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Ascribing Functions to Genes: Journey Towards Genetic Improvement of Rice Via Functional Genomics

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Abstract: Rice, one of the most important cereal crops for mankind, feeds more than half the world population. Rice has been heralded as a model cereal owing to its small genome size, amenability to easy transformation, high synteny to other cereal crops and availability of complete genome sequence. Moreover, sequence wealth in rice is getting more refined and precise due to resequencing efforts. This humungous resource of sequence data has confronted research fraternity with a herculean challenge as well as an excellent opportunity to functionally validate expressed as well as regulatory portions of the genome. This will not only help us in understanding the genetic basis of plant architecture and physiology but would also steer us towards developing improved cultivars. No single technique can achieve such a mammoth task. Functional genomics through its diverse tools viz. loss and gain of function mutants, multifarious omics strategies like transcriptomics, proteomics, metabolomics and phenomics provide us with the necessary handle. A paradigm shift in technological advances in functional genomics strategies has been instrumental in generating considerable amount of information w.r.t functionality of rice genome. We now have several databases and online resources for functionally validated genes but despite that we are far from reaching the desired milestone of functionally characterizing each and every rice gene. There is an urgent need for a common platform, for information already available in rice, and collaborative efforts between researchers in a concerted manner as well as healthy public-private partnership, for genetic improvement of rice crop better able to handle the pressures of climate change and exponentially increasing population.

Keywords: Functional genomics, Genetic improvement, Mutants, Rice, Omics, *Oryza sativa* L.

1. INTRODUCTION

The Rice (*Oryza sativa* L.) is staple food for more than half the world population, fulfilling 21% to 76% of daily calorie intake by South-east Asia and world, respectively [1, 2]. Rice is one of the most important cereal crops not only because of its vast acreage and importance as a food crop but also due to several other factors that makes it an ideal choice for research and breeding efforts. Rice includes its two cultivated species, *O. sativa* L. (Asian rice) and O. *glaberrima* Steud. (African rice) along with the wild relatives (22 wild species) and landraces, and has a rich germplasm repository at the International Rice Research Institute (IRRI) as well as in the individual collections with the major rice producing countries [3]. The IRRI alone has roughly 124,000 accessions of rice in its germplasm diversity collection (htpp://irri.org/ourwork/research/genetic-diversity). This offers a huge variation in terms of allelic diversity which can be tapped for expediting the breeding efforts to address the bottlenecks in genetic improvement of rice. Having a modest genome size of 430 Mbp, rice was also the first cereal crop plant to be fully sequenced with high precision [4-6]. A major update from the release 6 of rice psuedomolecules and annotation, release 7.1, was published in Oct. 2011 with the help of parallel efforts of researchers at the Agrogenomics Research Center at the National Institute of Agrobiological Sciences, Tsukuba, Japan and the Rice Annotation Project Database (RAP-DB). According to this release 373,245,519 bp of non-overlapping rice genome sequence from the 12 rice chromosomes has 55,986 genes (loci) of which 6,457 have 10,352 additional alternative splicing isoforms resulting in a total of 66,338 transcripts (or gene models). These predicted loci include 39,045 non-TE (transposable element) loci with 49,066 gene models and 16,941 TE loci with 17,272 gene models. Such genomic resources are continually being accumulated in rice and getting more refined by resequencing efforts via Next Generation Sequencing (NGS) platforms. Availability of high resolution linkage maps, high synteny and co-linearity with the other cereal crops, and amenability to high efficiency transformation techniques are few other characteristics which have provided rice with a status of model cereal.

Owing to the aforesaid features, rice has been at the forefront of the efforts in genetic improvement of cereals. Over the past six decades unprecedented gains have been realized in rice yield, mainly due to introduction of semi dwarf varieties and exploiting heterosis particularly in Asian subcontinent [7]. The green revolution almost doubled yields from

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1.9 tonnes a hectare in 1950-64 to 3.5 tonnes in 1985-98. Rice production increased by 130% from 257 million tons in 1966 to 600 million tons in 2000 [8]. Unfortunately, the exponentially increasing population and consequently the decrease in arable land have rendered these gains insufficient. This has also necessitated the utilization of marginal lands or areas unsuitable for rice cultivation, thus further declining the realized yield. Another debilitating factor in undermining the success of the achievements in increasing rice productivity is the gap in the actual and potential yield of rice varieties due to losses incurred by the action of abiotic and biotic stress factors. These factors impair the breeding efforts not only in rice but other crop plants as well. A suite of abiotic and biotic stresses result in 30%–60% yield losses in crop plants globally each year [9]. According to estimates, approximately a 40% increment in current average yield of rice (3.9 t/ha) will be needed to meet the requirements of 5 billion people by 2030 [10, 11]. This necessitates the development of varieties for breaking the current yield barrier either through a better response to stress factors or improved yield parameters. These collective features in rice plants were proposed the name 'green super rice' that would possess insect and disease resistance, high nutrient use efficiency, drought resistance, high grain quality and yield [12]. Concerted efforts should thus be directed towards strengthening the pace of rice research globally. Tailor-made rice varieties are the need of the hour to accomplish a leap in current yield parameters by virtue of better agronomic traits as well as an efficient stress response. However, engineering plants suited to our specific needs calls for a thorough understanding of complex plant responses dictated by its genetic makeup or the total complement of genes actively transcribing under range of conditions to which crop plants are exposed. We need to bring in best genomic regions and allelic counterparts governing the aforesaid traits in a single genotype, but unless we are able to differentiate best from the worst, such a mammoth task cannot be accomplished. Till two decades back, developing designer varieties seemed like a distant dream, but with the availability of near precise estimate of genomic loci in rice; research fraternity has a challenge as well as a launch pad to work out the significance of each one of these loci and associated regulatory genomic regions.

The study of functional expression of the genomic information of an organism using genome-wide or system-wide experimental techniques and computational/statistical approaches is referred to as functional genomics (FG) [13]. Genomics research has benefitted immensely from the various aspects of functional genomics. Tools and resources generated by functional genomics have been able to create considerable impact in unravelling gene function and interactions between genes in complex regulatory networks. Functional genomics employs the study of loss of function as well as gain of function mutants to precisely predict gene function and is aided by bioinformatics/data mining, transcriptomics, structural genomics, high throughput phenotyping as well as proteomics (Fig. **1**). In this paper, we will review the progress made in rice functional genomics along with tools and resources available to facilitate such research endeavors. We will also discuss future direction and prospects of functional genomic research in rice to bring desired genetic improvement in cultivated rice varieties.

1.1. Abolish Or Establish: Deciphering Gene Function Using Mutants

1.1.1. Approaches to Generate Mutants in Rice

Mutants (naturally occurring alleles, deletion mutants or insertion mutants) are pivotal to the functional genomics study for all organisms. Mutant analysis either through forward or reverse genetics is the most effective method to study gene function [14]. Gene function can be readily determined by altering the gene expression either by knocking out, knocking down, overexpression or ectopic expression, and study the corresponding effects on phenotype. Mutants which completely abolish the function of a specific gene also called as knock outs (KOs) give a direct "cause and effect relationship" based on the changes in phenotype. However, not all mutations exhibit the mutant phenotype due to redundancy of genes belonging to same family, expression of mu-

