The draft genome of the fast growing non-timber forest species Moso Bamboo (*Phyllostachys heterocycla***)**

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Supplementary Notes

Bamboo and the individual subjected to genome sequencing.

Bamboo is the general name of plants which belong to a Bambusoideae in Gramineae family, about 1,250 species and 90 genera widely distributed in the tropical and subtropical areas of Asia, Africa, America, and Pacific islands, and a few in the temperate and frigid zone¹.

According to the statistics, there are about 500 species of bamboo and totally 53,800 km^2 of bamboo forest in China, the largest cultivation area and the maximum gross output value of bamboo industry in the world. The [export](http://www.haodic.com/query/export/) [volume](http://www.haodic.com/query/volume/) [of](http://www.haodic.com/query/of/) bamboo products was US\$ 14 billion in 2009 and the gross output value of bamboo industry was about US\$ 130 billion in 2010. Bamboo is one of the most important non-timber forest resources and forest products in terms of their ecological conservation, feasible economic profit and social benefit, increasingly playing an important role in increasing farmer income and promoting local economic development. Fast growing, high productivity, strongly regeneration capability and various benefits of bamboo attracted attention in countries, where it was a sustainable material to supply works of the planting and industry to billions of people.

Moso bamboo is a large woody bamboo with the highest ecological, economic, and cultural values of all bamboos in Asia and accounting for ~70% of total area of bamboo growth and 5 billion US dollars of annual forest production in China¹. The moso bamboo is one of the plants with an incredible growth speed in the world. The height growth of its shoot is rapid and steady in suitable condition in spring depended on its strong rhizome-dependent system. At the growth peak, the shoot of the moso Bamboo can grow as long as 100-cm within 24 hours, and reach to its maximum height about 20 meters in shortly 45 to 60 days. It grows predominantly by vegetative propagation due to its unique rhizome-dependent system. Bamboo has a very term flowering interval as long as 60 to 120 years. All of these mysteries attracted our interesting in its biological research.

The moso bamboo genome contains 24 pairs of chromosomes, as well as basic number of chromosomes characters in other bamboos. Prior to the whole genome sequencing, we checked different moso bamboo communities growing in main planting areas, crossing Eastern, Southeastern, and Southern China. We finally chose the individual growing in the Tianmu-Mountain National Nature Reserve in Zhejiang Province of Eastern China (119º26'55.0"E, 30º19'13.4"N; 480-meter in elevation) where its growth had not been interrupted by human activities for a long time.

The tissues for the transcriptome sequencing.

Five vegetative tissues (young leaves, rhizome, root, tip of the 20cm-high shoot, and tip of the 50cm-high shoot) were collected in the Tianmu-Mountain National Nature Reserve in Zhejiang Province of China in spring, which was from the same individual used in genome sequencing. To perform the transcriptome sequencing of floral tissues, we spent over two years to look for the floral tissues of moso bamboo in 8 provinces of China because its flowering was too rare. Finally, in early summer of 2010, two reproductive tissues (panicles at early stage and panicles at flowering stage) were obtained in suburban Guilin (110º31'20.2"E, 25º10'42.7"N; 216 meter in elevation), Guangxi Province of Southern China, more than 1800 kilometers (1,100 miles) from our institute. The collected panicles from plant with no flowering or post-flowering spikelet were considered as panicle at early stage, while those from the plant growing at least 50% of flowering or post-flowering spikelet were considered as that at flowering stage.

Cytogenetic analysis of moso bamboo chromosomes.

Fluorescence In situ hybridization.

Meiotic pachytene chromosomes were prepared from the root tip of freshly germinated seedling. Individual pachytene chromosomes were identified by fluorescence *in situ* hybridization using rice 45s rDNA probe², according the method

described in Jiang et al.³. Digital images were recorded from an Olympus BX60 fluorescence microscope.

Estimation of genome size of the moso bamboo by the flow cytometry.

We put 2-month-old leaves from the sequenced bamboo individual into a flow cytometric analysis to estimate genome size as mentioned by Galbraith⁴. Finally, over 10,000 nuclei were analyzed per sample with a FACSAria flow cytometer (Becton, Dickinson and Company), equipped with 488 nm argon laser. 24 samples were analyzed using rice as the standard species. The software BDFACSDiva was used for data analysis with the coefficient variation controlled in 5%. The peak values of the fluorescence intensity of 24 bamboo and rice samples, the genome size of moso bamboo was estimated to be about $2,075.025 \pm 13.08$ Mb, or 2C DNA about 4.24 pg (1 pg DNA = 0.978×10^9 bp ⁵).

Estimation of genome size of the moso bamboo by the frequency of k-mer occurency.

