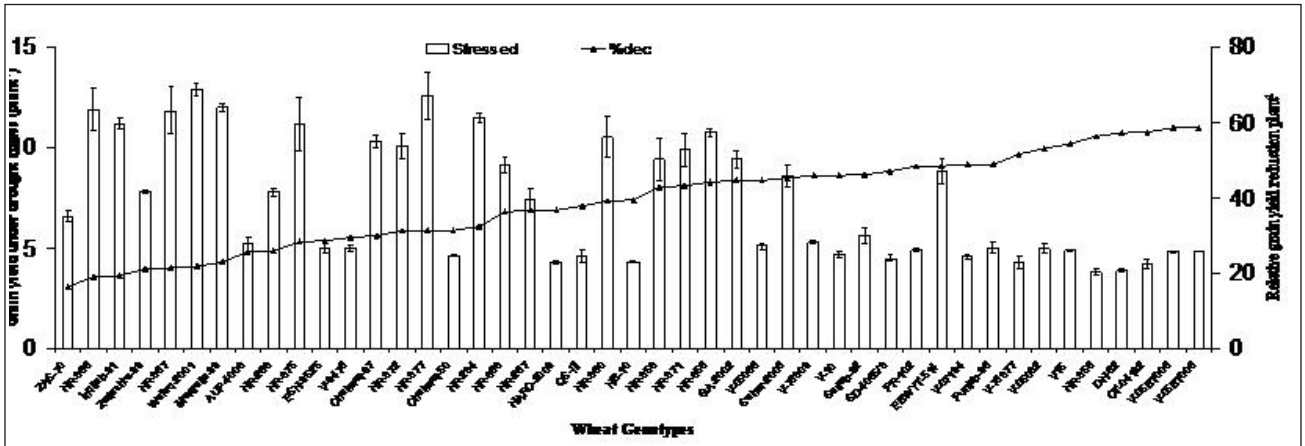

Fig. 32. The effect of drought stress on the relative grain yield reduction and grain yield (under drought stress) in wheat genotypes.

ITEMS FROM POLAND

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On breeding system in wheat and Brachypodium distachyon.

R. Kosina and P. Tomaszewska.

Studies of breeding systems in many plants proved that the level of auto- or allogamy may vary in terms of time and space and can be recognized as flexible. Any conclusions on the genetic structure of cultivated or wild populations cannot be made without the knowledge regarding the plant's breeding system. Wheat is considered as an autogamic plant; however, its mating system depends on a species, ecotype and location of cultivation. Percival's data on wheat blooming in England, in the oceanic climate, showed that natural hybridization is rare, but Meister's observations from Saratov, in the continental climate, presented a large number of hybridization events between wheat and rye (Vavilov 1949/50). Wheat, being a self-pollinated plant, frequently expresses chasmogamy during early morning up to noon hours (Fig. 1A), which opens the possibility of geitonogamic mating, or close outcrossing as it was evidenced by Waines and Hedge (2003). Low or very high temperatures and rains restrict chasmogamy and, thus, closed flowers are cleistogamic. Cleistogamy can be also determined by a structural mutation, e.g., of lodicules (Fig. 1B; Kosina 2010). Keydel (1972, 1973) proved great differences in the level of chasmogamy and production of pollen grains for many cultivars of winter wheat cultivated in Bavaria, Germany.

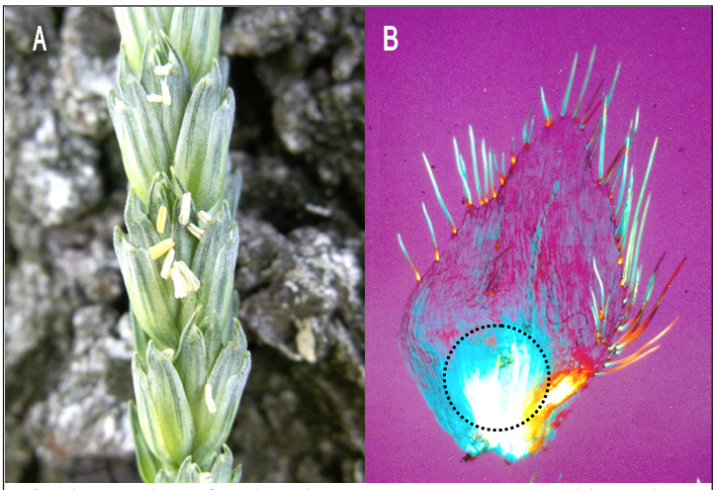

Fig. 1. Exertion of anthers in a *Triticum aestivum* cultivar (A) and a cushionless (encircled) mutation in lodicule of *T. compactum* (B).

Brachypodium distachyon, like wheat, is an autogamic plant and its chasmogamy is noted sporadically (Vogel et al. 2009; Jaroszewicz et al. 2012). Our last observations for many early blooming ecotypes of *B. distachyon* showed that chasmogamy is common in this species (Fig. 2A and B). The blooming occurred at temperatures ca 20–30°C, under windy and partially sunny weather, between 10:00 AM and 3:00 PM. The range of number of pollen grains/anther is 271–582. In *B. pinnatum*, a fully allogamic species, the range is between 5,000 and 7,640 grains. Thus, an input of pollen grains in the gene flow and heterozygosity of populations is 13 and many times larger in *B. pinnatum* than in *B. distachyon*.

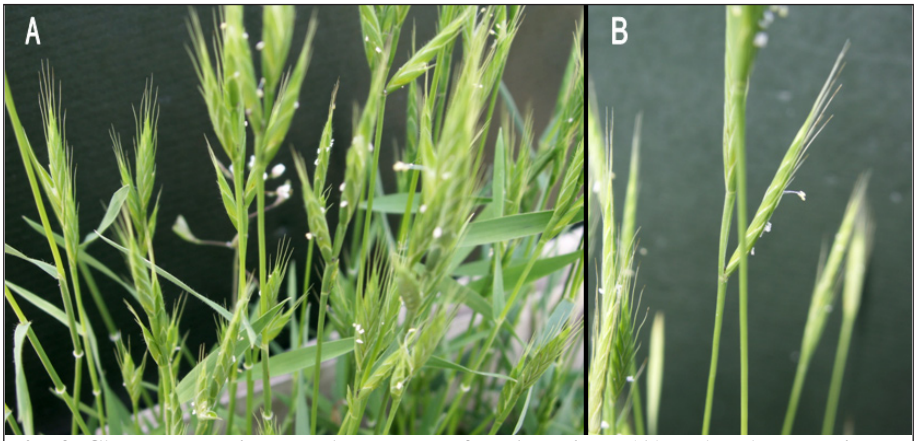

Fig. 2. Chasmogamy in an early ecotype of *B. distachyon* (A) and anther exertion in the species (B).

Therefore, chasmogamy in *B. distachyon* is common in suitable weather conditions, which occur frequently in the Mediterranean region, its area of origin. Such breeding behavior can be realized in the form of geitonogamy or allogamy. These data change our understanding on the genetic nature of *B. distachyon* populations.

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Regressional variation of wheat lodicules.

R. Kosina, M. Florek, and P. Tomaszewska.

Kosina (2004) proved that description of plant populations, individuals or other types of OTUs (Operational Taxonomic Units) by means of not only positional statistics (averages) but also by covariation parameters (correlation, regression) reveals new information on variability of studied objects. Such an approach was applied for wheat lodicule morphology, shapes of lodicule body and its main lobe (Kosina 2011a). The studied wheat species were as follows: diploids, *Triticum monococcum subsp. aegilopoides* (b, b') and subsp. *monococcum* (m); tetraploids, *T. timopheevii* subsp. *armeniacum* (a), subsp. *timopheevii* (t), and subsp. *militinae* (mi), *T. turdigum* subsp. *dicoccoides* (ds), subsp. *dicoccum* (d, d'), subsp. *durum* (dr), subsp. *carthlicum* (c), subsp. *georgicum* (g), and subsp. *ispahanicum* (i); and hexaploids *T. aestivum* subsp. *aestivum* (v), subsp. *macha* (ma), subsp. *compactum* (co), subsp. *sphaerococcum* (sph), subsp. *spelta* (sp), subsp. *vavilovii* (va), subsp. *petropavlovskyi* (p), and subsp. *zhukovskyi* (zh); and octoploids *T. fungicidum* (f) and *T. timonovum* (tv) (symbols in brackets in Fig. 3). *Triticum fungicidum*, *T. monococcum subsp. aegilopoides*, and *T. turgdum* subsp.*carthlicum* were extreme OTUs when described by regressional parameters related to the lodicules shape. The same three species also were the extremes for 'shape of the main lobe', however, they were positioned in different points of the ordination space. A diagram shows positions of the same 23 wheats

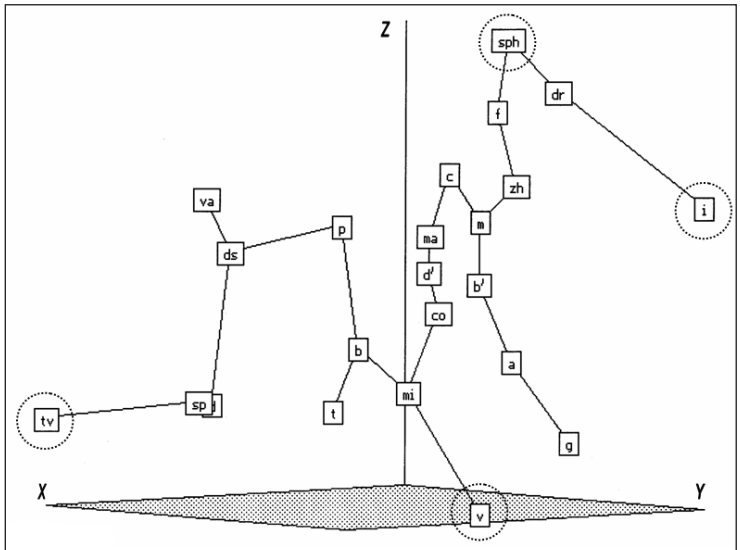

Fig. 3. A minimum spanning tree of wheat species. Operational taxonomic units (OTUs) in a nonmetric multidimensional scaling ordination space. OTUs are described by regressional parameters related to traits of lodicule (diploids, *Triticum monococcum subsp. aegilopoides* (b, b') and subsp. *monococcum* (m); tetraploids, *T. timopheevii* subsp. *armeniacum* (a), subsp. *timopheevii* (t), and subsp. *militinae* (mi), *T. turdigum* subsp. *dicoccoides* (ds), subsp. *dicoccum* (d, d'), subsp. *durum* (dr), subsp. *carthlicum* (c), subsp. *georgicum* (g), and subsp. *ispahanicum* (i); and hexaploids *T. aestivum* subsp. *aestivum* (v), subsp. *macha* (ma), subsp. *compactum* (co), subsp. *sphaerococcum* (sph), subsp. *spelta* (sp), subsp. *vavilovii* (va), subsp. *petropavlovskyi* (p), and subsp. *zhukovskyi* (zh); and octoploids *T. fungicidum* (f) and *T. timonovum* (tv)).

described by regression parameters of a relation between lengths of two lobes of the lodicule (Fig. 3). In fact, the ratio of both lengths presents differences in cell shapes of both lobes. Kosina (2011b) evidenced that these cells are different, depending on the number of cytokineses along main axis of the lodicule, and their longitudinal growth. *Triticum timonovum* (tv), *T. aestivum* subsps. *aestivum* (v) and *sphaerococcum* (sph), and *T. ispahanicum* (i) are extremes in the ordination space for the longitudinal development of the lodicule lobes. The visibility of structures is our common perception, but invisible covariation characteristics are no less important in plant biology considerations.

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On caryopsis crease and endosperm cavity in wheat and Brachypodium distachyon.

R. Kosina, P. Tomaszewska, and K. Kamińska.

For researchers studying internal structure of wheat caryopsis, it is obvious that shape of a crease is variable and dependent on the development of the main mass of starchy endosperm. The shapes of the crease and adjacent endospermal cavity (ec) dominate in exemplary wheats (Fig. 4). The crease is a complex structure composed of an outer pericarp, a pigment strand with a vascular bundle, a nucellar projection being a modified nucellus in the chalaza region, an endosperm cavity filled by substances derived from apoptosis of nucellus, and a modified aleurone layer adhering to endosperm cavity. In various wheats, this region can be organized as a symmetric or asymmetric structure, flat or penetrating deeply into the starchy endosperm, such as in *T. trugidum* subsp. *durum*. The endosperm cavity can have limited volume or can be large and filled by starch granules as in the cultivar Purdue. Hands and Drea (2011) expressed an opinion that the endosperm cavity is not developed in caryopsis of *Brachypodium distachyon*. Our observations of more than 20 accessions of this grass showed that in some ecotypes, the endosperm cavity is present (Fig. 5A and 5B). The crease is visible on the cross-sections of caryopsis as a symmetric structure (Fig. 5A) with two, separate, small cavities (see arrows) or with one cavity developed between nucellar projection and modified aleurone layer (Fig. 5B). The aleurone layer penetrates far between starchy cells, and its cell multiplication takes place in this area. In conclusion, the caryopsis creases of wheat and *B. distachyon* are qualitatively similar but quantitatively different.

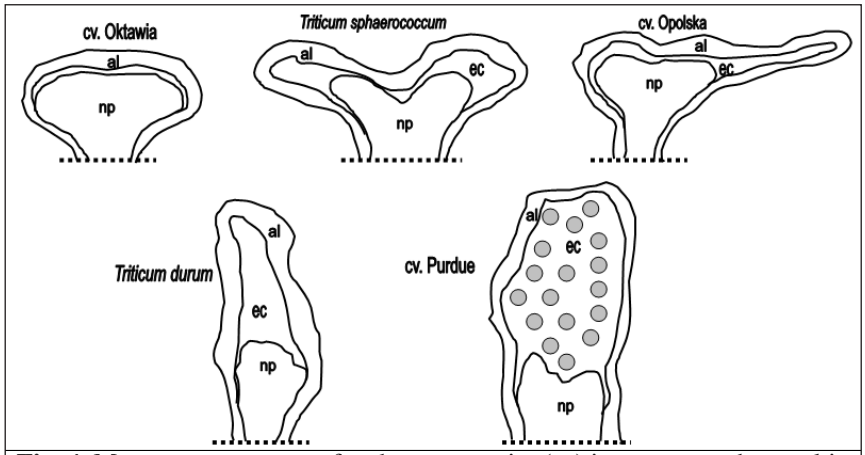

Fig. 4. Most common types of endosperm cavity (ec) in common wheat cultivars and in *T. aestivum* subsp. *sphaerococcum* and *T. turgidum* subsp. *durum* (np = nucellar projection, al = aleurone layer). In the endosperm cavity is filled by starch grains in the cultivar Purdue.

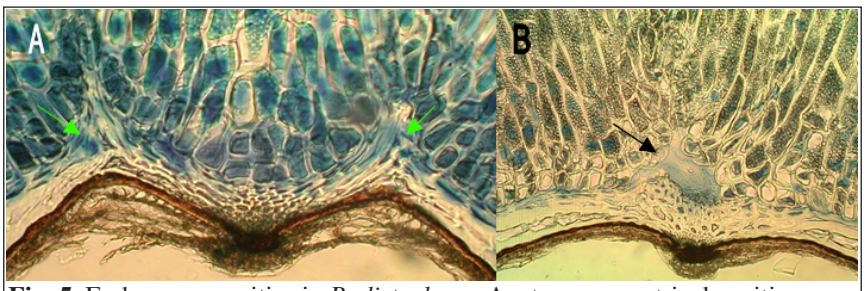

Fig. 5. Endosperm cavities in *B. distachyon*: A – two symmetrical cavities on the sides of the crease and B – one cavity penetrating the starchy endosperm.

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On caryopsis developmental events in wheat and Brachypodium distachyon.

R. Kosina, P. Tomaszewska, and K. Kamińska.

Development of wheat caryopsis varies between threshable and unthreshable forms. A 'floret cavity' formed by the lemma and palea determines the organization of caryopsis tissues. The development of ovary walls are especially restricted by sclerified glumellae. Such an interaction between inflorescence leaves and the young fruit results in the depression of the pericarp in unthreshable wheats. The pericarp in common or durum wheats is thicker and composed of more cell layers. The nucellar epidermis and integuments are restricted to thin, sometimes suberinized, layers and in the area of chalaza, they form pigment strand and nucellar projection. An endosperm is composed of unilayered aleurone and cylindrical (Fig. 6A) and isodiametric (Fig. 6B) starchy cells. The cell walls in the starchy endosperm are thin.

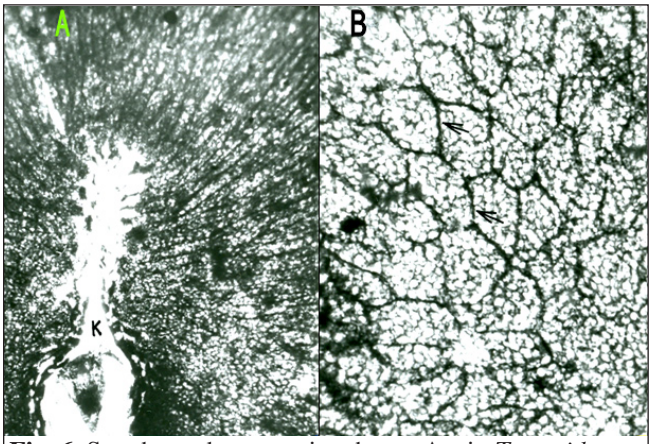

Fig. 6. Starchy endosperms in wheats: A – in *T. turgidum* subsp. *durum* with a dominance of cylindrical cells in the area of endosperm cavity, and B – in *T. aestivum* subsp. *aestivum* with isodiametric cells in a lateral part of caryopsis.

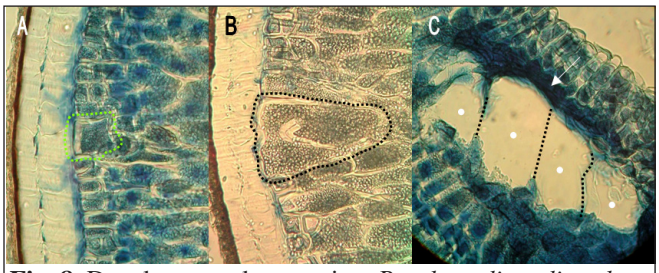

Fig. 8. Developmental events in a *Brachypodium distachyon* caryopsis: A – a large (polyploid ?) aleurone cell on the dorsal side, B – a large (polyploid ?) cell with starch granules on the dorsal side in the aleurone layer, and C – a clone of huge polyploid cells in the starchy endosperm, pressing the high-protein subaleurone layer (dark blue).

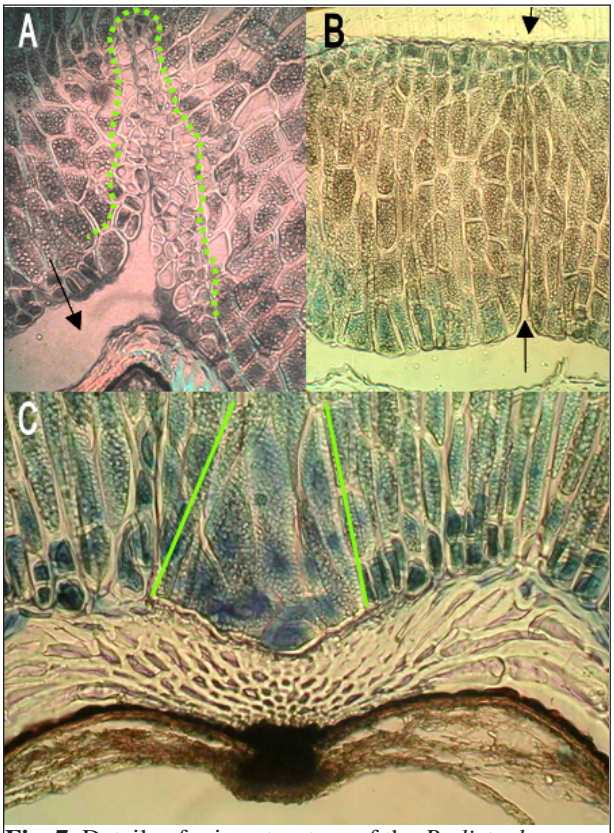

Fig. 7. Details of microstructure of the *B. distachyon* caryopsis: A – an endosperm cavity deeply penetrating starchy endosperm by multiplied aleurone cells, B – two large domains of starchy endosperm, and C – a large starchy cell segment adjacent to nucellar projection and a lack of aleurone layer in this area.

The development of the caryopsis in *Brachypodium distachyon* is a little different. The nucellar epidermis and starchy cell walls are very thick. Several developmental phenomena evidence the peculiarity of the caryopsis structure, which are that the aleurone layer in the crease area can deeply penetrate starchy endosperm and aleurone cells are multiplied there by means of frequent cytokineses (Fig. 7A). The starchy endosperm can be developmentally divided into macro-domains, which are of the same structure (or different) (Fig. 7B). This phenomenon is very rare. An island of

large starchy cells is often formed instead of aleurone layer in the crease area (Fig. 7C, p. 197). At the dorsal side, large cells are formed, probably polyploidized, being of aleurone or starchy nature (Fig. 8A and 8B, p. 197). In the starchy endosperm, a set of huge cells were observed (Fig. 8C, p. 197), which by pushing the adjacent proteinaceous subaleurone layers, ceased their development. It evidences an independent development of some parts of *B. distachyon* caryopsis. We suggest that such a fruit can be a good model for study of mosaicism.

Embryo relationships among wheat hexaploids.

R. Kosina.

The embryo of wheat hexaploids was described by 11 traits (scutellum, embryo axis, epiblast, coleoptile, and radicle). OTUs (wheats) were set into an ordination space (Fig. 9) using values of the first three principal components of each OTU. Cultivars of common wheat (labels with v plus large letters) are located in the center of the diagram together with items of *T. aestivum* subsps. *compactum* (co), one form of *petropavlovsky*i (p1), *vavilovii* (va), and an original form of *spelta* having a tough rachis (see an arrow). Variability of *T. aestivum* cultivars is quite large. *T. zhukovskyi* (zh), *T. aestivum* subsps. *sphaerococcum* (sph), *macha* (ma), and another accession of *T. petropavlovskyi* (p2) are situated outside the center. The spelta form with fragile spike rachis (sp2) is close to unthreshable wheats. *T. zhukovskyi* with genomes AAGGGG has a narrowest epiblast among hexaploids, while this organ is shortest in *T. sphaerococcum* (Kosina 1999). The latter has also the shortest radicle. The epiblast of *T. zhukovskyi* is similar to that in AAGG wheat tetraploids (Kosina 1995).

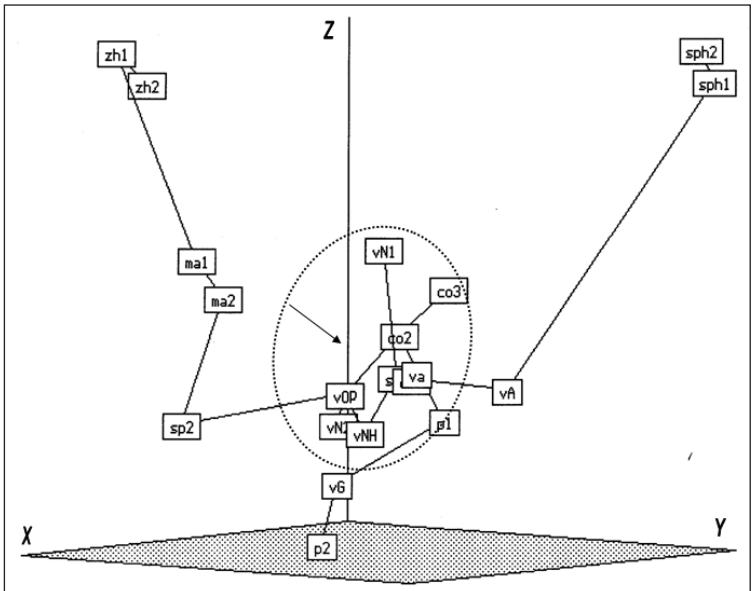

Fig. 9. A minimum spanning tree of wheat haxaploids. OTUs in a nonmetric multidimensional scaling ordination space. OTUs are described by arithmetic averages of embryo traits (*T. aestivum* subsps. *compactum* (co), *macha* (ma), *petropavlovsky*i (p1 and p2), a form of *spelta* having a tough rachis (see an arrow), *sphaerococcum* (sph), *vavilovii* (va), and *T. zhukovskyi* (zh).

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Kosina R. 1995. Tetraploids of the genus *Triticum* in the light of caryopsis structure. Acta Universitatis Wratislaviensis 1785, Prace Botaniczne 66. Wydawnictwo Uniwersytetu Wrocławskiego, Wrocław, s. 146 (In Polish).

Kosina R. 1999. Selected items of wheat variation – from palaeobotany to molecular biology. Acta Societatis Botanicorum Poloniae 68:129-141.

Caryopsis microstructure in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina, M. Markowska, and A. Koźlik.

Observations were made on semi-permanent microscopic slides of caryopsis cross-sections mounted in glycerine. Polarized (Amplival) and epifluorescence (Olympus) microscopes were used. Seeds of amphiploid parents were obtained from the IPK Gatersleben collection (Germany) and an amphiploid from Plant Germ-plasm Institute collection in Kyoto (Japan). In grains of demethylated amphiploid progeny, some changes in the pigment strands (suberinization) also have been recognized previously (Kosina and Markowska 2010). Differences between the parents (*Triticum* and *Aegilops*) and the amphiploid are noted for other fruit structures (Fig. 10, p. 199). The epidermis of the amphiploid pericarp is of an intermediate form (white arrows) between epidermis observed in either parent; a little thicker than that in *T. timopheevii*, but cellular walls and lumina are more similar to that of wheat. Aleurone cells are larger than those in *Ae. umbellulata*,

but not so high as in *T. timopheevii* subsp. *timopheevii*. Expression of the aleurone layer traits in the amphiploid is intermediate between both parents. The amphiploid expresses some paternal traits in the starchy endosperm structure. In the subaleurone layer, more protein is accumulated, as in *Ae. umbellulata* (see green arrows in Fig. 10). Both parents synthesize two types of starch granules, large and small, commonly identified in the literature as A and B, but variation in the diameter of the starch granules should be described quantitatively. In *Ae. umbelluata*, starch granules of the type A are significantly larger than those in *T. timopheevii*. Very small starch granules are synthesized additionally in *T. timopheevii*. The amphiploid synthesizes large granules, such as those in *Aegilops*, some of B type and, in addition, very small ones, such as those in *Triticum*. The starchy endosperms of the amphiploid and *Aegilops* look darker in epifluorescence because of the lack of fluorescence of abundant large starch granules (compare pictures in Fig. 10A with 10B and 10C). Cross-sections also show the accumulation of endospermal protein (a rose fluorescence) between starch granules in *T. timopheevii* and in the subaleurone layer in amphiploid and *Ae. umbellulata*. Demethylation did not change significantly the caryopsis structure in the amphiploid, with the exception of pigment strand.

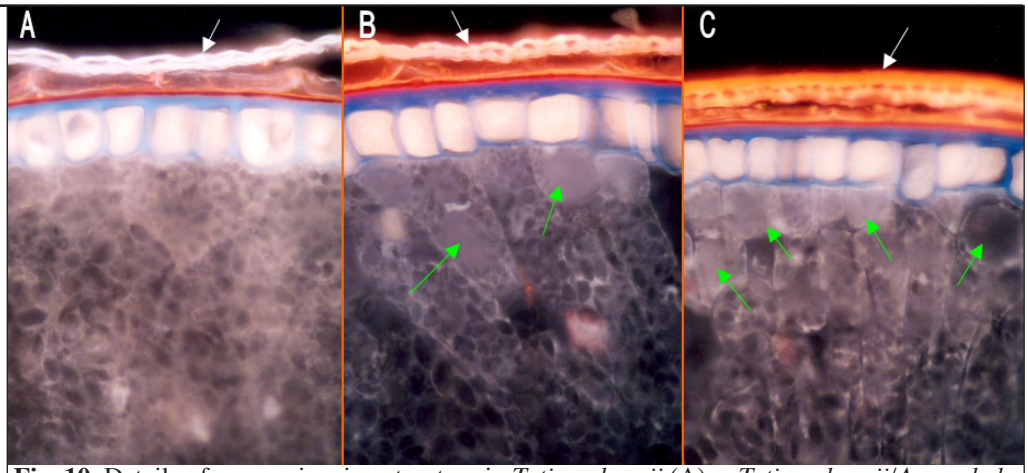

Fig. 10. Details of caryopsis microstructure in *T. timopheevii* (A), a *T. timopheevii*/*Ae. umbellulata* amphiploid (B), and *Ae. umbellulat*a (C). White arrows show an outer layer of pericarp, green arrows for the high-protein subaleurone layer.

Reference.

Kosina R and Markowska K. 2010. Patterns of variation in *Triticum timopheevii* x *Aegilops umbellulata* amphiploid after demethylation of genomes. Ann Wheat Newslet 56:207-208.

RAPD relationships in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina and K. Markowska.

We studied patterns of RAPD variation in two species, *T. timopheevii* subsp. *timopheevii* and *Ae. umbellulata*. Both species were obtained from the IPK collection in Gatersleben, Germany. Two accessions of amphiploid progeny *T. timopheevii* / *Ae. umbellulata* (A1 and A2) were kindly given by the Plant Germplasm Institute in Kyoto, Japan, and also investigated. Forty, 10-nucleotide primers were used to amplify DNA in an MJ Research thermocycler. Expressed bands were of 750–500 bp.

We found found species-specific, RAPD bands for both parental species (Kosina and Markowska 2010). Data from the 40 primers were elaborated numerically by nonmetric multidimensional scaling. The similarity of the Operational

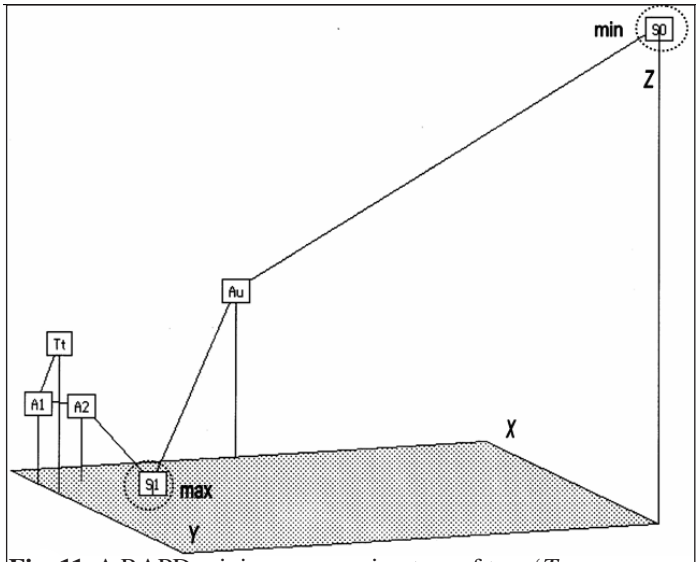

Fig. 11. A RAPD minimum spanning tree of two '*T. timopheevii* (Tt) / *Ae. umbellulata* (Au)' amphiploids (A1 and A2) and parents. The Operational Taxonomic Units (OTUs) are in a nonmetric multidimensional scaling ordination space. OTUs are described by Jaccard's coefficients calculated from 0, 1 RAPD data.

Taxonomic Units (OTUs) was calculated in the form of Jaccard's coefficients. For comparison, new OTUs, S0 with zero bands and S1 with maximal number of bands, were introduced into an ordination space (Fig. 11, p. 199). Both the A1 and A2 amphiploids are located close to *T. timopheevii,* the maternal parent. *Aegilops umbellulata* is closer to the OTU S0, which means that this species is less variable, with regard to RAPD markers. *Triticum timopheevii* and both forms of the amphiploid are more variable. These results are evidence of matroclinal dominance of RAPD patterns in the amphiploid.

Reference.

Kosina R and Markowska K. 2010. DNA RAPD variation in a *Triticum timopheevii* / *Aegilops umbellulata* amphiploid. Ann Wheat Newslet 56:203.

Inflorescence epidermal microstructure in a 'Triticum timopheevii subsp. timopheevii / Aegilops umbellulata' amphiploid.

R. Kosina and K. Markowska.

Variation of epidermal traits in the grass inflorescence is species specific and can be used for the taxonomic determination of contemporary and fossil materials (Kosina 1999a, b). The abaxial epidermis of the lower glume and lemma were analyzed in a '*T. timopheevii* subsp. *timopheevii* / *Ae. umbellulata*' amphiploid and its parental species. In the amphiploid, the epidermal traits of both parents are expressed. The following cells are typical in a lower glume epidermis: *T. timopheevii,* stomata, papillae, and duplexes composed of cork cells and micropapilla; *Ae. umbellulata*, stomata, macropapillae, hooks, duplexes composed of cork cells and large silica cells, and duplexes of macropapilla and cork cells; the amphiploid, stomata, macropapillae, and duplexes composed of cork cells and micropapilla (Fig. 12). Two types of cells, macropapillae, and duplexes with micropapillae were expressed in the amphiploid due to maternal and paternal inheritance.

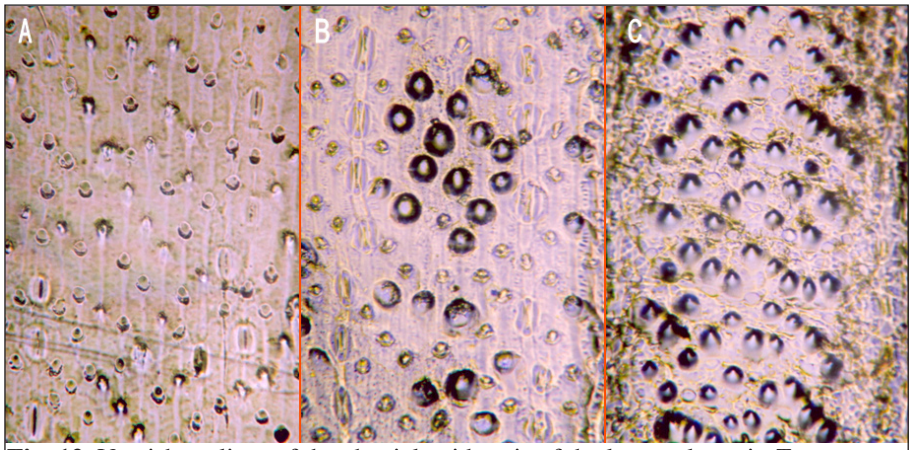

Fig. 12. Varnish replicas of the abaxial epidermis of the lower glume in *T. timopheevii* subsp. *timopheevii* (A), a *T. timopheevii*/*Ae. umbellulata* amphiploid (B), and *Ae. umbellulata* (C). Quantitative and qualitative differences between the replicas are seen.

The structural organization of the lemma epidermis seems to be simpler; *T. timopheevii,* papillae and small hooks; *Ae umbellulata*, macropapillae and single cells; and in the amphiploid, macropapillae and duplexes with micropapillae. Macropapillae are smaller than those in *Aegilops* and duplexes are rarely scattered when compared with those in the glume of *Triticum*. Such a cellular pattern of the lemma of amphiploid is intermediate between both parents, involving patterns of both organs, glume and lemma. Demethylation of amphiploid genomes increases the frequency of duplexes with micropapillae in lemma, however, this phenomenon should be presented quantitatively.

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Kosina R. 1999a. Patterns of flower microstuctural variation within the genus Bromus. Acta Societatis Botanicorum Poloniae 68:221-226.

Kosina R. 1999b. Selected items of wheat variation – from palaeobotany to molecular biology. Acta Societatis Botanicorum Poloniae 68:129-141.

Lodicule variability in a spiro-distichous spike of Lophopyrum elongatum.

R. Kosina, M. Florek, and P. Tomaszewska.

We noted a specimen of Lophopyrum elongatum with altered morphogenesis of spikes in a collection of Triticeae. In the lower part of the rachis, two spikelets instead of one developed. In the upper part of the inflorescence, densely packed spikelets were arranged spirally. *Lophopyrum elongatum* is an allogamic species with very distinct chasmogamy and the exertion of anthers on long filaments. There are no doubts that the opening of the grass flower is well correlated with the structure and metabolic nature of lodicules (Kosina 2005). In normal *Lophopyrum* plants, lodicules are very active and flowers open wide. Further progeny of the changed plant continued this type of morphogenesis, proving the genetic nature of this morphology.

The morphology of lodicules isolated from the changed spike was the purpose of this study. Lodicules were evaluated by four characters related to their dimension, shape, and hairiness. Morphotypes of lodicules are illustrated in the form of polygonal graphs (shown for extreme specimens in Fig. 13 by arrows). Lodicules (Operational Taxonomic Units (OTUs) were clustered into a dendrogram using Ward's method. The maximum linkage distance (Euclidean distance) was 170. Lodicules were scattered widely in the ordination space, created by the use of nonmetric, multidimensional scaling (see numbers in Fig. 13). Such a picture proves that the lodicules developed in the spirodistichous spike are very variable. They often present dysfunction because of a distinctly changed morphology. Spirally packed flowers remained closed like cleistogamic flowers. Such a changed structure decreases the number of pollen grains and ultimately the reproductive output of seeds. Such a type of inflorescence mutation can be a very good object for the study of morphology and physiology of the lodicule as well as its behavior during chasmogamy.