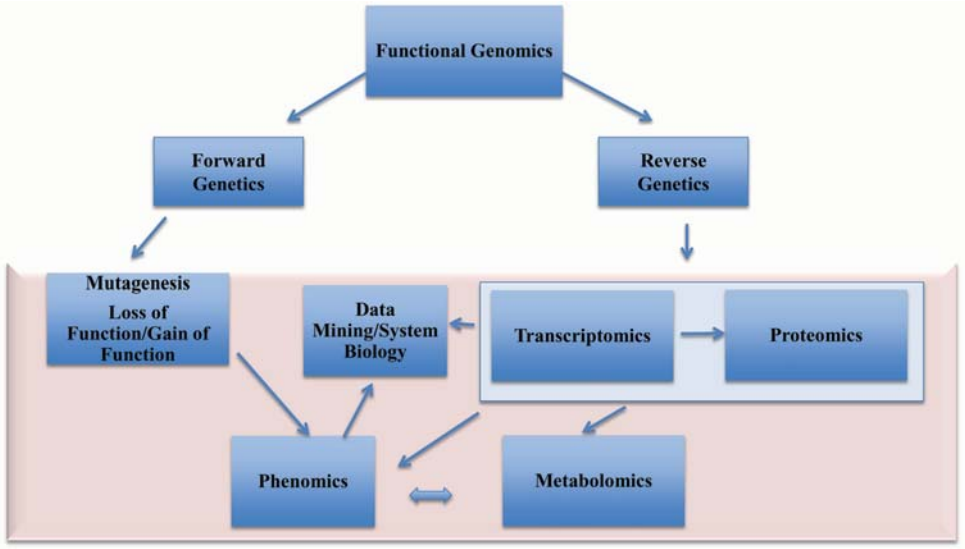

Fig. (1). Schematic diagram showing multidisciplinary approaches being used currently for functional genomics in rice.

tant phenotype only under specific condition or due to confluent phenotypes thus rendering it difficult to assign mutant phenotype for a given gene [15]. These limitations of mutant analysis can be overcome by analyzing mutants under a range of physiological conditions or by creating mutants for all the members of a gene family or by tagging the mutated genes using flanking sequences tags (FST) generated through the unique sequence flanking the insertional element. Besides loss of function mutations, specific insertional elements viz. T-DNA, transposons can be designed to bring changes in gene expression pattern which can be monitored through a reporter gene. Activation tagging (identifies genes as far as 10 Kb from the site of insertion), gene traps (to characterize adjacent promoter activity), and enhancer traps (to characterize adjacent enhancer activity), are commonly used vectors to discover redundant genes as well as those with lethal effects using gain of function studies [14].

Both insertional mutagenesis as well as conventional mutagens using chemical and physical mutagens has been used efficiently in rice [2]. Insertional mutagenesis entails disruption of gene function by insertion of a specific sequence in the genic region. This can be accomplished by using a T-DNA, transposons as well as retrotransposon owing to efficient Agrobacterium mediated transformation techniques in rice [16, 17]. T-DNA insertion lines constitute a large portion of all the mutants available in rice and they are preferred over other insertional elements due to their low copy number and stable inheritance over generations [18]. Besides T-DNA, transposons have been successfully utilized to generate mutants in rice. Maize class II transposable elements Ac/Ds, En/Spm-dSpm have been used to generate large scale mutations in rice. Genetic selection techniques for large scale transpositional activity of these elements have been well established [19-23]. Transposition of these elements has a tendency to be closely linked to the original location [24]. These transposable elements can produce large number of mutants from a smaller number of parental lines, and possibility of generating revertants renders tedious complementation studies unnecessary [25]. Native rice transposons like mPing, a miniature inverted repeat transposons element and nDart/aDart, a hAT type transposon, may prove promising in creating rice mutant populations [26-28]. Rice endogenous retroposon, Tos17, is activated during tissue culture, integrate at unlinked sites, becomes stable in regenerated plants, and the copy number is correlated with duration of tissue culture [21]. These features make it an ideal choice for generating saturated mutant populations. Mutant resources generated through transposable elements have been instrumental in unravelling the functions of rice genes. Jiang *et al.* 2012 [29] documented approximately 600 rice genes along with their chromosomal location which have been experimentally validated using insertional mutants.

Although insertional mutagenesis has been successful in rice, we are far from reaching the goal of tagged mutants for each and every rice genes. All the insertional elements used in rice so far show a bias for targeting specific genomic regions. Secondly, these methods cannot be employed in any variety of choice due to difficulty in transformation and regeneration. Therefore, random mutations created by chemical (Ethyl methanesulfonate:EMS, Sodium azide, N-methyl N-nitroso Urea:MNU, DEB: Diepoxybutane) and physical (ion beam, irradiation and fast neutrons) mutagens have immensely benefitted rice functional genomics [25]. Tagged sites make insertional elements more lucrative but with the advent of high throughput screening strategies for random mutants, chemically or physically induced mutants can be characterized as efficiently as the former. Screening of mutants based on Targeting Induced Local Lesions in Genomes (TILLING) [30], Tilling based sequencing [31], NGS platforms like MutMap [32], MutMap-Gap [33], MutMap+ [34], PCR based Deleteagene method [35], Microarray platforms [36] have greatly enhanced the significance of random mutagenesis in rice. In a recent review by Zhong-Hua *et al.* 2014 [2], a total of 64 genes functionally characterized by random mutagenesis have been summarized.

In addition to aforesaid methods, several promising alternatives have been developed to alter the expression of specific genes in rice. RNA interference (RNAi) is another quick, easy, sequence-specific way to 'knock-down' the expression of chosen genes by sequence-specific gene disruption induced by small non-coding double stranded RNA (dsRNA) i.e.,small interfering RNA (siRNA) and microRNA (miRNA) [37, 38]. Long dsRNA or short-hairpin RNA (shRNA) precursors homologous to the target gene to be silenced, initiate the process of RNAi [39, 40]. Similar to siRNA, artificial micro RNA (amiRNAs) can efficiently trigger gene silencing in rice and can be effectively used for candidate gene validation, comparative functional genomics between different varieties, and for improvement of agronomic performance and nutritional value [41]. Long hairpin (hpRNA) has been used to induce large scale gene silencing in rice plants [25]. Genes inaccessible to mutation owing to their small size, lethality or genome position can also be silenced by more directed gene specific approaches like amiRNA silencing or ectopic expression of genes as has been shown in a recent study to create genome wide mutants in rice using amiRNA [42]. RNAi has been successfully used for gene discovery and validation of gene function in rice (Table **1**). Novel technologies such as Zinc finger nucleases, Transcription activator like effector (TALE) nucleases, clustered regularly interspersed short palindromic repeats (CRIPSR/Cas9) technology have also been shown to be promising in rice to generate loss of function mutations [78-82]. In rice FOX project, gain of function mutants of rice genes developed by systematic overexpression of rice full length cDNAs in heterologous system Arabidopsis has been generated. This has been achieved through the joint efforts of National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan; the Research Institute of Biological Sciences at Okayama (RIBS); and RIKEN, Plant science center, Japan to characterize rice genes in a process called FOX hunting [83, 84]. Similar work has been done in rice system [85]. These gain of function mutants have been effectively used to elucidate the function of several rice genes [79]. Rice functional genomics has benefitted immensely from aforesaid mutation generation tools. A summary of breakthrough studies in functional validation of rice genes in the last five years has been provided in (Table **2**).

1.1.2. Mutant Resources For Functional Genomics in Rice

Concerted efforts to functionally characterize all the predicted rice genes were initiated by International rice

Table 1. List of selected studies utilizing RNAi for functional validation of rice genes.