Values of K-mers were plotted against the frequency at their occurency (**Supplementary Fig.3**). At a K-mer size of 51, the peak occurrency is at 36. Study of the panda genome used K-mer frequency to estimate the genome size⁶. To avoid potential over-estimation of genome sized introduced by base errors, we turned to the modified method as described in the paper of the Tasmanian devil genome⁷ to estimate the genome size of the moso bamboo. As the definition of genome size, the total number of effective K-mer words divided by the K-mer depth or the K-mer occurrence number at the peak kmer frequency D_{p} , $G_{s} = (K_{n} - K_{s})/D_{p}$. Here K_{n} is the total number of K-mer words and K_s is the number of single or unique K-mer words. So we estimated the genome size to be (80477036861 - 9537946584) / 36 = 1.97 Gb.

BAC and BAC-end sequencing.

The moso bamboo BAC library was constructed by Amplicon Express, USA,

composed of 165,888 clones harvested from a Hind-III BAC library with epicenter pCC1BAC Cloning-Ready Vector. The nuclear DNA was isolated from the same individual as used in genome sequencing. Average insert size of bamboo BAC is about 135 Kb. Eight randomly selected BACs were sequenced by using subcloning and standard Sanger sequencing methods. A total of 10,327 BACs were isolated with MACHEREY-NAGEL plasmid and large-construct DNA purification kit (NucleoSpin[®]96 Flash, Cat. No.740618.24). Both ends of all these BACs were sequenced by the dideoxy chain termination method using BigDye Terminator Cycle sequencing kit V3.1 (Applied Biosystems, Life Technologies). BAC end sequencing was carried out on ABI3730xl DNA analyzer. The assembling of the BACs and re-basecalling of raw BAC-end sequences were performed by the PHRED and PHRAP programs⁸. Manual editing was utilized to validate the accuracy of the re-basecalled reads.

Prediction of protein-coding genes.

We build a 7-step pipeline to construct the gene model set (**Supplementary Fig. 7**).

1) The prediction software program, FgeneSH++ with gene model parameters trained from monocots, was used in *ab initio* gene prediction to build the preliminary gene models.

2) Coding sequences of each predicted gene model were aligned to both the Repbase TE library and the moso bamboo TE library created by RepeatModeler, using the Blastn at E-value of 1e-5.

3) The Illumina RNA-seq sequences from five vegetative and two reproductive tissues were mapped onto the coding sequences of FgeneSH gene models by the aligner SMALT with parameters set to minimum Smith-Waterman (-m) at 80 (for 2*120 bp reads) or 60 (for 2*100 bp reads), maximum insert size (-i) at 1,500, and minimum insert size (-j) at 20. Only uniquely matched reads were selected in assistance with gene prediction. Information between unique matches and corresponding gene models were collected by using 2 thresholds to screen SMALT cigar outputs: A) cigar:S and cigar:A items with score at 50 or more were selected. B)

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for the selected cigar:A items, the available paired-end insert size should be at least 200 bp.

4) A total of 8,253 moso bamboo cDNAs, carrying entire coding sequences but not TE-derived, were selected from the 10,608 putative full-length cDNAs and were then mapped to the scaffolds by an mRNA/EST genome mapping program of GMAP⁹ with the parameters set to "-n 1 -f 2 -B 1 -A -t 4".

5) The gene models were screened by integrating the information from outputs of the step 2), 3), and 4), using the following 4 thresholds.

A, the gene models coding TE-elements or overlapping TE-elements greater than 10% of gene coding region were firstly discarded.

B, the models aligned to the full-length cDNAs were preferentially collected. The splicing sites were manually adjusted according to the alignment.

C, the models detected not by the FgeneSH++ but by the cDNAs were created and their coding information was added into gene model set.

D, candidate gene models without the evidence of full-length cDNAs should be supported by 2 different uniquely matched RNA-seq sequences. And at least 20% of their coding region was covered by RNA-seq reads. A pair of PE reads was treated as a single RNA-seq sequence when counting number of the mapped transcriptome reads for each model.

6) Information of cDNA-supporting UTR ends was attached to the gene model set.

7) The single-exon genes were manually checked by experts and the genes with no hits to homologs of grass genes were also discarded.

8) For the gene with different transcripts, the longest one was selected.

Comparison of parameters of gene models among plant genomes.

We did comparative analysis between the bamboo genes and the genes identified from *Arabidopsis*, Brachypodium, rice, sorghum, and maize. The bamboo gene models exhibited very high similarity to other grass species in all of these parameters, such as the distribution of gene length, coding sequences (CDS), exon length, intron length, GC content in coding region, and exon number per gene (**Supplementary Fig. 8**). Of compared species, only dicot *Arabidopsis* is obviously different from any other species in gene length, CDS GC content, and intron length.

Comparison of the assembled scaffolds to the available moso bamboo sequences in database.