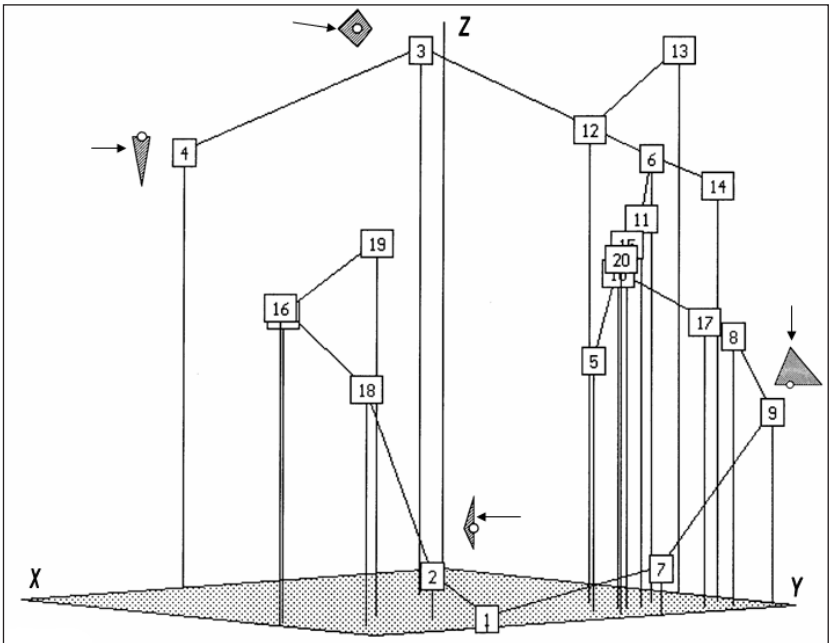

Fig. 13. A minimum spanning tree of *Lophopyrum elongatum* lodicule (numbers) Operational Taxonomic Units (OTUs) in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for four OTUs.

Reference.

Kosina R. 2005. A contribution to our knowledge on structure and function of the Pooideae lodicules. *In:* Biology of grasses (Frey L, Ed). Institute of Botany Polish Academy of Sciences, Kraków, pp. 245-256.

Lodicule variability in Elymus repens.

R. Kosina, M. Florek, and P. Tomaszewska

Spikes of *Elymus repens* were collected along two kilometers of field roads in the vicinity of Wroclaw, Poland. The material was gathered from several populations. Lodicules separated from flowers were investigated under a polarized microscope and described with four characteristics, dimensions of the organ, shape, and hairiness. Flowers of *E. repens* open by lodicules, show chasmogamy, and produce highly allogamic progeny. Lodicules (Operational Taxonomic Units (OTUs) were clustered into a dendrogram using Ward's method. The maximum linkage distance (Eulidean distance) was 255; 1.5 times greater than that of *Lophopyrum elongatum*. OTUs (plant number) were scattered in an ordination space after application of nonmetric, multidimensional scaling (Fig. 14, p. 202). An arrangement of OTUs is very regular

and directional, from minimum values in the x and z axes and maximum values in the y axis to maximum values in the y and z axes and minimum values in the x axis. This original arrangement can be a good characteristic of a 'lodicule pattern' in *E. repens* populations. Three polygonal graphs illustrating lodicule morphology in extremes and in an intermediate specimen (Fig. 14). Considering the importance of lodicule traits for breeding systems, we conclude that OTUs located in the upper part of the diagram are examples of obligatory allogamy and, vice versa, those in the lower part, such as 28, 24, 20, and 4, express facultative allogamy or even lack of chasmogamy.

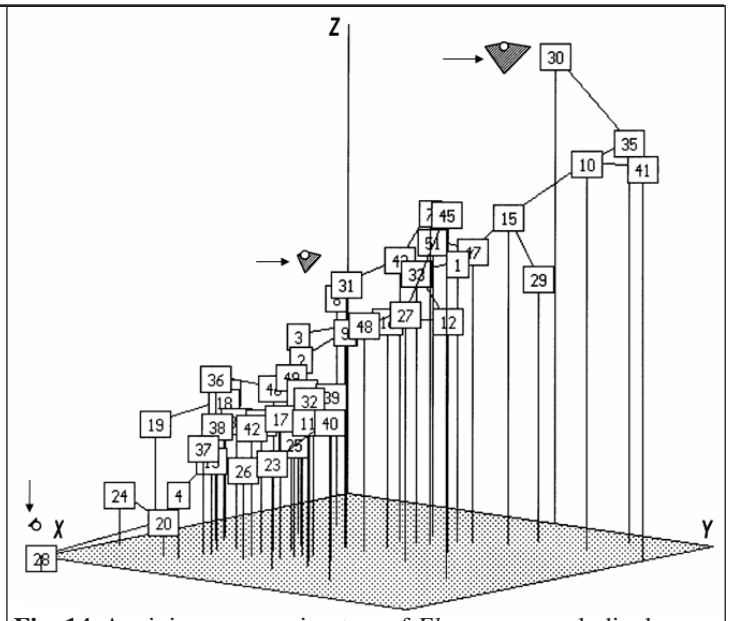

Fig. 14. A minimum spanning tree of *Elymus repens* lodicules (numbers). Operational Taxonomic Units (OTUs) are in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for three OTUs.

Lodicule variability in Dasypyrum villosum.

R. Kosina, M. Florek and P. Tomaszewska.

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Lodicules of *D. villosum* were separated from spikes of one plant. The studied material is a small random sample. *Dasypyrum villosum*, like *Lophopyrum elongatum* and *Elymus repens*, is an allogamic species presenting widely opened flowers with anthers on long filaments. An investigation of its lodicules was made to compare a range of variability of three objects; *L. elongatum* (one spike), *E. repens* (several populations), and *D. villosum* (one plant). Lodicules isolated from spikes were observed in an Amplival polarized microscope and evaluated with four characteristics, length and width of lodicule, shape, and length of hairs developed on the top. Lodicules (Operational Taxonomic Units (OTUs)) were clustered in a dendrogram using Ward's method. Maximal linkage distance (Euclidean distance) was 56; 4.5 times smaller than that in *E. repens* and 3.0 times smaller than that for *L. elongatum*. OTUs (lodicule number) were scattered in an ordination space after application of the nonmetric, multidimensional scaling (Fig. 15). Polygonal graphs (see arrows in Fig. 15) describe the large allogamic (No. 3) and small lodicule (No. 1). An arrangement of lodicules in an ordination space is not irregular as in *L. elongatum* (see above) but is directional and can be described by a significant regression.

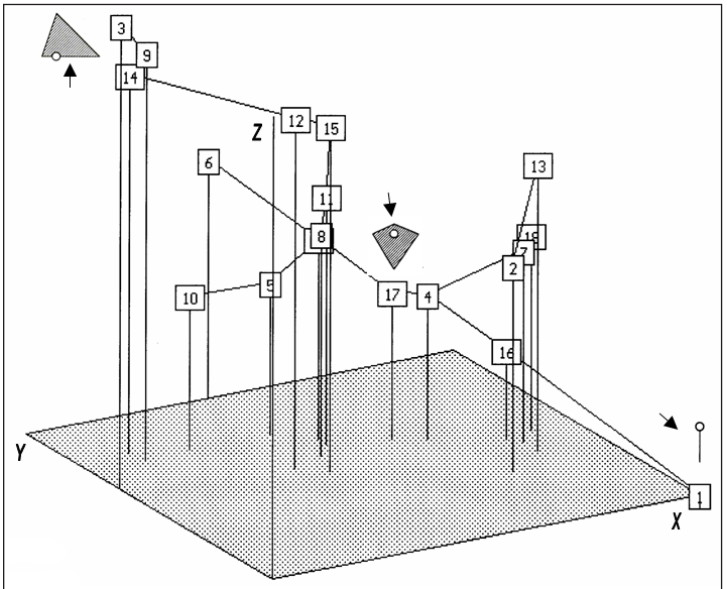

Fig. 15. A minimum spanning tree of *Dasypyrum villosum* lodicules (numbers). Operational Taxonomic Units (OTUs) are in a nonmetric, multidimensional scaling ordination space. OTUs are described by arithmetic averages of the lodicule traits. Polygonal graphs are shown for three OTUs.

On caryopsis development in Thinopyrum distichum versus wheat.

R. Kosina.

Several structures, formed by different tissues, can be identified in a wheat caryopsis, the pericarp, thicker and better preserved in threshable forms and thinner in unthreshable wheats; nucellar remnants, seen as a thin layer, commonly without cell lumina in embryo sac; and a unilayered aleurone in the embryo sac, cylindrical and isodiametric starch cells.

The aleurone layer is differentiated into three forms, one encompassing starchy cells, a second modified in the crease, and a third covering the embryo. Starchy cells develop due to periclinal cytokineses that produce new cells from the ventral and dorsal parts of the caryopsis. As a rule, isodiametric starch cells are formed in lateral parts of caryopsis. For a given species, variety, or cultivar, the volume ratio between cylindrical and isodiametric cells is characteristic. Caryopses of *Th. distichum* were kindly provided by R. de V. Piennar, the University of Stellenbosch, Republic of South Africa. Details of the structure and development were observed on microscopic slides in cross-sections of the caryopsis. Development of a multilayered aleurone is typical for this grass (Fig. 16A and 16B); however, this trait was not fully expressed in all the sections. Drawings present cellular clones in which sequential cytokineses form smallest (youngest) aleurone cells outside. Both clones, dorsal (A) and ventral (B), are of the same size, but the number of cytokineses differs, 17 versus 10, respectively.

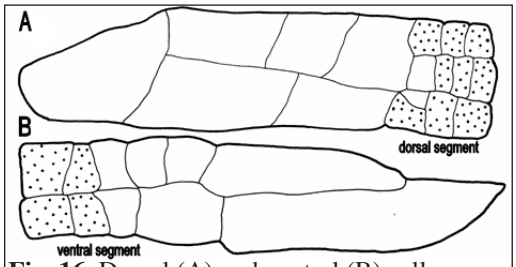

Fig. 16. Dorsal (A) and ventral (B) cell segments of endosperm in caryopsis of *Thinopyrum distichum*. The aleurone layer is dotted.

In caryopses showing some anomaly in development, this difference can be more pronounced (Fig. 17A and 17B) and it is due mainly to more periclinal and anticlinal cytokineses in outer parts of the caryopsis. The length of the cell cycle in wheat vs. *Thinopyrum* endosperm is not known. One can suggest that *Thinopyrum* caryopses are more active cytokinetically and express shorter cellular growth. There is a directional change of diameter of starch granules (S–S4), from the outside to the inside of the endosperm, vice versa of the protein content (Fig. 17B). Inner starch granules are older and distinctly larger, outer starchy cells form a subaleurone layer with a high protein content. In the developing grass endosperm, starch and protein sysnthesis are complementary.

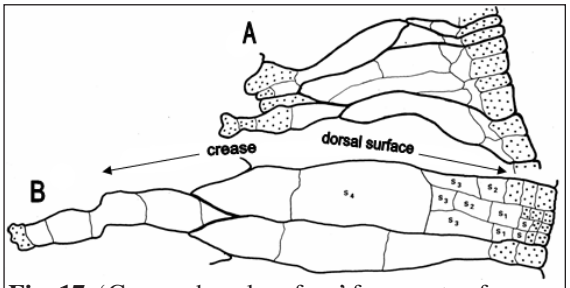

Fig. 17. 'Crease-dorsal surface' fragments of caryopsis cross-sections in *Th. distichum*. A, for some anomalous grain; B, showing a directional trend of starch synthesis (from S (small granules) to S4 (large granules)). Aleurone cells are dotted and cell segments are outlined by thick lines.

On caryopsis development in 'wheat/Thinopyrum distichum' true and partial amphiploids.

R. Kosina and P. Tomaszewska.

A study of caryopsis structure was made for amphiploids obtained from hybridization between *Triticum turgidum* subsp. *durum* (Td), *T. aestivum* subsp. *aestivum* (Ta), and *Th. distichum* (Thd) in the following associations: partial amphiploids, Td//Td/Thd, Td/Thd//Td, Td//Td/Thd/3/Agrotricum, and Ta/Thd//Ta, and true amphiploids, Td/Thd and Ta/Thd. All material was provided by R. de V. Piennar, the University of Stellebosch, Republic of South Africa.

It is well known that distant hybrids exhibit various developmental anomalies, observed also in caryopsis structure. Crosses of durum and common wheat with *Th. distichum* are interesting, because the wild grass is salt resistant. Their genomes were distinguished distinctly by GISH (Kosina and Heslop-Harrison 1995). Anomalous development of amphiploid caryopsis are known. A lack of a large part of the aleurone layer in the crease is often noted in partial amphiploids (Fig. 18A, 18B,and 18C, p. 204). The endosperm cavity deeply penetrates starch endosperm. All contact with nucellar projection lacks the aleurone (Fig. 18C, p. 204). A multilayered high protein subaleurone develops in this area. Anomalous development of the endosperm cavity (Fig. 18D, p. 204) was observed in the form of a multiplication

of small, defected aleurone cells inside the cavity.

An extremely thick, high-protein subaleurone layer develops on the dorsal side of endosperm (Fig. 19A, yellow line). Such a development is correlated with very thick, unilayered aleurone (note the green line). These aleurone cells express prolonged longitudinal growth and a lack of periclinal divisions. A multilayered aleurone, which is typical for *Th. distichum*, is partly expressed in the amphiploids. The development of the endosperm often occurs in the form of isolated clones of cells differing sharply from the adjacent tissue (Fig. 19B). Large, probably polyploid, cells of the aleurone are formed near the crease. In the endosperm, we can observe a mosaic of tissues, which is a result of its very variable development (Kosina 2007). Many defects in the development of the endosperm resulted in poor caryopses, were more often noted in partial and less in true amphiploids.

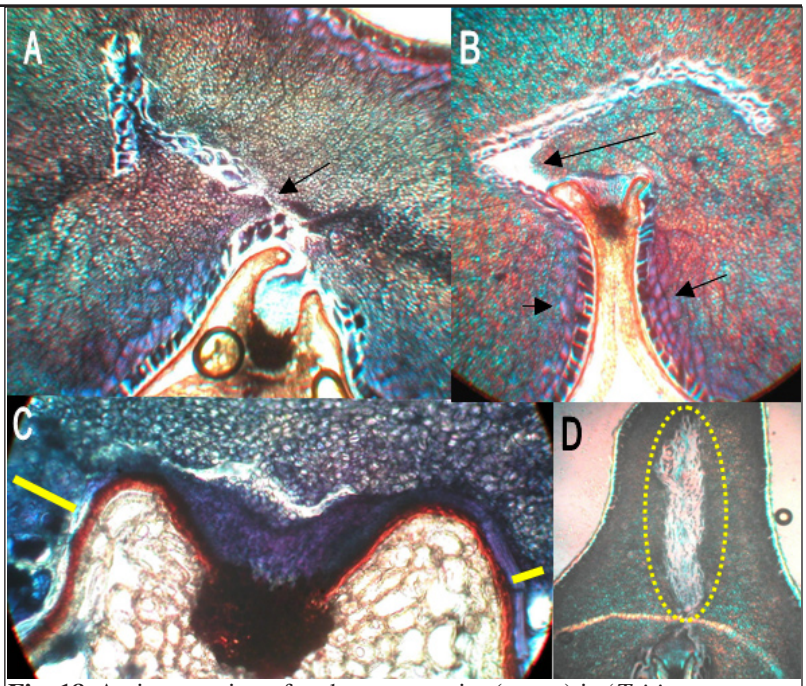

Fig. 18. An interruption of endosperm cavity (arrow) in '*Triticum turgidum* supbs. *durum* (Td)/*Thinopyrum distichum* (Thd)//Td' (A); a lack of aleurone layer in the area of endosperm cavity (a long arrow) and a multilayered, high-protein subaleurone layer adjacent to the crease (short arrows) in 'Td//Td/Thd/3/*Agrotricum*' (B); a large segment of starchy cells (see yellow lines), instead of an aleurone layer, adjacent to the nucellar projection in 'Td/Thd//Td' (C), and a large endosperm cavity (encircled), filled by anomalous aleurone cells, in a poor caryopsis of 'Td/Thd//Td (D)'.

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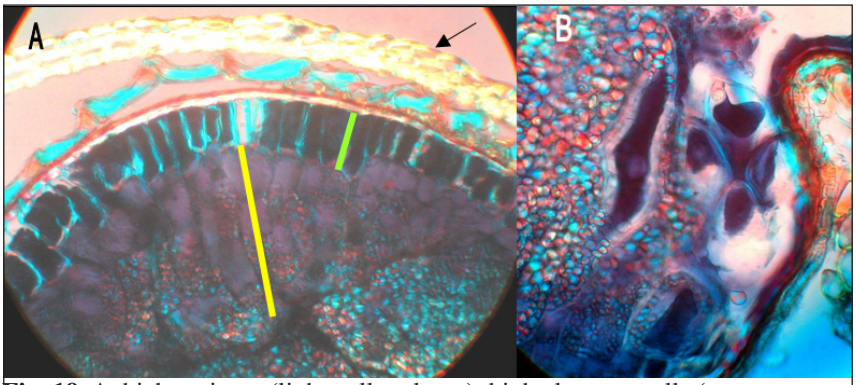

Fig. 19. A thick pericarp (light yellow layer), high aleurone cells (green line), and s thick high-protein subaleurone layer (yellow line) in a '*Triticum turgidum* subsp. *durum* (Td)/*Thinopyrum distichum* (Thd)//Td' cross (A) and huge, polyploid aleurone cells in the area of the crease in a '*T. aestivum* subsp. *aestivum* (Ta)/Thd//Ta' cross (B).

A case of endosperm mosaic in Triticum aestivum subsp. spelta.

R. Kosina and P. Tomaszewska.

The microscopic study was made for cross-sections of caryopsis of *T. aestivum* subsp. *spelta* var. *viridarduini* (VIR 45767). The sections were stained by bromophenol blue to show localization of the total protein. An original endosperm development was discovered in one caryopsis of the small random sample of seeds. In a large sector of the caryopsis, almost one-half of the seed (Fig. 20A, p. 205), there were extensive spots within the aleurone layer. The spots lacked aleurone cells. In this area, aleurone cells spread by a tangential growth with no anticlinal cytokineses and reached large dimensions (Fig. 20B, p. 205). This development also was associated with a differentiation of the aleurone cells inside a starchy endosperm and their strong vacuolization (Fig. 20C, p. 204). Similar observations were earlier provided by Becraft and Asuncion-Crabb (2000) in the genetics of aleurone development in mutated seeds of maize. Becraft

and Asuncion-Crabb (2000) suggested a hierarchy of gene functions, which determine the fate of aleurone cells. Large, mutated sectors of caryopsis also were observed in *Brachypodium retusum* (Kłyk 2005), '*Bromus commutatus/racemosus* (Skowrońska 2005), and *Bromus secalinus* (Kurek 2007; Kochmański 2008). As a rule, such sectors were formed at some level of tissue isolation and growth interaction between starchy and aleurone endosperm. Mutations in a grass endosperm can be evidenced in many aspects of the tissue development and metabolism, e.g., interaction between starch-protein cell phenotypes, vacuolization of aleurone cells, length of cell cycle, cellulose vs. hemicellulose or callose metabolism in cell walls, and synthesis of globoids (Kosina 2007; Kosina and Tomaszewska 2010; Kosina and Zając 2010).

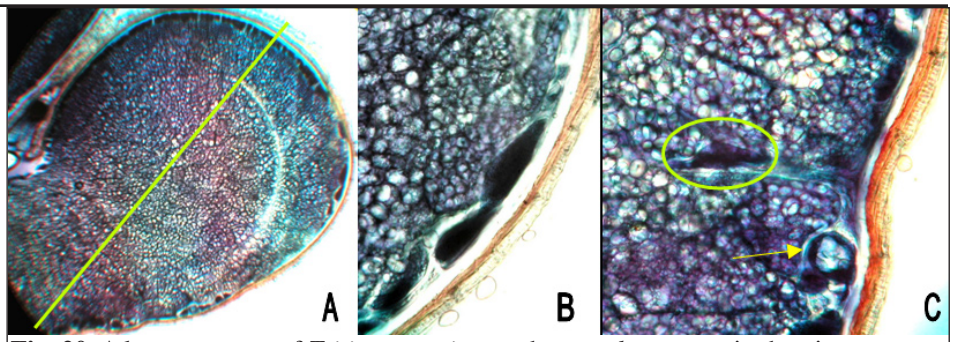

Fig. 20. A large segment of *Triticum aestivum* subsp. *spelta* caryopsis showing a patched aleurone layer (A), aleurone cells tangentially enlarged (B), an aleurone cell differentiated inside the starchy endosperm (outlined with a green line), and the aleurone cell with large vacuole (yellow arrow) (C).

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Skowrońska J. 2005. Mikrostrukturalna i cytogenetyczna analiza grupy *Bromus secalinus – B. commutatus – B. racemosus*. MSc Thesis, Department of Cytogenetics and Plant Speciation, Institute of Plant Biology, University of Wrocław, Wrocław, Poland (In Polish).

A mosaic aleurone layer in wheat and irradiated Hordeum vulgare.

R. Kosina.

We noted different cell segments on the surface of an isolated aleurone layer in *Triticum timopheevii* subsp. *timopheevii*. These segments, which expressed phenotypes in the form of small and large cells, were easily recognized (Fig. 21). Such a phenomenon also is seen in other wheat species, and we can correlate it with increased or decreased cytokinetic activity of the layer and some level of instability, typical for endospermal tissue. Under different stress conditions, this instability can be increased (Kosina 1989).

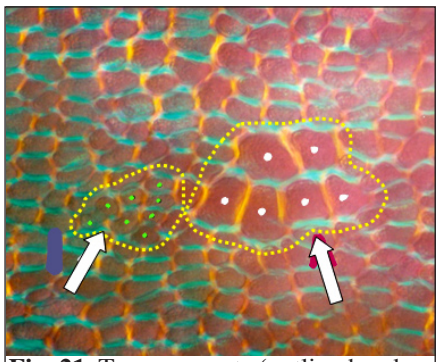

Fig. 21. Two segments (outlined and shown by arrows) of small and large aleurone cells in *Triticum timopheevii* subsp. *timopheevii*.

The same observations were made for plants of five old cultivars of Hordeum vulgare obtained from the IHAR collection in Radzików, Poland, which were irradiated with a dose of 0.03 Gy (X-rays). The irradiation was made at 8 DAF, at still existing cytokineses in "endosperm cambium" of main ears. Many morphological and anatomical changes were observed after irradiation. Looking at the surface of aleurone layer, several groups of smaller or distinctly larger cells were developed. The frequency of such, changed by irradiation, cell clones was significantly larger than in non-irradiated cultivars (compare A and B in Fig. 22). As it is visible in Triticum timopheevii (Fig. 21), an additional cytokinesis is typical for the segment of small aleurone cells – the length of their cell cycle is twice shorter. In the segment of large cells, the lack of two cytokineses is most often seen - the length of their cell cycle is three times longer. The visible morphological mosaic is, in fact, a metabolic mosaic of the cell cycle. Our data proved that the hybrid status of a plant or radiation environmental stress can cause the same changes related to metabolism, physiology, morphology or behaviour of any plant.

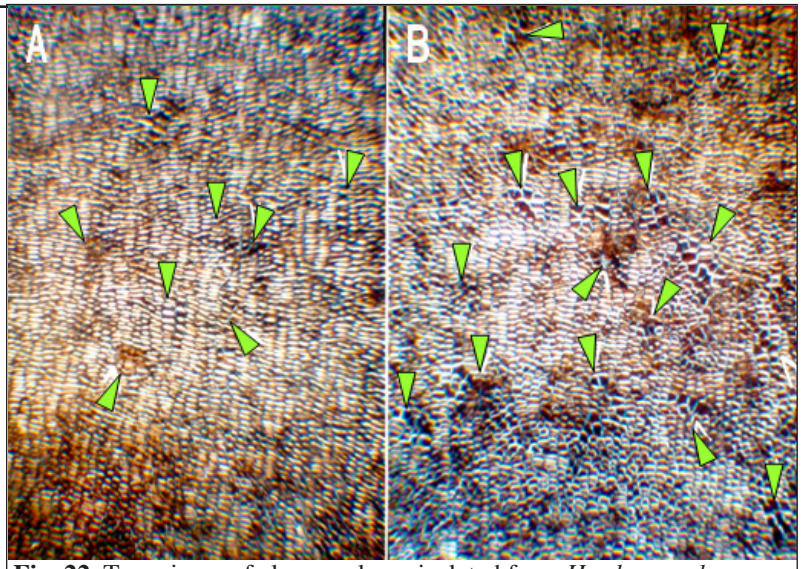

Fig. 22. Two pieces of aleurone layer isolated from *Hordeum vulgare* caryopses of normally growing (A) and irradiated (B) plants. Segments of cells with a changed cell cycle are indicated with arrowheads.

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ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE FOR THE SOUTH-EAST REGIONS
Department of Genetics, Laboratory of Genetics and Cytology, 7 Toulaikov St., Saratov, 410010, Russian Federation.

The influence of a translocation with the Lr19+Lr26 combination on grain productivity and bread-making quality in spring bread wheat.

S.N. Sibikeev, A.E. Druzhin, and S.A.Voronina.

At the Agricultural Research Institute for the South-East Regions (ARISER), NILs based on the Saratov-bred spring bread wheat cultivar L503 and carrying translocations with the *Lr19+Lr26* combination, prospective lines were produced and studied. The data from 2005–11 indicate that the interaction of these translocations positively influences grain yield (Table 1). During this period, leaf rust epidemics were observed three times (2005, 2006, and 2008) and drought conditions four times (2007, 2009, 2010, and 2011). Grain productivity significantly increased in two leaf rust epidemics in lines with *Lr19+Lr26*, and twice under drought conditions. In the hard drought of 2010, spring bread wheat plants died. The main limiting factor for the use of T1BL·1RS translocations in wheat breeding is the influence on bread-making quality. The decrease of bread-making quality is coupled with genes for disease resistance and *Sec1*. In the NILs produced in the genetic background of cultivar L503, the presence of *Lr19+Lr26* translocations did not influence the grain protein content or gluten values. The increase in grain yield of lines containing *Lr19+Lr26*-translocations was not accompanied by decreased of grain protein content. Dough extensibility (P) and strength of flour (W) were not significantly lower in the NILs and perspective lines with *Lr19+Lr26* translocations compared with L503. Similar results were obtained for bread-making qualities. Loaf volume and porosity also were not significantly lower than L503 in the NILs and lines with *Lr19+Lr26* translocations (Table 2). Thus, the *Lr19+Lr26* translocations had positive effects on resistance to disease anddrought and grain productivity with good bread-making quality.

Table 1. Grain productivity, grain protein content, and gluten deformation index of L503 NILs and perspective lines of spring bread wheat with *Lr19+Lr26*.

NIL / line	Grain yield (kg/ha)	Grain protein content (%)	Gluten value	
			content (%)	strength
L503 (*Lr19*)	1,755 a	17.78	42.17	81
L503 (*Lr19+Lr26*)	2,096 bc	17.94	40.53	78
L503(*Lr19+Lr26*)//L505	2,301 c	17.79	39.32	81
LSD	47.79	NS	NS	NS

Table 2. Bread-making qualities of L503 NILs and perspective lines of spring bread wheat with *Lr19+Lr26* (dough extensibility = P and strength of flour = W).

NIL / line	Physical traits of dough			Bread-making qualities		
	P	P/L	W	loaf volume (cm^3)	porosity	crumb color
L503 (*Lr19*)	93.4	1.5	192	788	4.5	yellow
L503 (*Lr19+Lr26*)	84.6	1.4	166	760	4.4	yellow
L503(*Lr19+Lr26*)//L505	89.3	1.3	185	750	4.3	yellow
LSD	NS	NS	NS	NS	NS	

The agronomic performance of Lr19+Lr37 translocations in the set of NILs in the genetic background of the spring bread wheat cultivar Dobrynya.

S.N. Sibikeev and A.E. Druzhin .

We used a set of NILs in the genetic background of the spring bread wheat cultivar Dobrynya to associate positive agronomical traits of *Lr19* and *Lr37* translocations. The presence of both translocations in the NILs was confirmed with molecular markers; STS marker Gb Lr19 for the *Lr19* translocation and primers VENTRIUP and LN2 for the *Lr37* translocation (Gultyaeva EI et al. 2012). In the droughty conditions of 2011, NILs with *Lr19+Lr37* translocations were significant higher than Dobrynya for grain productivity. The NILs also surpassed Dobrynya in grain protein content, however, the results for gluten content and gluten strength were ambiguous. One line was higher than that of Dobrynya, and another line was equal to Dobrynya (Table 3). For dough extensibility (P) and strength of flour (W), NIL 118 was equal to and NIL 113 exceeded those of Dobrynya. Loaf volume and porosity were lower in the NILs with *Lr19+Lr37* translocations than in Dobrynya (Table 4). We conclude that translocations with the *Lr19+Lr37* combination improve drought resistance but do not depress bread-making properties.

Table 3. Grain productivity, grain protein content, and gluten deformation index of Dobrynya NILs and perspective lines of spring bread wheat with *Lr19+Lr37*.

NIL / line	Grain yield (kg/ha)	Grain protein content (%)	Gluten value	
			content (%)	strength
Dobrynya (*Lr19*)	2,695 a	14.98	37.4	80
NIL 113 Dobrynya (*Lr19+Lr37*)	2,864 b	15.75	35.4	68
NIL 118 Dobrynya (*Lr19+Lr37*)	3,156 c	16.10	40.0	78
LSD	153			

Table 4. Bread-making qualities of Dobrynya NILs and perspective lines of spring bread wheat with *Lr19+Lr37* (dough extensibility = P and strength of flour = W).

NIL / line	Physical traits of dough			Bread-making qualities		
	P	P/L	W	loaf volume (cm^3)	porosity	crumb color
Dobrynya (*Lr19*)	108	1.9	249	810	5.0	yellow
NIL 113 Dobrynya (*Lr19+Lr37*)	156	2.7	366	700	4.5	yellow
NIL 118 Dobrynya (*Lr19+Lr37*)	110	1.7	249	710	5.0	yellow

Resistance screening to a local population of leaf rust among species and relatives of bread wheat.

S.N. Sibikeev, A.E. Druzhin, T.D. Golubeva, and T.V. Kalintseva.

Screening for resistance to a local population of leaf rust among 25 species and relatives of bread wheat gave the following results: 14 lines from 10 species/relatives of bread wheat and one triticale cultivar, Satu, were resistant. Temperature sensitivity for resistance to leaf rust was found in *T. turgidum* and *T. petropavloskyi* in 2006. With the purpose of enlarging the gene pool of bread wheat for *Lr* genes, hybrids were obtained between the spring bread wheat cultivars Saratovskaya 68, Saratovskaya 70, Saratovskaya 73, and Saratovskaya 74 and lines of the resistant species.

Evaluating spring bread wheat cultivars for resistance to tan spot (Pyrenophora tritici-repentis (Died.) Drechsler.) in 2011.

S.N. Sibikeev, A.E. Druzhin, T.D. Golubeva, and T.V. Kalintseva.

Tan spot, caused by the ascomycete fungus *Pyrenophora tritici-repentis*, is one of the most serious foliar diseases of wheat. The average yield loss caused by this pathogen is 5–10%, but in epipdemic years, losses can be up to 30%.

Moreover, tan spot also causes significant losses in grain quality by grain shriveling, red smudge, and black point. In recent years, this disease more often appear in the plantings of spring bread wheat in the Lower Volga Region of the Russian Federation. To screen for resistance to this pathogen, we evaluated 140 introgression lines of spring bread wheat in a nursery. Among the lines, 35 were resistant (% infection = 0) to the local tan spot population. NIL L400R, with a *Thinopyrum intermedium* 6Agi (6D) substitution was more resistant to tan spot than its sib lines L400S that lack 6Agi (Table 5). The combination of translocations T7DS·7DL-7Ae#1L (*T. elongatum*) and T6BS·6BL-6U#1L (*Ae. umbellulata*) and T7DS-7DL-7Ae#1L and 4BS·4BL-2R#1L (*S. cereale*) increase the resistance of the spring bread wheat lines to the tan spot, however, the combination T7DS·7DL-7Ae#1L and T1BL·1R#1S significantly increased susceptibility. NILs with a combination of T7DS·7DL-7Ae#1L and T3DS·3DL-3Ae#1L (*T. elongatum*) did not significantly differ on the extent of damage with respect to the cultivar (Table 5).

Table 5. The reaction of cultivars and NILs of spring bread wheat to *Pyrenophora tritici-repentis*.

Cultivar / NIL	Translocation	% infection
L 400 R	6Agi (6D)	5
L 400 S	—	30
L 503	T7DS·7DL-7Ae#1L	0
L 503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L	0
L 503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L + T1BL·1R#1S	5–10
L 505//L503 (*Lr26+Lr19*)	T7DS·7DL-7Ae#1L + T1BL·1R#1S	5
Dobrynya	T7DS·7DL-7Ae#1L	30
Dobrynya (*Lr19+Lr9*)	T7DS·7DL-7Ae#1L + T6BS·6BL-6U#1L	3
Dobrynya (*Lr19+Lr24*)	T7DS·7DL-7Ae#1L +T3DS·3DL-3Ae#1L	30
Dobrynya (*Lr19+Lr25*)	T7DS·7DL-7Ae#1L + 4BS·4BL-2R#1L	10

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The microclonal propagation of sterile triticale plants.

V.N. Akinina, T.I. Dyatchouk, and A.V. Pominov.

Applying biotechnology to crop improvement induces nonconventional plant breeding methodology. In biotechnology and plant breeding, preserving and propagating sterile plants, undoubled haploids, and remote hybrids for future use is important. The most frequent explant sources for morphogenetic callus induction in cereals are mature and immature embryos. To establish tissue culture systems in sterile plants, it is necessary to select the explant source.

Wheat–triticale hybrids (three genotypes) and haploids (nine genotypes) were grown in a phythotron. Somatic embryogenesis was used to produce the largest number of plantlets. Segments of immature inflorescence 2–3 cm and completely covered by leaves served as explants to establish callus cultures. A solid MS nutrient medium, supplemented with 2 mg/L 2,4-D and 2% sucrose was optimum for callus induction. Callus cultures were grown in the dark, with subculturing on fresh culture medium every 3–4 weeks. The subsequent elimination of 2,4-D from the culture medium and the addition of IAA (1 mg/L) allowed for embryogenesis. Centers of morphogenesis were milky white, compact, and modulary. The start of regeneration was observed within 7–10 days. The frequency of callus induction ranged from 76.5 to 84.0% and the frequency of morphogenetic callus was from 39.1 to 46.6%.

The microclonal propagation of sterile triticale sterile was elaborated using segments of immature inflorescence as source of totipotent cultures. Embryogenic callus and plant regeneration were formed. This technology allows one to obtain clones of each genotype in an unlimited amount for use in breeding work.

Identification of leaf rust resistance genes of lines of soft spring and winter wheats.

T.S. Markelova and O.V. Ivanova.

Leaf rust resistance genes were identified in 14 introgressed lines of soft wheat carrying noncompensating translocations from *Aegilops speltoides*, *Triticum turgidum* subsps. *dicoccum* and *persicum*, and wheat cultivars from the collection of the All-Russian Institute of Plant Breeding using phytopathological and molecular methods.

After molecular screening of wheat cultivars, we discovered three with gene *Lr9* and one with *Lr19* (Table 6, p. 197). The identification of *Lr9* used SCAR marker SCS5 (Gupta et al. 2005). A characteristic 550-bp fragment was found among breeding lines 6 and 7, Clez Alta, and a control line Tc *Lr9*. The results from the molecular screening of these species correspond with the phytopathological test. Gene *Lr9* in line 11 is unidentified despite the fact that it was used when the line was created. In addition to leaf rust resistance, this line also has mildew resistance from *T. turgidum* subsp. *persicum*.

Table 6. Leaf rust (*Lr*) genes identified using DNA markers.

#	Description	*Lr9*	*Lr10*	*Lr19*	*Lr20*	*Lr26 iag95*	*Lr26 SCM9*	*Lr34*	*Lr35*	*Lr37*
1	Saratovskaya 29/*T. persicum*//*T. dicoccum*				+				+	
2	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
3	Saratovskaya 55// *T. dicoccum*/ *Ae. speltoides*		+						+	
4	Saratovskaya 29//*T. persicum*/3/ *T. dicoccum*/*Ae. speltoides*								+	
5	Saratovskaya 29// *T. dicoccum*/*Ae. speltoides*								+	
6	Saratovskaya 29/*T. persicum*//*Lr9*	+								
7	Saratovskaya 29/*T. persicum*/*Lr9*	+								
8	Saratovskaya 29/*T. persicum*//*T. dicoccum*/*Ae. speltoides*								+	
9	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
10	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
11	Saratovskaya 29/*T. persicum*//*Lr9*								+	
12	Saratovskaya 29/*T. persicum*// *T. dicoccum*/*Ae. speltoides*								+	
13	Saratovskaya 55//*T. dicoccum*/*Ae. speltoides*								+	
14	Saratovskaya 29/*T. persicum*// *T. dicoccum*/*Ae. speltoides*								+	
15	Clez Alta	+								
16	K-34612			+						
17	W464//VEE/KOEL/3/PEG//MRL/BUC		+			+	+	+		
18	CROC-1/*Ae. tauschii* (205)//KAUZ/3/ATTILA					+	+			

A characteristic 130-bp fragment was found in sample 16 with the help of STS marker Gb *Lr19* (Prins et al. 2001). A 487-bp fragment was discovered in lines 1–5 and 8–14 with the help of marker Sr39#22 for gene *Lr35*. Gene *Lr35* is located on the short arm of chromosome 2B and is situated in the same translocation with seedling gene *Sr39*, which defends against *Puccinia graminis* race Ug99 (TTKS) (Singh et al. 2008). Diploid *Ae. speltoides* is the source of this translocation.

When marker PS10 for gene *Lr47* from *Ae. speltoides* was being applied its carriers were not discovered. Although gene *Lr35* has the potential for leaf rust resistance, examples of its application have not been given in the literature to date (McIntosh et al. 1995; Mago et al. 2009; Serfling et al. 2011). A good example of introgressing *Lr*/*Sr* genes from *Ae. speltoides* in Russia is the set of amphidiploid lines ((*T. dicoccum* x *Ae. speltoides*)*5//Saratovskaya 29) created at the ARJPB (Odintsova et al. 1991). These lines were widely used in many selection centres in the USSR, including SRJA of South-East. Thus, the existence of the given translocation in lines 1–5 and 8–14 is logical. Their resistance to

race *P. graminis* Ug99+*Lr24* (TTKST) (Sibikeev et al. 2011) is important to characteristics of these lines. Leaf and stem rust resistance, possibly caused by Lr and Sr cohesion, supports the existence of *Lr35*/*Sr39* genes in lines 1–5 and 8–14.