S. No.	Title of the Study	RNAi Against the Gene	Function Predicted	Year	Reference
1	Characterization of two rice DNA methyltransferase genes and RNAi-mediated reactivation of a silenced transgene in rice callus	*OsMET1-1*	maintenance of methylation	2004	[43]
2	RNAi-mediated silencing of OsGEN-L (OsGEN-like), a new member of the RAD2/XPG nuclease family, causes male sterility by defect of microspore development in rice	*OsGEN-like (OsGEN-L)*	microspore development	2005	[44]
3	RNAi knockdown of Oryza sativa root meander curling gene led to altered root development and coiling which were mediated by jasmonic acid signalling in rice	*Oryza sativa root meander curling (OsRMC)*	root development and curling	2007	[45]
4	Modification of plant height via RNAi suppression of OsGA20ox2 gene in rice	*OsGA20ox2*	Height	2007	[46]
5	Rice UDP-Glucose Pyrophosphorylase1 Is Essential for Pollen Callose Deposition and Its Cosuppression Results in a New Type of Thermosensitive Genic Male Sterility	*Ugp1*	Male Sterility	2007	[47]
6	RNAi-directed downregulation of OsBADH2 results in aroma (2-acetyl-1-pyrroline) production in rice (Oryza sativa L.)	*OsBADH2*	aroma accumulation	2008	[48]
7	RNAi-mediated suppression of hexokinase gene OsHXK10 in rice leads to non-dehiscent anther and reduction of pollen germination	*OsHXK10*	Anther development	2008	[49]
8	Hd3a and RFT1 are essential for flowering in rice	*Hd3a*	flowering	2008	[50]
9	RNAi-mediated knockdown of the XIP-type endoxylanase inhibitor gene, OsXIP, has no effect on grain development and germination in rice	*XIP-type endoxylanase inhibitor*	plant defense mechanisms against phytopathogens	2008	[51]
10	Amylase gene silencing by RNA interference improves recombinant hGM-CSF production in rice suspension culture	*alpha-amylase gene*	quantity of rice alpha-amylase	2008	[52]
11	Silencing by RNAi of the gene for Pns12, a viroplasm matrix protein of Rice dwarf virus, results in strong resistance of transgenic rice plants to the virus	*Pns12*	disease-resistance	2009	[53]
12	RNAi mediated silencing of a wall associated kinase, OsiWAK1 in Oryza sativa results in impaired root development and sterility due to anther indehiscence: Wall Associated Kinases from Oryza sativa	*wall associated kinase, (OsiWAK1)*	Plant growth and development	2011	[54]
13	Production of transgenic rice new germplasm with strong resistance against two isolations of Rice stripe virus by RNA interference	*Viral CP and viral SP*	Pathogen resistance	2011	[55]
14	Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress	*OsNAC5*		2011	[56]
15	RNAi-mediated disruption of squalene synthase improves drought tolerance and yield in rice	*Farnesyltransferase / squalene synthase (SQS)*	stomatal conductance and subsequent drought tolerance	2012	[57]
16	Production of high oleic rice grains by suppressing the expression of the gene	*OsFAD2-1*	Oleic acid	2012	[58]
17	Suppression of NS3 and MP is important for the stable inheritance of RNAi-mediated rice stripe virus (RSV) resistance obtained by targeting the fully complementary RSV-CP gene	*RSV-CP gene*	disease-resistance	2012	[59]
18	RNAi knockdown of rice SE5 gene is sensitive to the herbicide methyl viologen by the down-regulation of antioxidant defense	*PHOTOPERIOD SENSITIVITY 5 (SE5)*	antioxidant defense	2012	[60]
19	RNAi suppression of rice endogenous storage proteins enhances the production of rice-based Botulinum neutrotoxin type A vaccine	*endogenous storage proteins, 13 kDa prolamin and glutelin*	storage protein production	2012	[61]

(Table 1) contd….

S. No.	Title of the Study	RNAi Against the Gene	Function Predicted	Year	Reference
20	RNAi-directed down-regulation of RSV results in increased resistance in rice (Oryza sativa L.)	*coat protein gene (CP) and disease specific protein gene (SP) sequences from RSV*	viral infection resistance	2012	[62]
21	Gene silencing using the recessive rice bacterial blight resistance gene xa13 as a new paradigm in plant breeding	*Xa13*	viral infection resistance	2012	[63]
22	RNAi mediated down regulation of myo-inositol-3-phosphate synthase to generate low phytate rice	*myo-inositol-3-phosphate synthase*	seed phytate levels	2013	[64]
23	RNAi-directed downregulation of vacuolar H(+) -ATPase subunit a results in enhanced stomatal aperture and density in rice	*vacuolar H(+)-ATPase subunit A (OsVHA-A)*	stomatal aperture and density	2013	[65]
24	Production of marker-free and RSV-resistant transgenic rice using a twin T-DNA system and RNAi	*rice stripe virus (RSV) coat protein (CP) gene and the special-disease protein (SP) gene*	viral resistance.	2013	[66]
25	Development of low phytate rice by RNAi mediated seed-specific silencing of inositol 1,3,4,5,6-pentakisphosphate 2-kinase gene (IPK1)	*inositol 1,3,4,5,6-pentakisphosphate 2-kinase gene (IPK1)*	seed phytate levels	2013	[67]
26	RNA interference-mediated silencing of the starch branching enzyme gene improves amylose content in rice	*RBE3*	amylose content	2013	[68]
27	Repression of Lignin Synthesis in Rice by C4H and 4CL Using RNAi	*C4H and 4CL*	Lignin Synthesis	2013	[69]
28	Isolation and characterization of rice (Oryza sativa L.) E3-ubiquitin ligase OsHOS1 gene in the modulation of cold stress response	*OsHOS1*	cold stress response	2013	[70]
29	RNAi-mediated suppression of endogenous storage proteins leads to a change in localization of overexpressed cholera toxin B-subunit and the allergen protein RAG2 in rice seeds	*endogenous storage proteins 13-kDa prolamin and glutelin*	localization of overexpressed CTB and major rice allergens,	2014	[71]
30	Construction of rice stripe virus NS2 and NS3 Co-RNAi transgenic rice and disease-resistance analysis	*NS2 and NS3*	disease-resistance	2014	[72]
31	RNAi-directed downregulation of betaine aldehyde dehydrogenase 1 (OsBADH1) results in decreased stress tolerance and increased oxidative markers without affecting glycine betaine biosynthesis in rice (Oryza sativa)	*betaine aldehyde dehydrogenase 1 (OsBADH1)*	stress tolerance	2014	[73]
32	RNAi mediated silencing of lipoxygenase gene to maintain rice grain quality and viability during storage	*LOX*	grain quality	2014	[74]
33	Modification of Starch Composition Using RNAi Targeting Soluble Starch Synthase I in Japonica Rice	*SSSI*	Starch Synthesis	2014	[75]
34	Repression of OsEXPA3 Expression Leads to Root System Growth Suppression in Rice	*OsEXPA3*	Root Growth	2014	[76]
35	Constitutive expression and silencing of a novel seed specific calcium dependent protein kinase gene in rice reveals its role in grain filling	*OsCPK31*	grain filling	2015	[77]

functional genomics consortium (IRFGC) along with several national programs [21]. According to recent estimates 447,919 FSTs from independent mutant rice lines have been released. A systematic analysis indexed the precise location of 336,262 FSTs on japonica rice chromosomes [79]. A large proportion of these hits are found in the genic regions which are more likely to exhibit a mutant phenotype [25]. Given the success of these efforts in generating mutants for more than half the rice genes, IRFGC has recently proposed 'RICE 2020" a collaborative effort to systematically decipher the

Table 2. List of rice functionally validated through mutagenesis in the last five years.