The assemblies were compared with available sequences in the public database to assess the genomic coverage and assembling accuracy. In the GenBank till October of 2011, there have been 1,086 genome survey sequences, and 18 gene sequences. Alignment of the known genomic sequences with the length over 2 Kb to our assemblies showed that over 98% of sequence region were covered by the assembled scaffolds and 91% covered by a single best match (**Supplementary Table 2**). Similar coverage, 96% of all matches and 94% of the single best match, was observed in alignment of the rest 996 genome survey sequences less than 2 Kb. Most of the sequences with low coverage have putative sequencing errors because of more biases distribution within them. The known 18 gene coding sequences with total length of 28,741 bp were parallelly mapped onto the assemblies. Almost each one had a perfect match located on a single scaffold except unmatched bases at the end of the genes (**Supplementary Table 3**). The average sequence identities in aligned region were over 98%. Our manual check revealed that most unmatched bases at the end should be the low quality bases introduced by sequencing of amplified DNA fragments. Prior to this study, over ten thousand putative full-length cDNAs were cloned and sequenced for the moso bamboo. Of them, 8,253 cDNA sequences were picked up when those TE-derived, false ORF-coding, and non-moso-bamboo items were removed. We mapped the cDNAs onto the assemblies by means of GMAP. A total of 8118 (98.4% of 8,253) cDNAs were uniquely aligned to the assembled scaffolds with very high identities (averagely at 99.1%, **Supplementary Fig. 4**). All of the sequence comparison consisted with the estimated 98% coverage of genome assembly.

To evaluate the quality of whole genome shotgun assembly, the assembled scaffolds were aligned to 8 finished bacterial artificial chromosomes (BACs)

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sequences with average length 133 Kb by Sanger sequencing technology. Seven BACs were well aligned to a single scaffold and 1 BACs were aligned to 2 scaffolds each (**Supplementary Fig. 5**). The coverage of the scaffolds and initial contigs on the BACs were up to 88.8% and 98.8% (**Supplementary Table 4**), which supported our estimation of whole genome coverage of the assemblies. The frequency of single-base difference and insertion/deletion were approximately 0.19 and 0.09 per Kb, without regard to heterozygous single nucleotide polymorphisms (SNPs) and short indels detected by the annotation. The average PE read depth in aligned regions was at 100- to 132-fold coverage. The incompatible bases were inclined to located near the unclosed gaps, indicative of assembling quality were lower at the end of the initial contigs. Prior to sequence alignment, we have removed most of the sequence errors in assembly of the Sanger BACs. The detected SNPs or short indels were probably derived from potential heterozygosity or low rate assembling errors.

Repeat annotation.

The *de novo* repeat annotation revealed that the moso bamboo genome comprised approximately 59% transposable elements (TEs). Detection of the TEs in the Sanger-BACs showed 53% of TE content, similar with that in whole genome. With comparison to other grass species, the moso bamboo genome had similar TE content to that of the sorghum (62%) (main text ref. 36), and more TE content than rice (40%) (main text ref.18) and Brachypodium (28%)¹⁰, but lower than maize (84%) (main text ref.36, 26). Of the observed TEs, retrotransposons were the dominating repetitive sequences (39%), as well as 9.5% of DNA transposons. Like the rice, sorghum and maize genomes, the most abundant repeats in bamboo were long-terminal repeat elements (LTRs), 24.6% of *Gypsy*-type LTRs and 12.3% of *Copia*-type LTRs.

Bamboo genome has the highest copy numbers of TEs, *Gypsy/Copia*-type LTR retrotransposons and En/Spm transposons. Rice and sorghum have the highest copy numbers of MITE transposons (Tourist & Stowaway), and Harbinger transposons

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and *Gypsy/Copia*-type LTR retrotransposons, respectively (**Supplementary Table 10b and 10c**). It was inferred that insertion of the LTR retrotransposons played the most important role in expansion of the higher plant genome size, though some DNA transposons also had very high copies. The TEs covering approximately 11% of the bamboo genome were not classified. However, their average unit length was within the range of the LTRs, implying that there should still be some unknown TEs active in the bamboo. A total of 9,412 intact LTR retrotransposons were predicted in our genome assemblies. The average length was approximately 10.3 Kb, 3 times of the average gene length.

We performed *de novo* prediction for LTR retrotransposons with LTRharvest and LTR FINDER on the large-sized scaffolds (>10 Kb), using default parameter but -maxdistltr set at 30,000. The quality criteria were the existence of one or more typical retrotransposon protein domains and the simple-repeat/tandem-repeat content less than 35%. A total of 9,142 remaining candidates were considered as the full-length LTRs, including 3,103 *Gypsy*-type (PR-RT-INT), 2,677 *Copia*-type (PR-INT-RT), and 3,632 unclassified.

Identification of gene synteny and whole-genome duplication.

Detection of syntenic genes between bamboo and rice and between bamboo and sorghum.