Although *Lr10* was identified with the primers F 12245 and *Lr10*-6/r2 (Schachermayr et al. 1997), an amplicon with a molecular weight of 310 bp was discovered in lines 3 and 17.

Using the primers STS638-L and STS638-R for gene *Lr20* (Neu et al. 2002), a marker of 540 bp was found in line 1 and in the control Thatcher with *Lr20;* line 1 includes wild *T. persicum* and *T. dicoccum* in the pedigree. Today, *Lr20* has lost its efficiency practically everywhere (McIntosh et al. 1995; Khan et al. 2005).

To identify gene *Lr26*, two markers, SCM9 (Mago et al. 2002) and iag95 (Weng et al. 2007), were used. A characteristic 207-bp component of SCM9 and an ~1,000-bp iag95 marker were identified in lines 17 and 18, which corresponds to their gender lines.

By applying phytopathological and molecular methods and also analyzing pedigrees, we discovered *Lr* genes and their combinations in 14 introgressive lines and in four soft spring wheats. These results are very important for programs that use the application of certain *Lr* genes and their combinations in practical selection.

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The winter wheat cultivar Kalach 60.

J. Svistunov, A. Pryanichnicov, A. Zavorotina, A. Sergeeva, V. Uvarova, and N. Larionova.

Breeding of the winter wheat cultivars at ARISER began in 1915. The main task in the first stage of selection was creating cultivars adapted to the steppe zone of the Volga Region. Since the beginning, 26 cultivars have been developed from which seven are currently patented and registered.

Kalach 60 is an intensive type cultivar; to obtain high yield and high-quality grain, it requires the application of fertilizers and observing agronomical practices. Kalach 60 was developed using individual selection. The cultivar is of the variety lutescens. Plant height is 20–30 cm shorter compareed to that of Mironovskaya 808. The straw is thick. Kalach 60 has increased winter hardiness, lodging and drought resistance, early mature growth, high grain quality, and high grain yield (2.7 t/ha). These characters allow the use of Kalach 60 in intensive farming to obtain high-quality grain. Early ripening allows Kalach 60 to be used in a system used by large farms, which sow winter crops on large areas.

In 2011, Kalach 60 was planted in rhe Saratov region on 13.7 x 10^3 hectares (2.8%). In 2012, it was listed in the National Register of the Russian Federation in the lower Volga Region.

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The role aluminum salts ($AlCl_3$, $Al(NO_3)_3$, and $Al_2(SO_4)_3$ in the formation of spring wheat resistance to aluminum toxicity.

N.V. Poukhalskaya, Y.R. Ziangirova, and F.D. Davadov, and N.I. Pavlova (Pryanishnikov All Russian Research Institute of Agriculture and Solid Science, Pryanishnikova, 31. Moscow 127550, Russian Federation) and S.L. Ignatyeva (Russian State Agrarian University – MTAA named after K.S. Timiryazev).

We are researching the toxicity of aluminum, at present, because of insufficient data about the toxicity of various Al^{+3} salts (sulfates, nitrates, and chlorides). Our data show that aluminum toxicity changes in the presence of different ions. We studied the comparative degree of toxicity of aluminum ions when using sulfates, nitrates, and chlorides on spring wheat.

Materials and methods. Aluminum tolerance was observed at germination in laboratory experiments under controlled environmental conditions. Seedlings of two cultivars of spring wheat were grown under greenhouse conditions. Sprouts of plants were grown on solutions of $AlCl_3$, $Al(NO_3)_3$, and $Al_2(SO_4)_3$ with various concentration of aluminum (0, 0.4, 3, 5.13, 15, and 40 mg of Al/L. Absorption from the solutions was measured in vessels each containing the same number of plants. Plants were grown on plastic floats (rafts) on the surface of the solution. The seed was planted in apertures in the floats. Experiments also were made in rolled culture and in soil. The same aluminum salts were used at 1.3 mg/100 g soil for the vegetative trials.

Results and discussion. During germination, wheat plants reduce the absorption from solutions in different ways. Absorption decreases as follows: $Al(NO_3)_3 = Al_2(SO_4)_3 > AlCl_3$. Plants absorb a solution more actively if there are ions that do not interfere with metabolism, but apparently, more active absorption of a 'bad' aluminum ions also occurs.

Effects of aluminun toxicity. The root tip is deformed (Fig. 1, p. 213). The root becomes thickened; cell division continues but the stretching processes slow down. Our results show that growth in root tips is connected with the ability to

adapt to the aluminum toxicity than the reaction of the entire root system. The reaction of the shoot to aluminum toxicity is defined by the degree of growth inhibition of the root. However, we found a high correlation with leaf growth (Table 1). Such plants can have a stronger inhibition of root growth. The ability to respond to aluminum ions during the first stages of development corresponds to the genotypes that are capable of increased metabolic activity under stress. Genotypes with notable resistance to aluminum stress also had high yields.

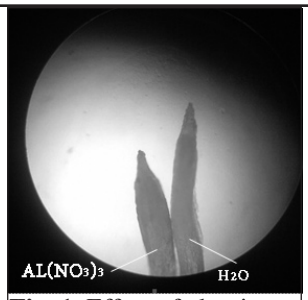

Fig. 1. Effect of aluminum toxicity on a root tip as seen under a microscope, left, growing in aluminum nitrate; right, water control.

Table 1. The apex (head) length at developmental stage VI after Kuperman of spring wheat.

Variant	Apex length (mm)	Seed/head
Yugo-vostochnaya 2		
Control	5.7	14
$AlCl_3$	10.5	14
$Al_2(SO_4)_3$	7.8	14
Kerba		
Control	17.8	17
$AlCl_3$	12.3	16
$Al_2(SO_4)_3$	16.0	17
LSD 0.05	0.3	0.7

Kernel number/spike was lower when aluminum ions were present. Aluminum sulfate caused a greater reduction in the length of roots compared to those grown in aluminum chloride. The length of the root system decreased more in an aluminum sulfate solution than those in an aluminum chloride solution.

Vegetative growth may be strengthened as a reaction to aluminum toxicity. Aluminum sulfate inhibited root growth to a greater degree and activated leaf growth leading to better adapted plants. In the variant with aluminum sulfate, the resistance of cultivar Kerba was 0.97 s/cm, an increase of 13.0% and 24.8 % in Yugo-vostochnaya 2, 25.6% higher in the different background (Fig. 2). Thus, we established that the ability of plants for active vegetative growth can be used to test adaptive potential. Apparently, we are unable to make conclusions about the stability of a genotype to aluminum toxicity or on the level of decrease in root length in aluminum solutions. We believe that the stability of plants can be made, connecting vegetative growth in plants to solutions of aluminum and loss of final productivity (Fig. 3).

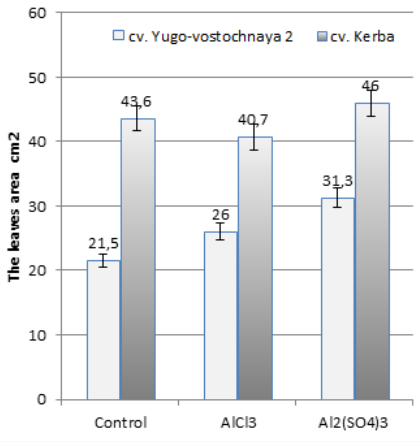

Fig. 2. Leaf area of plants of cultivar Yugo-vostochnaya 2 and Kerba grown in $AlCl_3$ and $Al_2(SO_4)_3$ (cm^2) solutions.

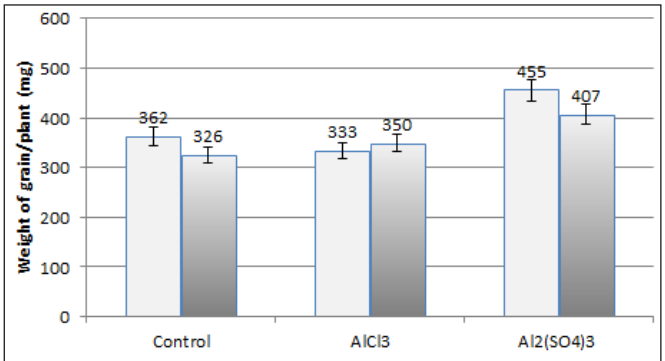

Fig. 3. The final productivity (grain/plant (mg)) of cultivars Yugo-vostochnaya 2 and Kerba under aluminun chloride and sulfate.

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Bacterial lipopolysaccharides in a culture of wheat calli.

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For several years, we studied the influence of outer-membrane lipopolysaccharide (LPS) in the nitrogen-fixing bacterium *Azospirillum brasilense* Sp245 on the cultivation of somatic wheat calli *in vitro*. Our working hypothesis for the possible effect of LPS on callus cells was based on their known ability to induce plant cell responses *in vitro* (Evseeva et al. 2011).

LPS at 1, 10, and 100 μg/mL was included in the composition of a nutrient medium for callus initiation from immature embryos of two model near-isogenic soft spring wheat lines (genetic background of cultivar Saratovskaya 29) differing in the *Rht-B1c* gene. The resultant morphogenic calli were transferred to a regeneration medium with the same LPS content.

No direct effect of the LPS on cellular proliferation was found, because callus formation occurred at a frequency close to 100% in all treatments. Likewise, no differences in callus weight were observed. LSP is known to stimulate the process of secondary differentiation of callus cells and the formation of morphogeneic loci. In the line with the *Rht-B1c* gene, whose morphogenic ability is greater than that of the sister line (Tkachenko and Lobachev 2008), the yield of morphogenic calli was found to increase in all treatments involving LPS. Yet the morphogenesis ability of the sister line increased significantly only on induction medium containing 1 μg/mL of LPS. During plant regeneration, no significant effect of LPS on the growth or weight characteristics of shoots has been recorded. According to our experimental data, the measures of shoot regeneration from morphogenic calli were associated with the genotype of the donor plants to a greater extent than they were with the LPS content in the nutrient medium.

In summary, the effect of Azospirillum on plant cells can be determined by components of the bacterial outer membranes (in this case, LPS). LPS not only can promote the mitotic activity of plant cells (which has been found in experiments *in vivo*) but also can regulate the differentiation of proliferating cells *in vitro*.

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ITEMS FROM UKRAINE

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The characteristics of primary callus NILs for PPD genes of winter wheat, Triticum aestivum L.

O.A. Avksentyeva and V.A. Petrenko.

Abstract. We studied primary callus isogenic for the *PPD* genes, controlling photoperiodic sensitivity, in lines of winter wheat. Genotypic dependence on the processes of callus formation, the rate of formation of callus tissue, size and number of cells, biomass accumulation, and water content was established. The genotype of the isolines, determining photoperiodic sensitivity *in vivo*, has an impact on the process of induction and on the characteristics of primary callus NILs *in vitro*.

Introduction. Methods of cultivating isolated plant cells are a unique tool for studying fundamental biological problems. Despite numerous research in cereal morphogenesis *in vitro*, many questions remain unresolved (Tyankova and Zagorska 2001; Wang and Wei 2004). The role of a particular genetic system or separate genes in the regulation of explants ability to cultivation *in vitro* is possible to study. To solve these problems, using near-isogenic lines (NILs) with a known connection between gene expression and its phenotype are a convenient system to use. Two basic gene systems in soft wheat define the type and rates of development (Stelmakh 1998). VRN genes (requiree for vernalization) determine spring/winter type, and *PPD* genes determine photoperiodic sensitivity. NILs of PPD genes differ in development rates, which are shown in short-day conditions (photoperiod). Isoline *PPD B1* shows the maximum sensitivity; *PPD D1a* and *PPD A1a* have a low degree of sensitivity, so they are almost photoperiodically neutral. These genetic systems are actively investigated at the molecular level in cereal flowering regulation (White et al. 2008; Wang et al. 2009; Bentley et al. 2010). The *PPD* system of genes also seems to take part in the control of callus initiation *in vitro*. This research investigated the influence of the *PPD* gene system on callus genesis processes and the cytological and morpho-physiological characteristics of primary callus from NILs of the wheat cultivar Mironovskay 808.

Materials and methods. Genotypes of soft winter wheat NILs for photoperiod sensitivity genes *PPD D1a*, *PPD B1a*, *PPD A1a*, and *PPD D1b PPD B1b PPD A1b* were used as objects of study. For callus production and quality explants, we used mature germs, which were cultivated on a nutrient Murashige-Skoog (MS) medium with a full set of macro and micro salts and containing 2,4 D (2 mg/l) as a growth regulator, 0.7% agar, 3% sucrose, and 10 mg/L $AgNO_3$. Explants were cultivated in an incubator at 26°C in the dark for 1.5 months. Growth rate was measured as the area of callus tissues per unit of time. Cytohistological observations of crushed preparations were made using a light microscope PZO (Warszava). Crude and dry biomass were defined at the end of cultivation, 45 days after the first passage. The quantity of soluble protein was calculated by the Loury method, allocating these fractions with tris-HCl buffer pH 5.6 and further photocolorimetrical analysis (730 nm) after reaction of the protein with Folin reactant. Results were from 4–5 independent experiments in not less than 4–5 Petri dishes or flasks (6–7 explants). Mean values and the least significant differences (LSD 0.5) are presented in the tables.

Results and discussion. Al investigated genotypes were capable of inducing primary callus, which we had shown previously (Avksentyeva et al. 2008). Primary callus from mature germ was dense, less watery, yellowish, and characterized by some elements of differentiation that were confirmed microscopically (Fig. 1A, p. 216). The most effective growth *in vitro* was detected in *PPD B1a*, 93.12%, less effective was *PPD A1a*, 61.30% (Table 1). The speed of growth was determined by the increase of callus area for a

Table 1. Growth of primary calluses NILs for *PPD* genes of wheat cultivar Mironovskay 808.

Genotype	% of callus genesis	Callus growth rate mm^2/day
PPD D1a	73.30	0.74
PPD B1a	93.12	0.85
PPD A1a	61.30	0.56
PPD D1b PPD B1b PPD A1b	84.55	0.41
LSD 0.5	19.30	0.11

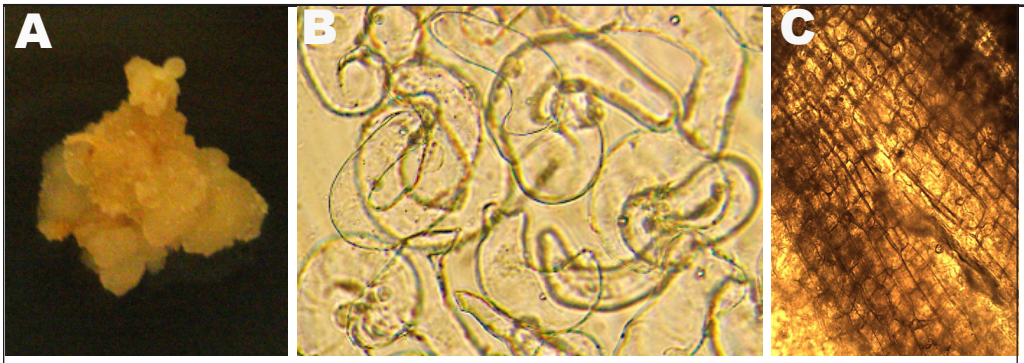

Fig. 1. Morpho-physiological characteristics of primary callus of wheat isogenic lines: A – callus general view, B – typical callus cells, and C – differentiation in callus tissue.

period of four weeks (mm^2/day). The maximum index for growth speed was detected in isoline *PPD B1a*, which also showed the maximum efficiency of callus genesis. Minimum values were found in Mironovskay 808.

Cytological investigations showed appreciable heterogeneity of callus tissues generated from mature germs. In addition to the typical callus cells of cereals, which are extended with round extremities, nontypical, bent cells, strongly vacuolated cells, meristematic cells, and large parenchymal cells were elements of differentiation (Fig. 1B and 1C).

The growth of primary callus tissues can occur either due to cell proliferation or to their growth by tension. Cytological results showed that among isolines, the minimum cell length and maximum quantity were detected in isoline *PPD B1a* (Table 2). Consequently, the growth of callus tissues of a given line seems to be defined only at the expense of their intensive division. Isoline *PPD D1a* had the opposite situation; a maximum cell size and a minimum number. We assume that the growth of primary callus in a given isoline is defined by the process of a vacuolization or growth by 'stretching'. In callus tissues of isoline PPD A1a, proliferation and vacuolization processes seems to be equal.

Table 2. Cytological characteristics of primary callus in NILs for *PPD* genes of the wheat cultivar Mironovskay 808.

Genotype	Cell length (μ)	Cell number (x 10^6/mg)
PPD D1a	167.1	2.0
PPD B1a	111.2	4.4
PPD A1a	113.9	1.9
PPD D1b PPD B1b PPD A1b	106.3	2.7
LSD 0.5	5.3	1.2

Biomass accumulation can be one indicator that characterizes the process of neoplasm in primary callus. Our results showed that the maximum biomass was detected in the primary callus of *PPD A1a* and the minimum in isoline *PPD B1a* (Table 3). Calculating callus tissue aqueousness showed that isoline *PPD B1a,* which accumulated the minimum biomass during the experiment also had the minimum aqueousness. The greatest aqueousness, 85.55%, was detected in isoline *PPD A1a*. Synthetic (metabolic) activity can aid in the maintenance of soluble protein in vegetative tissue. The fraction of soluble protein is mainly enzymes, which define metabolic activity. The maximum values were in isoline *PPD D1a* and *PPD A1*a callus. The callus tissue of these isolines was characterized by a minimum gain and maximum aqueousness. The genotype of a given isoline defines the fast transition from growth processes to develop the minimum gain and maximum synthetic activity.

Table 3. Morpho-physiological characteristics of primary callus from NILs for *PPD* genes of the wheat cultivar Mironovskay 808.

Genotype	Biomass (mg)		Aqueousness (%)	Protein (mg/g)
	crude	dry		
PPD D1a	45.75	6.50	84.38	5.70
PPD B1a	32.31	5.51	83.80	3.17
PPD A1a	47.02	7.25	85.55	8.84
PPD D1b PPD B1b PPD A1b	39.01	6.13	84.24	4.01
LSD0,5	6.30	0.80	0.35	2.20

Minimum maintenance of soluble protein fractions was shown for calluses of the isoline *PPD B1a*. Callus of this isoline were characterized by the minimum amount of crude and dry biomass accumulation, which also indicates a lower level of synthetic activity. Thus, our experiments showed that the genotype of an isoline determines photoperiodic sensitivity *in vivo* influences the callusogenesis processes and the characteristics of primary callus growth *in vitro*.

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ITEMS FROM THE UNITED STATES OF AMERICA

INDIANA

USDA–ARS AND PURDUE UNIVERSITY

Departments of Agronomy, Botany and Plant Pathology, Entomology, and the USDA–ARS Crop Production and Pest Control Research Unit at Purdue University, West Lafayette, IN 47907, USA.

C.E. Williams, S.E. Cambron, C. Crane, S.B. Goodwin, S. Scofield, B. Schemerhorn, R.H. Shukle (USDA–ARS); H.W. Ohm (Department of Agronomy); K. Wise (Department of Botany and Plant Pathology); and J. Stuart (Department of Entomology).

Wheat production. According to the USDA National Agricultural Statistics Service, harvested wheat acreage in Indiana in 2011 totaled 400,000 acres. Acreage seeded to wheat in fall of 2010 was 420,000 acres. Total production was estimated at 24.8 x 10^6 bushels, with an average yield at 59 bu/a. Winter survival of wheat during the winter of 2009–10 was excellent, but average temperatures from February to mid-April were significantly below normal and soil moisture was higher than normal due to frequent rainfall, resulting in delayed growth and development of wheat and limited uptake of nitrogen. Growth stage of wheat was 1 week later than normal at mid-April. However, from mid-April through June, temperatures were above normal and frequent rainfalls continued, so that wheat matured 1 week earlier than normal. Grain yields were below average, likely due to reduced plant development during the fall of 2009 and early spring of 2010.

Weather conditions were excellent for harvest of soybeans and corn in fall 2010, resulting in timely seeding of wheat and a return to typical acreage of wheat, estimated at 450,000 acres for the 2010–11 season. However, fall 2010 continued unusually dry throughout much of Indiana, especially southern Indiana; resulting in delayed and erratic emergence of wheat in some fields. Luckily there was good snow cover during cold weather periods resulting in little winterkill throughout Indiana, even given the late fall emergence and lack of wheat growth going into winter. Beginning in late January, the spring and summer through wheat harvest was unusually wet; and the unusually cool temperatures through April, together with the continually wet soil conditions resulted in loss of and limited uptake of nitrogen, causing

areas in fields to show signs of limited nitrogen and limited tillering and plant growth. Beginning mid- to late-April, temperatures were much higher than typical for that time of the season, and continued high into wheat harvest. Fortunately, rainfall was sufficient for wheat growth and development. Thus, by end of June, wheat maturity was on-schedule and wheat harvest was completed timely for Indiana. However, the unusually warm temperatures during May and June, cause wheat to mature sooner than normal, so test weight, and yield, was lower than typical.

Wheat disease summary. Fusarium head blight was present in most areas of the state, but severity of the disease varied widely. Other fungal diseases including glume blotch and leaf blotch were moderately severe, and more so in southern Indiana. Powdery mildew developed early in the season on susceptible varieties, but declined with onset of warm conditions. Leaf and stem rusts developed late and were not severe.

H.W. Ohm Laboratory.

New Cultivar. The new cultivar **INW1131** was performance tested as line 99751RA1-6-3-94 in multi-location tests in Indiana since 2007, and in tests in surrounding regions since 2009. INW1131 typically produces grain yield similar to or statistically not less than those of the current leading cultivars. INW1131 has acceptable pastry wheat milling and baking qualities, matures 2–3 days later than the early maturing cultivar Patterson, depending on latitude of the test location; has awnlets 1/16 to 5/16 inch long in the tip ½ of spikes, has yellow anthers, glumes are yellow at maturity, has strong straw that is typically 33 to 36 inches tall, and is moderately cold tolerant. An important contribution of INW1131 is it's effective resistance to Fusarium head blight (FHB), the same fungus that causes ear and stalk rot in corn, and that also produces the vomitoxin deoxynivalenol (DON). INW1131 has effective Type-I (reduced percentage of spikes that become infected) resistance, together with moderate Type-II (reduced spread of the disease within infected spikes) resistance to FHB; and DON content in the grain is consistently significantly less than that in susceptible cultivars. INW1131 has highly effective resistance to Hessian fly, and moderate resistance to Stagonospora glume blotch, Septoria leaf blotch, barley yellow dwarf virus, wheat spindle streak mosaic virus, and leaf- and stem rusts. Given its effective, but not complete, resistances to most of the important diseases, especially FHB, in Indiana and the eastern U.S. along with highly variable seasonal weather patterns, some being very favorable to disease organisms, wheat growers are strongly encouraged to monitor their wheat crop for presence and development of diseases, and apply fungicides when appropriate, to maximize crop performance and grain quality, particularly given the very low level of tolerance for DON in the food industry.

Breeding/Genetics: Combining multiple genes for resistance to foliar diseases, yellow dwarf, and Hessian fly in improved germ plasm and soft winter wheat cultivars adapted to Indiana.

Herb Ohm, Benjamin Campbell, Joshua Fitzgerald, Andy Linvill, Yanyan Liu, Samantha Shoaf, Jenae Skelton, Jin Sun, Shaylyn Wiarda and Xiangye Xiao.

Fusarium head blight. We are backcrossing the combination of *Bdv3* and *Qfhs.pur-7EL* on 7DL into elite winter wheat lines; *Qfhs.pur-7EL* is more distal than *Bdv3*. The combination of *Fhb1* and *Qfhs.pur-7EL* continues to be highly effective Type-II FHB resistance. We also are combining Type-I FHB resistance from combinations of three unrelated sources with the Type-II resistance to FHB.

Stem rust, yellow rust. We have identified and obtained germplasm lines that have resistance to stem rust race TTKS (Ug99) and yellow rust. In collaboration with USDA–ARS laboratory (Dr. Yue Jin) at St Paul, MN (Ug99) and at Purdue University for resistance to our local isolates of the causal fungal pathogens, we are mapping the resistance.

Marker-assisted selection (MAS). We have significantly expanded MAS as an integral part of the breeding program to combine a large number of desired QTL/genes for various important plant traits. MAS is a necessary technology to genotype parent lines for various desired traits and to plan parental combinations for efficiently combining a large number of desired plant traits.

Personnel. Ph.D. student Shaylyn Wiarda and M.S. students Melissa McDonald and Kirsten Thomas joined the Herb Ohm lab in 2011.

Publications.

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S. B. Goodwin laboratory.

Septoria tritici blotch: disease resistance in wheat and pathogen genomics.

Stephen Goodwin, Jessica Cavaletto, and Ian Thompson.

Disease resistance. Work on developing isogenic lines for STB resistance continued during 2011. The genes *Stb1–Stb8* are being backcrossed into the susceptible lines Taichung 29 and Apogee. Some erosion of resistance has been seen in the Taichung 29 crosses, which may indicate dominant toxin sensitivity genes rather than resistance and has slowed the progress. BCF_2 populations are being made to select homozygous resistant plants for successive crosses. Large recombinant-inbred populations segregating for the *Stb2* and *Stb3* genes are being made for fine-scale mapping. Recessiveness of the *Stb2* gene seen with an Indiana isolate of the pathogen has slowed progress. Ultimately these lines can be used to analyze the effects of each *Stb* gene in a common susceptible background and the progenies being developed can be used to validate previously published map locations.

Fungal genomics. The genome sequence of the Septoria tritici blotch pathogen *Mycosphaerella graminicola* was published during 2011. Comparative analyses with sequences from other fungal pathogens and non-pathogens identified many candidate genes that may be involved in pathogenicity. Functional analysis of specific genes identified through bioinformatics analyses of the *M. graminicola* genome sequence is being pursued by developing knock-out and over-expression mutants. A c-type cyclin gene from *M. graminicola* was involved in many cellular processes including secondary metabolite production and also affected pathogenicity. A homolog of the velvet gene was knocked out and had many effects on fungal growth and light signaling. It also affected the production of melanin, which has not been seen in other fungi, but surprisingly did not affect pathogenicity. Manuscripts describing both knockout mutants were published during 2011. Work to improve the efficiency of the transformation process is continuing and will be used on many additional genes that have been identified for functional analysis.

Personnel. No changes from last year.

Publications.

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R. H. Shukle Laboratory.

Hessian fly/wheat interactions: effects of Galanthus nivalis agglutinin on Hessian fly larvae.

Richard Shukle and Christie Williams (USDA–ARS), and Subhashree Subramanyam (Department of Agronomy, Purdue University).

We previously developed an *in planta* translocation feeding assay for Hessian fly larvae to screen antinutrient and toxic proteins as candidates for transgenic resistance. The most efficacious of the antinutrient proteins evaluated was *Galanthus nivalis* agglutinin (GNA) also known as snowdrop lectin. A property of GNA is its ability to cross the gut barrier in some insects and transport other toxic proteins into the hemocoel, resulting in toxic effects not displayed by either GNA or the toxin independently. Through a collaborative material transfer agreement, we have obtained a clone of GNA from John Gatehouse at Durham University as well as a chimeric construct expressing a fusion protein of GNA and an insect specific neurotoxin ButaIT. The GNA/ButaIT construct has been inserted into a vector for transformation of wheat and is currently being used to transform wheat to test the efficacy of the GNA/ButaIT fusion protein for transgenic resistance to Hessian fly and other major insect pests of wheat.

Cloning of genes for virulence in Hessian fly to R genes in wheat.

Brandi Schemerhorn and Richard Shukle (USDA–ARS), and Jeffrey Stuart (Department of Entomology, Purdue University).

In a collaborative interaction between Dr. Jeffrey Stuart and USDA–ARS scientists Brandi Schemerhorn and Rich Shukle genes controlling virulence in Hessian fly to R genes in wheat are being cloned. To date, genes for virulence to *H9*, *H13*, *H24*, and *Hdic* have been cloned. Using pheromone traps, samples of Hessian fly have been collected from fields across North Carolina, South Carolina, Georgia, and Alabama. Virulence to *H13* was assessed in the field collections using diagnostic PCR markers. Using this method, field populations can be monitored regularly to survey the efficacy of any R gene's ability to protect wheat by detecting the frequency of virulence in Hessian fly populations. With the identification of additional genes in Hessian fly for virulence to R genes in wheat, this quick and easy genotyping method can replace the current detection system which requires more time, effort, money, and flies.

Annotation of genes from Hessian fly.

Richard Shukle and Christie Williams (USDA–ARS), Jacob Shreve (Department of Entomology, Purdue University), and Subhashree Subramanyam (Department of Agronomy, Purdue University).

Using the Hessian fly genome sequence we are annotating genes essential in this insect's biology and interactions with wheat. Knowledge of such genes and gene networks will enhance our understanding of responses in Hessian fly such as its ability to elicit a systemic RNAi response and the feasibility of host induced gene silencing as a possible strategy for genetically engineered resistance in wheat.

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C. E. Williams laboratory.

Wheat resistance to Hessian fly.

Christie Williams, Jill Nemacheck, Shubha Subramanyam, Andrea Hargarten, and Jacob Shreve.

Hessian fly-induced changes in wheat surface permeability. Salivary secretions of neonate Hessian fly larvae initiate epidermal permeability in wheat plants that is detected by absorption of neutral red stain. This permeability allows larval elicitors to enter the plant where they can trigger plant processes leading to resistance or susceptibility. The rapid increase in cell permeability allows the delivery of either plant defense molecules as incompatible interactions proceed or nutrients for larval consumption during compatible interactions. Resistant plants remain permeable just long enough to deliver molecules that kill the larvae. In contrast, susceptible plants continue to increase in permeability until the entire crown of the plant becomes a nutrient sink capable of overwhelming any localized defense response. The three-dimensional spreading of permeability initiated by virulent larvae within a few hours of attack rescues genetically avirulent larvae that may be present and sustains larval growth until nutritive tissue is established.

Personnel. No changes from last year.

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KANSAS

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Internationalization of American science and its funding.

M.B. Kirkham.

Upon the 75th anniversary of the Soil Science Society of America in 2011, a session was held at its annual meeting to document how the field has changed over the years. I was asked to give the long-term perspective for soil physics. I surveyed soil-physics research published by the society over the past six years (2005–11) and compared it with a review done in 1961 upon the 25th anniversary of the society. The results are in press (Kirkham 2012).

I share a summary of the results, because they might be indicative of other areas of science. Of the 299 papers in my survey, 186 came from outside the U.S. (62% of the total). Twenty-nine countries were represented with the People's Republic of China having the most papers (27 papers). In the 1961 review, only five countries outside the U.S. were cited (The Netherlands, England, Australia, Belgium, and France). The results of the survey showed that soil-physics research has become heavily international.

Six percent of the papers were solely authored. About 14% of the papers had a woman as an author or co-author. Of the domestic (U.S.) papers, 13% (39 out of 299 papers) were published by federal laboratories (33 of these papers came from USDA laboratories) and 25% (74 out of 299 papers) were published by 35 university laboratories.

I surveyed the sources of funding for the papers. Of the non-U.S.A. papers, 27% gave no source of funding and the other 73% usually cited funding by the government of the corresponding author. Of the domestic papers, 47% cited no source of funding, and the other 53% usually cited multiple sources of funding for each paper. Of the 33 papers from USDA laboratories, 27 acknowledged no source of funding. The other six papers cited funds that were usually from the USDA. Of the 74 papers published by university laboratories, 26 acknowledged no source of funding. Funding for the other papers usually came from multiple sources. Twelve university papers cited support from State Agricultural Experiment Station (AES) funds, and only two of the 12 cited AES funds for sole support. This is in contrast to 1961 when essentially all research done at agricultural experiment stations was funded by the agricultural experiment station. The fact that in my survey only two out of the 74 papers published in the U.S. at universities acknowledged sole funding from a state AES shows the drop in federal support from agricultural experiment station funds for research.

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Ms. Kalaiyarasi Pidaran (kalai@ksu.edu) is continuing work toward the Master's degree and is currently writing her thesis.

Publications.

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KANSAS STATE UNIVERSITY
Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Department of Agronomy, and the USDA–ARS Hard Red Winter Wheat Genetic Research Unit, Throckmorton Plant Sciences Center, Manhattan, KS 66506-5501, USA.

Notice of release of KS13WGGRC60 (TA5657) stem rust-resistant wheat germ plasm.

B. Friebe, W. Liu (Laboratory of Cell and Chromosome Engineering, College of Life Sciences, Henan Agricultural University, Zhengzhou, Henan 450002, PR China), T. Danilova, D.L. Wilson, W.J. Raupp, J. Poland and R.L. Bowden (USDA–ARS Hard Winter Wheat Genetic Research Unit); A.K. Fritz (Department of Agronomy), M.N. Rouse (USDA–ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, USA), M.O. Pumphrey (Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA), and B.S. Gill.

The Agricultural Research Service, U.S. Department of Agriculture and the Kansas Agricultural Experiment Station announce the release of KS13WGGRC60 hard red winter wheat (*Triticum aestivum* L.) germ plasm with resistance to stem rust (*Sr44*) for breeding and experimental purposes.

KS13WGGRC60 is derived from the cross 'TA3061/TA3647' F_2, where TA3061 is a Chinese Spring wheat stock monosomic for chromosome 7D (CSM7D) and TA3647 is a disomic wheat–*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey chromosome addition line having *Th. intermedium* chromosome 7J#1 added to the wheat genome. KS13WGGRC60 has the short 7J#1S arm derived from *Th. intermedium* translocated to the long 7DL wheat arm in the form of a compensating, Robertsonian T7DL·7J#1S translocation. The 7J#1S arm in T7DL·7J#1S has the gene *Sr44* conferring resistance to stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn.) races TTKSK, TTSKT, and TTTSK. The compensating Robertsonian T7DL·7J#1S stock is cytogenetically stable and may be useful in wheat improvement.

Small quantities (3 grams) of seed of KS13WGGRC60 are available upon written request. We request that the appropriate source be given when this germ plasm contributes to research or development of new cultivars. Seed stocks are maintained by the Wheat Genetic and Genomic Resources Center, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506.

Publications.

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Liu W, Danilova TV, Jin Y, Rouse M, Friebe B, Gill BS, and Pumphrey MO. 2012. Development of a wheat-*Thinopyrum intermedium* Robertsonian translocation stock with *Sr44* resistance to stem rust (Ug99). PAG XX Abstract.
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MINNESOTA

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J.A. Kolmer, Y. Jin, M.N. Rouse, M.E. Hughes, and L.A. Wanschura.

Wheat rusts in the United States in 2011.

Wheat stem rust (*Puccinia graminis* f. sp. *tritici*). Wheat stem rust was first reported in mid-April in Texas and Louisiana. Extreme drought conditions in the southern and central plains limited stem rust development and inoculum production for areas further north. Generally, wheat stem rust was found at low levels in scattered plots and fields in the Great Plains, Ohio Valley, and Great Lakes regions in 2011. The exception was northeastern Wisconsin, where 1 to 40% severities were found in commercial soft red winter wheat fields located within 5 miles of Lake Michigan. Race QFCSC was the predominantly identified race from wheat, the only other race identified from wheat was race QCCDC from a collection made in a plot at Crowley, Louisiana (see wheat stem rust observation map, Fig. 1, p. 226-227).

Wheat stem rust was found in areas of Texas, Louisiana, Oklahoma, Kansas, Nebraska, North Dakota, Minnesota, Arkansas, Missouri, Kentucky, Illinois, Indiana, Wisconsin, and Michigan in 2011. Nationally, wheat only incurred a trace loss due to wheat stem rust (Table 3, p. 231, and Table 4, p. 232).

Texas. Wheat stem rust was first reported in southeastern Texas in McNair 701 plots on 15 April. By 18 April, stem rust had been found in McNair 701 plots at Castroville and Uvalde in south-central Texas and by 23 April, it was found in McNair 701 plots at McGregor in central Texas. Stem rust also was found on emmer, barley, and triticale used as windbreaks in watermelon fields in the Rio Grande Valley in southern Texas on 20–21 April. The infection was sparse on emmer and barley with severities from trace to 20%, whereas the triticale was highly susceptible with severities up to 80S. The persistent and widespread drought conditions limited the spread and development of stem rust in the state.

Louisiana. Trace amounts of wheat stem rust were found in plots of an unknown cultivar at Crowley in southern Louisiana on 22 April.

Oklahoma. Stem rust was found in a McNair 701 trap plot at Stillwater in north central Oklahoma the week of 9 May.

Arkansas. Stem rust was found late in the season in one plot at Keiser in northeastern Arkansas in mid-May.

Kansas. Low levels of stem rust (severity 1% or less, incidence 2%) were found on the susceptible cultivar Winterhawk in Barber County in south central Kansas on 25 May. Stem rust was found at trace to moderate levels in plots in Sumner, Labette, and Ellis counties in Kansas in early June. In mid-June, stem rust was found on Winterhawk at late milk stage in two locations in Republic County in north-central Kansas. The severities ranged from 1–20% on flag leaves with incidences in the 10–15% range. Wheat stem rust did not cause significant wheat yield loss in Kansas in 2011.

Missouri. Low levels of stem rust were found in a field in Chariton County in north-central Missouri in early June.

Nebraska. Stem rust was found on wheat and barley at the Havelock Farm in Lincoln in Lancaster County in southeastern Nebraska on 13 June.

Indiana. Stem rust was found at low levels in a plot in west-central Indiana on 6 June.

Illinois. Stem rust was found in plots in central and northern Illinois in late July.

Michigan. Light to moderate stem rust severities were observed in plots in two counties in south-central Michigan on the cultivar Jupiter and two nursery lines in late June and early July.