S. No.	Title	Mutated Gene	Phenotype	Year	Reference
1	A new rice dwarf1 mutant caused by a frame-shift mutation	C6PS (vitamin K-dependent coagulation factor)	Plant height	2011	[86]
2	Transcript profiling of crown rootless1 mutant stem base reveals new elements associated with crown root development in rice	CRL1	crown root development	2011	[87]
3	Genetic and molecular analysis of a purple sheath somaclonal mutant in japonica rice	OsC1	purple sheath trait	2011	[88]
4	A rice mutant displaying a heterochronically elongated internode carries a 100 kb deletion	SLR1	elongated internode	2011	[89]
5	Characterization and genetic analysis of a light- and temperature-sensitive spotted-leaf mutant in rice	spl30	biosynthesis or degradation of chlorophyll.	2011	[90]
6	A study of phyto hormone biosynthetic gene expression using a circadian clock-related mutant in rice	Os-GIGANTEA(GI)	phyto hormone biosynthesis	2011	[91]
7	Characteristics of pregelatinized ae mutant rice flours prepared by boiling after preroasting	IIb (starch branching enzyme)	Boiled grains are hard and non-sticky	2011	[92]
8	Complete loss of photoperiodic response in the rice mutant line X61 is caused by deficiency of phytochrome chromophore biosynthesis gene	se13	photoperiodic response	2011	[93]
9	Cell division and cell elongation in the coleoptile of rice alcohol dehydrogenase 1-deficient mutant are reduced under complete submergence	ADH1	coleoptile elongation	2011	[94]
10	Altered expression of auxin-related genes in the fatty acid elongase mutant oni1 of rice	beta-ketoacyl CoA synthase	entire shoot development was impaired	2011	[95]
11	Genetic analysis of cysteine-poor prolamin polypeptides reduced in the endosperm of the rice esp1 mutant	CysP	seed	2011	[96]
12	Generation of transgenic rice lines with reduced contents of multiple potential allergens using a null mutant in combination with an RNA silencing method	The alpha-amylase/trypsin inhibitors (14-16 kDa), alpha-globulin (26 kDa) and beta-glyoxalase I (33 kDa)	Allergens in seed	2011	[97]
13	Characterization of a novel high-tillering dwarf 3 mutant in rice	htd3	Tiller number and culm length (Plant Height)	2011	[98]
14	Abnormal endosperm development causes female sterility in rice insertional mutant OsAPC6	OsAPC6	Abnormal endosperm development	2012	[99]
15	Leaf variegation in the rice zebra2 mutant is caused by photoperiodic accumulation of tetra-cis-lycopene and singlet oxygen	CRTISO	photoperiodic accumulation of tetra-cis-lycopene and singlet oxygen	2012	[100]
16	Uptake of exogenous sugars and responses by rice root of young wild-type and ospk1 mutant seedlings	OsPK1	uptake of exogenous sugars	2012	[101]
17	Biomolecular analyses of starch and starch granule proteins in the high-amylose rice mutant Goami 2	SSI and SSIIa	starch branching and starch synthase activity	2012	[102]
18	Building a mutant resource for the study of disease resistance in rice reveals the pivotal role of several genes involved in defence	OsWRKY28, rTGA2.1 and NH1	disease resistance	2012	[103]

(Table 2) contd….

S. No.	Title	Mutated Gene	Phenotype	Year	Reference
19	Increased leaf photosynthesis caused by elevated stomatal conductance in a rice mutant deficient in SLAC1, a guard cell anion channel protein	SLAC1	leaf photosynthesis and stomatal conductance	2012	[104]
20	Reduced tillering in Basmati rice T-DNA insertional mutant OsTEF1 associates with differential expression of stress related genes and transcription factors	elf1	Reduced tillering, retarded growth of seminal roots, and sensitivity to salt stress	2012	[105]
21	Characterization and fine mapping of a novel rice albino mutant low temperature albino 1	Lta1	chlorophyll biosynthesis and chloroplast development (Leaf Color)	2012	[106]
22	Formation of two florets within a single spikelet in the rice tongari-boushi1 mutant	TOB1	plant development and morphology	2012	[107]
23	Morphological characteristics and gene mapping of a palea degradation(pd2) mutant in rice	REP1	plant height, total grain number per panicle, and sword leaf width	2012	[108]
24	Characterisation of a rice dwarf and twist leaf 1 (dtl1) mutant and fine mapping of DTL1 gene	DTL1	Plant Height	2012	[109]
25	A rice virescent-yellow leaf mutant reveals new insights into the role and assembly of plastid caseinolytic protease in higher plants	VYL	Leaf color	2013	[110]
26	Characterization and genetic analysis of a novel rice spotted-leaf mutant HM47 with broad-spectrum resistance to Xanthomonas oryzae pv. oryzae	HM47	Leaf color	2013	[111]
27	Isolation of a novel mutant gene for soil-surface rooting in rice (Oryza sativa L.)	sor1	root gravitropic response	2013	[112]
28	Characterization and fine mapping of a novel rice narrow leaf mutant nal9	ClpP	Leaf width	2013	[113]
29	Ospapst1, a useful mutant for identifying seed purity and authenticity in hybrid rice	Os01 g16040 (OsPAPST1)	male sterile associated with leaf color	2013	[114]
30	Isolation of a novel UVB-tolerant rice mutant obtained by exposure to carbon-ion beams	Os07g0264900 and Os07g0265100	UVB-tolerance	2013	[115]
31	Fine mapping and characterization of a novel dwarf and narrow-leaf mutant dnl1 in rice	dnl1	Plant height and leaf width	2013	[116]
32	Morphological characteristics and gene mapping of a novel bent pedicel branch (bpb1) mutant in rice	bpb1	pedicel development	2013	[117]
33	Characterization of OsMIK in a rice mutant with reduced phytate content reveals an insertion of a rearranged retrotransposon	OsMIK and OsIPK1	seed phytic acid content	2013	[118]
34	Relationships between starch synthase I and branching enzyme isozymes determined using double mutant rice lines	SSI and BEI or BEIIb	starch synthesis	2014	[119]
35	Selection and molecular characterization of a high tocopherol accumulation rice mutant line induced by gamma irradiation	OsVTE2	tocopherol accumulation	2014	[120]
36	Isolation and characterisation of a dwarf rice mutant exhibiting defective gibberellins biosynthesis	OsKS2	gibberellins biosynthesis	2014	[121]
37	Transcriptome analysis of grain-filling caryopses reveals the potential formation mechanism of the rice sugary mutant	OsAGPS2b	starch and sugar contents	2014	[122]
38	A proteomic study of rice cultivar TNG67 and its high aroma mutant SA0420	OsGAPDHB	aroma	2014	[123]

(Table 2) contd….

S. No.	Title	Mutated Gene	Phenotype	Year	Reference
39	Comparative metabolomic analysis of wild type and mads3 mutant rice anthers	MADS3	anther development	2014	[124]
40	The tillering phenotype of the rice plastid terminal oxidase (PTOX) loss-of-function mutant is associated with strigolactone deficiency	PTOX1	chloroplast function	2014	[125]
41	Reverse-genetic approach to verify physiological roles of rice phytoalexins: characterization of a knockdown mutant of OsCPS4 phytoalexin biosynthetic gene in rice	cps4	physiology	2014	[126]
42	Investigation of the rescue mechanism catalyzed by a nucleophile mutant of rice BGlu1	BGlu1	rescue mechanism and glycosylation mechanism	2014	[127]
43	Characterization and mapping of a spotted leaf mutant in rice (Oryza sativa)	spl30	leaf necrotic lesions	2014	[128]
44	The rice semi-dwarf mutant sd37, caused by a mutation in CYP96B4, plays an important role in the fine-tuning of plant growth	CYP96B4	developmental processes	2014	[129]
45	Production of superoxide from Photosystem II in a rice (Oryza sativa L.) mutant lacking PsbS	PsbS	Photosynthesis	2014	[130]
46	Characterization of a null allelic mutant of the rice NAL1 gene reveals its role in regulating cell division	NAL1	cell division	2015	[131]
47	Transcriptome profiling of the spl5 mutant reveals that SPL5 has a negative role in the biosynthesis of serotonin for rice disease resistance	SPL5	resistance to pathogens	2015	[132]
48	Transcriptional profile of genes involved in ascorbate glutathione cycle in senescing leaves for an early senescence leaf (esl) rice mutant	OsAPX	leaf senescence	2015	[133]
49	A T-DNA insertion mutant Osmtd1 was altered in architecture by upregulating MicroRNA156f in rice	SPL and OsmiR156f	Plant architecture	2015	[134]
50	Decreased photosynthesis in the erect panicle 3 (ep3) mutant of rice is associated with reduced stomatal conductance and attenuated guard cell development	EP3 (Os02g15950)	photosynthesis	2015	[135]

biological function of each and every rice gene by the year 2020 [136, 137]. To achieve whole genome saturation, 587,345 independent insertions are required so that at least one insertion per gene can be obtained with a probability of 0.99 [136]. There is thus, still a long way to reach the milestone of rice functional genomics by 2020 and it can be only possible with more involvement of rice researchers across the globe. An account of mutant resources available in different varieties of rice developed by diverse methods has been summarized in (Table **3**). Streamlining availability of these resources through a common platform, their enrichment and hassle free distribution system can go a long way in achieving the goal of rice 2020.