To generate a pair-wise alignment of gene models between bamboo and rice [\(MSU](http://rice.plantbiology.msu.edu/) [RGAP 6.1\)](http://rice.plantbiology.msu.edu/) and between bamboo and sorghum (v1.4), 30,379 bamboo genes located on the larger scaffolds (>50 Kb) were aligned to the reference gene models by Blastp with E-value < 1e-20. The evidence-based gene prediction approach scarcely concerned gene colinearity between bamboo and other grasses and also expected to miss some genes. So two criteria were used to call syntenic gene blocks in bamboo scaffolds: i) number of the genes in one syntenic block >= 5; ii) number of non-syntenic bamboo genes between two adjacent syntenic genes <5. A perl script and following manual check was applied to determine the syntenic blocks and breakpoints between the blocks.

Identification of the WGD by investigating the collinear orthologous genes between bamboo and rice.

According the location of rice genes in chromosome, the collinear gene blocks of bamboo were mapped to the rice chromosomes. The overlapped gene blocks were manually checked to remove redundancy. As shown in **Supplemental Fig. 10a**, moso bamboo seemed to carry as two duplicates as that of rice gene model sets, though lots of bamboo gene lost within these regions during the duplication. It was suggested that the large-scale genome duplication in bamboo resulted from whole genome duplication not from segmental duplication, which supported a tetraploid origin of bamboo. Interestingly, rice chromosomes are characteristic of diploid (2*n*=24) while moso bamboo chromosomes are 2*n*=48. There might be unknown connection between them. We collected the orthologous pairs of bamboo, which share unique rice ortholog in collinear blocks, to estimate the divergence time with universal substitution rate of 6.5×10^{-9} mutations per site per year. Thus, estimated 7 to 15 mya was the potential time when two bamboo porgenitors diverged **(Supplemental Fig.10b**), and the WGD should occur more recent. However, the moso bamboo chromosomes now exhibit to be a diploid according to the FISH analysis. Consequently, we speculated that there is a long progress from tetraploidization to diploidization in moso bamboo since approximately 7-12 mya. But for the bamboo species with different chromosome number or ploidy number, the process should be variable.

Annotation of gene function and comparison of fundamental pathways.

Prediction of gene function motifs and domains were performed by Interpro¹¹ against available databases, including ProDom, PRINTS, Pfam, Panther, Profile, PIR, Smart, and Pattern. The gene functional ontology was retrieved from the outputs of InterPro using Gene Ontology¹²

The bamboo gene models were aligned to entries of sorghum, rice, and maize from the KEGG database (release till April 2011) by Blastp under E-value 1e-10 to find the best hit for each gene. The similarity of each pathway is the ratio of number of shared enzymatic steps and sum of referenced enzymatic steps. For instance, similarity (bamboo vs. rice) = number of the enzymatic steps shared by rice and bamboo / sum of the rice-gene-involved enzymatic steps.

Annotation of conserved non-coding RNA (ncRNA) genes.

Identification of transfer RNAs (tRNA).

The $tRNAScan-SE^{13}$ algorithms with default parameters were applied to prediction of tRNA genes in the Arabidopsis, sorghum, maize, rice, Brachypodium, and bamboo genomes. Bamboo had 1,167 tRNA genes in the assemblies, nearly 0.5 times as many as that of maize because most of bamboo pseudogenes were not detected in the current assemblies (containing 10% unclosed gaps). The same analysis of the Arabidopsis¹⁴ and Brachypodium¹⁰ found 699 and 615 tRNA loci, respectively, very closed to 711 and 614 identified by their Genome Initiatives, suggesting that most of bamboo tRNAs had been found. Of all conserved tRNA genes, selenocysteine and suppressor tRNAs were involved in a special coding way by the stop codens. It was interesting that 6 Selenocysteine and 1 suppressor tRNAs were detected in the bamboo genome, which were only found in maize and sorghum of Panicoideae but not in its sister groups, Brachypodium of Pooideae and rice of Ehrhartoideae.

Identification of rRNA genes.

The rRNA fragments were identified by aligning the rRNA template sequences (Rfam database15, release 10.0) of *Arabidopsis thaliana, Oryza sativa, Sorghum bicolor*, and *Zea mays* using Blastn with E-value at 1e-10 and identity cutoff at 95% or more.

Identification of other non-coding RNA genes.

The miRNA and snRNA genes were predicted by INFERNAL 16 software against the Rfam database (release 9.1, 1,412 families). To accelerate the speed, a rough filtering prior to INFERNAL was performed by Blastn against the Rfam sequence database under E-value at 1. For the miRNA prediction, the assemblies were aligned to the precursor sequences of *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa, Sorghum bicolor, Saccharum officinarum, Triticum aestivum, Hordeum vulgar*e, and *Zea mays*, derived from the Rfam sequence database. The extended sequences covering detected loci region and 50 bp franking sequences from both ends of the region were put into the INFERNAL prediction with cutoff score at 30 or more. The predicted mature sequences of the bamboo miRNA were aligned to gene model set to detected miRNA target genes with Blastn under E-value at 1e-10. In the snRNA predictions, the assemblies were firstly aligned to the snRNA sequences of *Arabidopsis thaliana*, *Oryza sativa, Sorghum bicolor, Triticum aestivum* and *Zea mays* from Rfam database. The extended sequences, similar as that in miRNA prediction, were put into the INFERNAL prediction with cutoff score at 50 or more.