Minnesota. Stem rust was found at low levels in Panola and McNair 701 plots in southeastern Minnesota on 24 June. Low levels of stem rust were found scattered across southern Minnesota in late July.

Wisconsin. Stem rust was found in plots at Arlington in south-central Wisconsin and at Oconto in northeastern Wisconsin in late June; the infections at the latter site were not as full developed. By the time the plots reached maturity the fully susceptible cultivars (Ambassador, Envoy, and IL05-4236) were reading 100S. Stem rust also was observed on the check cultivar Pioneer 25R47, averaging 10–20 MR/MS throughout the nursery. This is the highest level of stem rust observed in the nursery in the last eight years. Significant levels of stem rust (1–40% severity) were found in commercial soft red winter wheat fields located within 5 miles of the Lake Michigan shoreline between Sturgeon Bay and Manitowoc in northeastern Wisconsin on 14 July. The crop was maturing rapidly and was harvested by the end of July.

North Dakota. Stem rust was found in some plots (overall uncommon in the nursery) at Williston in northwestern North Dakota on 14 July. Field scouts found no rust in the 137 commercial wheat fields scouted in North Dakota in early July. Race QCCJB and rye stem rust (*P. graminis* f. sp. *secalis*) were identified from collections on barley plots in Cass County.

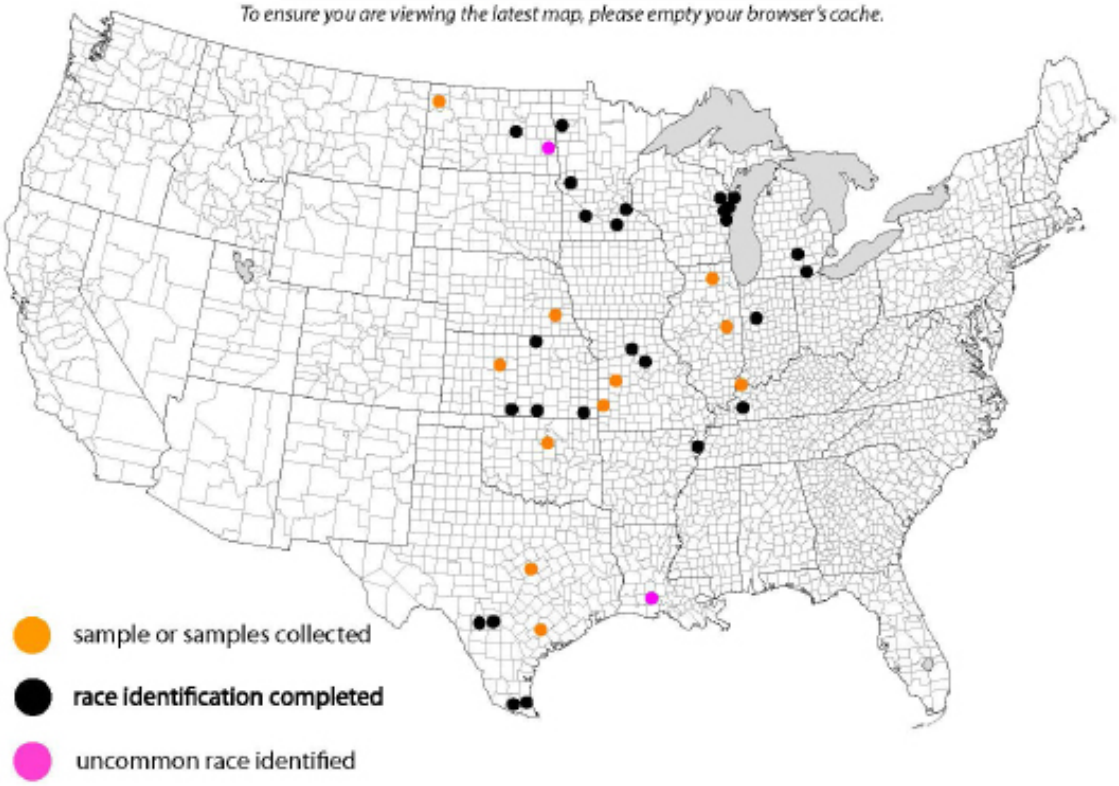
Wheat leaf rust (*Puccinia recondita*). Wheat leaf rust was first found in early March in commercial fields southwest of Houston, Texas, and in a nursery in north-central Oklahoma. Generally, leaf rust was widespread, but a low levels throughout plots and fields in the Great Plains and eastern U.S. in 2011. Extreme drought conditions in the southern and central Great Plains limited leaf rust development and inoculum production for areas further north. Additionally, widespread use of fungicide throughout commercial fields in the eastern states and northern spring wheat area further limited leaf rust development. Losses due to wheat leaf rust were minimal in the U.S. in 2011 (see Table 3, p. 231, and Table 4, p. 232).

Texas. Leaf rust was found on the cultivar Jackpot (*Lr39/41*) at high severity in commercial fields in two counties (Colorado and Jackson) southwest of Houston the first week of March. Fungicide was applied in this area and further south. Generally, trace to low levels of wheat leaf rust were found in plots in southeastern and south-central Texas in early March. However, wheat leaf rust was very active in irrigated plots at Yoakum in mid-April and could be found on flag leaves. By early April, leaf rust was increasing in irrigated plots at Castroville in south central Texas. Susceptible lines such as Jagger (*Lr17*), Jagalene (*Lr24*), and Bullet (*Lr39/41*) had 70S severity on 18 April. At Uvalde on 18 April, trace amounts of leaf rust were found in a few plots, but none higher than 10% severity. The plots were drying up due to lack of moisture. The persistent and widespread drought conditions limited the spread and development of leaf rust in the state.

Fig. 1. 2011 Wheat Stem Rust Observations in the U.S.

Prepared by USDA-ARS Cereal Disease Laboratory, St. Paul, MN

To ensure you are viewing the latest map, please empty your browser's cache.



Date*	Location	Field/Plot	Cultivar/line	Race(s) identified
4/7	Yoakum, Lavaca County, Texas	Plot	McNair 701	
4/18	Castroville, Medina County, Texas	Plot	McNair 701	QFCSC
4/18	Uvalde, Uvalde County, Texas	Plot	McNair 701, Wintex?	QFCSC
4/19	McAllen, Hidalgo County, Texas	Field	Unknown	QFCSC
4/20	McGregor, McLennan County, Texas	Plot	McNair 701	
4/21	Mission, Hidalgo County, Texas	Field	Unknown barley, Emmer	QFCSC
4/22	Lasara, Willacy County, Texas	Field	Unknown triticale	QFCSC
4/22	Crowley, Acadia Parish, Louisiana	Plot	Unknown	QFCSC, QCCDC
5/16	Stillwater, Payne County, Oklahoma	Plot	McNair 701	
5/17	Keiser, Mississippi County, Arkansas	Plot	Progeny PGX10	QFCSC
5/25	Isabel, Barber County, Kansas	Plot	Winterhawk	QFCSC
6/1	Parsons, Labette County, Kansas	Plot	Pioneer 25R40	QFCSC
6/1	Barton County, Missouri	Field	Unknown	
6/2	Anson, Sumner County, Kansas	Plot	Jagger	QFCSC
6/2	Ellis County, Kansas	Field	Unknown	
6/3	Posey County, Indiana	Field	Baker 200S, Pioneer 25R62	
6/4	Salisbury, Chariton County, Missouri	Field	Unknown	QFCSC
6/4	W. of Calhoun, Henry County, Missouri	Field	Unknown	
6/6	Battle Ground, Tippecanoe County, Indiana	Plot	Nursery line	QFCSC
6/6	Caldwell County, Kentucky	Plot	Unknown	QFCSC
6/8	Belleville, Republic County, Kansas	Field	Winterhawk	QFCSC
6/8	Cuba, Republic County, Kansas	Field	Winterhawk	QFCSC
6/13	Lincoln, Lancaster County, Nebraska	Plot	Unknown	
6/14	Columbia, Boone County, Missouri	Plot	Unknown	QFCSC
6/24	Rosemount, Dakota County, Minnesota	Plot	Panola, McNair 701, Pio 25R30	QFCSC

*if multipe observations at a site, earliest date listed.

Fig. 1 (continued). 2011 Wheat Stem Rust Observations in the U.S.

Prepared by USDA-ARS Cereal Disease Laboratory, St. Paul, MN

Date*	Location	Field/Plot	Cultivar/line	Race(s) identified
6/27	Oconto, Oconto County, Wisconsin	Plot	Pioneer 25R47, Envoy, line	QFCSC
6/27	Urbana, Champaign County, Illinois	Plot	Unknown	
6/28	DeKalb, DeKalb County, Illinois	Plot	Unknown	
6/29	East Lansing, Ingham County, Michigan	Plot	Jupiter, two nursery lines	QFCSC
7/3	Lenawee County, Michigan	Plot	Nursery line	QFCSC
7/7	Rosemount, Dakota County, Minnesota	Plot	McNair 701	QFCSC
714	Forestville, Door County, Wisconsin	Plot	Unknown	QFCSC
7/14	Two Creeks, Manitowoc County, Wisconsin	Field	Unknown	QFCSC
7/14	Francis Creek, Manitowoc County, Wisconsin	Field	Unknown	QFCSC
7/14	Cooperstown, Manitocowc County, Wisconsin	Field	Unknown	
7/14	Alaska, Kewaunee County, Wisconsin	Field	Unknown	QFCSC
7/14	Kewaunee, Kewaunee County, Wisconsin	Field	Unknown	QFCSC
7/14	New Franklin, Brown County, Wisconsin	Field	Unknown	QFCSC
7/15	Williston, Williams County, North Dakota	Plot	Unknown	
7/20	Morris, Stevens County, Minnesota	Plot	Baart	QFCSC
7/25	Lamberton, Redwood County, Minnesota	Plot	Unknown	QFCSC
7/26	Waseca, Waseca County, Minnesota	Plot	Unknown	QFCSC
8/2	Crookston, Polk County, Minnesota	Plot	Little Club	QFCSC
8/2	Carrington, Foster County, North Dakota	Plot	Baart	Rye stem rust
8/17	Fargo, Cass County, North Dakota	Plot	Land race, barley cvs	QFCSC, QCCJB, rye stem rust
8/17	Sheboygan, Sheboygan County, Wisconsin	Plot	Unknown	QFCSC

*If multipe observations at a site, earliest date listed.

Oklahoma. Trace levels of sporulating leaf rust pustules were noted in a strip of Jagalene (*Lr24*) in a nursery at Stillwater (north-central Oklahoma) in early March. By the end of March, the wheat leaf rust had increased only slightly. No rust samples were received at the Oklahoma State diagnostic lab by late March from western and southwestern Oklahoma where drought conditions were more severe. By mid-May, leaf rust increased to 65–80% severity in plots around Stillwater where there was more rain. However, leaf rust levels on susceptible cultivars were not consistent from field to field. In fields north and west of Stillwater, leaf rust was at low incidence. Despite rains in some areas, extremely dry to drought conditions persisted in much of the state, particularly the western half of the state that was classified as an exceptional drought area as of 24 May. Much like Texas, the persistent and widespread drought conditions limited the spread and development of leaf rust in the state.

Kansas. Trace amounts of overwintering leaf rust were found in plots near Manhattan (northeast Kansas) in mid-March. Low levels of wheat leaf rust were found in wheat at the jointing stage in southeastern Kansas and in the lower third of the canopy on known susceptible cultivars in Saline County in central Kansas in early April. By early May, low levels of leaf rust were found in central and south-central parts of the state. By late May, leaf rust was still at low levels in most areas in the state. Leaf rust increased slightly in plots and fields in north central Kansas in early June. The persistent dry conditions in many areas of the state limited the development of wheat leaf rust in the state.

Nebraska. Leaf rust was found in plots at Lincoln in Lancaster County in southeastern Nebraska on 3 June. Low levels of leaf rust were found in most fields surveyed in the southern tier of counties on 10 June.

Minnesota. Trace levels of leaf rust were found in plots at Rosemount in southeastern Minnesota on 26 May. By 24 June, leaf rust was heavy on flag leaves of susceptible cultivars and at lower severity in resistant cultivars in the plots. Low levels of leaf rust were found on the cultivar Marshall (*Lr2a*, *Lr10*, and *Lr34*) in plots in central and northwestern Minnesota in mid-July. The cultivars Faller and Prosper, which likely have *Lr21*, had low to moderate levels of leaf rust, whereas other cultivars with *Lr21* had no leaf rust. Low levels of leaf rust were found across southern–northern Minnesota in late July. Generally, leaf rust was at low levels in the northern hard red spring wheat region in 2011.

North Dakota. Trace amounts of leaf rust were found in a plot of Alsen spring wheat (*Lr2a*, *Lr10*, *Lr23*, and *Lr34*) at Fargo, in east-central North Dakota on 30 June. Trace amounts of leaf rust were found on Darrell winter wheat at Jamestown in central North Dakota on 30 June. These initial reports of leaf rust were about three weeks later than normal. In the second week of July, leaf rust was common in plots at Williston in northwestern North Dakota, but could not be

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2011 identified by virulence to 19 lines of wheat with single genes for leaf rust resistance. Lines tested were Thatcher lines with genes *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21, Lr28,* and winter wheat lines with gene *Lr41*.

Pheno-type	Virulences	AR, DE, GA, LA, MD, MS, NC, VA		NY		IL, IN, eastern MO, WI		OK, TX		IA, KS, western MO		MN, ND, SD		Total	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%
BBBDG	14a,28	1	1.1	0	0.0	0	0.0	0	0.0	3	3.8	2	1.5	6	1.4
LBBTG	1,B,10,14a,18,28	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MBBJG	1,3,10,14a,28	0	0.0	0	0.0	1	2.5	0	0.0	0	0.0	1	0.7	2	0.5
MBDSD	1,3,17,B,10,14a,39/41	1	1.1	0	0.0	1	2.5	1	1.4	3	3.8	2	1.5	8	1.8
MBJJG	1,3,11,17,10,14a,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
MBPNB	1,3,3ka,17,30,B,14a	0	0.0	0	0.0	1	2.5	0	0.0	0	0.0	0	0.0	1	0.2
MBPSB	1,3,3ka,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	0	0.0	2	2.6	1	0.7	3	0.7
MBTNB	1,3,3ka,11,17,30,B,14a	5	5.4	1	4.8	4	10.0	0	0.0	0	0.0	4	2.9	14	3.2
MBTSB	1,3,3ka,11,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
MCDSB	1,3,26,17,B,10,14a	0	0.0	0	0.0	2	5.0	0	0.0	1	1.3	2	1.5	5	1.1
MCDSD	1,3,26,17,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
MCGJG	1,3,26,11,10,14a,28	0	0.0	3	14.3	0	0.0	0	0.0	0	0.0	0	0.0	3	0.7
MCJSB	1,3,26,11,17,B,10,14a	0	0.0	0	0.0	2	5.0	0	0.0	0	0.0	0	0.0	2	0.5
MCLRG	1,3,26,3ka,B,10,18,28	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MCPSB	1,3,26,3ka,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
MCPSD	1,3,26,3ka,17,30,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	0	0.0	2	2.6	0	0.0	2	0.5
MCPTB	1,3,26,3ka,17,30,B,10,14a,18	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
MCRGG	1,3,26,3ka,11,30,10,28	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MCRKG	1,3,26,3ka,11,30,10,14a,18,28	1	1.1	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	2	0.5
MCTNB	1,3,26,3ka,11,17,30,B,14a	10	10.8	3	14.3	6	15.0	0	0.0	1	1.3	0	0.0	20	4.5
MCTQB	1,3,26,3ka,11,17,30,B,10	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MCTSB	1,3,26,3ka,11,17,30,B,10,14a	2	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
MDDSB	1,3,24,17,B,10,14a	0	0.0	0	0.0	0	0.0	2	2.8	0	0.0	0	0.0	2	0.5
MDPSB	1,3,24,3ka,17,30,B,10,14a	1	1.1	0	0.0	0	0.0	0	0.0	3	3.8	2	1.5	6	1.4
MDTSB	1,3,24,3ka,11,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
MFDSB	1,3,24,26,17,B,10,14a	2	2.2	0	0.0	0	0.0	10	13.9	3	3.8	6	4.4	21	4.8
MFGJG	1,3,24,26,11,10,14a,28	2	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
MFNSB	1,3,24,26,3ka,17,B,10,14a	4	4.3	0	0.0	0	0.0	2	2.8	0	0.0	7	5.1	13	3.0
MFNSL	1,3,24,26,3ka,17,B,10,14a,21	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
MFPSB	1,3,24,26,3ka,17,30,B,10,14a	5	5.4	0	0.0	0	0.0	0	0.0	1	1.3	4	2.9	10	2.3
MFQSB	1,3,24,26,3ka,11,B,10,14a	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
MFRJG	1,3,24,26,3ka,11,30,10,14a,28	5	5.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	1.1
MFTSB	1,3,24,26,3ka,11,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	2	2.8	0	0.0	1	0.7	3	0.7
MGDSD	1,3,16,17,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
MKDSB	1,3,16,24,26,17,B,10,14a	1	1.1	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	2	0.5
MLDSD	1,3,9,17,B,10,14a,39/41	10	10.8	3	14.3	0	0.0	8	11.1	2	2.6	10	7.4	33	7.5
MLNSD	1,3,9,3ka,17,B,10,14a,39/41	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MLPSD	1,3,9,3ka,17,30,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
MMDSD	1,3,9,26,17,B,10,14a,39/41	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
MMNSD	1,3,9,26,3ka,17,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
MMPSD	1,3,9,26,3ka,17,30,B,10,14a,39/41	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
NBBRG	1,2c,B,10,18,28	3	3.2	1	4.8	0	0.0	2	2.8	1	1.3	2	1.5	9	2.0
NBBSG	1,2c,B,10,14a,28	0	0.0	2	9.5	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
NBBTG	1,2c,B,10,14a,18,28	0	0.0	2	9.5	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
SBBGG	1,2a,2c,10,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBBBG	1,2a,2c,3,28	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
TBBBJ	1,2a,2c,3,28,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
TBBDJ	1,2a,2c,3,14a,28,39/41	0	0.0	0	0.0	1	2.5	0	0.0	0	0.0	0	0.0	1	0.2
TBBGD	1,2a,2c,3,10,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
TBBGG	1,2a,2c,3,10,28	0	0.0	0	0.0	0	0.0	0	0.0	3	3.8	1	0.7	4	0.9

Table 1. Number and frequency (%) of virulence phenotypes of *Puccinia triticina* in the United States in 2011 identified by virulence to 19 lines of wheat with single genes for leaf rust resistance. Lines tested were Thatcher lines with genes *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21, Lr28,* and winter wheat lines with gene *Lr41*.

Pheno-type	Virulences	AR, DE, GA, LA, MD, MS, NC, VA		NY		IL, IN, eastern MO, WI		OK, TX		IA, KS, western MO		MN, ND, SD		Total	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%
TBBGJ	1,2a,2c,3,10,28,39/41	0	0.0	0	0.0	3	7.5	12	16.7	16	20.5	20	14.7	51	11.6
TBBJG	1,2a,2c,3,10,14a,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBBQJ	1,2a,2c,3,B,10,28,39/41	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBGJG	1,2a,2c,3,11,10,14a,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBHKG	1,2a,2c,3,11,30,10,14a,18,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBJJG	1,2a,2c,3,11,17,10,14a,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	2	0.5
TBMKG	1,2a,2c,3,3ka,30,10,14a,18,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TBPSB	1,2a,2c,3,3ka,17,30,B,10,14a	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
TBRKG	1,2a,2c,3,3ka,11,30,10,14a,18,28	2	2.2	1	4.8	6	15.0	0	0.0	1	1.3	0	0.0	10	2.3
TCBGJ	1,2a,2c,3,26,10,28,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	2	1.5	3	0.7
TCBJG	1,2a,2c,3,26,10,14a,28	5	5.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	1.1
TCGJG	1,2a,2c,3,26,11,10,14a,28	0	0.0	1	4.8	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TCJSB	1,2a,2c,3,26,11,17,B,10,14a	2	2.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	3	0.7
TCJSG	1,2a,2c,3,26,11,17,B,10,14a,28	2	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
TCRKG	1,2a,2c,3,26,3ka,11,30,10,14a, 18,28	10	10.8	1	4.8	8	20.0	1	1.4	5	6.4	4	2.9	29	6.6
TCTBG	1,2a,2c,3,26,3ka,11,17,30,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TCTSB	1,2a,2c,3,26,3ka,11,17,30,B, 10,14a	0	0.0	0	0.0	3	7.5	0	0.0	0	0.0	0	0.0	3	0.7
TDBGG	1,2a,2c,3,24,10,28	0	0.0	0	0.0	0	0.0	1	1.4	5	6.4	13	9.6	19	4.3
TDBGJ	1,2a,2c,3,24,10,28,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
TDBGQ	1,2a,2c,3,24,10,21,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	15	11.0	15	3.4
TDBJG	1,2a,2c,3,24,10,14a,28	1	1.1	0	0.0	2	5.0	0	0.0	3	3.8	4	2.9	10	2.3
TDBJQ	1,2a,2c,3,24,10,14a,21,28	0	0.0	0	0.0	0	0.0	3	4.2	0	0.0	1	0.7	4	0.9
TDDJG	1,2a,2c,3,24,17,10,14a,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
TFBGG	1,2a,2c,3,24,26,10,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.5	2	0.5
TFBGJ	1,2a,2c,3,24,26,10,28,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	1	0.7	2	0.5
TFBGQ	1,2a,2c,3,24,26,10,21,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	5	3.7	6	1.4
TFBJG	1,2a,2c,3,24,26,10,14a,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TFBJQ	1,2a,2c,3,24,26,10,14a,21,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	2	0.5
TFBKG	1,2a,2c,3,24,26,10,14a,18,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
TFJGG	1,2a,2c,3,24,26,11,17,10,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
TFLJJ	1,2a,2c,3,24,26,3ka,10,14a,28, 39/41	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	1	0.2
TFPSB	1,2a,2c,3,24,26,3ka,17,30,B, 10,14a	2	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.5
TFRJG	1,2a,2c,3,24,26,3ka,11,30,10, 14a,28	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
TGBJG	1,2a,2c,3,16,10,14a,28	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	0	0.0	1	0.2
THBJG	1,2a,2c,3,16,26,10,14a,28	1	1.1	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	2	0.5
TJBGQ	1,2a,2c,3,16,24,10,21,28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.5	2	0.5
TLBJJ	1,2a,2c,3,9,10,14a,28,39/41	0	0.0	0	0.0	0	0.0	0	0.0	1	1.3	1	0.7	2	0.5
TNBGJ	1,2a,2c,3,9,24,10,28,39/41	0	0.0	0	0.0	0	0.0	8	11.1	12	15.4	6	4.4	26	5.9
TNBJJ	1,2a,2c,3,9,24,10,14a,28,39/41	3	3.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.7	4	0.9
TNGFJ	1,2a,2c,3,9,24,11,14a,18,28,39/41	0	0.0	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0	1	0.2
TNRJJ	1,2a,2c,3,9,24,3ka,11,30,10,14a, 28,39/41	3	3.2	0	0.0	0	0.0	3	4.2	0	0.0	0	0.0	6	1.4
TPBGJ	1,2a,2c,3,9,24,26,10,28,39/41	0	0.0	0	0.0	0	0.0	2	2.8	1	1.3	1	0.7	4	0.9
Total		93		21		40		72		78		136		440	

Table 2. Number and frequency (%) of isolates of *Puccinia triticina* in the United States in 2011 virulent to 19 lines of wheat with single resistance genes for leaf rust resistance.

Resistance gene	AR, DE, GA, LA, MD, MS, NC, VA		NY		IL, IN, MO, WI		OK, TX		IA, KS, NO		MN, ND, SD		Total	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Lr1	92	98.9	21	100.0	40	100.0	72	100.0	75	96.2	134	98.5	434	98.6
Lr2a	37	39.8	3	14.3	23	57.5	38	52.8	55	70.5	86	63.2	242	55.0
Lr2c	40	43.0	8	38.1	23	57.5	40	55.6	56	71.8	88	64.7	255	58.0
Lr3	89	95.7	15	71.4	40	100.0	70	97.2	73	93.6	132	97.1	419	95.2
Lr9	18	19.4	3	14.3	0	0	23	31.9	16	20.5	21	15.4	81	18.4
Lr16	2	2.2	0	0	0	0	3	4.2	1	1.3	2	1.5	8	1.8
Lr24	32	34.4	0	0	2	5.0	39	54.2	29	37.2	77	56.6	179	40.7
Lr26	60	64.5	10	47.6	21	52.5	24	33.3	15	19.2	45	33.1	175	39.8
Lr3ka	55	59.1	8	38.1	28	70.0	12	16.7	16	20.5	30	22.1	149	33.9
Lr11	48	51.6	11	52.4	29	72.5	10	13.9	10	12.8	13	9.6	121	27.5
Lr17	52	55.9	7	33.3	19	47.5	30	41.7	20	25.6	49	36.0	177	40.2
Lr30	50	53.8	7	33.3	28	70.0	9	12.5	17	21.8	20	14.7	131	29.8
LrB	53	57.0	14	66.7	19	47.5	33	45.8	21	26.9	48	35.3	188	42.7
Lr10	76	81.7	17	81.0	28	70.0	69	95.8	74	94.9	130	95.6	394	89.5
Lr14a	88	94.6	18	85.7	37	92.5	41	56.9	37	47.4	65	47.8	286	65.0
Lr18	17	18.3	7	33.3	14	35.0	5	6.9	8	10.3	8	5.9	59	13.4
Lr21	1	1.1	0	0	0	0	3	4.2	1	1.3	25	18.4	30	6.8
Lr28	45	48.4	14	66.7	21	52.5	40	55.6	59	75.6	90	66.2	269	61.1
Lr39/41	19	20.4	3	14.3	5	12.5	43	59.7	38	48.7	47	34.6	155	35.2
Total	93		21		40		72		78		136		440	

found in fields in the Dickinson area in the southwestern part of the state. Field scouts found no rust in the 137 commercial wheat fields scouted in the state the second week of July. On 28 July, high levels of leaf rust (20–30S) were found in plots of the cultivars Faller (*Lr21*) and Prosper (*Lr21*) at Carrington in east-central North Dakota. Until the summer of 2010, these cultivars were resistant to leaf rust since, because carry the gene *Lr21*. However, RB07, which also carries *Lr21*, had lower leaf rust severity. In plots at Fargo, both Faller and the Thatcher line with *Lr21* had susceptible leaf rust reactions. This follows the identification of new races of *P. triticina* in 2011 carrying *Lr21* virulence in the North Dakota and Minnesota.

Montana. Leaf rust was present in the Yellowstone Valley by mid-June. Low levels of leaf rust were found on the cultivar Yellowstone near Manhattan in the Gallatin Valley in southwestern Montana on 23 June. Leaf rust reports had come in from throughout the state by late June. On 12 July, leaf rust (100% incidence, 5% severity on flag leaves) was found in winter wheat fields south of Malta in north-central Montana. Generally, leaf rust was at low levels throughout the state in 2011.

Louisiana. Leaf rust was actively increasing, but at relatively low levels in plots throughout the state on 25 March. There were no reports of rust issues in commercial fields by late March. Leaf rust was still very active in plots of susceptible wheat at Baton Rouge and Winnsboro (northeastern Louisiana) in mid-April. Generally, wheat leaf rust arrived late in the state and caused little damage to commercial fields that matured earlier than normal.

Mississippi. Low levels of wheat leaf rust were found in edges of a Croplan 8868 field (near boot stage) in northwestern Mississippi in late March. On 15 April, low levels of leaf rust were found in a field of Pioneer 26R22 (*Lr11*) in Winston County in east central Mississippi. Generally, low levels of leaf rust were found in most wheat producing areas in the state in 2011.

Alabama. Low levels of leaf rust were observed in plots in southern and central Alabama in early May. No rust was found in the northeastern part of the state at that time.

Georgia. Wheat leaf rust was found in early planted plots and had spread to susceptible plots in Plains (west-central Georgia) by early March. Low levels of leaf rust were found in susceptible plots by mid-April. No leaf rust was found in surveys of several commercial fields in south central Georgia. Traces of leaf rust were observed in a field in Lee County

Table 3. Estimated losses in winter wheat due to rust in 2011 (T = trace, less than 1% loss statewide). Stripe rust disease pressure was great in the Pacific Northwest (even greater than in 2010), however, the widespread use of fungicides greatly mitigated potential yield losses in this area. — No reports or surveys conducted in the state by CDL staff. Total does not include states for which loss or production data is not available.

State	1,000 acres harvested	Yields in bushels per acre	Production in 1,000 of bushels	Losses due to:					
				Stem rust		Leaf rust		Stripe rust	
				%	1,000 bu	%	1,000 bu	%	1,000 bu
AL	195	73.0	14,235	0.0	0	T	T	T	T
AZ	6	70.0	420	—	—	—	—	—	—
AR	520	58.0	30,160	0.0	0	T	T	T	T
CA	420	85.0	35,700	0.0	0	T	T	6.0	2,279
CO	2,000	39.0	78,000	T	T	T	T	T	T
DE	75	69.0	5,175	0.0	0	T	T	0.0	0
FL	8	45.0	360	0.0	0	0.0	0	0.0	0
GA	200	55.0	11,000	0.0	0	T	T	T	T
ID	770	82.0	63,140	0.0	0	0.0	0	7.0	4,752
IL	765	61.0	46,665	0.0	0	T	T	0.0	0
IN	400	62.0	24,800	T	T	1.0	251	T	T
IA	16	45.0	720	0.0	0	T	T	0.0	0
KS	7,900	35.0	276,500	T	T	T	T	T	T
KY	440	70.0	30,800	0.0	0	T	T	0.0	0
LA	235	63.0	14,805	T	T	T	T	T	T
MD	190	66.0	12,540	0.0	0	T	T	T	T
MI	680	75.0	51,000	T	T	T	T	T	T
MN	26	56.0	1,456	T	T	T	T	T	T
MS	335	64.0	21,440	0.0	0	0.0	0	T	T
MO	680	50.0	34,000	0.0	0	2.0	694	0.0	0
MT	2,190	41.0	89,790	0.0	0	0.0	0	10.0	9,977
NE	1,450	45.0	65,250	0.0	0	T	T	T	T
NV	9	115.0	1,035	—	—	—	—	—	—
NJ	31	49.0	1,519	—	—	—	—	—	—
NM	95	22.0	2,090	—	—	—	—	—	—
NY	93	56.0	5,208	0.0	0	T	T	0.0	0
NC	610	68.0	41,480	T	T	T	T	0.0	0
ND	375	37.0	13,875	0.0	0	1.0	140	1.0	140
OH	850	58.0	49,300	--	—	—	—	—	—
OK	3,200	22.0	70,400	0.0	0	1.0	711	0.0	0
OR	825	77.0	63,525	0.0	0	T	T	7.0	4,781
PA	170	51.0	8,670	—	—	—	—	—	—
SC	180	60.0	10,800	T	T	1.0	109	0.0	0
SD	1,590	42.0	66,780	0.0	0	0.0	0	0.0	0
TN	310	69.0	21,390	—	—	—	—	—	—
TX	1,900	26.0	49,400	0.0	0	1.0	499	0.0	0
UT	124	50.0	6,200	0.0	0	0.0	0	T	T
VA	250	71.0	17,750	0.0	0	1.0	179	T	T
WA	1,730	75.0	129,750	0.0	0	0.0	0	3.5	4,706
WV	6	59.0	354	—	—	—	—	—	—
WI	335	65.0	21,775	T	T	T	T	0.0	0
WY	130	34.0	4,420.0	—	—	—	—	—	—
Total above	32,314	46.2	1,493,677		0		2,583		26,635
U.S. % loss				T		0.2		1.8	
U.S. total	32,314	46.2	1,493,677		T		2,583		26,635

Table 4. Estimated losses in spring and durum wheat due to rust in 2011 (T = trace, — = states for which loss or production data is not available).

SPRING WHEAT									
State	1,000 acres harvested	Yields in bushels per acre	Production in 1,000 of bushels	Losses due to:					
				Stem rust		Leaf rust		Stripe rust	
				%	1,000 bu	%	1,000 bu	%	1,000 bu
CA	—	—	—	0.0	0	T	—	1.0	—
CO	44	87.0	3,828	T	T	T	T	T	T
ID	620	84.0	52,080	0.0	0	0.0	0	4.0	2,170
MN	1,500	46.0	69,000	T	T	T	T	T	T
MT	2,400	32.0	76,800	0.0	0	T	T	5.0	4,042
NV	3	90.0	270	—	—	—	—	—	—
ND	5,500	31.5	173,250	0.0	0	0.0	0	0.0	0
NY	—	—	—	0.0	0	T	—	0.0	0
OR	157	70.0	10,990	0.0	0	T	T	7.0	827
SD	20	46.0	920	0.0	0	0.0	0	0.0	0
UT	1,220	31.0	37,820	—	—	—	—	—	—
TX	—	—	—	0.0	0	1.0	—	0.0	—
WA	615	61.0	37,515	0.0	0	0.0	0	2.5	962
Total above	12,079	38.3	462,473		0		T		8,001
U.S. % loss				T		T		1.7	
U.S. total	12,079	38.3	462,473		0		T		8,001
DURUM WHEAT									
State	1,000 acres harvested	Yields in bushels per acre	Production in 1,000 of bushels	Losses due to:					
				Stem rust		Leaf rust		Stripe rust	
				%	1,000 bu	%	1,000 bu	%	1,000 bu
AZ	79	101.0	7,979	—	—	—	—	—	—
CA	115	109.0	12,535	0.0	0	0.0	0	T	T
ID	11	69.0	759	0.0	0	0.0	0	0.0	0
MT	390	30.0	11,700	0.0	0	0.0	0	5.0	616
ND	720	26.0	18,720	0.0	0	0.0	0	0.0	0
OR	—	—	—	0.0	0	T	T	0.0	0
SD	7	28.0	196	0.0	0	0.0	0	0.0	0
Total above	1,322	39.3	51,889		0		0		616
U.S. % loss				0.0		T		1.2	
U.S. total	1,322	39.3	51,889		0		0		616

in southwestern Georgia in late April. Otherwise, no rust was found in commercial fields in several counties surveyed in the southwestern part of the state by late April.

South Carolina. Leaf rust was found in the susceptible plots of USG 3209 (*Lr26*, +) and Panola (*Lr11*) in Barnwell County in the southern coastal plain on 22 April.

North Carolina. Wheat leaf rust was very light or absent in commercial fields in late May in eastern North Carolina due to widespread fungicide use. Leaf rust was heavy in susceptible plots in eastern North Carolina on 20 May, indicating inoculum was present. Generally, leaf rust was at low levels throughout the state in 2011. However, a late, moderate leaf rust epidemic occurred in Robeson County in south-central North Carolina.

Virginia. Traces of wheat leaf rust were found in plots at Warsaw in eastern Virginia on 14 April. Wheat leaf rust was very light or absent in commercial fields in eastern Virginia due to widespread fungicide use. Leaf rust was heavy in susceptible plots in eastern Virginia on 20 May, indicating inoculum was present.

Delaware, Maryland. Low levels of leaf rust were found on lower leaves in a plot in southern Delaware on 9 May and on the eastern shore areas of Delaware and Maryland on 20 May.

Arkansas. Heavy amounts of leaf rust were found in a 120-acre field of Jackpot grown for seed in central Arkansas in mid-March. This was the only known leaf rust in the state by 22 March. Trace levels of leaf rust were found in plots at Kibler in northwestern Arkansas in early May and by 18 May, severities up to 70% were observed on flag leaves in the plots (late soft dough stage). Trace amounts of leaf rust were found on some cultivars (no leaf rust found on most cultivars) in northeastern Arkansas in mid-May. In late May, trace amounts of leaf rust were found in plots at Fayetteville in northwestern Arkansas. Generally, leaf rust was at low levels in the state in 2011.

Missouri. Low levels of leaf rust were found in fields throughout much of the state in early June.

Iowa. Low levels of leaf rust were found in a field at mealy ripe stage in Wayne County in south-central Iowa on 4 June.

Kentucky. In early June, leaf rust was widespread on susceptible cultivars (at late grain fill) not treated with fungicide. However, the rust arrived too late to cause any significant yield loss.

Illinois. Low levels of leaf rust were found in a soft red winter wheat nursery (at soft dough stage) in Pope County in southeastern Illinois on 1 June.

Indiana. Leaf rust was found throughout the state and was moderately severe on some cultivars in early June.

Wisconsin. Leaf rust was found at trace levels in fields in Dodge and Jefferson Counties in southeastern Wisconsin in early June. Plots of unsprayed soft red winter wheat at the University of Wisconsin Experiment Station at Sturgeon Bay in northeastern Wisconsin had leaf rust severities 0 to 40% on 8 July. Unsprayed soft red winter wheat fields (soft dough growth stage) located within 5 miles of the Lake Michigan shoreline from Sturgeon Bay to Manitowoc, had leaf rust severities of 40% on 14 July.

New York. Leaf rust from a suspected overwintering site was found in Cayuga County in north central New York on 2 June. Low incidences of leaf rust were found across central, western, and southern New York by 20 June. Most fields had infections on the upper leaves, but a small number of fields with more severe rust had infections all over the plants typical of overwintering local infections. In western New York, a field of the cultivar Richland was nearly killed by leaf rust and an adjacent field of the cultivar Caledonia had green leaf tissue remaining only on the flag leaves. Low levels of wheat leaf rust were found on various soft red winter wheat lines in mowed alleyways in the nursery at Aurora in central New York in mid-July. Very little rust was found in the plots.

Washington. A few leaves with wheat leaf rust pustules were found in a field in south-central Washington in mid-March. A single wheat leaf rust pustule was found on the cultivar Farnum in a field in southeastern Washington on 15 April. In 2010, leaf rust was severe on Farnum in the same county.