1.2. Omics Tools For Functional Genomics in Rice

1.2.1. Transcriptomics in Rice Gene Functional Analysis

The transcriptome refers to the entire set of transcribed regions within a genome. Predicting the function of a gene is greatly facilitated by analyzing its spatial and temporal expression patterns under range of physiological condition. Expression of a specific portion of genome also helps in its annotation. Most often than not, transcript levels are directly correlated to the protein amount and activity, thus helping us predict the possible function and further validation by technique of choice. Co-expression of genes, comparison between different species/cultivars and analyzing effect of altered gene expression on other genes enables us to identify genes associated with specific regulatory pathway. Besides these obvious facets of transcriptome study, transcriptome resources are also gaining importance in accelerating Marker Assisted Selection (MAS) by establishing rapid marker trait linkages and delineating molecular tags such as haplotypes, (Quantitative trait loci) QTLs, alleles, novel genes and novel markers [141]. Techniques employed to deduce and quantify transcriptome include hybridization based methods viz. Northern, microarray and sequence based methods like RT-PCR, cDNA/(Expressed cDNA tags) ESTs, Serial Analysis of Gene Expression (SAGE), Massively parallel signature sequencing (MPSS), mRNA seq, DDRT-PCR, qRT-PCR etc.

Table 3. Mutant resources available in various rice varieties and associated databases (-represents unavailability of data in the relevant field).

S. No.	Rice Variety	Mutation Type	Mutagen	FST Lines /Mutant Lines	Mutant Loci	Database Website/Reference
1	Nippon-bare (Ja-ponica)	Insertional Mutagenesis	T-DNA, Tos 17, Ac-Ds, GT/ET, Spm/dSpm	81721	712141	http://www.pi.csiro.au/fgrttpub http://orygenedb.cirac.fr http://urgi.versailles.inra.fr/OryzaTagLine http://tos.nias.affrc.go.jp (RMD) http://rmd.ncpgr.cn http://ship.plantsignal.cn/home.do http://www.plb.ucdavis.edu/Labs?Sundar http://www.genomics.zju.edu.cn/ricetdna
		Chemical	Sodium Azide, MNU	few selected lines	6000	http://www.plb.ucdavis.edu/Labs?Sundar
		Physical Mutagenesis	Gamma ray, ion beam	few selected lines	15000	http://tos.nias.affrc.go.jp
2	Katy (Japonica)	Chemical	EMS (Ethyl Meth-ane Sulfonate)	4880	-	http://www.ars.usda.gov/Main/docs.htmdocid
		Physical Mutagenesis	Fast neutrons, gamma ray	17961	-	http://www.ars.usda.gov/Main/docs.htmdocid
3	IR64 (Indica)	Chemical	DEB (Diepoxybu-tane) , EMS (Ethyl Methane Sulfonate)	-	500000	http://www.iris.irri.org/cgibin/Mutanthome.pI
		Physical Mutagenesis	Fast neutron, Gamma ray	-	-	http://www.iris.irri.org/cgibin/Mutanthome.pI
4	Dongjin Byeo (Japonica)	Insertional Mutagenesis	T-DNA, Ac/Ds, ET/GT	63763	37000	(KRDD) http://www.niab.go.kr/RDS (RISD) http://an6.postech.ac.kr/pfg
5	Hwayoung (Japonica)	Insertional Mutagenesis	T-DNA	-	550000	(RISD) http://an6.postech.ac.kr/pfg
6	Zhongua 11 (Japonica)	Insertional Mutagenesis	T-DNA, ET	45840	211508	http://www.genomics.zju.edu.cn/ricetdna (RMD) http://rmd.ncpgr.cn
7	Zhongua 15 (Japonica)	Insertional Mutagenesis	T-DNA, ET		14197	(RMD) http://rmd.ncpgr.cn
8	Tainung 67 (Japonica)	Insertional Mutagenesis	T-DNA AT	31000	30000	(TRIM) http://trim.sinica.edu.tw
9	Kasalath (Japonica)	Physical Mutagenesis	Gamma ray	-	-	http://www.genomics.zju.edu.cn/
10	SSBM (Japonica)	Chemical Mutagenesis	EMS (Ethyl Meth-ane Sulfonate)	-	-	http://www.genomics.zju.edu.cn/
11	Taichung 65 (Japonica)	Chemical Mutagenesis	MNU (N-methyl, N-nitroso Urea)	-	-	[138]
12	Kitaake (Japonica)	Insertional Mutagenesis	T-DNA	14000	6758	[139]
		Physical Mutagenesis	Fast neutrons	4000	2357	https://pag.confex.com/pag/xxiii/webprogram/Paper15189.html
13	Nagina 22 (Indica)	Chemical Mutagenesis	EMS (Ethyl Meth-ane Sulfonate)	22292	-	[140]

Traditionally, gene expression analysis was done by Northern blot analysis, which gives data for few selected genes. With the advent of PCR based methods like RT-PCR, qRT-PCR and DDRT-PCR, possibility of analyzing several selected genes became a reality. However, all these methods are low throughput, show cloning bias and low sensitivity. Sequencing of short tags based on the expressed portion of genome has also been useful in commenting about the kinetics of gene expression [142]. EST datasets have been generated for rice on a large scale. Currently, there are 1,253,557 ESTs in NCBI dbEST collection as per the latest statistics (www.ncbi.nlm.nih.gov/dbEST/). Generation of ESTs via myriad methods, despite ahigh throughput, do not give an accurate picture of transcript levels and the benefits are also mired by the presence of redundant ESTs, and inability to detect low level transcripts [141, 143]. Advanced tag based techniques such as SAGE, Cap Analysis of Gene Expression (CAGE) [144] and MPSS are much better in terms of reduced sequencing cost, identification of novel and rare transcripts. A comprehensive expression atlas of rice sequences based on MPSS tags was established for rice (Rice MPSS) and 46,971,553 mRNA transcripts from 22 libraries, and 2,953,855 small RNAs from three libraries were documented [145]. This approach captured sense expression of at least 25,500 annotated genes and antisense expression of nearly 9,000 annotated genes [146]. Using publicly available MPSS dataset of rice, 82 highly expressed pollen-specific, 12 developing seed-specific, and 19 germinating seed-specific genes were identified [147]. Similarly, SAGE and its modifications have also been used effectively for expression profiling in rice [143, 148-150]. These approaches however only identify the start or end of transcription sites, but cannot reveal gene structures and cannot distinguish between isoforms [151, 152]. These tag based techniques were superceded by the genome wide array based transcript profiling based on hybridization. Besides tags generated on the basis of expressed cDNA, full length cDNA resources are also useful for functional genomics studies to predict exons, annotate and identify gene products and their corresponding promoters [29]. Current database search depicted the availability of nearly 38,000 full length rice clones from japonica rice variety nipponbare (http://cdna01.dna.affrc.go.jp/cDNA/), ~ 10,081 and 12,727 from indica varieties Guang Lu ai 4 and Ming Hui 63, respectively (http://202.127.18.221/ricd/). Full length cDNAs (~19000) have also been isolated from wild rice *O. rufipogon* [153], which are made available at rice database RICD (www.ncgr.ac.cn/ricd). Microarrays rely on EST or full length cDNA data to generate probes required for profiling gene expression data. Studies based on microarrays have been successfully used to characterize genes involved in a specific pathway in rice [154-158]. Microarrays have been used to decipher complex regulatory and interactive pathways by generating a snapshot of global genes expression under a given experimental condition. There are several microarray platforms available for rice including BGI/Yale, NSF20 and 45K, Agilent 22 and 44K, Affymetrix 57 K. Microarray data for 40 cell types for vegetative growth, 25 stages of reproductive development, and 39 tissues representing the entire life cycle of rice has been documented in rice atlas [159-162]. Microarray have some drawbacks which limit their utility viz. high background signals, low coverage of complete genome set, requirement of predetermined probes and high development cost which render them unable to identify novel transcribed regions [163]. NGS platforms have led to the discovery of mRNA-seq, a novel method to quantify, map and identify even hitherto unknown transcribed part of transcriptome. RNA-seq has shown advantages over microarrays by allowing accurate, efficient and reproducible estimations of transcript abundance of either known or unknown transcripts with a larger dynamic range using less RNA sample [164]. RNA-seq can detect genes expressed at low levels in a cost effective manner and refine the structure of transcripts [165, 166]. Deep mRNA seq has been employed for comparing gene expression, discovering UTRs, novel splice junctions, rare transcripts, as well as alternative splice forms [167-169]. Several novel transcripts and their spliced variants have been identified from various tissues, cell types, developmental stages and cultivars of rice using RNA seq [159, 163, 167, 170-173]. Data generated from genome wide profiling experiments is publicly available and can be accessed through several databases including rice oligonucleotide array database (http://www.ricearray.org/matrix.search.shtml), RiceXpro (http://ricexpro.dna.affrc.go.jp/), RiceGE, PLEXdb (http://www.plexdb.org/plex.php?database=Rice), Oryza express (http://bioinf.mind.meiji.ac.jp/OryzaExpress/, Rise array net (http://www.ggbio.com/arraynet/), RED (http:// cdna02.dna.affrc.go.jp/RED/) NGS RGAP (http://rice.plantbiology.msu.edu/expression.shtml), and UniVio (http://univio.psc.riken.jp/).