The C/D snoRNA were predicted using snoScan¹⁷ with the yeast rRNA 16 methylation sites and yeast rRNA sequences provided by the snoScan distribution. The minimum cutoff score was based on the settings which yield a false positive rate of 30 bits. Similarly, H/ACA snoRNAs were detected by snoGPS using the yeast score tables and target pseudouridines 18 .

Quantity of all predicted non-coding RNA genes was listed in **Supplementary Table 9**.

Reconstruction of phylogeny among 6 fully sequenced grass genomes.

The OrthoMCL clustered a total of 968 single-copy gene families among 6 fully grass and 2 dicot genomes, which was used to reconstruct the phylogeny. The coding sequences of the genes were concatenated to a supergene for each species. When the best substitution model (GTR+gamma+I) were determined by Modeltest¹⁹, the supergene sequences were subjected to phylogenetic analyses by Mrbayes (main text ref.54) with the parameter set to 1,000,000 (1 sample / 100 generations) and the first 250 sample were burned in. Two independent runs reached the same result using Arabidopsis as an outgroup. Branch-specific *dN* and *dS* were estimated with codeml of PAML²⁰. The output of OrthoMCL and phylogenic tree structure were subjected to a computational analysis of changes in gene family size with the software CAFE (Online methods ref. 55).

Estimation of divergence time of paralogous pairs.

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To estimate the divergence time of the paralogs, we selected the gene families consisting of exactly 2 members to calculate the Ks of the pairs because the 2-member gene families had a single divergence relationship between two members. Thus, 3,786 bamboo 2-member gene clusters were put into the calculation of Ks, together with 2,552 rice, 2,161 sorghum, 1,874 Brachypodium, 3,285 maize, and 2,665 foxtail millet clusters. The Ks was calculated by a model that averages parameters across 14 candidate models $(P$ -value ≤ 0.001 ³⁰ and then converted to the divergence time using a substitution rate of 6.5 \times 10⁻⁹ mutations per site per year.

Estimation of divergence time between orthologous genes.

To estimate the divergence time, the Ks values were calculated between orthologs of bamboo and other genome from the 968 single-copy gene clusters determined by the OrthoMCL calculation. The Ks distribution of the one-to-one orthologous pairs of bamboo-Brachypodium, bamboo-rice, bamboo-foxtail-millet, bamboo-sorghum, bamboo-maize, and bamboo-wheat suggested the different divergence time between bamboo and other grass genome, which was consisted with the phylogenic relationship generated by Mrbayes analysis. The mean Ks was used to estimated the divergence time between different genomes. The internal duplication during the WGD was estimated by calculating the Ks of the paralogs in 2-member gene families of bamboo and maize, which was then converted to the divergence time to indicate the WGD time.

Calling of heterozygous SNPs and small indels.

To detect the heterozygous sequence polymorphism, all of the used PE reads (around 120× coverage) were firstly mapped to the assembled scaffolds by aligner SMALT. The SNPs were then called by SSAHA_Pileup (version 0,8) and 6 thresholds were used to post-filter unreliable SNPs: 1) SSAHA_Pileup SNP score >= 20; 2) ratio of two alleles between 3:17 to 17:3; 3) the highest sequencing depth of SNP position \le 240; 4) the lowest sequencing depth for each allele \ge 5; 5) the minimum distance for adjacent SNPs >= 10 bp; 6) only one polymorphism detected at each SNP position. The small indel (length \leq 6 bp) were called by the Pindel²¹ and 4 thresholds were used to remove unreliable small indels: 1) length of indels \le =6 bp; 2) the highest sequencing depth of indel position \le 240; 3) the lowest sequencing depth for each allele >= 5; 4) ratio of gapped and ungapped reads at the indel position between 3:17 to 17:3.

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Supplementary Figures

a

b

Supplementary Figure 1 Cytogenetic analysis of bamboo chromosomes. (**a**)

Fluorescence *in situ* hybridization of the moso bamboo at mitotic metaphase with the probe of rice 45S rDNA. The chromosomes were dyed by DAPI. The 45S rDNA probe was labeled with digoxin. Signals were detected by FITC with a magnification of microscope at 200 times. Two copies of chromosome sets were displayed. (**b**) Test result of *Oryza sativa* and *Phyllostachys heterocycla* mixed samples by using flow cytometry. The term C-value refers to the amount (picograms) of DNA contained within a haploid nucleus or one half the amount in a diploid somatic cell of a eukaryotic organism. Blue peak indicated 2 C DNA of *Oryza sativa* at 16,378. Pink peak indicated 2 C DNA of *Phyllostachys heterocycla* at 79,892. Compared with that of rice (430 Mb), genome size of the moso bamboo was estimated to be 2 075.025 \pm 13.08 Mb.