California. Leaf rust was moderately severe in Dirkwin plots at Colusa in the Sacramento Valley in early April and by late April it was severe in Dirkwin and Mika plots. Leaf rust was found in plots, particularly on the hard white spring cultivar Blanca Grande and some advanced breeding lines, at the UC Davis Agronomy Farm in the Sacramento Valley on 23 May. Leaf rust was observed in nurseries in California's Central Valley where normal crop maturity was delayed by cooler and wetter than normal weather.

Ontario, Canada. In early July plots in southwestern Ontario (Windsor to north of London), had leaf rust incidence of 10% and trace to 40% severities on flag leaves. The late development of leaf rust was expected to have limited impact on yield.

The number and frequency of virulence phenotypes of *P. triticina* found in 2011 in the U.S. can be found in Table 1 (pp. 228-229) and Table 2 (p. 230).

The 2011 wheat leaf rust observation map can be found at http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrustbulletins/2011wlr.pdf.

Lr gene postulations of current soft red winter, hard red winter, and hard red spring wheat cultivars are available in a searchable database at http://160.94.131.160/fmi/iwp/cgi?-db=Lr%20gene%20postulations&-loadframes.

Wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*). Generally, wheat stripe rust was widespread and severe in the Pacific Northwest, Sacramento Valley in California, and Montana in 2011. Stripe rust was active very early in the Pacific Northwest, e.g. mid-February in Washington and Oregon. Very little stripe rust was found in Texas and Oklahoma, whereas it was at low levels in Kansas and Nebraska except for scattered hot spots in some fields. Stripe rust was mostly at low levels and widely scattered in the eastern U.S. Significant wheat yield losses due to stripe rust occurred in the Pacific Northwest, California, and Montana (see Tables 3 and 4).

Texas. Stripe rust was found in south central Texas plots on 8 March, but by mid-April development and spread had ended.

Kansas. Low levels of stripe were found in many locations in the state by late May. By early June, stripe rust could be found in most locations in the state and the levels had increased slightly. However, a few hot spots were found in fields in Ellis (central Kansas) and Smith County (north-central Kansas). Susceptible cultivars in an irrigated nursery in Hays had nearly 100% incidence with severities in the range of 5 to 70%. The cultivars with severe disease depended on *Yr17* for resistance. Moderate levels of stripe rust (90% incidence, 10–50% severity) were found in a field in Smith County in north-central Kansas in early June. By mid- June, stripe rust was inactive in north-central Kansas. Dry conditions in many areas of the state limited stripe rust development in 2011.

Nebraska. A small focus of stripe rust (trace to 35% severity) was found in a commercial field (Feekes 10.5.1) in Polk County in southeastern Nebraska on 30 May. Severe levels of stripe rust were found scattered throughout a field in Adams County in south-central Nebraska on 10 June.

Colorado. Very low levels of stripe rust were found in plots of susceptible cultivars at Fort Collins in north-central Colorado on 13 June.

Louisiana. Stripe rust was found at very low levels in very susceptible plots at Winnsboro (northeastern Louisiana) on 3 March. By late March the stripe rust was very active in the plots. Some lines and several commercial cultivars heavily infected in 2010 were clean in 2011, whereas some lines and cultivars that were clean in 2010 were susceptible in 2011 suggesting perhaps a population change in the area. By mid-May, stripe rust had developed around the state, but was not a significant problem for growers.

Mississippi. Stripe rust hot spots were detected in a commercial field of Croplan 8868 (near boot stage) in northwestern Mississippi in late March. Much lower levels were detected in a field of Dixie 427 two miles away.

Arkansas. Stripe rust was the most prevalent wheat disease in the state in mid-April, but levels were low due a combination of dry, warm weather, effective resistance in many cultivars and fungicide use. It appears most stripe rust development was restricted to the fields where it overwintered. Rains and cool evenings in late April were favorable for stripe rust development, but development generally slowed by late May. There were, however, some areas where spore production persisted in late May.

Missouri. Traces of stripe rust were found in many areas of the state in early June.

Kentucky. Low levels of stripe rust were detected in a commercial field of Pioneer 25R35 (Feeke's 6 growth stage) in southwestern Kentucky in late March.

Illinois. Low levels of stripe rust were found in plots in east central Illinois on 13 May. Stripe rust was found at very low incidence in plots (near soft dough stage) in Pope County in southeastern Illinois on 1 June.

Indiana. Stripe rust was found in a southern Indiana field (Feekes 10.5.3 to 10.5.4 stage) at low incidence and severity the second week of May. Low levels of stripe rust were found in plots and fields in west-central and central Indiana, respectively in early June.

California. Cool, wet weather combined with late fall planting of fall-sown spring wheat and barley extended the time of exposure for wheat and barley to stripe rust in the Central Valley (Sacramento and San Joaquin Valleys) and surrounding areas in 2011. Stripe rust was severe on some plots at heading at Colusa in early March. A commercial field of Joaquin (heading stage) in the same county had severe stripe rust (80% severity, 100% incidence) despite two fungicide applications applied too late for control. A severe natural stripe rust infection developed in the UC Davis Agronomy farm in mid-April where spreader rows had 60–100% severities. Stripe was severe on several cultivars in plots at Grimes and Clarksburg (early dough and anthesis to early dough, respectively) in the Sacramento Valley in late April. The commonly grown hard red wheat Joaquin (reported at 139,000 acres in commercial production) incurred severe levels of stripe rust throughout the area. Relatively few other commercial wheat cultivars were affected. There was an estimated 6% winter wheat loss and 1% spring wheat loss to stripe rust in California in 2011.

Washington. Generally, stripe rust was active much earlier in 2011 than 2010 throughout the Pacific Northwest with active sporulation noted in areas in Washington and Oregon in mid-February. Despite the cold weather in late February, stripe rust was active in mid-March in many fields in southeastern and central Washington. In mid-April, stripe rust was found at low incidence on lower leaves in winter wheat fields in southeastern Washington. This was the earliest detection of stripe rust in the area in many years. Stripe rust was found in nearly every field checked in Adams and Franklin Counties in central Washington. Incidences ranged from 1 to 10% (except for one field with incidence greater than 30%) and the rust was appearing on some upper leaves. Many fields in central and south-central Washington were sprayed with fungicides. Stripe rust severities up to 60% (normal for the area) were found in the Mount Vernon winter nursery in northwestern Washington in late April. Low levels of stripe rust were found in fields in eastern Washington by late April. Fungicide applications had stripe rust under control in many commercial winter wheat fields in southeastern Washington in mid-May where conditions had been favorable for stripe rust development. Up to 30% of the winter wheat in Garfield County in southeastern Washington was affected by stripe rust by mid-June. Fungicides continued to be applied to control stripe rust. Stripe rust increased rapidly in much of the state, particularly the Palouse region in southeastern Washington by late June. Susceptible winter wheat entries in plots around Pullman (southeastern Washington) had 100% severities, all from natural infection. Due to the extended rust season and extremely high spore load this year fungicide treatment was economical even on resistant cultivars. High-temperature-adult-plant resistance held up, but not to its full extent due to the early season low temperatures and heavy spore load. There was an estimated 3.5% winter wheat loss and 2.5% spring wheat loss to stripe rust in Washington in 2011.

Oregon. Stripe rust was widespread in western and northeastern Oregon in mid-April. There was an estimated 7% winter wheat loss and 7% spring wheat loss to stripe rust in Oregon in 2011.

Idaho. Stripe rust was found in a row of the hard red winter wheat Moreland in southeastern Idaho in late March. The rust had overwintered, something uncommon in this area. Stripe rust was widely distributed in northern and southern Idaho in mid-April. Stripe rust was increasing on lower leaves in plots at jointing stage at Aberdeen (southeastern Idaho) on 11 May and was increasing in commercial winter wheat fields throughout southern Idaho in mid-May. The cool, wet weather was very conducive for stripe rust development and fields not sprayed with fungicides were likely severely impacted. Fungicide applications had stripe rust under control in many commercial winter wheat fields (Feekes 5) in Latah County in northwestern Idaho in mid-May. Unsprayed fields in the county had 10% severity and 40% prevalence. Stripe rust continued to be an issue in northwestern Idaho in mid to late June. Stripe rust development in winter wheat nearly ceased by mid-August, particularly in cultivars with high-temperature adult plant resistance. Some spring wheat cultivars were exhibiting higher than expected stripe rust infection (e.g., UI Pettit), whereas others (e.g., Alturas) were still holding up. Spring wheat fields sprayed with fungicides at herbicide timing had reduced infection compared to unsprayed fields; however, stripe rust redeveloped when a second application was not applied. There was an estimated 7% winter wheat loss and 4% spring wheat loss to stripe rust in Idaho in 2011.

Montana. Stripe rust was found in plots and fields in northwestern Montana on 10 May. No stripe rust was found in Pondera, Choteau, and Teton counties east of the Rockies. In mid-May stripe rust was found at very low incidence on an unknown cultivar in Choteau County. By mid-June, stripe rust was severe in many areas of the state including Hill, Prairie, Big Horn, Lake, and Flathead Counties. The resistance in the cultivar Yellowstone was holding up, whereas the reactions on the cultivars Genou and Jagalene varied by location. Stripe rust was very active and severe throughout most wheat producing counties in the state by late June. According to a retired plant pathologist, this is the worst stripe rust he had seen in 30 years. The resistance in the cultivar Yellowstone and AP503 were holding up, whereas the cultivar Genou was very susceptible. Stripe rust was widely prevalent in both winter and spring wheat in Fergus, Phillips, and Valley counties in north-central Montana in mid-July. Despite the high daytime temperatures (90–100+°F) in mid-August, stripe

rust was still active in much of the state. Nighttime temperatures ranged from 55–70°F with high humidity and significant dews. Stripe rust was widespread and severe in Montana in 2011. Most wheat fields were fungicide sprayed at least once. There was an estimated 10% winter wheat loss and 5% spring wheat loss to stripe rust in Montana in 2011.

Utah. High levels of stripe rust were found in commercial winter wheat fields in Weber and Box Elder Counties in north central Utah the second week of June. Most fields had been treated with fungicides, but some untreated fields were significantly impacted. Some irrigated fields in the Bear River Valley of northern Utah likely experienced yield reductions due to stripe rust. Many producers sprayed fungicides to mitigate the possible damage.

Alberta, Canada. Stripe rust was found at Vulcan in south central Alberta on the winter wheat AC Intrepid (full boot to 50% headed) and the hard white spring wheat Snowstar (6 leaf, 3 tillers) in early July.

Ontario, Canada. Stripe rust was found in a winter wheat nursery at Ridgetown in southwestern Ontario in late June. Incidences ranged from trace to 20% with severities up to 30%.

The 2011 stripe rust observation map can be found at http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrustbulletins/2011wstr.pdf.

NEBRASKA

UNIVERSITY OF NEBRASKA AND THE USDA–ARS GRAIN, FORAGES AND BIOENERGY UNIT.
Lincoln, NE, USA.

The 2009–10 Nebraska wheat crop.

Wheat production. In 2011, 1,500,000 acres of wheat were planted in Nebraska and 1,400,000 were harvested with an average yield of 45 bu/acre for a total production of 63,000,000 bu. In 2010, 1,600,000 acres of wheat were planted in Nebraska and 1,490,000 were harvested with an average yield of 43 bu/acre for a total production of 64,070,000 bu. In 2009, 1,700,000 acres of wheat were planted in Nebraska and 1,600,000 were harvested with an average yield of 48 bu/acre for a total production of 76,800,000 bu. Despite continued genetic improvement, the main determinant in wheat production seems to be acres harvested, government programs, the price of corn, and weather (which also affects disease pressure and sprouting). This is an economic reality in understanding wheat yields and productivity in Nebraska.

Cultivar distribution. In 2011, Overland was the most widely grown wheat cultivar in Nebraska (10.8%), closely followed by Pronghorn (10.4%). Pronghorn and Goodstreak are tall (conventional height) wheat cultivars that have consistently done well in the drought-prone areas of western Nebraska. Interestingly, the Buckskin acreage increased slightly, indicating that tall wheats, which are adapted to drought in the west, remain very popular. TAM 111 became the third most popular wheat in Nebraska, followed by Millennium, Buckskin, Jagalene, and Goodstreak (Table 1, p. 237).

New cultivars. Two new cultivars were increased and formally released in 2010. No new line was released in 2011. The two lines released in 2010 were **NE01481** and **NI04421**.

NE01481 will be marketed as Husker Genetics Brand McGill in honor of a legendary professor of genetics at the University of Nebraska. McGill is recommended for release, primarily due to its superior adaptation to rainfed wheat production systems in eastern and west-central Nebraska and its excellent resistance to wheat soil borne mosaic virus (WSBMV), a trait that is very rare in recent Nebraska releases. Additional information can be found at: http://agronomy.unl.edu/c/document_library/get_file?uuid=af82c455-7c15-48b7-ac84-c84ec9a4332f&groupId=4128273.

The second line is NI04421,which will be marketed as Husker Genetics Brand Robidoux, in honor of a pioneer French trapper who had a trading post between Nebraska and Wyoming. Robidoux was released primarily for its superior

Table 1. Cultivars grown in Nebraska between 2004 and 2011

Cultivar	Percent							
	2004	2005	2006	2007	2008	2009	2010	2011
2137	7.8	4.3	3.5	1.4	2.1	1.7		
2145			1.0	1.2	2.2			
Above			1.3					
Agripro Abilene	1.7	1.7		1.0				
Agripro Art							2.4	
AgriPro Dumas				1.4	1.2			4.3
Agripro Hawken						1.2	2.1	
Agripro Jagalene	4.5	16.8	23.8	33.4	20.9	13.8	8.5	5.4
Agripro Ogallala	2.4	2.0	1.4	1.0	1.1			
Agripro Postrock					1.1	4.1	4.4	3.3
Agripro Thunderbird							1.1	
Agripro Thunderbolt	3.0	1.9	1.9	2.0	2.4	1.6	1.5	2.2
Alliance	13.6	10.1	10.1	7.2	6.1	6.1	6.0	3.9
Arapahoe	6.8	5.2	2.9	2.0	3.4	2.2	2.1	1.5
Armour								1.0
Buckskin	4.9	3.7	5.0	3.5	3.4	3.3	4.5	5.9
Camelot								1.1
Centura	2.1	2.4	1.9	1.3	1.0			
Goodstreak		1.7	3.7	3.6	5.1	5.0	6.5	4.4
Hatcher						1.2	1.5	1.8
Hawken								1.5
Infinity CL					2.3	3.5	3.7	3.3
Jagger	2.8	3.1	2.5	1.7	1.5	1.1		
Karl/Karl 92	3.3	2.7	2.7	1.6	2.9	2.5	1.6	2.1
Millennium	11.1	10.7	9.5	7.2	9.4	13.2	11.9	7.6
Niobrara	3.5	2.2						
Overland						3.4	5.6	10.8
Overly				1.0	1.1			
Platte	1.3	1.6						
Pronghorn	10.4	11.4	10.1	12.2	10.6	12.1	13.7	10.4
TAM 111			1.2	1.6	3.2	6.5	7.4	8.1
TAM 112								1.2
Wahoo	1.7	1.8	1.8	1.1	1.5	1.1		
Wesley	5.9	5.5	5.8	7.2	7.7	4.8	4.1	4.2
Winterhawk								1.3
Other Private Cultivars	4.4	4.0	3.8	2.8	4.1	5.0	3.6	5.4
Other Public Cultivars	8.8	7.2	6.1	4.6	5.7	6.6	7.8	9.3
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

performance under irrigation and rainfed conditions in western Nebraska (west of North Platte, where drought is common) and irrigated production sites in western Nebraska and eastern Wyoming. Robidoux seems to have good drought tolerance and does best in irrigated environments in the drier areas (eastern WY). Additional information can be found at: http://agronomy.unl.edu/c/document_library/get_file?uuid=5d4eff93-65f7-4920-9849-2fd42919cccb&groupId=4128273.

Use of wheat synthetics to expand our genepool.

K. Onweller, R. Ward, P.S. Baenziger, Y. Jin, R. Bowden, S. Wegulo, C. Baker, R. Graybosch, S. Haley, and P. Byrne.

A collaborative effort with Colorado State University to use the CIMMYT-developed, wheat synthetic lines as sources for drought tolerance led us to further characterize six synthetic CIMMYT wheat lines. In our characterization studies, we discovered some lines were resistant to *Puccinia graminis*, *P. striiformis*, and *Schizaphis graminum*. Two of the six lines possessed resistance to stem rust races in the Ug99 family. Studies to determine the identity of the genes are underway. Based on phenotyping of the synthetic parental lines at the Cereal Disease Laboratory in Minnesota, it has been

hypothesized that the resistance in the synthetic parental lines may be from *Sr33*. *Sr45* will be tested for as well as *Sr33* and *Sr45* are both derived from *Aegilops tauschii* and are located on chromosome 1DS. Both genes have been shown to confer resistance to numerous races of stem rust, including Ug99. All synthetic lines exhibited seedling resistance and five lines exhibited adult resistance to *P. striiformis* race PST-100. Two different synthetic lines conferred excellent resistance to greenbug biotypes E, I, and K. A detailed inheritance study was undertaken with the assistance of Cheryl Baker (USDA–ARS, OK) to identify the genetic constitution of the resistance. Preliminary data suggest that single, dominant genes are acting in each synthetic line. In addition to resistance, the synthetic lines were assayed for high-molecular-weight glutenin and gliadin composition. The work revealed protein subunit compositions not commonly found in the Great Plains wheat cultivars.

Understanding the stem rust resistance in Gage wheat.

T. Kumsa, P.S. Baenziger, S. Wegulo, M. Rouse, and Y. Jin.

In this project we are interested in understanding the *Sr2* complex in Gage (a Nebraska cultivar released in 1965), which historically was superior to Scout 66 (a wheat cultivar that also carried the *Sr2* gene). Our goal is to understand the nature of Gage's superior resistance to stem rust when compared to that found in many other *Sr2* cultivars. We have created $F_{2:3}$ families for genotyping and phenotyping at seedling and adult-plant stage. These families have been planted in the field at research stations in Mead, Nebraska, and at Haymana, Turkey, for evaluation in summer 2012. Phenotyping at seedling stage was done at Cereal Disease Laboratory using QFCSC (a mild race of stem rust) and TTKSK (Ug99) stem rust races. The DNA sample from the same families was sent to Dr. Jesse Poland, USDA–ARS, Kansas State University for genotype by sequencing. The preliminary marker and phenotype data indicated that resistance in Gage is conditioned by *Sr2* and other gene/s. The effectiveness and phenotypic expression of resistance gene *Sr2* is only at the adult-plant stage (called an adult-plant resistance gene), whereas the phenotypic effect from other resistance genes can be detected at both the seedling and adult-plant stages. Based on evaluation at the seedling stage with 14 pathotypes, including variants of Ug99, Gage was postulated to carry a major resistance gene in addition to *Sr2*. The F_3 families seedling infection phenotype signify segregation between susceptible and resistance parents supporting involvement of a gene other than *Sr2*. However, almost all families showed less seedling resistance reaction to Ug99 (TTKSK) than Gage, whereas the resistance reaction of families to QFCSC is comparable to that of Gage. A heterozygous, resistant type of reaction is the maximum resistance observed to Ug99 infection. The seedling infection type data from both TTKSK and QFCSC does not fit either single or two gene models, perhaps due to low number of plants represented per family. We are advancing the population using single-seed descent to increase the homozygosity. The genotyping and adult-plant field stem rust evaluation will be done this year.

Association mapping for important biotic and abiotic related traits in a structured wheat breeding population.

I. Salah, D. Wang, K. Eskridge, J. Crossa, and P.S. Baenziger.

This study focuses on applying DNA molecular markers in plant breeding process, using different statistical methods to increase wheat productivity. The field part of this study was carried out in two successful seasons 2009–10 and 2010–11 using two $F_{3:6}$ wheat populations. The first population contained 276 lines and two local checks genotyped using DArT markers, and the second population consisted of 278 plus the same local checks and genotyped using the same marker system. The populations were phenotyped in nine different locations throughout Nebraska. Our results showed that the $F_{3:6}$ populations had sufficient genetic diversity that would make the selection effective in improving the population productivity and adaptability to Nebraska environmental condition.

The second objective was to apply association-mapping approaches to identify DArT markers associated with important traits in $F_{3:6}$ wheat populations. Those markers could be used in further studies to investigate the genetic architecture of important traits such as yield and quality. Based on the yield, grain volume weight, disease resistance, plant height, maturity and molecular marker data, we applied different statistical methods to identify markers that have significant correlation with the previous phenotypic data. We have been successful in identifying potential QTL for those traits. Some of the QTL have been published in spring wheat populations which was surprising, but indicated that there are QTL important in two growth habits of wheat. Others identified QTL were novel.

The third objective was applying genomic selection methods in our breeding program using different statistical approaches to build new applicable protocol that could be used to improve our selection processes. Our results indicated that, when factors such as heritability, relative costs of genotyping versus field evaluation, and the number of cycles of selection per year are taken into account, the efficiency of GS becomes favorable in comparison with phenotypic selection.

Preharvest sprouting derived from red/white wheat mating populations.

Juthamas Fakthongphan, R. Graybosch, and P. S. Baenziger.

Preharvest sprouting of wheat, the premature germination of wheat heads, takes place in a field under conditions of delayed harvest, high humidity or wet conditions. This problem has a high economic impact on farmers and end-users. Wheat breeders have tried to diversify the wheat production system in Nebraska by introducing hard white winter wheat cultivars. The grain yield potential and disease resistance have been increased but the current germplasm of hard white winter wheat lacks some essential quality traits such as low levels of grain enzyme polyphenol oxidase, and resistance to pre-harvest sprouting. Both traits will be important issues once the U.S. exports white wheat to the world markets. This research will focus on (1) identifying red wheat parents capable of donating genes for tolerance to PHS; (2) mapping or confirming using markers applicable for the Great Plains hard white wheat gene pool using populations derived from Jagalene/RioBlanco, Jagalene/NW99L7068, Niobrara/RioBlanco, Niobrara/NW99L7068, NE98466/RioBlanco, and NE98466/NW99L7068 crosses; and (3) analyzing the ABA sensitivity in these materials to correlate the misting assay for PHS to ABA response.

Fusarium head blight (FHB) research.

Ali Bakhsh, Stephen Wegulo, Guihua Bai, Bill Berzonsky, and P. S. Baenziger.

For many years, we have been perplexed why we make so many crosses to FHB resistant material, only to have very few resistant lines survive our selection procedures. In this study, we developed a population of lines carrying the *Fhb1* gene and a population of lines not carrying the *Fhb1*. We also created mechanical mixtures (blends) of each of the two populations to determine if the *Fhb1* gene was pleiotropic or linked to genes that may reduce grain yield. In this study, the *Fhb1* lines survived the winter better than the non-*Fhb1* lines, but the blends had very similar grain yields. We interpreted these results as *Fhb1* was not pleiotropic or linked to genes that reduce grain yield. The difficulty with creating high-yielding, *Fhb1* lines is most likely due to the wide diversity and poor adaptation of many of parents lines used as *Fhb1* sources. In a second study, we evaluated a number of Wesley BC_2 lines with the *Fhb1* gene. We identified seven high-yielding, Wesley *Fhb1* lines for use as parents in crossing. Four lines were very similar to Wesley, and three lines were similar for most traits but were significantly earlier than Wesley. These lines have been used heavily as parents in our breeding program.

Nitrogen use efficiency.

Katherine Frels, Mary Guttieri, Teshome Regassa, Brian Waters, and P. Stephen Baenziger.

As part of a multistate effort, we began a major experiment on nitrogen use efficiency (NUE) at Mead, NE. Although too early to report any results, we have worked very hard to develop an excellent NUE testing site, protocols for canopy spectral reflectance (CSR, high throughput phenotyping), and data collection. We expect the efficient use of nitrogen and other major inputs in modern agriculture will be important areas for future research in agricultural profitability and sustainability.

Studying the role of roots in drought in wheat.

Sumardi bin Haji Abdul Hamid, Harkamal Walia, and P.S. Baenziger.

We began a study to investigate the role of roots to confer drought tolerance in wheat. The initial studies are laying the groundwork for future studies. So far, we have developed the needed protocols to look at the effect of drought at seeding and seedling emergence by studying germination, seed vigor, shoot and root ratios, and how best to induce controlled drought. The 2-D root phenotyping has been employed to compare the different root system architecture parameters of different cultivars in order to evaluate their drought tolerance. This study will help to evaluate the effectiveness of the previous selection of cultivars for dryland versus irrigated production systems.

Hybrid wheat.

P. S. Baenziger, MengYuan Wang, and friends.

In 2010, we began a small hybrid wheat program using two cytoplasmic male-sterile systems. The goal of this effort is to provide a system for hybrid wheat. The story of hybrid rice is inspirational where scientists worked very hard to overcome the barriers to hybrid rice (30 years of difficult research) that led to one of the great hybrid crop successes. Hybrid wheat and traits (syn. transgenes, genetically modified wheat) are two of the last great frontiers in wheat research. However, although there is considerable private- and public-sector research in traits, there is relatively little public-sector research in hybrid wheat. Hence we decided to begin a program so that should hybrid wheat become a reality, there would be public sector research looking at heterosis and heterotic pools, hybrid production systems, and pollinators. Both hybrid systems are still under evaluation, but it appears that we have excellent restorers and male sterility.

On the genetics of grain polyphenol oxidase.

S. Nilthong, R.A. Graybosch, and P.S. Baenziger.

Grain polyphenol oxidase (PPO) activity can cause discoloration of wheat food products. Five crosses (PI 117635/Antelope, Fielder/NW03681, Fielder/Antelope, NW07OR1070/Antelope, and NW07OR1066/OR2050272H) were selected to study the genetic inheritance of PPO activity. STS markers, PPO18, PPO29, and STS01, were used to identify lines with putative alleles at the *Ppo-A1* and *Ppo-D1* loci conditioning low or high PPO activity. ANOVA showed significant genotypic effects on PPO activity ($P < 0.0001$) in all populations. The generation and 'generation × genotype' effects were not significant in any population. A putative third (null) genotype at *Ppo-A1* (no PCR fragments for PPO18) was discovered in NW07OR1066- and NW07OR1070-derived populations, and these had the lowest mean PPO activities. Results demonstrated both *Ppo-A1* and *Ppo-D1* loci affect the kernel PPO activity, but the *Ppo-A1* locus had the major effect. In three populations, contrary results were observed to those predicted from previous work with *Ppo-D1* alleles, suggesting the markers for *Ppo-D1* alleles might give erroneous results in some genetic backgrounds or lineages. Results suggest selection for low or null alleles only at *Ppo-A1* is sufficient to allow development of low PPO wheat cultivars.

Effects of single and double infections of winter wheat by Triticum mosaic virus and wheat streak mosaic virus on yield determinants.

E. Byamukama, T. Satyanarayana, G.L. Hein, R.A. Graybosch, P.S. Baenziger, R. French, and S.N. Wegulo.

Triticum mosaic virus (TriMV) is a recently discovered virus of winter wheat in the Great Plains region of the United States. TriVM is transmitted by wheat curl mites (*Aceria tosichella*), which also transmit wheat streak mosaic virus (WSMV) and wheat mosaic virus. In a greenhouse experiment, winter wheat cultivars Millennium (WSMV-susceptible) and Mace (WSMV-resistant) were mechanically inoculated with TriMV, WSMV, TriMV+WSMV, or sterile water at the 2-leaf growth stage. At 28 days after inoculation, final soil plant analysis development (SPAD) readings (an indicator of chlorophyll content), the number of tillers/plant (TPP), shoot weight, root weight, TriMV and WSMV titers, total nitrogen, and total carbon were determined. In Millennium, all measured variables were significantly reduced by single

or double virus infections, with the greatest reductions occurring in the double infection treatment. In Mace, only SPAD readings and total nitrogen were significantly reduced by single or double virus infections, and these reductions were smaller than those in Millennium. SPAD readings and shoot weight were linearly and positively related in Millennium but not in Mace. Total nitrogen was linearly and positively related to shoot weight in both cultivars. TriMV and WSMV titer was linearly and negatively related to shoot weight in Millennium, but not in Mace. TriMV titer was linearly and negatively related to SPAD readings in Millennium but not in Mace. WSMV titer was linearly and negatively related to SPAD readings in both cultivars. The results from this study indicate that 1) Mace, a WSMV-resistant cultivar, also is resistant to TriMV, and 2) double infection of a susceptible cultivar by TriMV and WSMV exacerbates symptom expression and loss of biomass.

Genetic improvement in winter wheat grain yields – redux.

R.A. Graybosch and C.J. Peterson (Limagrain).

A previous investigation, using region-wide data from Great Plains wheat breeding trials, indicated a possible plateau in the rate of genetically determined yield potential. Data from the same USDA–ARS-coördinated, long-term, regional performance nurseries was used to further examine the rate of genetic improvement of Great Plains winter wheats in specific agroecological or production zones over the time period 1987 to 2010. The absolute grain yield of all entries and of the top five most productive entries increased in the majority of production zones over this time period. The relative rate of genetic improvement, obtained by comparing grain yields to those of the long-term control cultivar Kharkof, ranged from not significantly different from zero to 1.98%/yr. This rate of change, however, was statistically significant ($\alpha = 0.05$) in only two of the 12 zones evaluated. Variance components identified production zone and locations within production zone as being the largest sources of variation in grain yields. Variance due to either genotype or 'genotype × environmental' factors remained both constant over the 24-year time period and small, relative to the environmental variances. Genetic progress for enhanced wheat yield in the region might be limited by the magnitude of these environmental variances and by constraints arising from continuous evolution of pest and pathogen populations.

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Microspore culture for production of doubled haploid plants of Nebraskan winter wheat cultivars.

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Introduction. Microspore culture is a cell (haploid) culture-based approach for producing completely homozygous doubled haploid (DH) plants from immature pollen grains in a single generation. In plant breeding, the single-seed descent (SSD) method is often used to hasten the development of homozygous breeding lines; however, six generations of self-pollination are required to reach 98% homozygosity. Microspore culture is more efficient than the SSD method, results in the recovery of genotypes with 100% homozygosity in a single generation, and can be performed at any stage of the breeding process. Because winter wheat requires 6 to 8 weeks of vernalization to induce flowering in every generation of advancement, microspore culture may prove useful for improving the efficiency of winter-wheat breeding programs, because only one vernalization cycle is required to obtain completely homozygous lines. Therefore, DH technology enables shortening the time required for developing cultivars when applied in traditional plant breeding (Forster and Thomas 2005).

Two basic methods of androgenesis for the production of DH plants are (i) anther and (ii) isolated microspore (immature pollen) cultures. Microspore culture is defined as isolating the microspores from the anther prior to culture, whereas anther culture involves culturing the whole anther (Ferrie and Caswell 2011). The advantage of isolated microspore culture is that microspores can be isolated in greater amounts, providing large number of potentially embryogenic single haploid cells, which can undergo androgenesis, thereby producing thousands of genetically different homozygous plants in one season from a single hybrid plant. Microspore culture consists of three major steps (i) pretreatment (process of sporophytic development from immature microspores), (ii) induction (developing embryoids from embryogenic microspores), and (iii) regeneration (regeneration of microspore-derived embryos).

Our long-term objective is to develop a high-throughput procedure for production of DH plants from major winter wheat cultivars of Nebraska to complement the current breeding program in Nebraska. We report here preliminary results of androgenic response of three Nebraskan winter wheat cultivars to two different pretreatment methods.

Materials and methods. ***Plant materials and growing conditions.*** The three Nebraskan winter wheat cultivars used in the study were Anton, Antelope, and Camelot. Plants were grown in pots in a controlled green house regulated with a photoperiod of 16–17 h light/7–8 h dark. Temperature was set at 22°C day and 15°C night. Humidity was not maintained. Plants were watered on alternate days and fertilized once a week.

Microspore culture. The whole procedure of microspore culture was according to Kasha et al. (2003) except that the pretreatment method was modified. Anthers from four sterilized spikes (half emerged when most of the microspores were at the late uninucleate stage) were removed and put in '60 x 15 mm' sterile petri dishes containing 4 mL of solution B. The petri dishes were kept at 25°C for 4–5 days (no cold treatment). For a cold treatment, the plates were incubated for additional five days at 4°C. The cell density was counted with a haemocytometer and adjusted to a range of 2–4 x 10^5 cells/mL. The suspended microspores were cultured in 35-mm (2.0 mL) or 60-mm (4.0 mL) petri dishes depending on the volume of microspores obtained. Ten to twelve ovaries were put into each petri dish. The petri dishes were incubated in dark at 28°C for 10 days and then transferred to a shaker in the dark at 28°C. The microspore-derived, multicellular structures were observed after 7–10 days. After 21 days, 1–2 mm size embryos were transferred to 90-mm petri dishes containing a modified, semisolid, MMS5 media fortified with ascorbic acid (Santra et al. 2012). The petri dishes were kept at 25°C in the dark for 4 days and then transferred to a light cabinet at 25°C. After 1–2 weeks, plantlets with well-developed roots and shoots were transferred to magenta boxes containing a modified MS media without hormones, which were kept at 22°C in light cabinets for 2–3 weeks.

Results and discussion. The correct microspore development stage is the most important step of androgenic response in isolated microspore culture (Ferrie and Caswell 2011). The right stage of the spike (Fig. 1a, p. 243) was determined based on mid- to late uninucleate stage of the microspores (Fig. 1b, p. 243) in anthers from the middle part of the spike.

The morphological marker for the embryogenic microspores is appearance of star-like structures (Fig. 2a; Shariatpanahi et al. 2006). After putting such star-like microspores in induction media, three things were observed among the isolated microspores (i) more than ~90% of the cells shrunk in the medium, (ii) ~4–5% of the cells did not progress but remained static, and (iii) ~1–2% of the cells enlarged and developed into multicellular structures, which subsequently advanced to embryo-like structures (ELS) (Fig. 2b) and pro-embryoids. The induced pro-embryoids eventually developed into 1–2 mm sized embryos (Fig. 2c), which were transferred to 90 mm petri dishes (Fig. 2d). The regenerated green plantlets (Fig. 2e) from the transferred embryos were transferred to magenta boxes (Fig. 2f).

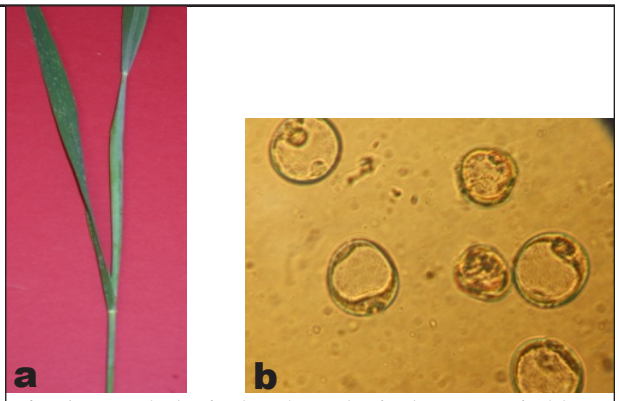

Fig. 1. Morphological and cytological stages suitable for isolated microspore culture in winter wheat. (a) Ideal morphological stage of spike, which carries late, uninucleate microspores in the middle portion; (b) microspores at late, uninucleate stage as seen by the vacuole being formed.

The androgenic response towards two different pretreatment methods of the three winter wheat cultivars is summarized in Table 1. Compared to no cold pretreatment, a cold pretreatment increased the number of embryogenic microspores in Anton by two fold, but no such differences were observed between the two pretreatments in Camelot and Antelope. *In vitro* development of microspores into multicellular and embryo-like structures was quicker in Camelot than in Anton and Antelope. Green plants were regenerated in all three cultivars following both cold and noncold pretreatments (Table 1). In Antelope, the number of green plantlets was higher with a cold (8) than with a noncold (4) pretreatment; because the number was much less, a further experiment is in progress.

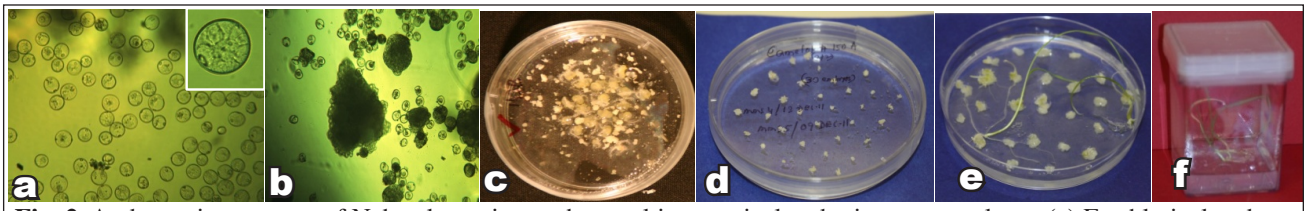

Fig. 2. Androgenic response of Nebraskan winter wheat cultivars to isolated microspore culture. (a) Freshly isolated embryogenic microspores (star-like structure; inset is an enlarged view of a star-like cell; (b) embryo-like structures after two weeks in induction medium; (c) embryoids formed after 20–21 days in induction medium; (d) 1–2-mm embryos transferred to MMS5, semisolid media; (e) regeneration of plantlets in petri dishes, and (f) transfer of green plantlets into magenta boxes.

Table 1. Androgenic response of three Nebraskan winter wheat cultivars to isolated microspore culture.

Cultivar	Treatment	Total number of microspores cultured (x 10^5)	Number of multicellular + embryo-like structures	Number of transferred embryos	Number of regenerated plantlets	Number of regenerated green plantlets
Anton	No cold	5.74	3,776	7	1	1
	Cold	10.8	736	7	1	1
Antelope	No cold	10.0	576	17	5	4
	Cold	8.84	960	37	13	8
Camelot	No cold	11.0	544	57	7	1
	Cold	6.48	640	59	1	1

Because isolated microspore culture depends on the genotype and a number of other factors (Ferrie and Caswell 2011), we studied the androgenic potential of the cultivars to produce DH plants. The higher number of embryogenic microspores woth a cold pretreatment in Anton was not regenerated into a proportional number of green plants. Repli-

cated experiments are under progress to comprehensively compare the two pretreatments in the three cultivars. We hope to establish DH production methods on these and other major Nebraskan winter wheat cultivars so that this method will be a beneficial tool in our wheat breeding efforts.