1.2.2. Role of Proteomics in Rice Functional Genomics

The analysis of total array of proteins in an organism at a given time and physiological condition is the most direct approach to define the function of their associated genes, thus a valuable tool for functional genomics [174]. Understanding the proteome also requires analysis of post translational modifications viz. glycosylation, phosphorylation of proteins; secretome or secretory proteins and the complex networks of protein-protein interactions [175]. Transcriptomics tools although popular and informative do not yield information on protein levels and their state of modification [176] mainly due to post-translational regulation, thus leading to absence of one to one correlation between mRNA and protein abundance [177, 178]. Both gel-based (1-DE and 2-DE) and gel-free shotgun techniques such as Multidimensional Protein Identification Technology (MudPIT), Isotope coded affinity tag (ICAT), and isobaric tags for relative and absolute quantification (iTRAQ) approaches have been successfully used for proteomics studies [179]. Along with traditional methods of 1-DE, 2-DE/(Difference Gel Electrophoresis) DIGE in conjunction with Edman sequencing, advent of MS based methods and coupled gel-free shotgun liquid chromatography tandem mass spectrometry (LC–MS/MS) for analysing the global proteome changes and comparative genomics have revolutionized the area of proteomics and it has emerged as a complementary technique to transcriptomics as well as metabolomics [86, 180, 181]. As per a recent review, proteomics is one of the useful approaches to understand plant biology and owing to the importance of rice crop, rice proteomics has been labelled as a cornerstone for cereal food crop proteomics [179]. Beginning of rice proteomics in the year 2000 was marked by efforts involved in cataloguing proteins which was followed by more robust techniques and tools for functional proteomics i.e. to reannotate proteins along with comparative proteomics. Gel based techniques viz. 1D, 2DE were the methods of choice during the initial

years of proteomics. 2DE methods still account for 75% studies in rice proteomics [179]. Although, they are popular even today for their ease of execution and resolution but these methods are inefficient at capturing less abundant proteins/hydrophobic proteins. Discovery of less abundant proteins is often masked by high abundant plant proteins like Rubisco in plants. These shortcomings can be overcome by using modified protocols for protein isolation viz. TCA precipitation, affinity chromatography. Their use in combination with latest MS based techniques is more fruitful in terms of information content and reproducibility. Over the last 15 years, tremendous progress has been made to understand rice proteome and these studies have been discussed in several recent reviews [174, 179, 180, 182-185]. Snap shots of rice proteomes have been generated for various environmental stresses, tissues/organs and developmental stages. Within the span of last two years, 2014-2015 more than a dozen studies have been published directly pertaining to rice proteomics. These studies have been summarized in (Table **4**), along with their major findings. Rice proteomics data generated by various research groups is also publicly available through various online databases. The Rice Proteome Database contains 23 reference maps based on 2D-PAGE of proteins from various rice tissues and subcellular compartments comprising 13,129 identified proteins involved in growth or stress responses along with the amino acid sequences of 5092 proteins [204]. OryzaPG-DB, a database for rice proteome based on shotgun proteogenomics contains peptides obtained from 27 rounds of nanoLC-MS/MS performed using a hybrid ion trap-Orbitrap mass spectrometer, which offers high accuracy for analyzing tryptic digests from undifferentiated cultured rice cells. It hosts data about 3,182 gene and 5,034 protein products with a total of 15,121 non-redundant peptides. Proteogenomic analysis performed using the 166 novel peptide sequences revealed 40 new genomic features of rice [205]. Besides these dedicated rice proteomics databases, few plant databases also provide access to rice proteomics data. The plant phosphoproteome database RIPP-DB (https://database.riken.jp/sw/en/Plant_Phosphoproteome_Database/ria102i/) provides information on phosphopeptides, which were identified in rice cells by LC-MS/MS-based shotgun phosphoproteomics approach. It allows users to predict conserved phosphorylation sites in proteins. Current database houses information for 6,919 phosphopeptides from rice. Another phosphoproteome database P3DB (http://p3db.org/index.php) harbours data about phosphorylation sites for rice proteins. Rice proteomics data generated by the individual efforts of scientists across the globe needs to be accessible via a common platform. The ProteomeXchange consortium has been set up to provide a coordinated submission of MS proteomics data (http://www.proteomexchange.org/). Although a couple of online resources have clubbed the data generated from these studies, a dedicated database for rice proteome, secretome, and interactome data along with post-translational modifications such as phosphorylation, glycosylation, and other modifications, obtained experimentally is need of the hour, which can give a true picture of progress made in understanding rice proteome. Such a resource base can greatly aid functional genomics as well as genetic improvement of rice. Moreover, since genomic databases are being updated frequently, proteomic databases should keep pace with these changes.

1.2.3. Metabolomics As a Tool For Functional Genomics

Plant metabolites constitute a diverse array of low molecular weight natural products, biologically formed in a suite of catabolic and anabolic pathways in plants. There are ~200,000 metabolites catalogued in plant kingdom alone [206]. The complex interactions between these metabolites are responsible for driving a plethora of plant activities including tolerance to biotic and abiotic stresses (anthocyanins, flavanoids, phenolics), plant growth and development (phytohormones), yield attributes (starch, sugars, proteins), quality parameters like color, taste and aroma attributes (caretenoids, volatiles), as well as overall physiology [207]. Spatial and temporal changes in both primary and secondary metabolites occur in response to environmental, developmental or genetic perturbations which are ultimately a reflection of changes in the expression of genome. Therefore, metabolomics in conjunction with transcriptomics and proteomics generates an unambiguous picture of gene function. Metabolomics provides a direct link between genome and phenome by validating gene and protein function experimentally [208]. Thus, metabolomics is an invaluable tool for functional genomics as well as systems biology. Owing to the diverse nature of plant metabolites, there is a need for sophisticated methods to isolate/separate, detect, quantify and analyze them [209]. Usually crude extracts are subjected to chromatographic analysis, which are then analyzed by mass spectrometry (MS) and/or nuclear magnetic resonance (NMR) spectroscopy. Using a combination of these methods often proves to be more advantageous viz. Gas chromatography GC–MS, liquid chromatography LC–MS, Capillary electrophoresis CE–MS, Fourier transform ion cyclotron resonance (FT-ICR) MS, LC/MS/NMR, GC/MS/MS, LC/MS/MS, LC/NMR/MS [207]. Ultrahigh-resolution MS (LC-MS) combined with Fourier transform ion cyclotron resonance–MS increases the possibility of precisely estimating the molecular formula of the peaks of secondary metabolites in plants [210]. Liquid chromatographic quadrupole time of flight tandem mass spectrometry (LC/QTOFMS/MS) has the ability to accurately determine the mass and product ion information of chromatographically separated metabolites [207]. Metabolic studies leads to discovery of huge proportion of hitherto unknown metabolites, therefore there is often a lack of reference molecule for comparison. Identification of unknown peaks is facilitated by MS, MS/MS libraries and NMR shift libraries [211, 212]. There are a number of online resources for reference metabolites reviewed in several recent reports [207, 213-215]. Due to low sensitivity of most of the techniques for metabolomics, usually a large amount of tissue is required which is not favorable for determining cell type specific metabolites. Techniques such as laser micro-dissection, fluorescence-activated cell sorting (FACS;) or Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) based mass spectral imaging can prove instrumental for highly spatially resolved analyses [216]. Once the preliminary data is obtained, hierarchical clustering and principle component analysis is employed to generate a new metabolic profile based on comparative profiling which can be correlated to determine linkages and correlations [217].