Supplementary Figure 2 Phusion-meta pipeline of short read assembly. To complete assembly of this highly heterozygous genome, we developed an integrated *de novo* assembly pipeline for large-sized genomes using short read sequencing data. Although this pipeline used existing algorithms and assembler, there were three critical variables introduced into the assembling strategy: i) filtering of the paired-end (PE) reads by K-mer occurrency to lower sequence error; ii) clustering of the reads before assembling to reduce assembling errors derived by direct assembling of the short read; iii) multiple use of reads and contigs in different cycles to make up for deficiencies of different algorithms. Thus, we generated comparatively high-quality assemblies by using nearly entire short reads. This pipeline therefore can be efficiently used in *de novo* assembly of complex and large-sized genomes.

Supplementary Figure 3 Distribution of Kmer frequency. Distribution of 51-mer frequency in the reads of short insert size libraries (350 - 400 bp). Values of K-mers were plotted against the frequency (*y*-axis) at their occurrency (*x*-axis). The leftmost truncated peak at low occurrence (1-2) was mainly due to random base errors in the raw sequencing reads. The frequency exhibited a bi-modality caused by heterozygosity.

Supplementary Figure 4 Mapping of the full-length cDNA sequences to the moso bamboo genome. The cDNA sequences were aligned to the assembled scaffolds. Totally, 8,118 of 8,253 (98.4%) cDNAs were uniquely mapped onto the assemblies with high identities at 99.1% using the GMAP. The *Y* axis showed accumulative frequency of the aligned cDNAs. The *X* axis exhibited the identity of sequence alignment.

Supplementary Figure 5 Alignment of assembled scaffolds to the BACs sequenced by Sanger method**.** Depth of reads in blue was calculated by mapping PE reads onto the BAC sequences. Repeats in red showed the RepeatMasker-annotated TEs on the BAC sequences. The blocks in white indicated the unfilled gaps on the scaffolds. The grey blocks showed aligned region between Sanger BACs and scaffolds. GC contents were shown in green curves.

b. Number of insertion and deletion

Supplementary Figure 6 Comparison of detected assembling errors by different assembling methods.

Supplementary Figure 7 Simplified pipeline of gene prediction with the combination of *ab initio* gene prediction, mapped RNA-seq reads, and cDNA sequences. Using both RNA-seq data and 8,253 cDNA sequences, we initially detected 35,378 transcribed loci in the genome. By applying the stringent criteria to gene prediction, a total of 31,987 high-confidenced genes were finally identified in the annotation, which were in the same range as those of other grass families.

Supplementary Figure 8 Comparison of gene parameters among fully sequenced genomes. Gene structure of the *Phyllostachys heterocycla* (phe) showed highly consistent with that of other grass speices, including *Arabidopsis thaliana* (ath), *Brachypodium distachyon* (bdi), *Oryza sativa ssp. Japonica* (osa), *Sorghum bicolor* (sbi), and *Zea mays* (zma), in distribution of gene length, exon number per gene, coding sequence length, GC content in coding region, exon and intron length.

Supplementary Figure 9 Quantitative comparison of single-copy genes and gene families consisting of 2 to 4 members among foxtail millet, maize, sorghum, Brachypodium, rice, and bamboo. The gene families and single-copy genes were categorized by OrthoMCL analysis. For the *y* axis, number of gene member at 1 indicated the single-copy gene and 2 to 4 meant that the gene families consisted of 2 to 4 member(s). The *x* axis indicated their proportion (%).

a

Supplementary Figure 10 The bamboo WGD identified by analysis of gene collinearity between bamboo and rice orthologs. (**a**) Collinear gene blocks between bamboo and rice genome. The rice genes are arranged according to their gene order. Rice gene sets on different chromosomes were exhibited in blue rough lines. The ordinal number of the genes were measured by the bar in the left. The collinear gene blocks of bamboo were shown in red blocks, which implicated that moso bamboo carried nearly two duplicates of rice genome. (**b**) Estimated divergence time of bamboo orthologous pairs. Only the orthologous pairs of bamboo sharing unique rice orthologs in collinear blocks were used to estimate the divergence time. The 7 to 15 mya was the potential time when two bamboo porgenitors diverged, and the WGD should occur more recent than it.