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2011 Wheat Production in the Commonwealth of Virginia.

Growing conditions. Following an extremely dry summer and corresponding low yields in most of the Commonwealth in 2010, small grain growers experienced a generally drier and warm early start to planting (Fig. 1 and Fig. 2, p. 245). Many farmers were able to get an early start on wheat planting, since the harvest season for corn and soybeans was abbreviated greatly. By 20 September, about 9% of the wheat crop was seeded, compared to the average of 4%. By 20 October, most areas had received enough rainfall so that 65% of the state was rated adequate for topsoil moisture. The trend toward early seeding and early emergence continued with 46% of intended acreage reported as already planted, and 18% of acres emerged compared with the 5-year average of 8% by this date. The end of the first week of November showed continued cool and relatively wet weather throughout much of the state. Still growers managed to have 77% of acres planted. Conditions for early season growth

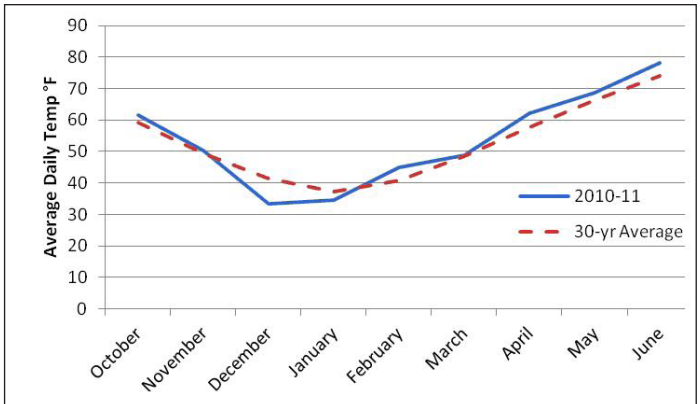

Fig. 1. 2010–11 and 30-yr mean monthly average temperatures in the Commonwealth of Virginia.

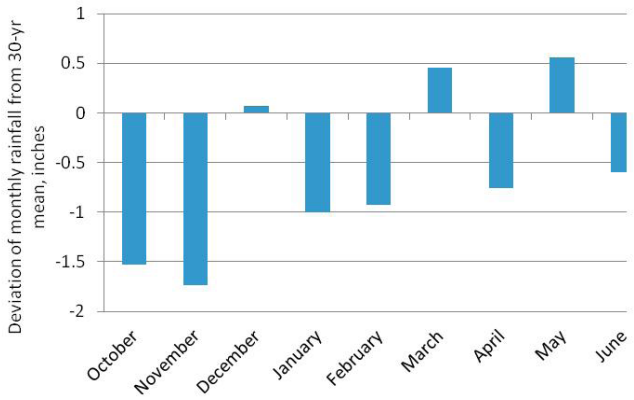

Fig. 2. Deviation of 2010–11 season rainfall from the 30-yr mean by month in the Commonwealth of Virginia.

were favorable, especially for the early planted wheat and the Virginia Agricultural Statistics Service reported that 81% of wheat had emerged compared to the 5-year average of 53%. Mid-winter was relatively dry and cold with little snowfall, which resulted in more winter injury to some small grain fields but did allow producers to access their fields. Rain in March was welcome and helped improve condition of wheat throughout the state.

By early April, wheat was rated at greater than 80% good or excellent. Crop condition remained quite good in most locations in late April however some areas were beginning to feel the effects of dry weather. By the end of the first week of May, 64% of the wheat crop was headed, compared to 41%, the 5-year average for this timeframe. The wheat harvest was estimated to be 30% finished by 12 June. This, combined with a relatively dry grain-fill period and harvest season, allowed producers to harvest a large wheat crop.

Disease and insect incidence and severity. Entries in Virginia's 2011 state wheat variety trials were rated (0 = no infection to 9 = severe infection) for disease severity in five environments at four locations. Mean disease severity scores for powdery mildew (*Blumeria graminis*) varied from 1 to 3 in four environments. The 87 entries in the 2011 trial had powdery mildew ratings that varied from 0 to 7 at the Northern Piedmont, Eastern Virginia, and Eastern Shore Agricultural Research and Extension Center (AREC) test sites. Mean test scores for leaf rust (*Puccinia triticina*) varied from 2 to 4 in four environments. Wheat entries received mean ratings from 0 to 9 in the Eastern Virginia (Warsaw) no-till test and from 0 to 8 in the Eastern Virginia conventional test, the Eastern Shore (Painter) test, and the Southwest Virginia (Blacksburg) test. Cultivars having only genes *Lr24* or *Lr26* were susceptible to leaf rust at most locations, and cultivars having only gene *Lr9* or this gene combined with *Lr24* were susceptible at Blacksburg. Race surveys conducted by the USDA–ARS Cereal Disease Lab on 27 isolates from four regions in Virginia indentified 11 races of leaf rust. Six races having virulence for gene *Lr26* but avirulent to gene *Lr24* were identified and included MCTNB and TCTBG (Painter, VA); MCTQB, TCJSB, and TCRKG (Warsaw, VA); and TCJSG (Painter and Warsaw). Four races identified with virulence for genes *Lr24* and *Lr26* include TFRJG (Painter), MFDSB (Warsaw), and MFGJG and MFRJG (Painter and Warsaw). Race TNRJJ having virulence for genes *Lr9* and *Lr24* was identified only at Blacksburg, VA. Stripe rust (*Puccinia striiformis*), was found only at Warsaw, VA, in 2011 and a sample was sent to Dr. Xianming Chen at USDA–ARS in Pullman, WA, for race identification. Two races were identified including PSTv34 having virulence for genes *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr44*, *YrTr1*, and *YrExp2*, and PSTv37 with virulence for genes *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*. Barley/cereal yellow dwarf virus infection was moderate at Blacksburg (0–2) and Blackstone (1–4).

Production. According to the United States Department of Agriculture's National Agriculture Statistical Service (http://www.nass.usda.gov/Statistics_by_State/Virginia/index.asp), in autumn 2010, Virginia wheat growers planted 270,000 acres (109,350 ha). In the spring of 2011 an estimated 250,000 acres (101,250 ha) was harvested in the state of Virginia. The average yield was 71 bu/acre (4,770 kg/ha), which was a 20 bu/acre (1,344 kg/ha) increase over that of the previous year. In all, Virginia growers produced 17.7 x 10^6 bushels (481,191 metric tons), which was a significant increase from the previous year.

State cultivar tests. In the 2010–11 tests, there were a total of 87 entries planted in eight environments across Virginia (http://www.grains.cses.vt.edu/). The test included 51 cultivars and 36 experimental lines. No-till tests, planted after corn, were conducted at Warsaw and Holland, VA. Mean grain yields varied from 72 bu/ac (4838 kg/ha) at Holland, VA to 105 bu/acre (7,055 kg/ha) in the no-till test at Warsaw, VA, with a mean yield over all eight environments of 91 bu/acre (6,094 kg/ha). Commercial cultivars Featherstone VA258, W1566, Progeny 870, Dyna-Gro 9171, SS 520, Pioneer Brand 26R10, Shirley, SS 8340, USG 3438, 12V51, Branson, 5187J, Progeny 125, and Merl all produced yields (94–99 bu/acre, 6,316–6,652 kg/ha) that were significantly higher than the overall trial average. Average grain yields among the 87 entries ranged from 74 bu/acre (4,972 kg/ha) for the long-term check cultivar Massey to 99 bu/acre (6,652 kg/ha) for Featherstone VA258. Test weight means among the eight trials varied from 56.3 lb/bu (72.5 kg/hl) in the Shenandoah Valley test to 62.0 lb/bu (79.8 kg/hl) in the Warsaw no-till test. The average test weights of the 87 entries over all eight

environments ranged from 56.4 lb/bu (72.6 kg/hl) to 61.7 lb/bu (79.4 kg/hl) with an overall trial average of 59.4 lb/bu (76.4 kg/hl).

Other tests. In 2010–11, tests were initiated by Dr. Maria Balota at the Tidewater AREC in Holland, VA, to evaluate the response of cultivars to moisture stress under rain exclusion shelters. Based on recorded weather at Holland, spring rainfall has diminished in the last five years compared with the 70-year average with possible effects on crops yields including wheat. To know which cultivars are the most drought tolerant and the mechanisms governing this tolerance, six wheat cultivars were planted in replicated trials under two rain exclusion shelters. One shelter was kept under moisture stress, no rain or irrigation from two weeks after flower until physiological maturity, and one was irrigated twice with one inch of water every time. The irrigated plots also received rainfall, as the shelter was left uncovered. The cultivars were Jackson, Merl, Pioneer 2580, Roane, Shirley, and SS 5205. Under moisture stress, yields ranged from 56 to 70 bu/ac (4,240–5,190 kg/ha). With approximately 2.5 inches of irrigation and rainfall from two weeks after flower to maturity, yields were from 63 to 76 bu/acre (4,742–5,688 kg/ha). Shirley was the highest yielding cultivar (72.3 bu/acre; 5,439 kg/ha), followed by SS 5205 (66.4 bu/acre; 4,996 kg/ha), Roane (64 bu/acre; 4,842 kg/ha), Pioneer 2580 (63.3 bu/acre; 4,759 kg/ha), Jackson (61.8 bu/acre; 4,648 kg-ha), and Merl (59.7 bu/acre; 4,491 kg/ha). Under moisture stress, higher yields were associated with increased spike and straw weight. Under irrigation, higher yields were related to a higher number of spikelets per spike.

2011 Virginia Wheat Yield Contest Results. The 2011 contest was conducted statewide and the results are presented (Table 1). Top yields were 58.5 to 47.0 bu/acre (3,191–3,973 kg/ha) higher than the 2011 state average yield. All growers planted their wheat no-till following corn except for Frank Hula, whose wheat crop was planted no-till following full season soybean. All growers planted fungicide treated seed at rates of 24 to 32 seed/row foot in 7.5-inch rows, applied herbicides and foliar fungicides and insecticides, and used N rates from 90 to 150 lbs/acre applied over three to four application times. Congratulations to our winners.

Table 1. 2011 Virginia Wheat Yield Contest Winners (http://www.vdacs.virginia.gov/news/releases-b/080211vgpawinners.shtml)

Rank	Grower	Farm	County	Bushels/acre	Cultivar
1st	Frank Hula	Riverside Farm	Charles City	129.5	Shirley
2nd	John Shepherd	Tri-County Grain Farms	Nottoway	120.9	USG 3555
3rd	Bill Nelson	Colonial Acres Farm	Henrico	120.3	Roane
4th	Craig Brann	Brann Farms	Richmond	118.0	Shirley
Other entries in the 2011 Virginia Wheat Yield Challenge					
	Chris Clarke	Ridgefield Farms	Lancaster	114.3	Shirley
	Jason Benton	Benton Farms	Middlesex	109.7	USG 3555
	David Hudnall, Sr.	Roadview Farm	Lancaster	98.4	Pioneer Brand 26R15
	Sparky Crossman	Laurel Springs Farm	Richmond	90.9	Vision 40 (HRW)

Release of the soft red winter wheat cultivar 5187J.

Cultivar 5187J, formerly designated and tested as VA05W-151, was derived from the cross 'Pioneer Brand 26R24 (PI 614110 PVPO)/McCormick (PI 632691)'. Cultivar 5187J is a broadly adapted, high-yielding, early maturing, short height semi-dwarf (gene *Rht2*). Plant color is blue green. At maturity, 5187J has white-colored, slightly tapering strap, awnletted spikes, and purple colored straw. In the eastern SRW wheat region, average head emergence is 129 to 135 d (Julian). Mature plant height is 84 to 86 cm. On average, straw strength (0 = erect to 9 = completely lodged) is moderate (2.6–3.4). Among entries in Virginia's State Variety Trials, 5187J had the highest three year average (2008–10) grain yield (5,778 kg/ha) and test weight (77.7 kg/hl). On the basis of winter kill ratings (0 = no injury to 9 = complete kill) reported at 5 of 28 locations in the 2008–09 USDA–ARS Uniform Eastern SRW Wheat Nursery (UESRWWN), winter hardiness of 5187J is good (2.1). Cultivar 5187J has notably strong gluten strength for SRW wheat and has exhibited milling and baking qualities that are most similar to those of other strong gluten SRW wheat cultivars.

Cultivar 5187J is moderately resistant to powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*), and stem rust (*Puccinia graminis*) conferred by gene *Sr24* and the T1AS·1RL wheat–rye translocation. It is moderately

resistant to Barley and Cereal Yellow Dwarf Viruses, Wheat Soil Borne Mosaic Virus, Wheat Spindle Streak Mosaic Virus, *Septoria tritici* leaf blotch, and *Stagonospora nodorum* glume blotch. 5187J is susceptible to stripe rust (*Puccinia striiformis*). This cultivar expresses an intermediate level of resistance Fusarium head blight (*Fusarium graminearum*). In seedling growth chamber tests of 2009 UESRWWN entries conducted by USDA–ARS at West Lafayette, IN, cultivar 5187J was resistant to Hessian fly (*Mayetiola destructor*) biotype O and susceptible to biotypes C, D, and L. In the 2010 tests, it was susceptible to biotypes B, O, and L.

Release of the soft red winter wheat cultivar 12V51.

Cultivar 12V51, formerly designated and tested as VA05W-251, was derived from the cross 'VA98W-130//VA96W-348/Pioneer Brand 26R61 (PI 612153 PVPO)'. Parentage of VA98W-130 is 'Savannah/VA87-54-558//VA88-54-328/GA-Gore'. Parentage of VA87-54-558 is 'Massey/Holley' and parentage of VA88-54-328 is 'Lovrin 29/Tyler//Redcoat*2/Gaines'. Parentage of VA96W-348 is 'IN81401A1-32-2/FFR555W', and the parentage of IN81401A1-32-2 is 'Arthur 71/Caldwell/4/Arthur 71/3/Benhur//Riley*2/W62-63-119A'.

Cultivar 12V51 is a short height, semi-dwarf (gene *Rht2*) that is mid-season maturity, broadly adapted, and high yielding. Plant and spike color is blue green. At maturity it has creamy white colored, awnletted spikes that are strap in shape and recurved. Straw color at maturity is predominantly yellow with trace anthocyanin present. In the southern SRW wheat region, average head emergence is 114 to 118 d (Julian). Mature plant height is 79 to 86 cm. Straw strength (0 = erect to 9 = completely lodged) is moderate (1.7–3.4). In Virginia's State Variety Trials, cultivar 12V51 had a three year (2008–10) average grain yield (5,644 kg/ha) similar to that of the highest yielding cultivar. In the same tests, it had a three year average test weight of 74.7 kg/hL.

Cultivar 12V51 is resistant to *Stagonospora nodorum* glume blotch, leaf rust, and Wheat Soil Borne Mosaic Virus. It is moderately resistant to powdery mildew, Barley and Cereal Yellow Dwarf Viruses, and *Fusarium* head blight. 12V51 is susceptible to stem and stripe rust and has expressed an intermediate reaction to Wheat Spindle Streak Mosaic Virus and *Septoria tritici* leaf blotch. In seedling growth chamber tests of entries in the 2009 and 2010 Uniform Southern SRW Wheat Nurseries, conducted by USDA–ARS at West Lafayette, IN, cultivar 12V51 was heterogeneous in reaction (resistant and susceptible plants) to Hessian fly biotype O, and susceptible to biotypes B, C, D, and L.

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Engineering wheat for celiac patients.

Wheat is one of the elementary nutritional elements of human civilization. Wheat has accompanied human beings since the dawn of civilization, and so has celiac disease. Removing wheat from the daily diet has severe consequences not only for human diet but also for social life. Celiac disease is a complex autoimmune disorder of humans and apes and is represented by a large variety of phenotypic manifestations in different patients. In celiac sprue, genetically predisposed individuals develop intolerance to wheat and wheat products. After decades of research, the complex mixture of seed storage proteins designated as glutens were determined to be responsible for celiac disease. The glutens comprise a huge family of proteins including gliadins (alpha/beta, omega, and gamma) and high- and low-molecular-weight glutenins. Of these proteins, gliadins and low-molecular-weight glutenins (LMWgs) account for most of the celiac-causing epitopes (a peptide that can serve as an antigen) and need to be eliminated or detoxified for making celiac safe wheat. According to the Codex definition, only about 1 mg of total gluten in 50 g of food is labeled or considered to be gluten free. With the advent of better diagnostic methods, more and more celiac cases are registered ever year,.resulting in an excess of 3 millon registered cases alone in the United States, making it a serious concern for human health.

In the genetically predisposed individuals, initial presentation of gluten peptides by antigen presenting cells expressing human leukocyte antigen (HLA)-DQ2 or -DQ8 to $CD4^+$T cells results in production of interferon-gamma (IFN-γ) that causes higher expression of HLA-DQ molecules and results in increased presentation of gluten peptides. The epitopes from gluten proteins on activation to a highly immunotoxic form via enzymatic deamidation, exert both innate and immunogenic effects in susceptible individuals, triggering the onset of disease.

Gliadins and LMW glutenins are not only pathogenic in nature they also are deficient in lysine content. Eliminating these lysine-deficient proteins from the grain can improve lysine availability in wheat grains. Lysine is an essential amino acid not produced by primates, thus the need to be acquired through dietary sources. Deficiency of lysine in the diet can lead to the creation of kidney stones and other health-related troubles including fatigue, slow growth, anemia, nausea, dizziness, loss of appetite, agitation, bloodshot eyes, and reproductive disorders. Because lysine helps in the absorption of calcium, a deficiency also can lead to defective bone growth. In view of producing celiac-safe wheat lines and to improve the nutritional quality of wheat grains in general, we employ three different strategies: i) epigenetic elimination of gliadins and LMWgs by silencing DEMETER gene(s) involved in their transcriptional de-repression, ii) post-transcriptional elimination by targeting transcripts of gliadins and LMWgs using a chimeric RNAi construct, and iii) post-translational detoxification of 'gluten' proteins by ectopic expression of an endoprotease in combination with an endopeptidase.

Epigenetic elimination of gliadins and low-molecular-weight glutenins. The concept behind targeting wheat DEMETER (5-methyl cytosine DNA glycosylase/lyase) homoeologues is inspired from studies conducted in barley and wheat, where it has been shown that promoters of gliadins and LMWgs need to be demethylated in seeds for their accumulation. DME regulates activation of LMWgs and gliadins by demethylation of their promoters, thus knocking down DME genes in principle will epigenetically eliminate most of the immunogenic prolamins (~150 in total) from wheat grains. These proteins also have been shown to be superfluous for baking.

i) Cloning and sequencing of DEMETER (DME) homoeologues. A pair of degenerate DME-specific primers (potentially amplifying homoeologous copies of DME from wheat genome covering nucleotide pos. 13263-13618 on FM164415.1) was designed and used to amplify fragment(s) from wheat genomic DNA. The PCR fragment was used to screen the hexaploid wheat Chinese Spring BAC library containing 1.3 million clones. Macroarray hybridizations led to the identification of three unique BAC clones. The three BAC clones (1946D08, 2106P11, and 2159B03) were sequenced at >60-fold coverage by a 454 sequencing method at the DNA Sequencing Core, WSU, Pullman. A total of 38.9 Mb of good-quality sequences were obtained. Analysis of sequences obtained from the above three BAC clones revealed that each harbors a full-length DME sequence (accession numbers JF683316-JF683318). The three DME sequences differ in length, ranging from 12.27 kb for 2106P11 to 12.63 kb for 1946D08. The observed differences in the length of DME homoeologues are mostly due to insertions and deletions (InDels) in the introns. A large number of point mutations and small InDels between DME homoeologous also exist in exons and contribute to the observed diversity in the protein sequences. A total of 135 homoeologous sequence variants (HSVs) giving a frequency of 22.7 HSVs/kb in exons and 584 HSVs giving a frequency of 90 HSVs/kb in introns. Of the 135 HSVs, 60 (44.44%) contain amino acid substitutions in at least one of the three DME homoeologues. Comparison of DME homoeologues with mapped wheat ESTs showed high levels of homology with BE471039 and allowed their assignment to the long arm of wheat group-5 chromosomes (5AL, 5BL, and 5DL). Assignment of DME homoeologues to specific subgenomes of bread wheat was confirmed using homoeologues-specific primers derived by tagging their 3'-ends at HSVs. The homoeologue-specific primers allowed unambiguous assignment of 2159B03 to chromosome 5A, 1946D08 to chromosome 5B, and 2106P11 to chromosome 5D. Subgenome assignment of DEMETER homoeologues TaDEM-5B was validated further by the use of wheat group-5-specific nulli-tetrasomic lines, whereas, the subchromosomal location of TaDEM-5B to a subcentromeric-bin encompassing 98.78 Mb of genomic DNA on 5BL, was determined using 5B-specific, terminal and interstitial deletion lines. Comparison of the full-length DEMETER sequences with wheat ESTs available in the public domain suggested that all three copies of DEMETER are transcriptionally active.

ii) TILLING of DEMETER homoeologues in tetraploid and hexaploid wheats. In cooperation with Arcadia Biosciences, we screened for DEMETER mutations in tetraploid Kronos and hexaploid Express wheat M_2 populations by Targeting Induced Local Lesions in Genome (TILLING). The average mutation density in *ethyl methanesulfonate* (EMS) mutagenized M_2 population of Kronos was 1 mutation per 40 kb DNA, and the mutation density for Express was 1 mutation per 24 kb DNA. The mutations are mostly single nucleotide polymorphisms or small deletions. Two runs, one each, with a set of subgenome specific primers were executed on Kronos and Express M_2 DNA-bulks. The subgenome-specific DEMETER primers amplified 1,050 bp from the A subgenome and 1,044 bp from the B subgenome of tetraploid and hexaploid wheats. In total, 39 mutations in the A homoeologue and 35 mutations in the B homoeologue of TdDEM were detected in tetraploid wheat. Similarly, 42 mutations in the A homoeologue and 53 mutations in the B homoeologue of TaDEM were detected in hexaploid wheat. Heterozygous and homozygous M_3 mutants were propagated in glasshouse to obtain M_4 seeds. These were analyzed for the effect of mutations on DME transcription using qRT-PCR and accumulation of gliadin and low molecular glutenins using SDS-PAGE gene analyses, followed by RP-HPLC.

Single mutations in the A and B subgenome DEMETER homoelogues of bread and durum wheat identified as above were recently crossed in combinations to obtain DEMETER double mutants. All crosses were made reciprocally and in duplicates. The F_1 and resulting F_2 seeds were obtained for eight different mutant combinations. The F_2 grains obtained from the aforementioned crosses were propagated in 48 well flats and are currently being tested for homo-/heterozygous double mutations by PCR followed by sequencing. The preliminary analysis allowed identification of seven double mutations in the Kronos background and 14 double mutants in the Express background. These selected double mutants were transferred to larger pots and are currently being cultivated in glasshouse to obtain F_3 grains to study effect of mutations on transcriptional and translational profiles of DEMETER homoeologues.

Recently, a subgenome-specific primer pair amplifying a 1,008 bp fragment from the active site of a D subgenome DEMETER homoeologue of hexaploid wheat was used to screen the Express TILLING library. In total, 25 mutations in the DEMETER D subgenome homoeologue were detected. Some of these selected D-subgenome mutations will be crossed with the DEMETER double mutations identified from the analysis to obtain DEMETER triple mutations.

iii) Silencing wheat DEMETER genes using artificial microRNAs (amiRNAs) and hairpin constructs. A total of 342 putative transformants were obtained by four rounds of biolistic transformations using five different constructs, where three express artificial microRNAs (pRB104, pRB105, and pRB106) and two express hairpin RNA (p728 and pDRB6). All of the above single-cassette vectors were co-transformed in 2:1 proportion with another single cassette vector (pDPG165) expressing the *Bar* gene cloned under the control of 35S promoter and Nos terminator using biolistic transformation of

scutellar calli derived from soft white winter wheat varieties (Brundage 96 and Simon) adapted to Pacific Northwest United States. The co-transformation provides the option for elimination of false positives by regenerating calli on selective media with increasing quantities of herbicide (bialaphos; up to 5 µg/ml). And in principle has greater opportunity for independent integration(s) of the two cassettes at different loci, thus allowing removal of the undesirable marker gene(s) by random assortment. The plants recovered from the tissue culture were transferred to soil and vernalized for 8 weeks. After vernalization the plants were transplanted to 6-inch pots, and their leaves were painted with a 2% Ignite solution (active ingredient biolaphos) and data were recorded for injury on 0–5 scale, where 0 represents no injury and 5 represents dead tissue.

Two weeks after transplanting, leaf tissue was collected from 153 putative transformants (T_0) of the first round of transformations, and DNA was extracted to study integration of ami/hpRNA expressing cassettes in the wheat genome. Clear integrations were observed in the genomic DNA of twenty (13.07%) of the above 153 T_0 plants. The integrations were confirmed by sequencing of PCR products obtained using construct-specific primers in 11 (55%) cases. However, good quality sequencing reads could not be obtained in nine cases (plants transformed with p728), even after multiple attempts. The failure to obtain good a quality sequence can be attributed to the nature (hairpin) of construct used to transform these plants.

In the T_0 generation, transgene integrations are in hemizygous state, and plants obtained from transformed calli mostly represent chimeric plants, which also is apparent in our case by the number of tillers produced per plant. Thus, we have not discarded any plants at this stage on the basis of PCR results. T_0 spikes were collected from all 153 plants to extract RNA from immature grains to study transcriptional suppression of wheat DEMETER homoeologues.

RNA was extracted from developing T_1 grains harvested 17 (±3 days) days post anthesis (DPA) from the T_0 spikes of 153 putative transformants. The spikes were collected in liquid nitrogen. Between 0.15 to 0.3 g of the developing grains were pulverized to isolate RNA using TRIZOL reagent (Invitrogen Corp., Carlsbad, CA, USA) following the manufacturer's recommendations. The qRT-PCR analysis of TaDME transcripts was performed using the DNA Master SYBR Green 1 chemistry on the *LightCycler® 480 Real-Time PCR* System (Roche Diagnostics, Indianapolis, IN, USA) using degenerate wheat DEMETER (amplifying three DEMETER homoeologues) and Actin specific primers (used as internal control). TaDME mRNA level was normalized to Actin using the DDC_T method (Livak and Schmittgen 2001 Methods 25:402). Transcript levels were expressed as a ratio of TaDME transcripts (normalized to Actin) in control (Brundage 96) and other putative transformants (co-transformed with pDPG165 and vectors expressing hairpin and artificial micro RNAs; see above).

Of the 153 putative transformants obtained from the first round of transformations, suppression of the DMETER transcript abundance was observed in 37 cases (24.18%), where the suppression levels range from ~30% to 65%. Five of the above 37 plants also showed integration of DEMETER silencing cassettes in the wheat genome using PCR, however, integration(s) cannot be confirmed in the remaining 32 cases due to a high level of chimerism in plants and possibly due to a low copy number of integrated cassettes. The low level of DEMETER suppression observed in the transformants can also be attributed to high level of chimarism in plants.

On the basis of the results of PCR and qRT-PCR analysis, a set of 52 (33.98%) transformants was selected for further analysis. Of the above 52 plants, 50 gave seeds (T_1) and were used for protein extraction following the protocol described in Wieser et al. (1998) with minor modifications. The three different fractions albumins/globulins (salt soluble fraction), gliadins (aqueous alcohol) and glutenins (aqueous alcohol with reducing agents) were extracted from the T_1 grains obtained from each transformant, and were analyzed by loading on appropriate (SDS- or A-PAGE) gels.

The extracted proteins were first quantified using Bradford colorimetric assay followed by quantitative (by loading equal volume of extracted proteins obtained from equal amount of starting seed material) and qualitative analysis (by loading equimolar amounts of proteins) on gel and/or HPLC. Preliminary results of PAGE gel analysis and RP-HPLC revealed elimination of specific gliadins (in gamma and alpha/beta fractions) and glutenins (in LMW glutenin fraction) instead of mass eliminations. These observations have been attributed to the bulk harvest of all T_1 grains from individual T_0 plants that are chimeric in nature, thus diluting the effect in the protein gels as well as RP-HPLC profiles. In order to deal with the problem of chimerism we are currently propagating ~2,700 T_1 progeny plants in the glasshouse and equal number of plants at Cook Agronomy Farm, Washington State University, Pullman. To determine the identity of the eliminated proteins and the novel peaks that appeared in the HPLC profiles we are currently working on establishing a standard procedure for the analysis of intact proteins on MALDI-TOF MS and LC-ESI MS.

Post-transcriptional elimination of gliadins and LMWgs. In order to achieve the transcriptional silencing, we designed a novel hairpin construct which contain a chimeric stem derived from a number of miRNAs. Each are designed from a conserved region indentified by individually aligning different kind of gliadins and LMWgs. A truncated version of wheat TAK14 intron was used as the loop. The construct was cloned in gamma sub-genome of barley streak mosaic virus (BSMV) to be used in virus induced gene silencing (VIGS) and also with 1Dy HMWg promoter and Nos terminator for RNAi. The VIGS results showed significant reduction in the amount of gliadins and LMWgs. The putative transformants obtained using this construct are currently in glasshouse and will be analyzed for elimination of celiac causing gliadins and glutenins.

Post-translational detoxification of gluten proteins. Post-translational detoxification of gluten proteins by a combination of glutamine and proline specific endo-peptidase/proteases is currently considered as a therapeutic alternative for celiac patients. In view of the therapeutic potential of this approach we decided to express thermostable and codon optimized forms of these enzymes in the wheat endosperm. This anticipates obtaining transgenic grains expressing large amounts of these enzymes, which in turn can be mixed with the dough of nontransgenic wheat and baked into whole/cracked grain breads to be consumed by the celiac patients with nutritional benefits of whole grains and gluten detoxifying enzymes. The grains with these enzymes are also beneficial for the healthy individuals as it expedites and improves digestion of gluten proteins.

i) Virtual digestion of prolamins with endopeptidases and endoprotease under simulated gastro-intestinal conditions. A total of 1,336 prolamin sequences including wheat α/β- (151), γ- (272), and ω- (13) gliadins; LMW- (457) and HMW- (318) glutenins; barley B- (26), C- (22), γ- (30), and D- hordeins (4); and rye γ/ω- (26) and HMW- (17) secalins were virtually digested under simulated gastric conditions with barley EP-B2 or a mixture of wheat endopeptidases followed by *Flavobacterium meningosepticum* prolyl endopeptidase (FM-PEP) or *Aspergillus niger* prolyl endopeptidase (AN-PEP) treatment. Virtual digestion with pepsin, trypsin and chymotrypsin left a significantly large number of peptides with ≥10 residues undigested. The length of peptides left undigested ranged from 2–144 residues for γ-gliadins followed in order by ω-gliadins (2–132 residues), HMW-glutenins (2–122 residues), α/β-gliadins (2–119 residues), and LMW-glutenins (1–70 residues). The length of proteolytically resistant hordein peptides fall within the range of undigested peptides reported for wheat prolamins. For instance, the length of proteolytically resistant hordein peptides ranged from 2–130 residues for D-hordeins, 2–118 residues for C-hordeins, and 2–74 residues for B- and γ-hordeins. However, γ/ω-secalins were among the least properly digested prolamins leaving large peptides up to 339 residues undigested, in comparison with HMW-secalins (2–99 residues) and other prolamins from wheat and barley.

The optimal length of peptides stimulating T-cell response is 10–15 residues has been documented in the literature. Thus, any peptide ≥10 residues in length would potentially elicit immune response. The proteolytically resistant peptides when compared with 44 immunogenic peptides documented in the literature, encountered 413 cases showing similarity in γ-gliadins, 179 cases in α/β-gliadins, and two cases in ω-gliadins. Similarly, 17 cases were encountered in γ- and ω-secalins. However, the number of immunogenic-peptides detected in each group is biased as most of the studies conducted so far were based on wheat α/β-, γ- and ω-gliadins, thus listing only the immunogenic peptides underlying these gliadins. We consider it likely that a systematic study conducted for immunogenic-peptides underlying other prolamins will significantly add to the repertoire of immunogenic peptides.

Prolamins digested with barley EP-B2 or a mixture of wheat endoproteases significantly reduce the size of proteolytically resistant peptides leading to a greater reduction in the number of immunogenic peptides, leaving only 76 out of 179 immunogenic peptides detected in α/β-gliadins and 1 out of 2 immunogenic peptides detected in ω-gliadins. Although wheat endoproteases work better on γ and ω-gliadins, EP-B2 works better for the rest. In view of the results of the virtual digestion, and considering the fact that EP-B2 is one of the best-characterized endoproteases from Triticeae, it has been proposed as a component of combined therapy. Both FM-PEP and AN-PEP were equally active against the peptides digested with EP-B2 or wheat endoproteases under simulated gastro-intestinal conditions, except for ω-gliadins where FM-PEP performed better than AN-PEP. In view of the results of in silico analysis we undertook a nutraceutical approach to express barley EP-B2 and FM-PEP in large quantities in the wheat endosperm to detoxify immunogenic gluten proteins.

ii) Transformation of wheat scutellar calli. Biolistic bombardment of wheat scutellar calli resulted in a total of 91 putative transformants that survived bialaphos treatment. Of the 91 putative transformants, 54 were transformed with pDPG165: pHMWg+Fmen+nos: pHMWg+EP-B2+nos used in a molar ratio of 1:2:2, and 37 were transformed with pDPG165: pBSK^{+}(HMWg+Fmen+ nos/HMWg+EP-B2+nos) used in a molar ratio of 1:2. When screened us-

ing gene specific primers to confirm transgene integration(s), 20 putative transformants showed integration(s) only for pHMWg+Fmen+nos, four showed integration(s) only for pHMWg+EP-B2+nos, and six plants showed integrations for both. Of the six plants showing integrations for both genes, two plants were obtained using the double cassette linear construct and four were obtained using co-transformation of single cassette circular constructs. Results of the PCR analyses were validated by sequencing of the PCR products obtained from the positive transformants, all of the products showed perfect sequence identity with the genes used for transformation, further confirming the transgene integration(s). The results showed that the minimal gene cassettes (which were linear DNA fragments lacking vector sequences), excised from the plasmids, function as efficiently as whole plasmids containing the suitable gene constructs for wheat transformation. The linear constructs are totally devoid of the vector backbone thus allowing isolation of marker and vector free transformants, and serving as a perfect example of 'clean' DNA technology for the production of plants expressing agronomically important traits.

The isolated transformants will be raised to maturity and expression of desired enzymes will be verified at transcript level using qRT-PCR. Their activity will be determined using activity assays: FM-PEP activity will be determined as described in Yoshimoto et al. (1980; J Biological Chem 255:4786) and Chevallier et al. (1992; J Biol Chem 267:8192), for EP-B2, as described in Marti et al. (2005; J Pharmacol Exp Ther 312:19) and Bethune et al. (2006; Chem biol 13:637). The grains expressing high amounts of the two enzymes will be tested in whole grain breads prepared by the addition of whole/cracked transgenic grains to the dough (prepared from normal wheat flour) just prior to baking process to avoid hydration and thermal denaturation of the enzymes.

Establishment of a novel transformation procedure based on microspore culture and electroporation. Similar to barley, time-lapse tracking of wheat microspores clearly showed three developmental pathways for microspore development, and the fate of the developing microspore depends upon the choice of the developmental pathway. All of these developmental pathways have their hallmarks, which allow identification of the fate of microspore cultures in advance. For instance, the ultra-structural study of microspores using transmission electron microscopy revealed three cell types, where the first type displayed a thin intine layer and an undifferentiated cytoplasm, the second type showed a thick intine layer and a starch-rich cytoplasm (similar to developing pollen grains) and the third type showed an intermediate phenotype. Accumulation of starch in the pollen amyloplasts marks the commitment to the pollen developmental pathway. Our observations indicate the enlarged cells that evolved to pollen morphology after treatment with specific conditions were still committed to the gametophytic pathway, probably representing the type III developmental pathway as identified by time-lapse tracking study in barley (de Maraschin et al. 2005; Planta 220:531). On the other hand, microspores with undifferentiated cytoplasm have been associated with the repression of the gametophytic pathway. Prior to induction of androgenesis, wheat and barley uninucleate microspores are characterized by the lack of specialized morphological structures in the cytoplasm and a thin intine layer. This suggests that, in wheat and barley, the microspores with undifferentiated cytoplasm and a thin intine layer after specific treatment, are associated with the repression of the gametophytic pathway (developmental types I and II). The maintenance of a thin intine layer after specific treatment seems to represent an early morphological marker for induced microspores in wheat. Tracking showed that the first developmental change associated with dividing microspores (developmental types I and II) was a star-like morphology, which was characterized as a transitory stage between vacuolated microspores after pretreatment and the initiation of cell division. Although the star-like morphology appears to be a morphological marker for the initiation of cell division in treated microspores, a star-like morphology per se does not assure that a microspore will ultimately commit to the embryogenic pathway. The occurrence of a star-like morphology is a dynamic process, in which the time of occurrence will depend on the type of treatment applied and the stage of microspore development. In wheat, microspores of developmental type I displayed the tendency to form a star-like morphology relatively later than type-II microspores.

The morphological markers identified from the time-lapse and ultrastructural studies have significantly improved the efficiency of getting viable calli from wheat microspore cultures, which in turn influence the number of putative transformants obtained per experiment. To obtain the optimal conditions for microspore-based transformations we transformed wheat microspores with constructs expressing three different marker genes [*green fluorescence protein* (*GFP*), *beta-glucuronidase (GUS*), and endochitinase] using different electroporation and culture conditions. A wide range of electroporation voltages ranging from 150–1,000 V were tested and a range of 250–500 V was found optimal with a peak ~375 V, at which maximum number of transformants were recovered. Similarly, pretreatment of immature spikes with $CuSO_4$ solution (500 mg/L) at 4°C for 10 days and incubation of microspores after transfection at 24°C resulted in recovery of large number green plants. Following these optimal conditions, we recently performed transformations of wheat microspores using *GUS*, *GFP*, and *endochitinase* expressing cassette and confirmed integration of these cassettes in the wheat genome by PCR followed by sequenceing of the PCR fragments.