Huge germplasm collection in rice offers the possibility of identifying distinct metabolite profiles specific to a particular genotype. Advances in chromatographic techniques,

Table 4. Selected breakthrough rice proteomics studies undertaken in the last two years (2014-2015) to understand multifarious variety and physiological condition specific response.

S. No.	Tissue/developmental Stage/Physiological Condition	Technique Used	Major Finding	Reference
1.	Roots and leaves/ Seedlings	2-DE, nanospray liquid chromatography/tandem mass spectrometry	104 salt responsive differentially expressed protein spots in rice roots and 59 in leaves identified by 2-DE, 83 proteins in rice roots and 61 proteins in rice leaves by MS, Glycolysis, photosynthesis and purine metabolism major pathways to counteract salinity stress	[186, 187]
2	Spikelets	2-DE with Coomassie-brilliant blue (CBB) and Pro-Q Diamond phosphoprotein fluorescence stain	revealed that 123 proteins in abundance and 43 phosphoproteins generated from phosphorylation were significantly different	[188]
3	Germinating seeds	Gel free methods	12 protein modification-related proteins showing peak abundance of phosphoproteins at 12 h after imbibitions, brassinosteroid signal transduction likely triggers seed germination	[189]
4	Rice embryo	Both gel-based and gel-free strategies	343 differentially expressed proteins were identified	[190]
5	Early meiotic cells	nLC-MS/MS	1316 different proteins have been identified in rice isolated meiocytes in early meiosis, being 422 exclusively identified in early prophase I	[191]
6	Black vs white glutinous seeds	2-DE	13 differentially expressed proteins	[192]
7	Seeds of notched-belly mutant (DY1102) in a Chinese Japonica rice Wuyujing3	iTRAQ	A total of 113 differentially expressed proteins responsible for chalkiness in rice seeds were identified amongst which major proportion were metabolic (27.4%) proteins.	[193]
8	Leaves of Thai jasmine rice (*Oryza sativa* L. ssp. *indica* cv. KDML105) under drought vis a vis NSG19 and IR20	GeLC-MS/MS shotgun	623 proteins identified, 53 proteins showed significant difference in protein expression	[194]
9	Contrasting rice mutants during Vegetative stage	2-DE, Tandom MS	854 protein spots identified through 2-DE, 63 were highly responsive, 83 identified through tandem MS	[195]
10	Seeds of *O. rufipogon*, *O. officinalis*, *O. meyeriana* vs *O. sativa japonica* Hexi35 and *O. sativa indica*Dianlong201	2-DE	35 differential protein spots were found for glutelin acidic subunits, glutelin precursors and glutelin basic subunits in wild rice species	[196]
11	Leaves of 93-11 and Nipponbare	2-DE, MS	47 differentially expressed proteins 7 unique to nipponbare and one to 93-11	[197]
12	Rice selenoproteome in leaves	2-DE, apHPLC with the dual ICP MS and electrospray Orbitrap MS detection.	Selenomethionine (SeMet) and selenocysteine (SeCys) residues in a dozen proteins	[198]
13	Seedling cDNA library	yeast two-hybrid system and bimolecular fluorescence complementation	12 novel nonredundant interacting protein pairs (IPPs) representing 11 nonredundant interactors using 12 rice MAP3Ks as bait and a rice seedling cDNA library as prey.	[199]
14	Leaves of contrasting rice cultivars	Comparative protein profiling	26 and 16 MeJA-modulated proteins in resistant and susceptible cultivars	[200]
15	Young panicles of TGMS lines	2-DE, MALDI-TOF-TOF MS	Eighty-three protein spots were found to be significantly changed in abundance	[201]
16	Foliar proteome of Pusa Basmati I vs. *O. longistaminata*	2-DE, MALDI-TOF	29 unique spots identified	[202]
17	Proteomic profiles of two contrasting rice cultivars, TN1 (susceptible) and PTB33 (resistant)	2-DE, LC MS/MS	Differentially expressed proteins identified using 2-DE, Lignin production by activated glycolysis connected to a phenylpropanoid pathway may be responsible for rice resistance to the BPH mechanism.	[203]

chemometrics, and structural estimation methods can greatly aid this objective. However, handling such large populations is not amenable to high throughput metabolomics. Therefore, core collections representing the total diversity in rice germplasm have been made to study rice metabolomics e.g. Rice Germplasm Core Set from the International Rice Research Institute (623 accessions; http://iris.irri.org/germplasm/), the GCore collection (18 collections each of which consists of approximately 130 accessions) [218], the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Rice Core Collection (242 accessions) [219], and the rice diversity research set (67 accessions) [220]. Significant amount of data has been generated by carrying out metabolomics studies in rice, as have been documented in a series of reviews [213, 215, 221]. Genome-wide association study (GWAS) is a method for mapping the loci responsible for natural variations in a target phenotype by the identification of significantly associated genetic polymorphisms in a large population [222] [223]. Loci correlated to various agronomically important traits can be effectively identified by GWAS [224-227]. Availability of comprehensive metabolic profiles has allowed the prediction of genotype-metabolite associations by metabolome-GWAS in rice [228, 229]. GWAS conducted by analyzing the aerial part of 175 Japanese diverse rice cultivar seedlings using LC-MS/MS for the non-targeted analysis of known and unknown metabolites revealed that there are two types of genetic architectures responsible for the natural variations of secondary metabolites in the rice population. While the small number of metabolite associated quantitative trait loci (mQTL) tightly associated with levels of one-third of analyzed metabolites, levels of other one-third of metabolites were under the smaller effect of multiple QTL [230]. QTL analysis studies to identify useful QTLs that control metabolite accumulation i.e. mQTL analyses, have focused mostly on primary metabolites in rice [231]. Trait-metabolite association analyses have been successfully used in rice [214, 232-234]. The effective use of metabolomics and QTL analysis using mapping populations to delineate the genetic control of metabolism in rice has been reported. Key genes regulating flavonoid biosynthetic pathway have been identified via mQTL analysis [235, 236]. Understanding the metabolic profiles offer an opportunity to reconstruct metabolic pathways in genetically modified rice to increase or decrease a specific metabolite. Gain-of-function genetic mutations can add new and useful characteristics to improve the quality of rice. For example, ''golden rice,'' generated with a genetic engineering approach, can accumulate β-carotene in the endosperm [237, 238]. Engineering feedback insensitive Anthranilate synthase gene has been used to increase tryptophan content in rice seeds [239]. Although considerable progress has been made in rice metabolomics, but we need to tread ahead with caution. Plant metabolites are highly susceptible to changes in environment therefore quality control should be a primary concern. Although metabolic engineering seems lucrative, complete metabolic profiling to avoid perturbations in important physiological processes and allergenicity should be accounted for. New metabolites are being discovered consistently which calls for a need to strengthen the databases for reference molecules. Few databases have been set up for rice metabolites including Platform for Riken metabolomics (http://prime.psc.riken.jp/?action=drop_index), rice fox database (http://ricefox.psc.riken.jp/index.php? contents=top&subcontents=research&research=metabo), Plant metabolic network (http://www.plantcyc.org/), Fiehn lab (http://fiehnlab.ucdavis.edu/projects/FiehnLib/index_html). More efforts to accumulate metabolic profiles in publicly available databases are required. Moreover, databases for rice metabolomics should be regularly updated keeping in pace with other omics branches.