Supplementary Figure 11 Ks distribution of orthologous genes between bamboo and grass species. The bin size of Ks value was 0.05. Frequency was quantity of the one-to-one gene clusters.

a

Supplementary Figure 12 Phylogenic tree of *CesA* and *Csl* gene families among Arabidopsis, poplar, rice, maize, sorghum, Brachypodium, and bamboo. (**a**) NJ tree of *CesAs*. A, B, C, D, E, F, and G indicated 7 clades where the bamboo genes were located. (**b**) NJ tree of *Csls*. Different subfamilies were shown in different colors. (**c**) NJ tree of CCR genes*.* (**d**) NJ tree of HCT genes*.* The bamboo genes were labeled by red point. The numbers beside the branches were bootstrap percentage.

C

Supplementary Figure 13 A hypothesized pathway in activation of flowering. (**a**) Quantified expression levels of bamboo *Dof* (the homolog of the *OsDof12*) and *MADS14* (one of homologs of the *OsMADS14*, involved in the FMI) in different tissues. The expression is indicated as the normalized quantified transcript levels (RPKM). (**b**) A predicted pathway in controlling of flower-time in bamboo. Of the identified floral genes, the homologs of *OsMADS14* (main text ref.30) (bamboo *MADS14s*, Identity >70%, FMI genes) were highly expressed in panicles (16- to 84-fold over vegetative tissue, Q-value < 0.001). However, the expressions of the homologs of its upstream regulatory genes, such as *OsSOC1*23, and *Ehd1*24, were not detected (**Supplementary Table 17**) except for a homolog of *OsDof12*²⁵ (bamboo *Dof,* Identity >80%, **Supplementary Table 16**) with significantly higher expression in panicles (5- to 26-fold, Q-value < 0.001), implying that the bamboo *Dof* might also be functional in regulating *MADS14*s at the flowering of bamboo. As the previous studies in rice, the *OsDof12* can be induced under long-day conditions²⁵ or drought stress²⁶. The flowering bamboo were grown in the typical short-day growing area in Southern China where a severe drought had just occurred, suggesting that the bamboo *Dof* was likely induced by drought stress. Taken together, a pathway of drought-*Dof*-*MADS14*-flowering might be active during the flowering.

Supplementary Figure 14 Distribution of insert sizes in paired-end sequencing libraries with inserts at around 3 to 18 Kb. (**a**) Distribution of the library with insert sizes around 3Kb. (**b**) Distribution of the library with insert sizes around 7-8 Kb. (**c**) Distribution of 16-18 Kb insert library. The insert sizes and their distribution were estimated by counting the number of read pairs located on existed initial contigs.

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Supplementary Figure 15 Number of detected non-TE-derived loci by transcriptome sequences from 7 different tissues, tip of 20cm-high shoot (shoot20), tip of 50cm-high shoot (shoot50), rhizome, root, leaf, panicle at early flowering stage (panicle_1), and panicle at late flowering stage (panicle_2). The number indicated quantity of the detected loci in corresponding tissues. Some of the detected loci were discarded in filtering of gene models, which resulted in the detected number in some tissues was more than final gene models.

Supplementary Figure 16 Transcriptome evidence for gene prediction in moso bamboo. A potential gene model should be supported by at least 20% coverage of transcriptome reads in gene coding region. Over 27,000 (87% of 31,987) genes' coding regions were strongly supported by transcriptome sequences.

Supplementary Tables

Supplementary Table 1 Summary of sequence assembly.

Supplementary Table 1b Summary of sequencing and assembly of the moso bamboo genome.

† Final scaffolds with less than 500 bp were excluded.

Supplementary Table 1c Comparison of length of contigs and scaffolds assembled by different methods.

Supplementary Table 2 Comparison of assembled scaffolds and genomic sequences in Genbank. The known genome sequences with the length at 2 - 40 Kb were downloaded from GenBank (Accession NO. GQ252841 - 252869). Of the downloaded sequences, gi|284434746|gb|GQ252834.1| was probably from chloroplastic DNA. Blast with E-value at less than 1e-05 was used in alignment.

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Supplementary Table 3 Comparison of assembled scaffolds and 18 available genes' mRNA/coding sequences in the public database. The mRNA and coding sequences were downloaded from GenBank. Poly(A) sequences at the end of mRNA were removed anterior to the alignment. Alignment indicated that most of the unmatched bases were located at the end of the GenBank genes, which were probably introduced by DNA amplification using homologous genes' primers of different species.

Supplementary Table 4 Comparison of the assembled scaffolds 8 independently sequenced Sanger BACs. The assemblies were aligned to the BACs using an aligner MUMmer²⁷ and Blastn with 98% or more identity. The difference of single-base and insertion/deletion in each aligned block was counted by manual checked. Content of TE-element were estimate by running RepeatMasker against constructed bamboo repetitive sequence library.

Supplementary Table 5 Comparison of detected assembling errors by different methods.

Supplementary Table 6 Statistics of heterozygous polymorphisms. The potential sites of SNPs and short indels were detected by unique read coverage in genic and intergenic regions.