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H.E. Bockelman, Agronomist and Curator.

Recent PI Assignments in Triticum, Secale, Aegilops, and X Triticosecale.

Passport and descriptor data for these new accessions can be found on the Germplasm Resources Information Network (GRIN): http://www.ars-grin.gov/npgs. Certain accessions may not be available from the National Small Grains Collection due to intellectual property rights, quarantine, or insufficient inventories. Accessions registered in the *Journal of Plant Registrations* or *Crop Science* are available by contacting the developers.

Table 1. Recent PI assignments in *Triticum, Aegilops*, and *X Triticosecale*. There were no PI assignments in *Secale* in the past year.

PI number	Taxonomy	Cultivar name or identifier	Country	State/Province
660666 PVPO	*Triticum aestivum* subsp. *aestivum*	Bridgeport	United States	New York
660667 PVPO	*Triticum aestivum* subsp. *aestivum*	Hopkins	United States	New York
660669	*Triticum aestivum* subsp. *aestivum*	Daws High PPO	United States	Washington
660670	*Triticum aestivum* subsp. *aestivum*	Daws Low PPO	United States	Washington
660671	*Triticum aestivum* subsp. *aestivum*	Centennial High PPO	United States	Washington
660672	*Triticum aestivum* subsp. *aestivum*	Centennial Low PPO	United States	Washington
660981 PVPO	*Triticum aestivum* subsp. *aestivum*	Duclair	United States	Montana
660987	*Triticum aestivum* subsp. *aestivum*	MT06X424-B6	United States	Montana
660988	*Triticum aestivum* subsp. *aestivum*	MT06X424-B20	United States	Montana
661061 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Mayville	United States	North Dakota
661062 PVPO	*Triticum aestivum* subsp. *aestivum*	Pivot	United States	North Dakota
661099 PVPO	*Triticum aestivum* subsp. *aestivum*	12303W	United States	Indiana
661100 PVPO	*Triticum aestivum* subsp. *aestivum*	W00109G	United States	Indiana
661101 PVPO	*Triticum aestivum* subsp. *aestivum*	112310W	United States	Indiana
661102 PVPO	*Triticum aestivum* subsp. *aestivum*	112306W	United States	Indiana
661103 PVPO	*Triticum aestivum* subsp. *aestivum*	112311W	United States	Indiana
661104 PVPO	*Triticum aestivum* subsp. *aestivum*	Blanca Grande 515	United States	California
661105 PVPO	*Triticum aestivum* subsp. *aestivum*	Malbec	United States	California
661114 PVPO	*X Triticosecale spp.*	718S	United States	California
661152	*Triticum aestivum* subsp. *aestivum*	WA8059	United States	Washington
661153	*Triticum aestivum* subsp. *aestivum*	Vision 30	United States	Virginia
661154	*Triticum aestivum* subsp. *aestivum*	Vision 40	United States	Virginia
661159 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Idamax	United States	Montana
661160 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Fuzion	United States	Montana
661215 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Matlock	United States	North Dakota
661991 PVPO	*Triticum aestivum* subsp. *aestivum*	Ruby Lee	United States	Oklahoma
661992 PVPO	*Triticum aestivum* subsp. *aestivum*	Garrison	United States	Oklahoma

Table 1. Recent PI assignments in *Triticum*, *Aegilops*, and *X Triticosecale*. There were no PI assignments in *Secale* in the past year.

PI number	Taxonomy	Cultivar name or identifier	Country	State/Province
661995 PVPO	*Triticum aestivum* subsp. *aestivum*	Tiger	United States	Kansas
661996 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Cedar	United States	Kansas
661997 PVPO	Triticum turgidum subsp. durum	WB-Belfield	United States	North Dakota
662035 PVPO	*Triticum aestivum* subsp. *aestivum*	Red Ruby	United States	Michigan
662036 PVPO	*Triticum aestivum* subsp. *aestivum*	Jewel	United States	Michigan
662047 PVPO	*Triticum aestivum* subsp. *aestivum*	SY Ovation	United States	Washington
662048 PVPO	*Triticum aestivum* subsp. *aestivum*	SY Soren	United States	North Dakota
662049 PVPO	*Triticum aestivum* subsp. *aestivum*	SY 1526	United States	Indiana
662050 PVPO	*Triticum aestivum* subsp. *aestivum*	SY Wolf	United States	Colorado
662051 PVPO	*Triticum aestivum* subsp. *aestivum*	SY Tyra	United States	Montana
662053	*Aegilops tauschii*	TKM02-010	Turkmenistan	
662055	*Aegilops tauschii*	TKM03-016	Turkmenistan	
662056	*Aegilops tauschii*	TKM08-038	Turkmenistan	
662058	*Aegilops tauschii*	TKM11-067	Turkmenistan	
662060	*Aegilops tauschii*	TKM12-085	Turkmenistan	
662062	*Aegilops tauschii*	TKM13-100	Turkmenistan	
662063	*Aegilops tauschii*	TKM13-101	Turkmenistan	
662064	*Aegilops tauschii*	TKM14-114	Turkmenistan	
662065	*Aegilops tauschii*	TKM16-126	Turkmenistan	
662066	*Aegilops tauschii*	TKM18-141	Turkmenistan	
662067	*Aegilops tauschii*	TKM26-178	Turkmenistan	
662068	*Aegilops tauschii*	TKM28-193	Turkmenistan	
662069	*Aegilops tauschii*	TKM29-198	Turkmenistan	
662070	*Aegilops tauschii*	TKM31-219	Turkmenistan	
662072	*Aegilops tauschii*	TKM34-253	Turkmenistan	
662076	*Aegilops tauschii*	TKM39-345	Turkmenistan	
662078	*Aegilops tauschii*	TKM46-397	Turkmenistan	
662084	*Aegilops tauschii*	TJK2006:042	Tajikistan	Khujand
662091	*Aegilops tauschii*	TJK2006:078	Tajikistan	Khujand
662095	*Aegilops tauschii*	TJK2006:092	Tajikistan	Khujand
662105	*Aegilops tauschii*	TJK2006:154	Tajikistan	Khujand
662106	*Aegilops tauschii*	TJK2006:159	Tajikistan	Khujand
662111	*Aegilops tauschii*	TJK2006:185	Tajikistan	Khujand
662112	*Aegilops tauschii*	TJK2006:191	Tajikistan	Khujand
662116	*Aegilops tauschii*	TJK2006:200	Tajikistan	Khujand
662149	*Triticum monococcum* subsp. *aegilopoides*	TUR-05-BJS-HB-025	Turkey	Urfa
662221	*Triticum monococcum* subsp. *aegilopoides*	GR05-052	Greece	Central Greece
662222	*Triticum urartu*	IG 44827	Jordan	
662223	*Triticum urartu*	IG 44831	Syria	
662224	*Triticum urartu*	IG 44832	Syria	
662225	*Triticum urartu*	IG 44943	Jordan	
662226	*Triticum urartu*	IG 110748	Syria	
662227	*Triticum urartu*	IG 110753	Syria	
662228	*Triticum urartu*	IG 110766	Syria	
662229	*Triticum urartu*	IG 110784	Syria	

Table 1. Recent PI assignments in *Triticum, Aegilops*, and *X Triticosecale*. There were no PI assignments in *Secale* in the past year.

PI number	Taxonomy	Cultivar name or identifier	Country	State/Province
662230	*Triticum urartu*	IG 110834	Lebanon	
662231	*Triticum urartu*	IG 110835	Lebanon	
662232	*Triticum urartu*	IG 110840	Lebanon	
662233	*Triticum urartu*	IG 110844	Lebanon	
662234	*Triticum urartu*	IG 113240	Iran	
662235	*Triticum urartu*	IG 113241	Iran	
662236	*Triticum urartu*	IG 113243	Iran	
662237	*Triticum urartu*	IG 113245	Iran	
662238	*Triticum urartu*	IG 113247	Iran	
662239	*Triticum urartu*	IG 113249	Iran	
662240	*Triticum urartu*	IG 113250	Iran	
662241	*Triticum urartu*	IG 113251	Iran	
662242	*Triticum urartu*	IG 113252	Iran	
662243	*Triticum urartu*	IG 45212	Lebanon	
662244	*Triticum urartu*	IG 45219	Armenia	
662245	*Triticum urartu*	IG 45260	Syria	
662246	*Triticum urartu*	IG 45261	Syria	
662247	*Triticum urartu*	IG 45262	Syria	
662248	*Triticum urartu*	IG 45263	Syria	
662249	*Triticum urartu*	IG 45278	Syria	
662250	*Triticum urartu*	IG 45281	Syria	
662251	*Triticum urartu*	IG 45282	Syria	
662252	*Triticum urartu*	IG 45283	Syria	
662253	*Triticum urartu*	IG 45284	Syria	
662254	*Triticum urartu*	IG 45475	Lebanon	
662255	*Triticum urartu*	IG 45476	Lebanon	
662256	*Triticum urartu*	IG 45484	Syria	
662257	*Triticum urartu*	IG 45485	Syria	
662258	*Triticum urartu*	IG 45486	Syria	
662259	*Triticum urartu*	IG 45487	Syria	
662260	*Triticum urartu*	IG 45488	Syria	
662261	*Triticum urartu*	IG 45489	Lebanon	
662262	*Triticum urartu*	IG 109087	Iraq	
662263	*Triticum urartu*	IG 113304	Iran	
662264	*Triticum urartu*	IG 115813	Jordan	
662265	*Triticum urartu*	IG 115814	Jordan	
662266	*Triticum urartu*	IG 115816	Jordan	
662267	*Triticum urartu*	IG 115817	Jordan	
662268	*Triticum urartu*	IG 45285	Syria	
662269	*Triticum urartu*	IG 45286	Syria	
662270	*Triticum urartu*	IG 45287	Syria	
662271	*Triticum urartu*	IG 45288	Syria	
662272	*Triticum urartu*	IG 45292	Syria	
662273	*Triticum urartu*	IG 45293	Syria	
662274	*Triticum urartu*	IG 45298	Syria	
662275	*Triticum urartu*	IG 45299	Syria	
662276	*Triticum urartu*	IG 45300	Syria	

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PI number	Taxonomy	Cultivar name or identifier	Country	State/Province
662277	*Triticum urartu*	IG 45301	Syria	
662278	*Triticum urartu*	IG 45462	Syria	
662279	*Triticum urartu*	IG 45470	Lebanon	
662280	*Triticum urartu*	IG 45471	Lebanon	
662281	*Triticum urartu*	IG 117911	Syria	
662282	*Triticum urartu*	IG 118182	Syria	
662283	*Triticum urartu*	IG 118183	Syria	
662386 PVPO	*Triticum aestivum* subsp. *aestivum*	Fineway 2	United States	Washington
662387 PVPO	*Triticum aestivum* subsp. *aestivum*	Prosper	United States	North Dakota
663156 PVPO	*Triticum aestivum* subsp. *aestivum*	Lucin CL	United States	Utah
663157	*Triticum aestivum* subsp. *aestivum*	Curlew	United States	Utah
663158	*Triticum aestivum* subsp. *aestivum*	Harry-H9	United States	Washington
663159	*Triticum aestivum* subsp. *aestivum*	Wesley-*Lr37-Yr17-Sr38*	United States	Washington
663160	*Triticum aestivum* subsp. *aestivum*	Goodstreak-*Dn4*	United States	Washington
663161	*Triticum aestivum* subsp. *aestivum*	Alliance-*Lr37-Yr17-Sr38*	United States	Washington
663162	*Triticum aestivum* subsp. *aestivum*	Arrowsmith-*Lr37-Yr17-Sr38*	United States	Washington
663163	*Triticum aestivum* subsp. *aestivum*	Millenium-*Lr37-Yr17-Sr38*	United States	Washington
663164	*Triticum aestivum* subsp. *aestivum*	Millenium-*Dn4*	United States	Washington
663165	*Triticum aestivum* subsp. *aestivum*	Harry-*Lr37-Yr17-Sr38*	United States	Washington
663166	*Triticum aestivum* subsp. *aestivum*	Harry-*Dn4*	United States	Washington
663167	*Triticum aestivum* subsp. *aestivum*	Wahoo-*H9*	United States	Washington
663168	*Triticum aestivum* subsp. *aestivum*	Wahoo-*Dn4*	United States	Washington
663169	*Triticum aestivum* subsp. *aestivum*	Wahoo-*Lr37-Yr17-Sr38*	United States	Washington
663170	*Triticum aestivum* subsp. *aestivum*	Wesley-*H9*	United States	Washington
663205 PVPO	*Triticum aestivum* subsp. *aestivum*	Spika	United States	Idaho
663206 PVPO	*Triticum aestivum* subsp. *aestivum*	NC-Yadkin	United States	North Carolina
663207 PVPO	*Triticum aestivum* subsp. *aestivum*	Accipiter	Canada	Saskatchewan
663208 PVPO	*Triticum aestivum* subsp. *aestivum*	Peregrine	Canada	Saskatchewan
663216	Triticum turgidum	DGE-2	United States	North Dakota
663870	*Triticum aestivum* subsp. *aestivum*	KS12WGGRC55	United States	Kansas
663945	*Triticum aestivum* subsp. *aestivum*	Kaixian-luohanmai	China	Sichuan
663950	*Triticum aestivum* subsp. *aestivum*	Fieldstar	Canada	Manitoba
664028	*Triticum aestivum* subsp. *aestivum*		Kharoba	Morocco
664077	*Triticum aestivum* subsp. *aestivum*	PI350078-sel-awl	United States	Idaho
664078	*Triticum aestivum* subsp. *aestivum*	E5024	United States	Michigan
664079	*Triticum aestivum* subsp. *aestivum*	Jupiter	United States	Michigan
664088 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Tucson	United States	Montana
664089 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Perla	United States	Arizona
664090 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Patron	United States	Arizona
664091 PVPO	Triticum turgidum subsp. durum	WB-Mead	United States	Arizona
664092 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-1070CL	United States	Montana
664093 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Quake	United States	Montana
664094 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-249	United States	Indiana
664095 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-196	United States	Indiana
664096 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-139	United States	Indiana

Table 1. Recent PI assignments in *Triticum, Aegilops*, and *X Triticosecale*. There were no PI assignments in *Secale* in the past year.

PI number	Taxonomy	Cultivar name or identifier	Country	State/Province
664097 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-112	United States	Indiana
664098 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Joaquin Oro	United States	Arizona
664099 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-1066CL	United States	Montana
664100 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Junction	United States	Montana
664101 PVPO	*Triticum aestivum* subsp. *aestivum*	WB-Arrowhead	United States	Montana
664102 PVPO	Triticum turgidum subsp. durum	Tipai	United States	California
664103 PVPO	*Triticum aestivum* subsp. *aestivum*	Dinero	United States	Colorado
664250	*Triticum aestivum* subsp. *aestivum*	NC10-23603	United States	North Carolina
664251	*Triticum aestivum* subsp. *aestivum*	NC8889-8	United States	North Carolina
664252	*Triticum aestivum* subsp. *aestivum*	NC8860-5	United States	North Carolina
664253	*Triticum aestivum* subsp. *aestivum*	NC8889-2A	United States	North Carolina
664254	*Triticum aestivum* subsp. *aestivum*	NC8889-3	United States	North Carolina
664255 PVPO	*Triticum aestivum* subsp. *aestivum*	Brawl CL Plus	United States	Colorado
664256	*Triticum aestivum* subsp. *aestivum*	Denali	United States	Colorado
664257	*Triticum aestivum* subsp. *aestivum*	Byrd	United States	Colorado
664268 PVPO	*Triticum aestivum* subsp. *aestivum*	New Dirkwin	United States	California
664269 PVPO	*Triticum aestivum* subsp. *aestivum*	W000582Z2	United States	Indiana
664270 PVPO	*Triticum aestivum* subsp. *aestivum*	XW09H	United States	Indiana
664271 PVPO	*Triticum aestivum* subsp. *aestivum*	Rimrock	Canada	Ontario
664272 PVPO	*Triticum aestivum* subsp. *aestivum*	VA258	United States	Virginia
664301	*Triticum aestivum* subsp. *aestivum*	CO08RWA050	United States	Colorado
664304 PVPO	*Triticum aestivum* subsp. *aestivum*	Bruneau	United States	Idaho
664480 MAP	*Triticum aestivum* subsp. *aestivum*	Becker	United States	Virginia
664481 MAP	*Triticum aestivum* subsp. *aestivum*	Massey	United States	Virginia
664482	*Triticum aestivum* subsp. *aestivum*	Advance	United States	South Dakota
664483	*Triticum aestivum* subsp. *aestivum*	Forefront	United States	South Dakota
664549	*Triticum aestivum* subsp. *aestivum*	UC1110 (5+10) + *Yr36*-GPC	United States	California
664556 PVPO	*Triticum aestivum* subsp. *aestivum*	SY 314	United States	California
664557 PVPO	*Triticum aestivum* subsp. *aestivum*	SY Summit 515	United States	California
664558 PVPO	*X Triticosecale spp*.	SY 115T	United States	California
664559 PVPO	*X Triticosecale spp*.	SY 158T	United States	California
664560 PVPO	*X Triticosecale spp*.	SY 141T	United States	California

V. CATALOGUE OF GENE SYMBOLS FOR WHEAT: 2012 SUPPLEMENT

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The most recent version of the Catalogue, compiled for the 11Th International Wheat Genetics Symposium held in Brisbane, Australia, and the 2009, 2010, and 2011 Supplements (*Annual Wheat Newsletter* 55:256-278; 56:256-278; 57:303-321) are available from the Komugi (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp) and GrainGenes (http://wheat.pw.usda.gov/GG2/*Triticum*/wgc/2008/) websites. The Wheat Gene Catalog is not included as part of the IWGS proceedings and, therefore, cannot be cited as part of them.

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Morphological and Physiological Traits

10. Boron Tolerance

Bo1. **v:** Additional genotypes {10833,10834}.
tv: Kalka {10834}; Linzhi 10834}; Niloticum {10834}; additional genotypes {10834}.
ma: Add: Co-dominant PCR marker AWW5L7 co-segregated with *Bo1* and was predictive of of the responses of 94 Australian wheat genotypes {10833}; *Xbarc32-7B* – 2.4 cM – *Xaww5L7* – 1.2 cM – *Xbarc182-7B*/*Bo1* – 1.2 cM – *Xpsr680*/*Xmwg2062-7B* {10833}; *Xbarc32-7B* – 2.6 cM – *Xaww7L7*/*Bo1* {10834}.

QTL: 'Cranbrook (moderately tolerant) / Halberd (tolerant)': DH population; QTL for tolerance were identified on chromosomes 7B and 7D {10832}.
Add note:
For a review of boron tolerance in wheat, see {10835}.

At be beginning of the last paragraph in the exiting file insert:
Boron efficiency

11. Cadmium Uptake

Low uptake is dominant.

Cdu1. Add: '; corrected to 5BL {10894}.' **tv:** Fanfarran {10894}.
bin: 5BL9 0.76-0.79.
ma: *Xfcp2-5B* – 12 cM – *Cdu1* – 3 cM – *ScOPC20* {10894}; *ScOPC20*/*Xrz575-5B*/*XBG608197* – 0.5 cM – *Cdu1*/*XbF293297*/*XBF474090*/*Os03g53590* (*Xusw15-5B*) – 0.2 cM – *XBF474164* {10895}. *Cdu1* is close to *Vrn-B1* {10895}.

cdu1. **tv:** DT369 {10894}.

12. Chlorophyll Abnormalities

12.2. Chlorina

cn-A1a. **i:** ANK-32 {10820}.
cn-A1d. **itv:** ANW5A-7A {10820}.
Two mutants in diploid wheat are reported in {10820}.
ma: Hexaploid wheat: *Xhbg234-7A* – 8.0 cM – *cn-A1* – 4.3 cM – *Xgwm282*/*Xgwm332-7A* {10820}; Tetraploid wheat: *Xbarc192-7A* – 19.5 cM – *cn-A1* – 11.4 cM – *Xgwm63-7A* {10820}; Diploid wheat: *Xgwm748-7A* – 29.2 cM – *cn-A1* – 33.3 cM – *Xhbg412-7A* {10820}.

17. Dormancy (Seed)

17.1. Vivipary

Insert above the present entry for *Vp-A1*.
Alleles of *Vp-A1* were recognized using STS marker A17-19 {10919}.

Vp-A1{10919}. 3AL {10919}.

Vp-A1a {10919}. **v:** Nongda 311 {10919}. **c:** 599 bp {10919}.
Higher germination index.

Vp-A1b {10919}. **v:** Wanxianbaimaizi {10919}; Yannong 15 {10919}.
c: 596 bp {10919}.
Lower germination index.

Vp-A1c {10919}. **v:** Jing 411 {10919}. **c:** 593 bp {10919}.
Higher germination index.

Vp-A1d {10919}. **v:** Xiaoyan 6 {10919}. **c:** 590 bp {10919}.
Lower germination index.

Vp-A1e {10919}. **v:** Zhengzhou 6 {10919}; Bainong 64 {10919}.
c: 581 bp {10919}.
Higher germination index.

Vp-A1f {10919}. **v:** Yumai 34 {10919} **c:** 545 bp {10919}.
Higher germination index.

Insert after the present *Vp-B1* entry.

Vp-D1 {10919}. 3DL {10919}. AJ400714 {10919}.
Vp-D1a {10919}. **v:** 81 Chinese wheat cultivars {10919}.
c: 5 pairs of primers {10919}.

17.2. Pre-harvest sprouting

Continue under the Rio Blanco cross:
Qphs.psweru-3A was fine mapped to a 1.4-cM region flanked by two AFLP markers and was tightly linked to *Xbarc57-3A* and seven other AFLP markers {10893}.

26. Glaucousness (Waxiness/Glossiness)

26.1. Genes for glaucousness

26.2. Epistatic inhibitors of glaucousness

Add to existing comment:
Although maps constructed from three tetraploid crosses suggested that *w1* and $Iw1^{DIC}$ could be at different loci, allelism of *w1*, *W1*, and $Iw1^{DIC}$ = *Vir* remain unresolved {10815}.

40. Height

40.1. Reduced Height : GA-insensitive

At end of section add:
…….are given in {10404} and those for eastern and central U.S. eastern and central winter wheat cultivars are given in {10868}.

40.2. Reduced Height : GA-sensitive

Rht8.
Add at end of section:
Allele sizes for *Xgwm261* in U.S. eastern and central wheat winter cultivars are given in {10868}.

Rht14. To the note add ‘,10818’ to the reference.

Rht16. To the note add ‘,10818’ to the reference.

Rht18. To the note add ‘,10818’ to the reference.

Rht22 {10857}. 7AS {10857}. **tv:** Aiganfanmai {10857}.
ma: *Xgwm471-7A* – 29.5 cM – *Rht22* – 20.1 cM – *Xgwm350-7A* {10857}.

46. Leaf Tip Necrosis

Ltn. **c:** Putative ABC transporter {10862}.

48. Male Sterility

48.1. Chromosomal

Ms1376 {10814}. Sterility is dominant. **v:** TR1376A {10814}.

Male fertile counterpart: TR1376B {10814}.
Ms1376 was discovered among progenies of a transgenic family of Xinong 1376 containing the leaf senescence-inhibiting gene P_{SAG12}-*IPT* {10814}.

54. Nuclear-Cytoplasmic Compatability Enhancers

scs. Add: *scs*^{ti} {10878}. **ma:** *Xbcd1449.2-1A* – 0.6 cM – *scs* – 2.3 cM – *Xbcd12-1A* {10878}.

60. Red Grain Colour

Correct and add to the first paragraph: '……Himi & Noda {10107} provided evidence that the *R* genes were wheat forms of Myb-type transcription factors (*Tamyb10-3A*, *Tamyb10-3B*, and *Tamyb10-3D*). Genetic evidence is provided in {10838}'.

R-A1. **v:** Rio Blanco {10839}.
ma: *Xwmc559-3A* – 16.3 cM – *R-A1*/*Xgwm155-3A* – 4.5 cM – *Xwmc153-3A* {10839}.

R-A1a. **ma:** Based on *Tamyb10-A1* sequences this allele in CS lacks the ability to bind DNA due to deletion of the first half of the R2 repeat of the MYB domain {10838}. The *R-A1a* allele in Norin 17 has a 2.2-bp insertion in the second intron that appears to prevent transcription {10838}.

R-B1. **ma:** *Xgwm4010-3B* – 1.6 cM – *R-B1* – 4.6 cM – *Xgwm980-3B* {10839}.

R-B1a. **ma:** Based on the *Tamyb10-B1* sequence this allele in CS has a 19-bp deletion of the CCG repeat region causing a frameshift mutation {10838}.

R-D1. **ma:** *Xgwm2-3D* – 15.4 cM – *R-D1* – 3.2 cM – *Xgwm4306-3D* {10839}.

R-D1a. **ma:** No *Tamyb10-D1* sequence was detected in lines with this allele indicating that it may be a deletion {10838}.

Add note at the end of this section:
Functional markers based on *Tamyb10* sequences are given in {10838}.

62. Response to Photoperiod

The following sections are updated on the listing in the 2009 supplement.

Ppd-A1.

Ppd-A1a {10612}. t**v:** GS100, Kofa (1,027-bp deletion in the promoter) {10612}; GS105, Svevo (1,117-bp deletion in the promoter) {10612}. A survey of *Ppd-A1* alleles is reported in {10915}.

GS100 and GS105 had different deletions relative to GS101 and GS104, respectively, and both were consistently a few days earlier flowering than their near-isogenic counterparts with *Ppd-A1b* {10612}.

Ppd-B1.

Ppd-B1a {0063}. [*Ppd2* {1566}]. 2BS {1566,1268,1269}.
i: H(C) = Haruhikari*5 / Fukuwasekomugi {10611}. H(D) = Haruhikari 5*/ Fukuwasekomugi *Ppd-D1a* {10611}.
s: Cappelle-Desprez*/CS 2B {0058}.
v: CS {1268}; Spica {557}; Timstein {1269}.
v2: Sharbati Sonora *Ppd-A1a* {887}. Fukuwasekomugi *Ppd-D1a* {10611}.
c: Varieties with the photoperiod insensitive allele have more than one *Ppd-B1* copy per chromosome 2B: two copies in Récital, three copies in Sonora 64, Timstein and C591, and four copies in Chinese Spring {10881}.

Ppd-B1b [{10611}],{10881}.
v: Cappelle-Desprez {10881}; Cheyenne {10881}; Norstar {10881}; Renan {10881}; Paragon {10881}; Beaver {10881}.
v2: Haruhikari Ppd-D1b [{10611}].
c: Varieties with the photoperiod sensitive allele have a single *Ppd-B1* copy per chromosome 2B {10881}.

63. Response to Salinity

63.3. Sodium exclusion

Add after *Nax1* and *Nax2*:

QTL for Na^+ exclusion and seedling biomass under salt stress were detected in the cross 'Berkut / Krichauff' on chromosomes 2A (*Nax1* region) and 6A (*cfd080-barc171-6A*) {10917}.

65. Response to Vernalization

Vrn-A1.

Vrn-A1. **ma:** *Xgwm271-5A* – 6.5 cM – *Vrn-A1* – 12.6 cM – *Xbarc232-5A* {10880}.

Insert heading:

Dominant spring habit alleles at the *Vrn-A1* locus

As currently listed based on the 2010 Supplement and earlier lists:

Recessive winter habit alleles at the *Vrn-A1* locus

vrn-A1. Copy number variation for *vrn-A1* was detected in IL369 (two copies) {10202}, Malacca (two copies) and Hereward (three copies). Higher copy number was associated with later flowering or with increasing requirement for vernalization (i.e., longer exposure to cold is needed to achieve full vernalization) {10881}.

vrn-A1a [{10198}]. *vrn-A1a* {10198}. **v:** Claire {10880}; Triple Dirk C {10880}.
v2: Chinese Spring *Vrn-D1a* {10880}.
c: GenBank AY616455 {10198}.

vrn-A1b {10881}. **v:** IL369 {10202}; Malacca {10881}.
c: GenBank JF965396 {10881}.

This allele has two copies of the gene, possibly arranged in tandem although the physical structure is unknown. Both copies are distinguished from Chinese Spring *vrn-A1a* by a SNP in exon 7 (T in Malacca, C in Chinese Spring). One copy also has a SNP in exon 4 (T in Malacca, C in Chinese Spring). Sequenced cDNAs from Malacca show that both copies are expressed {10881}.

vrn-A1c {10881}. **v:** Hereward {10881}.
c: GenBank JF965397 {10881}.

A comparison of Claire (*vrn-A1a*), Malacca {*vrn-A1b*}, and Hereward (*vrn-A1c*} indicated that increasing gene copy number is associated with lateness{10881}.

Two winter alleles were identified based on an SNP in exon 4 {10656}:

vrn-A1v {10916}. **v:** Don Ernesto INTA {10916}; Jagger {10916}; Norin 61 {10916}; Opal {10916}.

vrn-A1w {10916}. **v:** Bezostaya {10916}; Bavicora M 92 {10916}; Kavkaz {10916}; Gennson 81 {10916}; Seri M 82 {10916}; Wichita {10916}.

Vrn-B1. **ma:** *Tsn1* – 14.8 cM – *Vrn-B1* – 0.7 cM – *Xwmc75-5B* {10880}.

Vrn-B1c {10880}. **tv:** *T. turgidum* subsp. *carthlicum* PI 94749 {10880}.
c: GenBank JN817430, contains a 5,463 retrotransposon insertion in the 5' UTR region {10880}.

Proteins

80. Proteins

80.2. Enzymes

80.2.33. Phytoene synthase

Psy-A1.

Psy-A1t {10920}. **v:** WAWHT2074 {10920}.
ma: *Xgwm344-7A* – 3.9 cM – *Psy-A1t* – 9.9 cM – *Xcfa2257a-7A* {10920}.
c: HM006895 {10920}.

Associated with a higher flour b* value.

80.2.38. Flavone 3-hydroxylase (EC 1.14.11.9)

F3h-A1 {10823}. 2AL {10823}. **v:** CS {10823}.
ma: *Xgwm1067-2A* – 2.1 cM – *F3h-A1* – 11.4 cM – *Xgwm1070-2A* {10823}.

F3h-B1 {10823}. 2BL {10823}. **v:** CS {10823}.
ma: *F3H-B1/Xgwm1067-B1* – 11.4 cM – *Xgwm1070* {10823}.

F3h-D1 {10823}. 2DL {10823}. **v:** CS {10823}.
ma: *Xgwm877-2D* – 1.8 cM – *F3h-d1/Xgwm1264-2D* – 22.7 cM – *Xgwm301-2D* {10823}.

F3h-B2 {10823}. 2AL {10823}. **v:** CS {10823}.
ma: *Xgwm1070-2B* – 30.1 cM – *F3h-B2* {10823}. Located in the terminal region near *Xgwm1027-2B* {10823}.

80.2.39. Zeta-carotene desaturase

Zds-A1 {10905}. 2A {10905}. **tv:** Langdon {10905}

Zds-B1 {10905. 2B {10905}. **tv:** Langdon {10905}.

Zds-D1 {10906}. 2DL {10906}. **v:** CS {10906}.
Zds-D1a {10906}. *TaZDS-D1a* 10906}.
v: CA9632 {10906}. Many Chinese wheat and 80 CIMMYT lines {10906}.
Zds-D1b {10906}. *TaZDS-D1b* {10906}.
v: Ning 99415-8 {10906}; Zhengzhou 9023 {10906}; Zhongyou 9507 {10906}; Zhoumai 13 {10906}.

Cv. Zhongyou 9507 has lower yellow flour pigment content, preferred for Chinese steamed bread and dry Chinese noodles. A QTL in the *Zds-D1a* region explained 18.4% of the variation in yellow pigment content in 'Zhongyou 9507 / CA 9632' {10906}.

80.2.40. Carotenoid beta-hydroxylase (non-heme di-iron type)

HYD are non-heme di-iron b-hydroxilases that act primarily on b-carotene

Hyd-A1 {10913}. 2AL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

Hyd-B1 {10913}. 2BL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

Hyd-D1 {10913}. 2DL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

Hyd-A2 {10913}. 5AL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

Hyd-B2 {10913}. 4BL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

Hyd-D2 {10913}. 4DL {10913}. **tv:** Kronos {10913}.
v: UC1041 {10913}.

80.3. Endosperm storage proteins
80.3.1. Glutenins
80.3.1.3. *Glu-3*

Glu-A3.
Due to an error made in an earlier update, add:
Glu-A3ax [{10116}]. 6.1 {10116}. **tv:** Buck Cristal {10116}.
The designation of this protein (subunit 6.1) as an allele of *Glu-A3* was deduced from its electrophoretic mobility and awaits confirmation through mapping studies.

Glu-B3.
Due to an error made in an earlier update, delete:
Glu-B3z [{10116}].6.1 {10116}. **tv:** Buck Cristal {10116}.

80.3.3. Other endosperm storage proteins
80.5.8. Puroindolines and grain softness protein
After the second last paragraph of notes starting 'In *T. monococcum* the gene order…….' Add a new paragraph:
The soft kernel trait was transferred to durum {10899}.

80.5.9 Endosperm-specific wheat basic region leucine zipper (bZIP) factor storage activator
Spa-A1 (10908}. 1AL {10909}. **v:** Recital {10909}.

Spa-B1 {10908}. 1BL {10909}. **v:** Recital {10908}.
ma: *Glu-B1* – 1.3 cM – *Spa-B1* {10909}.
Spa-B1a {10908}. **v:** Chinese Spring {10909}; Recital {10908}; Australian genotypes listed in {10908}.
Spa-B1b {10908}. **v:** Renan {10909}; Australian genotypes listed in {10908}.

Spa-D1 {10908}. 1DL {10909}. **v:** Recital {10909}.

After testing an earlier hypothesis that SPA genes affected wheat quality, analyses conducted by both {10908} and {10909} obtained no evidence supporting a significant effect and attributed any variation to the closely linked *Glu-B1* locus.

Pathogenic Disease/Pest Reaction

81. Reaction to Barley Yellow Dwarf Virus

Bdv3. Add note:
Further translocations lines with *Bdv3* are described in {10882}.

82. Reaction to *Bipolaris sorokiniana*

Sb1 {10855}. Partial resistance. 7DS {10855,10856}.
i: HUW234Ltn+ {10855}.
v: Saar {10856}; Lines with *Lr34/Yr18/Pm38/Sr57* – see Reaction to *Puccinia triticina*, Reaction to *Puccinia striiformis*, Reaction to *Blumeria graminis*, Reaction to *Puccinia graminis*, and Leaf tip necrosis.

ma: Pleiotropic or closely linked with *Lr34*/*Yr18*/*Pm38*/*Sr57* located between *Xgwm1220-7DS* and *Xswm10-7DS* (1.0 cM interval) {10856}; see also Reaction to *Puccinia triticina*, Reaction to *Puccinia striiformis*, Reaction to *Puccinia graminis,* and Reaction to *Blumeria graminis*.
c: Putative ABC transporter {10862}.

83. Reaction to *Blumeria graminis* DC.

83.1. Designated genes for resistance

Pm3.

Pm3a. **v:** Madrid {10843}; Merker {10843}; Robigus {10843}; Tabasco {10843}.

Pm3b. **v:** Enorm {10843}.

Pm3d. **v:** Vergas {10843}.

Pm3e. **v2:** Cortez *Pm5* allele {10843}.
ma: *Pm3e* – 7.1 cM – *Xwmc818-1A* {10843}.

Pm3f. **v:** Viza {10843}.

Pm21. **bin:** 6VS 0.45-0.58 {10859}.
ma: Potentially useful markers are provided in {10918}.
c: *Pm21* is likely the serine/threonine kinase gene *Stpk-V* {10859}.

Pm31. This gene designation {0301}is not valid; subsequent studies {10918} showed the gene was *Pm21*.

Pm46 {10847}. Partial resistance. 4DL {10847,10678}.
bin: Distal to break point 0.56 FL {10678}.
i: RL6077 = 'Thatcher*6 / PI 250413' {10847,10678}.
ma: Pleiotropic or closely linked with *Lr67*/*Yr46*/*Sr55* and associated with *Xgwm165-4D* and *Xgwm192-4DL* {10847,10678}.

Pm47 {10912}. Recessive. *PmHYLZ* {10912}. 7BS {10912}.
bin: 7BS-1 c-0.27. **v:** Hongyanglazi {10912}.
ma: *Xgpw2097-7B* – 0.9 cM – *Pm47* – 3.6 cM – *Xgwm46-7B* {10912}.

83.2. Suppressors of *Pm*

SuPm8. Add comment following the present entry:
Pm8 was suppressed when locus *Pm3* is transcribed (including Chinese Spring and Thatcher which have no currently detectable *Pm3* resistance alleles {10828}.

83.3. Temporarily designated genes for resistance to *Blumeria graminis*

PmG16 {10886}. 7AL {10886}. **bin:** 7AL16 0.86-0.90.
tv: *T. turgidum* subsp. *dicoccoides* G18-16 {10886}.
ma: *Xgwm1061*/*Xgwm344-7A* – 1.2 cM – *PmG16*/*wPt-1424*/*wPt6019* – 2.4 cM – *wPt-0494*/*wPt9217*/*Xwmc809-7A* {10886}.

PmHNK54 {10897}. 2AL {10897}. **bin:** 2AL1 C-0.85.
v: Zheng 9754 {10897}.
ma: *Xgwm372-2A* – 5 cM – *PmHNK54* – 6.0 cM – *Xgwm312-2A* {10897}.

Ml3D32 {10892}. 5BL {10892}. **bin:** 5BL 0.59-0.76.
tv: *T. turgidum* subsp. *dicoccoides* I222 {10892}. **v:** 3D232 {10892}.
ma: *Xwmc415-5B* – 1.3 cM – *Ml3D232* – 3.3 cM – *CJ832481* {10892}. Co-segregation with eight EST markers including an NBS-LRR analogue {10892}.