1.2.4. Joining the Dots Through Phenomics

Following closely behind the vast repository of sequence data, unprecedented leaps have been seen in various techniques dealing with omics as well as mutant analysis. NGS platforms and array based methods have revolutionized genotyping, transcriptomics and proteomics. The advent of NGS technologies has greatly enhanced rice functional genomics and molecular breeding studies [240, 241]. Besides rendering whole genome sequencing economically and technologically feasible, NGS is being used for sampling genetic diversity within and between germplasm pools, deep sequencing, epigenetics, transcriptomics, megagenomics as well as genotyping by sequencing/restriction site associated DNA sequencing (GBS/RAD) and genome wide association studies (GWAS) [242]. Resequencing of more than 1,500 accessions of rice germplasm and thousands of lines of biparental populations has contributed to evolutionary analysis, genomics and functional genomics studies [3]. Phenotyping constitutes an essential component of all the approaches of functional genomics. Phenotyping establishes a direct correlation of gene expression to that of its function, either through analysis of phenotype of gain of function/loss of function mutants or change in physiology of plants in response to a subset of proteins/genes. However, scoring phenotypes to study complex quantitative traits like yield and abiotic stress tolerance is still in its infancy [243]. A majority of phenotyping is still done manually or with very basic equipment which is time consuming as well as labor intensive. Traditional phenotyping tools, which measure a limited set of phenotypes, have become a bottleneck in functional genomics and plant breeding studies [244]. A substantial collection of mutants available in crop plants is lacking a description w.r.t phenotype. The frequency with which the annotation 'no visible phenotype' occurs is sometimes a reflection of inability to analyze the subtle or complex phenotypic effects of genetic modification [245]. This is where high throughput phenotyping with state of the art technology, phenomics, has catapulted into view as a promising avenue. Phenomics refers to cataloguing plant physiology, performance and architecture, through a combination of high throughput novel multidisciplinary technologies such as non-invasive imaging, spectroscopy, image analysis robotics, and high performance computing [246]. Forward phenomics uses phenotyping tools to 'sieve' collections of germplasm for valuable traits, while reverse phenomics is the detailed dissection of traits through a hypothesis driven approach to reveal mechanistic understanding by correlating a physiological trait to biochemical or biophysical processes and ultimately to a gene or genes [244]. Majority of the phenotyping systems rely on intensive measurements platforms that combine robotics and image analysis of individual plants grown under controlled-environment systems [247]. However, con-

trolled environments often fail to mimic the natural field conditions of crop plant. Therefore, more emphasis has been laid on field based phenotyping that can transform the characterization of plant populations for genetic research and crop improvement [248].

Rice phenomics has gained from number of plant phenotyping tools developed within a short span for measuring root morphology [249], leaf characteristics [250], biomass [251], yield-related traits [252, 253], photosynthetic efficiency [254], and abiotic stress response [253]. Phenotype databases of rice have been developed in many countries, e.g., Oryzabase [255], Rice Mutant database (RMD) [256], and IRRS [257], Tos17 mutants phenotype data [245] (http://tos.nias.affrc.go.jp/), South Green Bioinformatics platform (http://www.southgreen.fr/category/data-types/phenotype), OryzaSNP (http://oryzasnp.org/iric-portal/), and Oryza Tag Line [258]. Public-private partnership has been undertaken for Global Rice Phenotyping Network (GRiSP) initiated in 2011 to conduct a joint effort in the measurement of ~300 accessions each from indica and japonica genotypes to dissect important traits such as yield, resource use efficiency, and responses to major environmental stresses (http://ricephenonetwork.irri.org/). In a recent review by Yang *et al.* 2013 [259], a comprehensive account of rice phenomics has been documented along with the high throughput techniques used to generate large dataset for various physiologically important traits. During the last two years, several studies have employed phenomics platforms to comment on the different traits of rice plants. A high-throughput phenotyping tool was developed for imaging techniques with a "yield traits" scorer to automatically screen for 15 agronomic traits in rice [242]. Infrared imaging and non-destructive imaging was used for phenotyping rice to describe salinity related traits [260, 261]. C4 rice has been in the limelight because of its obvious advantages and phenotyping the associated plant architecture has been a challenge for phenomics. An automated, high-throughput phenotyping system (LUNTIAN) is being developed for the greenness and biomass of C4 rice [262]. These studies emphasize the importance of rice phenomics as well as the need for novel technology driven tools to strengthen the efforts in plant phenotyping. There is a substantial progress in phenomics tools in developed countries but developing countries are still lagging behind. Concerted efforts are therefore required to make techniques more user friendly, cost effective and accessible to rice scientists from developing countries as well. Moreover, data being generated from studies across the globe should be made accessible on a common platform.

CONCLUSION

The end goal for any functional genomics study is to utilize the information thus obtained for crop improvement. There is no need to emphasize that it is imperative to improve rice yields to sustain the exponentially growing population and economies which significantly rely on it. One of the major breakthroughs in improving rice yield has been brought about through conventional breeding by introduction of a single gene, sd1 which heralded the first green revolution. The gene, *sd1,* is responsible for altering plant architecture through hormonal perturbations. Hybrid rice was another turning point for rice productivity. Both these exemplary achievements validate the importance of understanding gene function as well as breeding efforts. However, rice yields now have reached a plateau and are declining in many parts of the world. This decline can be attributed to poor culture practices like monoculture of same variety year after year on the same agricultural land till it is superseded by a better variety; thus robbing the land of its nutrients. Secondly, injudicious use of inputs like fertilizers, pesticides, insecticides and deterioration of land due to industrialization and urbanization leads to poor yields. Adding to our woes is the continuous challenge of climate change evident by aberrant temperatures, precipitation and flooding, and sea level rise. To counteract these issues, we need climate smart super rice with insect, pest and disease resistance, high nutrient use efficiency, drought resistance, high grain quality and yield. Rice varieties with introduction of *sub1* which tolerate submergence conditions and offer a yield advantage of 1.6 t/ha is just a small step in this direction [263]. Similarly, a major QTL identified for drought on Chromosome 1, qDTY1.1, gives a 27% yield advantage than the susceptible varieties [264, 265]. Several such promising studies are being reported through conventional as well as modern approaches. The last decade has been witness to development of ingenious technologies and innovations in science which have been successfully adopted in understanding plant functional biology. Rice functional genomics is now at the crossroads of multidisciplinary approaches to address issues pertaining to ease in generating gene function data. Every year breakthrough studies are being published generating usable information to decipher varied aspects of rice functional genomics. However, the aim of delineating the function of each and every gene is far from reach as yet. Even if we generate this data, making sense of this data to engineer ideal plant types is still a distant vision. However, we are moving in the right direction with global efforts; there is a further need to establish a global resource platform to bring all the data under the auspices of a common platform. A recent effort to integrate the genome variant information, gene expression data, literature annotation and homologue sequence information in rice on a common platform is first step towards combining the available data (http://202.127.18.221/RiceHap3/home.php). Similar efforts in collaboration can strengthen the functional genomics research in rice. More and more data should be made publicly available and public-private partnership should go hand in hand in both developing and developed countries. Rice being a model cereal will not only benefit from such functional genomics studies but would benefit other cereal crops too. The benefits of rice functional genomics can be fully realized by engineering ideal genotypes only by cooperation and collaboration between researchers globally.

LIST OF ABBREVIATIONS

IRGSP	=	International rice genome sequencing project
IRRI	=	International Rice Research institute
GRiSP	=	Global Rice Phenotyping Network
mQTL	=	Metabolite associated quantitative trait loci

GWAS = Genome-wide association study

RAP-DB = Rice Annotation Project Database

EMS = Ethyl methanesulfonate

MNU = N-methyl N-nitroso Urea

DEB = Diepoxybutane

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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