MlAB10 {10873}. 2BL {10873}. **bin:** 2BL6 0.89-1.00.
v: NC97BGTAB10 PI 604036 {10873}.
tv: *T. turgidum* subsp. *dicoccoides* PI 471746 {10873}.
ma: *Xwmc445-2B* – 7 cM – *MlAB10* {10873}.

New: Reaction to *Cephalosporium gramineum*
Disease: Cephalosporium stripe
QTL:
'Coda (more resistant) / Brundage (less resistant)': RIL population: seven QTL identified based on whiteheads; three from Coda – *QCs.orp-2D.1* (nearest marker C, $R^2 = 0.11$), *QCs.orp-2B* (nearest marker *Xwmc453-2B*, $R^2 = 0.08$), and *QCs.orp-5B* (nearest marker *Xgwm639-5A*, $R^2 = 0.12$) and four from Brundage (*QCs.orp-2D.2* (nearest marker *Xbarc206-2D*, $R^2 = 0.04$), *QCs.orp-48* (nearest marker *wpt-3908*, $R^2 = 0.05$), *QCs.orp-5A.1* (nearest marker *wpt-3563*, $R^2 = 0.08$), *QCs.orp-5A.2* (nearest marker *B1*, $R^2 = 0.05$) {10836}.

87. Reaction to *Fusarium* spp.
87.1. Disease: Fusarium head scab, scab

Fhb4 {10884}. *Qfhi.nau-4B* {10282}. 4BL {10282,108831}.
bin: 4BL5-0.86-1.00. **i:** 'Mianyang 99-323*4/Nanda 2419/Wangshibai' {10885}.
v2: Wangshuibai *Fhb5*{10884}.
ma: Located in a 1.7-cM segment flanked by *Xhbg226-4B* and *Xgwm149*/*Xmag4580-4B* {10883}.
Although plants with *Fhb4* were taller than the recurrent parent, the height difference was not associated with the *Rht-B1* locus {10885}.

Fhb5 {10896}. *Qfhi.nau-5A* {10282}. 5AS {10896}.
bin: C-5AS3 0.75. **i:** Mianyang 99-323 and PH691 backcross derivatives selected for *Qfhi.nau-5A* {10896}.
v2: Wangshuibai *Fhb4* {10896}.
ma: Mapped to a 0.3-cM interval between *Xbarc117*/*Xbarc358*/*gwm293*/*Xgwm304-5A* and *Xgwm415-5A* {10896}.

'Ernie (I) / MO 94-317 (S)': RIL population: three QTL on chromosomes 3BSc, 4BL, and 5AS accounted for 31 and 42% of the total phenotypic variances for DON and Fusarium damaged kernels (FDK), respectively. A minor QTL ($R^2 = 0.04$) for FDK was on chromosome 2B {10831}.

Add at end of this section:
Six of nine NIL pairs made by MAS for *Xgwm0181-3B* earlier located near a FCR QTL on 3BL.

'Grandin (S) / PI 277012 (I)': DH population: Two QTL, *Qfhb.rwg-5A.1* on 5AS ($R^2 = 0.06$-0.2) and *Qfhb.rwg-5A.2* on 5AL ($R^2 = 0.12$–0.20) conferred type I and II resistance and reduced DON content {0147}. The new QTL on 5AL was closely but not completely linked with gene *q*, which is present in PI 277012 {10860}.

'Nanda 2419 / Wangshuibai': Above Type IV resistance add:
Backcross-derived NILs with *Qfh.nau-2B*, *Qfhs.nau-3B*, *Qfhi.nau-4B* (syn. *Fhb4*), and *Qfhi.nau-5A* were developed with Mianyang 99-323 as the recurrent parent {10884}.

'Wheaton (I) / Haiyanzhong': RIL population: Four QTL, *Qfhb.uhgl-7D* (syn. *Qhb.hyz-7D*), nearest marker *Xwmc121-7D*, $R^2 = 0.16$–0.20), *Qfhb.uhgl-6B.1* (*Qhb.hyz-6B.1*), $R^2 = 0.4$), *Qfhb.uhgl.6B.2* (*Qhb.hyz-6B.2*), $R^2 = 0.07$), and *QFhb.uhgl-5A* (*Qhb.hyz-5A*), $R^2 = 0.04$–0.07) were from Haiyanzhong, and *QFhb.uhgl-1A* (*QFhb.ughl-1A*), $R^2 = 0.05$) was from Wheaton {10837}.

To the paragraph beginning: In a reciprocal backcross of Chris……..{10398}' add: Further study of the 3A, 6A, and 4D reciprocal substitution lines indicated that chromosome 3A of Frontana had the largest effect on incidence, severity, spread, and kernel damage, 4D less so and 6A possibly not at all (10900}.

87.2. Disease: Crown rot caused by *Fusarium pseudograminearum*, *F. culmorum*, and other *Fusarium* species

'2-49 / W21MMT70': DH lines: Three QTL for seedling resistance, viz. *QCr.usq-1D.1*, and a weaker QTL on chromosome 7A from 2-49 and *QCr.usq-3B.1* ($R^2 = 0.41$) from W22MMT70 {10883}.

Following the entry 'Lang (S) / CSCR6' add:
Six of nine NIL pairs made by MAS for *Xgwm01081-3B* earlier located near the 3BL QTL {10703} in CSCR6 showed significant differences ($P<0.01$) in crown rot response {10891}.

'Sunco / 2-49': DH population: Three QTl for seedling resistance, viz. *QCr.usq-1D.1* and *QCr.usq.4B.1* ($R^2 = 0.19$) from 2-49 and *QCr.usq-2B.11* from Sunco {10883}.

90. Reaction to *Mayetiola destructor* (Say) (*Phytophaga destructor*) (Say)

H26. **bin:** 3DL3-0.81-1.00.

Add note:
H26 is very close to *H32* {10846}.

H32. **bin:** 3DL3-0.81-1.00.
ma: *Xrwgs10-3D* – 0.5 cM – *H32*/*Xrwgs11-3D* – 0.5 cM – *Xrwgs12-3D* {10846}.

Add note:
H32 is very close to *H26* {10846}.

Add to temporary symbols:
HNC09MDD14 [*Hf-NC09MDD14* {10844}]. 6DS {10843}.
v: NC09MDD14 PI 656395 {10843}.
dv: *Ae. tauschii* TA2492 and/or TA2377 {10843}.
ma: *Xgdm36-6D* – 1.5 cM – *HNC09MDD14*/*Xcfd123-6D* {10843}. *HNC09MDD12* could be allelic to, but is different from, *H13* {10843}.

91. Reaction to *Meloidogyne* spp.
92. Reaction to *Mycosphaerella graminicola* (Fuckel) Schroeter

Stb9 {10027}. Culture IPO89011. 2BL {10027}.
v: Courtot {10027}; Tonic {10027}.
ma: *Xfbb226-2B* – 3 cM – *Stb9* – 9 cM – *XksuF1b-2B* {10027}.

Stb16 [{10879}]. Seedling and adult-plant resistance. *Stb16q* {10879}.
3DL {10879}. **v2:** Synthetic W-7976 *Stb17* {10879}.
ma: Associated with *Xgwm494-3D* and mapped as a QTL, $R^2 = 0.4–0.7$ in seedling tests and 0.28–0.31 in mature plants {10879}.

Stb17 {10879}. Adult plant resistance. 5AL {10879}.
v2: Synthetic W-7976 Stb16 {10879}.
ma: Associated with *Xhbg247-5A* and mapped as a QTL, $R^2 = 0.12–0.32$ {10879}.

Stb18 {10827}. Confers resistance to IPO0323, IPO98022, IPO98046 {10827}.
6DS {10827}. **v2:** Balance *Stb6 Stb11* {10827}.
ma: Mapped as a QTL located in a 8.8-cM region spanned by *Xgpw3087-6D* and *Xgpw5176-6D* {10827}.

QTL: Add at end of section:
'Apache / Balance': Analyses with a panel of *M. graminicola* cultures identified QTL on chromosomes 1BS (Apache, considered to be *Stb11*), 3AS (Balance, considered to be *Stb6*), 6DS (Balance, named as *Stb18*), 7DS (Apache, considered to be *Stb4*), and 7DL (Apache) {10827}.

'Florett / Biscay (S)': RIL population: two QTL for APR located on chromosomes 3B and 6D {10901}.

'Tuareg / Biscay (S)': RIL population: two QTL for APR were located on chromosomes 4B and 6B {10901}.

93. Reaction to *Phaeosphaeria nodorum* (E. Muller) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano).

93.1. Genes for resistance

QTL
'Salamouni / Katepwa': RIL population: Two QTL, *QSnb.fcu-1A* (*Snn4*) ($R^2 = 0.24$) and *QSnb.fcu-7A* ($R^2 = 0.16$) were associated with SNB response to isolate Sn99CH 1A7a {10867}.

93.2. Sensitivity to SNB toxin

Snn4. Add: **v:** Salamouni {10867}.
Snn4. Add: **v:** Katepwa {10867}.

95. Reaction to *Puccinia graminis* Pers.

Sr6. **ma:** Add: *Xgwm102-2D* – 0.9 cM – *Xgpw94049-2D* – 5.6 cM – *Sr6* – 1.5 cM – *Xwmc453/Xcfd43-2D* {10870}.

Sr21. **dv:** After the Einkorn entry insert: Dv92 *Sr35*; G2919 *Sr35* {10876}.

Sr22. **bin:** Add: 7AL-13 0.83-0.89 {10869}.
ma: Add: Recombined lines with shortened introgressions from diploid wheat are reported in {10869}; the shortest was U5616020-154.

Sr24. **v:** Ernest {10845}; Keene {10845}.
ma: *Xbarc71-3Ag* was considered a better marker for *Sr24* than STS Sr24#12 {10845}.
1BL. **tr:** Add: Millenium {10845}.

Sr30. **ma:** *Xcfd12-5D* – 9.0 cM – *Sr30* – 16.6 cM – *Xgwm292-5D* {10858}.
Add note:
According to {10858} Webster RL6201 carries a second gene *SrW* that confers resistance to the race Ug99 group.

Sr31. **ma:** *Xscm09-1R*$_{208}$ {10845}.

Sr35. **bin:** 3AL8 0.85-1.00. **i:** 'Marquis*5 / G2919' {10876}.
dv: DV92 *Sr21* {10876}; G2919 Sr21 {10876}.
ma: Add: Mapped in diploid wheat within to a 2.2–3.1-cM region between *Xbf483299* and *XCJ656351* and corresponding to a 174-kb region in *Brachypodium* {10876}.

Sr36. **ma:** *Xgwm429-2B* – 0.8 cM – *Sr36*/*Xstm773-2*/*Xgwm319*/*Xwmc477-2B* {10824}; *Xgwm319-2B* – 0.9 cM – *Sr36*/*Xstm773-2*/*Xwmc477-2B* {10824}; of four markers *Xwmc477-2B* was the best, but it is not a perfect marker {10845}.

Sr39. Add note:
A Ti2BL·2BS-2SS-2BS translocation (10872} separated from *Sr47* in DAS15 could contain *Sr39* – see *SrAEs7t*.

Sr40. **ma:** *Xwmc661-2B* – 6.4 cM – *Sr40* – 0.7 cM – *Xwmc344-2b* – 2.0 cM – *Xwmc477-2B* {10825}; *Xwmc661-2B* – 7.8 cM – *Sr40* – 2.5 cM– *Xwmc474-2B* – 1.0 cM – *Xwmc477-2B* {10825}.

Sr47. Add to chromosome location: ', 2BS {10872}'.
Add note: Further chromosome engineering on DAS15 showed that the alien segment carried two resistance genes. The gene on 2BL was considered to be *Sr47* based on low infection type. The second gene located in 2BS produced a low infection type similar to *Sr39* and was located in a similar position to that gene {10872}.
2B = T2BL-2SL-2BL·2BS **tv:** RWG 35 {10872}; RWG 36 {10872}.
ma: Located in the interval *Xgwm47-2B – Xgpw4165-2B* {10872}.

Sr48. Update: **v:** To be provided. **v2:** Arina *Sr56* {10851}.

Sr54 {10816}. 2DL {10816}. **v2:** Norin 40 *Sr42* {10816}.

Sr55 {10847}. Adult-plant resistance. 4DL {10847,10678}.
bin: Distal to break point 0.56 FL {10678}.
i: RL6077 = 'Thatcher*6 / PI 250413' {10847,10678}.
ma: Pleiotropic or closely linked with *Lr67* and *Yr46* and associated with *Xgwm165-4D* and *Xgwm192-4DL* {10847,10678}.

Sr56 {10851}. Adult-plant resistance. *QSr.Sun-5BL* {10565}.
5BL {10565,10851}. **bin:** 5BL16.
v: AF533 {10851}. **v2:** Arina *Sr48* AUS 91457 {0138}.
ma: *Xgwm118-5BL* – 13.6 cM – *wPt9116* – 5.4 cM – *Sr56* – 6.9 cM – *wPt0484* {10851}.
In the earlier QTL analysis of an 'Arina / Forno' population, *QSr.Sun-5BL* accounted for 12% of the PVE {10565}. In the present study of an 'Arina / Yitpi' RIL population stem rust response segregated as a single gene. The response phenotype was 40-50 MS–S.

Sr57 {10861}. Adult-plant resistance. 7DS {10861}.
bin: 7DS4.
su: Lalbahadur (Perula7D) GID 5348503 and GID 5348496 {10861,10862}.
v: Chinese Spring {10861}; Wheats with *Pm*/*Lr34*/*Yr18*, see Reaction to *Blumeria granminis*, Reaction to *Puccinia striiformis*, Reaction to *Puccinia triticina*, Leaf tip necrosis.
ma: See Reaction to *Puccinia triticina*.
c: Putative ABC transporter {10862}.
Further evidence for the effects of this gene on stem rust response can be found in {299, 10565,10733,10863,10864, 10865,10866}.

SrAes7t {10872}. 2BS = T2BL·2BS-2SS-2BS {10872}.
v: Line 0797 {10872}. **ma:** *Sr39#50s* {10741,10872}.
SrAes7t may be identical to *Sr39* {10872}.

SrWeb {10858}. 2BL {10858}. **v2:** Webster RL6201 *Sr30* {10858}.
ma: *Xgwm47-2B* – 1.4 cM – *SrWeb* – 12.5 cM – *Xwmc332-2B* {10858}.

Sr1RS^{Amigo} {10845}. 1AS (T1AL·1RS) {389,1624}.
v2: Amigo *Sr24* {1464,10845}.
ma: *Xscm09-1R224* {10845}.
This alien segment also carries *Pm17* – see *Pm17*.

QTL:
'RL6071 / RL6058' (R): RIL population: RL6058, a Tc backcross line with *Lr34*/*Sr57* is more resistant than Tc. Enhancement of resistance in both Kenya and North America was attributed to a QTL in the region *wPt5044 – Xgwm-2B* in chromosome 2BL {10902}.

96. Reaction to *Puccinia striiformis* Westend.

96.1. Designated genes for resistance to stripe rust

Yr5. **ma:** *Xwmc175-2B* – 1.1 cM – *YrSTS-7/8* – 0.3 cM – *Yr5* – 0.4 cM – *Xbarc349-2B* {10826}.

Yr15. **ma:** *Xwmc128/Xgwm273/Xgwm582-1B* – 0.4 cM – *Yr15/Xwgp34/Xgwm413/Xbarc8* {10826}.

Yr18. **c:** Putative ABC transporter {10862}.

Yr46. Add note:
Pleiotrophic or closely linked with *Sr55* and *Lr67*.

Yr47.
Update the existing entry to the following:
Yr47 {10679}. 5BS {10679}. **bin:** 5BS5-0.71- 0.81.
v: AUS28183 = V336 {10679}; AUS28187 {10679}.
ma: *Xgwm234-5B* – 10.9 cM – *Lr52* – 4.1 cM – *Yr47* – 9.6 cM – *Xcfb309-5B* {10679}; *Xgwm234-5B* – 10.2 cM – *Lr52* – 3.3 cM – *Yr47* – 8.2 cM – *Xcfb309-5B* {10679}.

Update:
Yr48 {10705}. Adult-plant resistance. Syn. *Qyr.ucw-5AL* {10705}. 5AL {10705}.
bin: 5AL23 0.87-1.00.
v: UC1110 (S) / PI 610750 RIL 4 = GSTR 13504 & RIL 167 = GSTR 136 {10705}.
ma: *Xwmc727-5AL* – 3.7 cM – *Vrn-A2* – 0.1 cM – *Yr48/BE444566-5AL/Xcfa2149-5AL/Xgpw2181a-5AL/Xwmc74-5AL/Xwmc410-5AL* {10705}.
PI 610750 = Synthetic 205 (Croc 1 / *Ae. tauschii*) / Kauz) {10705}.

Yr50 {10849}. Derived from *Th. intermedium*. 4BL {10849}.
v: CH233 {10849}. **ma:** cent….*Xbarc1096-4B* – 6.9 cM – *Yr50* – 7.2 cM – *Xbarc-4B* {10849}.

Yr51 {10850}. 4AL {10850}. **bin:** 4AL4-0.80-100.
v: Line 5515 AUS 91456 {10850} **v2:** AUS 27858 Gene 2 {10850}.
ma: *wPt4487* – 9.8 cM – *Yr51* – 4.4 cM – *wPt0763* – 7.9 cM – *Xgwm160-4B* {10850}.

Yr52 {10852}. Adult-plant resistance. 7BL {10852}.
bin: 7BL-3 0.86-1.00. **v:** PI 183527 {10852}; PI 660057 = 'Avocet S / PI 183527' F4-41{10853}.
ma: *Xbarc182-7B* – 1.2 cM – *Yr52* – 1.1 cM – *Xwgp5258* – 5.7 cM – *Xcfa2040-7B* {10852}.

Yr53 {10854}. 2BL {10854}. **tv:** PI 480148 {10854}.
v: 'Avocet S / PI 480148' F5-128{10854}.
ma: *Xwmc441-2B* – 5.6 cM – *Yr53* – 2.7 cM – *XLRRrev/NLRRrev*$_{350}$ – 6.5 cM – *Xwmc149-2B* {10853}. *Yr53* was estimated to be 35 cM distal to *Yr5* based on an F_2 allelism test, but on an integrated map this distance was about 20 cM.

96.2. Temporarily designated genes for resistance to stripe rust

YrAS2388 {10822}. **dv:** *Ae. tauschii* AS2388 {10822}.
ma: *Xwmc617-4DS* – 34.6 cM – *YrAS2388* – 1.7 cM – *Xwmc285-4DS* {10822}.

YrR61 {10914}. *QYr.uga-2AS* 10914}. 2AS {10914}.
v: Pioneer 26R61 = PI 612056 {10914}.

Yrxy1 {10829}. High temperature resistance.
v: 'Mingxian 169 / Xiaoyan 54' F3-4-14 {10829}.
v2: Xiaoyan 54 *Yrxy2* {10829}.
ma: *Xbarc49-7AS* – 15.8 cM – *Yrxy1* with closer flanking RGA markers {10829}.

Yrxy2 {10829}. High temperature resistance.
v: 'Mingxian 169 / Xiaoyan 54' F3-4-30 {10829}.
v2: Xiaoyan 54 *Yrxy1* {10829}.
ma: *Xwmc794-2AS* – 4.0 cM – *Yrxy2* – 6.4 cM – *Xbarc5-2AL* {10829}.

96.3. Stripe rust QTL

'Pioneer 26R61 (R) / AGS2000 (S)': RIL populations: Two QTL, *QYr.uga-2AS* ($R^2 = 0.56$) flanked by *Xbarc124-2A* and *Xgwm359-2A* (also named *YrR61*) and *QYr.uga-6AS* ($R^2 = 0.06$) {10914}. Minor QTL were also on other chromosomes.

'UC1110 (MR) / PI 610750 (MR)': RIL population: *Qyr.ucw-3BS* ex UC1110, $R^2 = 0.22$, associated with *Xgwm522-3B.1*. This marker differs from *Xgwm533-3B.2* that is associated with *Yr30* {10705}; *Qyr.ucw-5AL*, $R^2 = 0.1$, ex PI 61075 – syn. *Yr48* {10705}; *Qyr.ucw-2BS*, $R^2 = 0.045$, ex UC1110, located in the centromeric region near *Xwmc474-2BS* {10705}; and *Qyr.ucw-2AS*, $R^2 = 0.023$, ex PI 61725, near wPt-5839 (10705}.

'Stephens I / Platte (S)': RIL population; 13 QTL were identified across several environments; significant 'QTL x environment' interactions suggested that plant stage specificity, pathogen genotype and temperature as well as host genotype were important in determining rust response {10890}.

92.Reaction to *Puccinia triticina*

92.1. Genes for resistance

Lr11. **v:** Panola {10830}. **v2**: Jamestown *Lr18* {10830}.

Lr17.
Lr17a. **v:** Santa Fe {10830}.

Lr18. **v2:** Jamestown *Lr11* {10830}.

Lr19. **v:** Dobrynya {10821}; Ekada 6 {10821}; L505 {10821}; Samsar {0108}; Volgouralskaya {10821}.
v2: Kinelskaya Niva *Lr23* {10821}.

Lr32. **i:** RL6086 = 'Tc*7 // Rl5713 / Marquis K' {10874}; BW196 = 'Katepwa*6 // RL5713 / 2*Marquis K' {10874}.
ma: *Xbarc128-3D* – 9.1 cM – *Lr32* – *Xwmc43*/*Xbarc235-3D* {10874}.

Lr34. **v:** 2174 {10888}. List of U.S. hard wheats in {10888}. Pedigree charts showing the presence of *Lr34* in various Canadian wheat classes are given in {10889}.
ma: Further markers for *Lr34* and various marker-positive haplotypes that lack leaf rust resistance are described in {10887,10888}.
c: Putative ABC transporter, GenBank FJ436983, in CS {10862}. Further confirmation of the ABC transporter is provided in {10887}.

Lr39. **v:** Postrock {10830}.

Lr42. Change the current listing to the following:
1D {218}. **v:** AR93005 {10840}; Fannin {10595}, but not confirmed with markers {10840}.
v2: KS91WGRC11 *Lr24* {218,10840}. **dv:** TA2450 {218}.
ma: *Lr42* – 0.8 cM – *Xwmc432-1D* – 1.6 cM – *Xcfd-D1* {10840}.

Lr48. Add: , 2BS {10842}.
ma: *Xwmc175-2B* – 10.3 cM – *Lr48* – 2.5 cM – *Xwmc332-2B* {10842}.
Centromere – 27.5 cM – *Lr48* (est.) {10842}.

Lr52. **v:** Add: AUS28183 = V336 {10679}; AUS18187 {10679}.
ma: Add: *Xgwm234-5B* – 10.9 cM – *Lr52* – 4.1 cM – *Yr47* – 9.6 cM – *Xcfb309-5B* {10679}; *Xgwm234-5B* – 10.2 cM – *Lr52* – 3.3 cM – *Yr47* – 8.2 cM – *Xcfb309-5B* {10679}.

Lr58. **ma:** Add: A codominant STS marker *Xncw-Lr58-1* was based on the sequence of *XksuH16* {10819}.

Lr63. Under *Lr63* change reference 10550 to 10875.
ma: Replace existing text by '*Xbarc321*/*Xbarc57-3A* – 2.9 cM – *Lr63* {10875}.'

Lr65 {10848}. *LrAlt* {10739}. 2AS {10739,10848}.
v: Selection ARK 0; {10848}; *T. aestivum* subsp. *spelta* Altgold Rotkorn {10739,10848}.
ma: *Lr65* – 1.8 cM – *Xbarc212-2A*/*Xwmc382-2A* – 2 cM – *Xgwm636* {10739}; *XE41M57-165* – 3 cM – *Lr65* – 2 cM – *Xbarc124*/*Xbarc222*/*Xgwm614-2A* {10848}. *Lr65* was estimated to be about 10 cM from *Lr17* {10848}.
Some plants of Altgold Rotkorn possess a second gene conferring IT 12C {10848}.

Lr67. Correct chromosome location to 4DL {10675}.
bin: C-0.53 {10675}; Distal to 0.56 {10678}.
ma: Replace first sentence with: *Xcfd71-4D* – 1.5 cM – *Lr67* {10675}.
Lr67 is pleiotropic or closely linked with *Sr55* and *Yr46*.

Lr68 {10817}. Adult-plant resistance. 7BL {10817}.
v: Arula 1 CIMMYT GID 1847450 {10817}; Arula 2 CIMMYT GID 1847422 {10817}.
v2: Parula *Lr3b Lr34 Lr46* {10817}.
ma: Close linkage with several markers in chromosome arm 7BL and *Lr14b* in the 'Apav / Arula' populations. Flanking markers are *Xpsy1-1* and *Xgwm146-7BL* at 0.4 and 0.6 cM. Gamma-irradiation induced deletion stocks of Arula 1 that lack *LrP* but have *Lr14b* were identified showing that the two genes are located at different closely linked loci {10817}.

Lr69 {10903}. 3DL {10903}. **v:** Toropi-6.3 {10903}.

Lr70 {10904}. 5DS {10904}. **v:** Yet to be named selection of cross or backcross to Tc {10904}.
v2: KU3198 LrXX {108221}. **ma:** *Lr70* – *Xgwm190-5D* {10904}.
LrXX is believed to be a known gene for resistance.

Lr71 {10911}. *LrARK12c* {10910}. 1B centromere region not resolved {10911}.
v: LrARK12c = *T. aestivum* subsp. *spelta* Altgold Rotkorn selection {10910}. Common wheat reference line under increase {10911}.
ma: *Xgwm11-1B* – 3.3 cM – *Xgwm18-1B* – 1.0 cM – *Lr71* – 1.3 cM – *Xbarc187-1B* – 0.5 cM – *Xbarc137-1B* {10911}.

LrAlt. Delete this section.

98. Reaction to *Pyrenophora tritici-repentis* (anomorph: *Drechlera tritici-repentis*)
98.2. Insensitivity to tan spot toxin (chlorosis)

Tsc2. **v:** Add: Katepwa (10871}. **bin:** 2BS3 0.84-1.00.

***tsc2*.** **v:** Salamouni {10871}. **tv:** Altar 84 {10871}.
ma: *Xmag681-2B/XTC339813* – 2.7 cM – *Tsc/XBE444541* – 0.6 cM – *XBE517745* {10871}. An *XBE444541* EST-STS co-segregating marker for *Tsc2* was developed and lines with *tsc2* produced a 505-bp fragment, whereas those with *Tsc2* produced a 340-bp band {10871}.

QTL
'Salamouni / Katepwa': RIL population: variation at the *Tsc2* locus explained 54% of the variation in response to race DW5 {10871}.

NEW SECTION XX Reaction to *Sitobion avenae*
English grain aphid.
Sa1 [{10877}]. *RA-1* {10877}. 6AL {10877}. **tv:** C273 {10877}.
ma: *Xwmc179-6A* – 3.37 cM – *Sa1* – 4.73 cM – *Xwm580-6A* {10877}.

99. Reaction to *Sitodiplosis mosellana* (Gehin)
Add:
QTL:
'Reeder I / Conan': RIL population: *QSm.mst-1A*, flanked by *Xwmc59-1A* and *Xbarc1022-1A* was the most effective and constant QTL for reduced larval infection over two years (R^2 = 0.17 and 0.34) {10841}. RILs with this QTL in three genetic backgrounds had reduced infestations of 42% {10841}.

100. Reaction to *Schizaphis graminum* Rond. (*Toxoptera graminum* Rond.)

***Gb3*.** **bin:** 7DL3 0.82-1.00. **v:** TAM 112 {0194}.
tv: *Ae. tauschii* PI 268210 {10907}.
ma: At the end of the present entry add: ….. – 0.8 cM – *Xbarc76-7D* {10169}. *H1067J6-R* – 0.7 cM – *Gb3* – 0.4 cM – *H1009B3-F* {10907}.

107. Reaction to Wheat Streak Mosaic Virus

Wsm2 {10802,10898}. **v:** RonL {10898}.
ma: *Xgwm389-3B* – 30.8 cm – *Wsm2* – 45.2 cM – *Xgwm566-3B* {10898}.

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VI. ABBREVIATIONS USED IN THIS VOLUME.

PLANT DISEASES, PESTS, AND PATHOGENS:

BYDV = barley yellow dwarf virus
BMV = barley mosaic virus
CCN = cereal cyst nematode, *Heterodera avenae*
FHB = Fusarium head blight
RWA = Russian wheat aphid
SBMV = soilborne mosaic virus
SLB = Septoria leaf blotch
TMV = *Triticum* mosaic virus
WDF = wheat dwarf mosaic
WSBMV = wheat soilborne mosaic virus
WSMV = wheat streak mosaic virus
WSSMV = wheat spindle streak mosaci virus
WYMV = wheat yellow mosaic virus
E. graminis* f.sp. *tritici = *Erysiphe graminis* f.sp. *tritici* = the powdery mildew fungus
F. graminearum = *Fusarium graminearum* = head scab fungus
F. nivale = *Fusarium nivale* = snow mold fungus
H. avenae = *Heterodera avenae* = cereal cyst nematode
P. graminis = *Polymyxa graminis* = wheat soilborne mosaic virus vector
P. striiformis* f.sp. *tritici = *Puccinia striiformis* f.sp. *tritici* = strip rust fungus
P. triticina = *Puccinia triticina* = *P. recondita* f.sp. *tritici* = leaf rust fungus
R. cerealis = *Rhizoctonia cerealis* = sharp eyespot
R. solani = *Rhizoctonia solani* = Rhizoctonia root rot
R. padi = *Rhonpalosiphum padi* = bird cherry-oat aphid
S. tritici = *Septorai tritici* = Septoria leaf spot fungus
S. graminearum = *Schizaphus graminearum* = greenbug
St. nodorum = *Stagonospora nodorum* = Stagonospora glume blotch
T. indica = *Tilletia indica* = Karnal bunt fungus

SCIENTIFIC NAMES AND SYNONYMS OF GRASS SPECIES (NOTE: CLASSIFICATION ACCORDING TO VAN SLAGEREN, 1994):

A. strigosa = *Avena strigosa*
Ae. cylindrica = *Aegilops cylindrica* = *Triticum cylindricum*
Ae. geniculata = *Aegilops geniculata* = *Aegilops ovata* = *Triticum ovatum*
Ae. longissima = *Aegilops longissima* = *Triticum longissimum*
Ae. markgrafii = *Aegilops markgrafii* = *Aegilops caudata* = *Triticum caudatum*
Ae. speltoides = *Aegilops speltoides* = *Triticum speltoides*
Ae. tauschii = *Aegilops tauschii* = *Aegilops squarrosa* = *Triticum tauschii*
Ae. triuncialis = *Aegilops triuncialis* = *Triticum triunciale*
Ae. umbellulata = *Aegilops umbellulata* = *Triticum umbellulatum*
Ae. peregrina = *Aegilops peregrina* = *Aegilops variabilis* = *Triticum peregrinum*
Ae. searsii = *Aegilops searsii* = *Triticum searsii*
Ae. ventricosa = *Aegilops ventricosa* = *Triticum ventricosum*
D. villosum = *Dasypyrum villosum* = *Haynaldia villosa*
S. cereale = *Secale cereale* = rye
T. aestivum* subsp. *aestivum = *Triticum aestivum* = hexaploid, bread, or common wheat
T. aestivum* subsp. *macha = *Triticum macha*
T. aestivum* subsp. *spelta = *Triticum spelta*
T. militinae = *Triticum militinae*
T. monococcum* subsp. *aegilopoides = *Triticum boeoticum*
T. timopheevii* subsp. *timopheevii = *Triticum timopheevii*
T. timopheevii* subsp. *armeniacum = *Triticum araraticum* = *T. araraticum*
T. turgidum* subsp. *dicoccoides = *Triticum dicoccoides* = wild emmer wheat
T. turgidum* subsp. *dicoccum = *Triticum dicoccum*

T. turgidum* subsp. *durum = *Triticum durum* = durum, pasta, or macaroni wheat
T. urartu = *Triticum urartu*
Th. bessarabicum = *Thinopyrum bassarabicum*
Th. elongatum = *Thinopyrum elongatum* = *Agropyron elongatum*
Th. intermedium = *Thinopyrum intermedium* = *Agropyron intermedium*

Scientific journals and publications:

Agron Abstr = Agronomy Abstracts
Ann Wheat Newslet = *Annual Wheat Newsletter*
Aus J Agric Res = *Australian Journal of Agricultural Research*
Can J Plant Sci = *Canadian Journal of Plant Science*
Cereal Chem = *Cereal Chemistry*
Cereal Res Commun = *Cereal Research Communications*
Curr Biol = *Current Biology*
Eur J Plant Path = *European Journal of Plant Pathology*
Funct Integ Genomics = *Functional Integrative Genomics*
Ind J Agric Sci = *Indian Journal of Agricultural Science*
Int J Plant Sci = *International Journal of Plant Science*
J Agric Sci Technol = *Journal of Agricultural Science and Technology*
J Cereal Sci = *Journal of Cereal Science*
J Hered = *Journal of Heredity*
J Phytopath = *Journal of Phytopathology*
J Plant Phys = *Journal of Plant Physiology*
Mol Gen Genet = *Molecular and General Genetics*
Nat Genet = *Nature Genetics*
PAG = Plant and Animal Genome (abstracts from meetings)
Phytopath = *Phytopathology*
Plant Breed = *Plant Breeding*
Plant, Cell and Envir = *Plant, Cell and Environment*
Plant Cell Rep = *Plant Cell Reporter*
Plant Dis = *Plant Disease*
Plant Physiol = *Plant Physiology*
Proc Ind Acad Sci = *Proceedings of the Indian Academy of Sciences*
Proc Natl Acad Sci USA = Proceedings of the National Academy of Sciences USA
Sci Agric Sinica = *Scientia Agricultura Sinica*
Theor Appl Genet = *Theoretical and Applied Genetics*
Wheat Inf Serv = *Wheat Information Service*

Units of measurement:

bp = base pairs
bu = bushels
cM = centimorgan
ha = hectares
kDa = kiloDaltons
m^2 = square meters
m^3 = cubic meters
μ = micron
masl = meters above sea level
me = milli-equivalents
mL = milliliters
mmt = million metric tons
mt = metric tons
Q = quintals
T = tons

MISCELLANEOUS TERMS:

Al = aluminum
AFLP = amplified fragment length polymorphism
ANOVA = analysis of variance
A-PAGE = acid polyacrylamide gel electrophoresis
APR = adult-plant resistance
AUDPC = area under the disease progress curve
BC = back cross
BW = bread wheat
CHA = chemical hybridizing agent
CMS = cytoplasmic male sterile
CPS = Canadian Prairie spring wheat
DH = doubled haploid
DON = deoxynivalenol
ELISA = enzyme-linked immunosorbent assay
EMS = ethyl methanesulfonate
EST = expressed sequence tag
FAWWON = Facultative and Winter Wheat Observation Nursery
GA = gibberellic acid
GIS = geographic-information system
GM = genetically modified
GRIN = Germplasm Resources Information Network
HPLC = high pressure liquid chromatography
HMW = high-molecular weight (glutenins)
HRSW = hard red spring wheat
HRRW = hard red winter wheat
HWSW = hard white spring wheat
HWWW = hard white winter wheat
ISSR = inter-simple sequence repeat
IT = infection type
kD = kilodalton
LMW = low molecular weight (glutenins)
MAS = marker-assisted selection
NSF = National Science Foundation
NILs = near-isogenic lines
NIR = near infrared
NSW = New South Wales, region of Australia
PAGE = polyacrylamide gel electrophoresis
PCR = polymerase chain reaction
PFGE = pulsed-field gel electrophoresis
PMCs = pollen mother cells
PNW = Pacific Northwest (a region of North America including the states of Oregon and Washington in the U.S. and the province of Vancouver in Canada)
PPO = polyphenol oxidase
QTL = quantative trait loci
RAPD = random amplified polymorphic DNA
RCB = randomized-complete block
RFLP = restriction fragment length polymorphism
RILs = recombinant inbred lines
RT-PCR = real-time polymerase-chain reaction
SAMPL = selective amplification of microsatellite polymorphic loci
SAUDPC = standardized area under the disease progress curve
SCAR = sequence-characterized amplified region
SDS-PAGE = sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE-HPLE = size-exclusion high-performance liquid chromatography
SH = synthetic hexaploid

SNP = single nucleotide polymorphism
SRPN = Southern Regional Performance Nursery
SRWW = soft red winter wheat
SRSW = soft red spring wheat
STMA = sequence tagges microsatellite site
SWWW = soft white winter wheat
SSD = single-seed descent
SSR = simple-sequence repeat
STS = sequence-tagged site
TKW = 1,000-kernel weight
UESRWWN = Uniform Experimental Soft Red Winter Wheat Nursery
VIGS = virus-induced gene silencing

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IX. VOLUME 59 MANUSCRIPT GUIDELINES.

Manuscript guidelines for the *Annual Wheat Newsletter*, volume 59. The required format for Volume 59 of the *Annual Wheat Newsletter* will be similar to previous editions edited from Kansas State University.

CONTRIBUTIONS MAY INCLUDE:

—Current activities on your projects.
—New cultivars and germ plasm released.
—Special reports of particular interest, new ideas, etc., normally not acceptable for scientific journals.
—A list of recent publications.
—News: new positions, advancements, retirements, necrology.
—Wheat stocks; lines for distribution, special equipment, computer software, breeding procedures, techniques, etc.

FORMATTING & SUBMITTING MANUSCRIPTS:

Follow the format in volume 44–58 of the *Newsletter* in coördinating and preparing your contribution, particularly for state, station, contributor names, and headings. Use Microsoft Word™ or send an RTF file that can be converted. Use Times 12 CPI and 1.0" (2.5 cm) margins. Please include a separate jpg, gif, or equivalent file of any graphic in the contribution. Submit by E-mail to jraupp@k-state.edu.

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The *Annual Wheat Newsletter* will continue to be available (Vol. 37–58) through the Internet on GrainGenes, the USDA–ARS Wheat Database at http://wheat.pw.usda.gov/ggpages/awn/.