Supplementary Table 7 Overview of gene prediction in some fully sequenced higher plants.

Supplementary Table 8 Pathway similarity between bamboo and selected grass genomes.

Supplementary Table 9 Predicted non-coding RNA genes.

Supplementary Table 9a Summary of tRNA genes identified in maize (Z. ma), rice (O. sa), sorghum (S. bi), Brachypodium (B. di), bamboo (P. he), and Arabidopsis (A. th).

Supplementary Table 9b Conserved non-coding RNA genes in the moso bamboo genome.

Supplementary Table 9c Prediction of microRNA target genes. The INFERNAL-predicted miRNAs were aligned to the bamboo gene models by Blastn with e-value at 1e-10. The microRNAs with were clustered into the different families according to outputs of the INFERNAL prediction against Rfam database. The functional domain of each gene was searched by InterproScan against pfam database.

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Supplementary Table 10 Statistics of Repetitive sequences.

Supplementary Table 10a Repetitive sequences in the moso bamboo genome.

Supplementary Table 10b Comparison of TEs with highest copies among moso bamboo, rice, and sorghum.

Supplementary Table 10c Comparison of TEs occupying most genome size among the moso bamboo, rice, and sorghum.

Supplementary Table 11 Mean Ks and divergence time for the bamboo versus grass species. Mean Ks and divergence time between the bamboo and fully sequenced grass species were calculated from the Ks distribution of obtainned 968 single-copy gene families. Calculation of Ks were performed by MA model that averages parameters across 14 candidate models³⁰. Divergence time were calculted using a substitution rate of 6.5 \times 10⁻⁹ mutations per site per year. The Ks of internal dupliation was estimated by calculating the Ks of the paralogous pair from the 2-member gene clusters, of which the derived divergence time was indicative of the WGD time.

 1 The wheat gene models were downloaded at ftp://ftp.ncbi.nih.gov/repository/UniGene/Triticum_aestivum/.

Supplementary Table 12 Change of gene family size in bamboo, with comparison to different plant genomes. Fields highlighted in green represented the families with expanding gene number. Fields highlighted in red represented the families with contracting gene number. The gene families were generated by the OrthoMCL analysis. The estimation of gene familiy size change was perfromed by a CAFE calculation with *P*-value < 0.01.

Supplementary Table 13 Gene synteny.

Supplementary Table 13a Statistics of syntenic bamboo loci on the aligned rice genome

Supplementary Table 13b Statistics of syntenic bamboo loci on the aligned sorghum genome

Note: A total of 30,379 (94.8% of 31,987) Bamboo loci located on the scaffolds with length over 50 KB were aligned to the rice and sorghum gene models, respectively. At least 5 genes are required to call synteny. Within a syntenic gene block, the maximum number of non-syntenic genes between two adjacent syntenic genes should be less than 5.

Supplementary Table 14 Quantity of cell wall biosynthesis genes in plant genomes.

Supplementary Table 14a Comparison of copy numbers of cellulose synthase (*CesA***) and cellulose synthase-like (***Csl***) genes among grasses, Arabidopsis, and poplar.** The subfamilies *CslA, C, D, E, F*, G, H, and *J* were classified by the referenced genes located on the same clade.

Supplementary Table 14b Copy number of genes involved in phenylpropanoid and lignin biosynthetic pathways.

* Two interrupted bamboo CCR genes were not included.

Abbreviation of the encoded proteins: Phenylalanine amonnia lyase (PAL), Cinnamate-4-hydroxylase (C4H), p-Coumaroyl shikimate 3'-hydroxylase/Coumaroyl 3-hydroxylase (C3H), 4-Coumarate:CoA Ligas (4CL), Hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase (HCT), Cinnamoyl-CoA reductase (CCR), Trans-caffeoyl-CoA 3-O-methyltransferase (CCoAOMT), Cinnamyl alcohol dehydrogenase (CAD), Ferulate 5-hydroxylase (F5H), Caffeic acid 3-O-methyltransferase (COMT).

Supplementary Table 15 Expression of cell wall genes in bamboo.

Supplementary Table 15a Gene expression of the *CesA* **subfamilies.** The expression level was shown as the quantified transcript level (RPKM) in the 7 sequenced tissues.

Supplementary Table 15b Gene expression of the *Csl* **subfamilies.** The expression

level was shown as the quantified transcript level (RPKM) in the 7 sequenced tissues.

Note: The plant cell walls represented the most predominant determinant of overall form, grow, and development. The younger moso bamboo shoot has fastest growth speed in the world, which can grow around 4 feet within 24 hours. Our phylogenic analysis and RNA-seq data revealed gene expansion in *CslCs*, *CslAs*, and *CesAs* and many duplications involved genes with higher expression in shoot. The *CslC* has been identified to encode a xyloglucan glucan synthase to produce β-1-4-glucans backbone of xyloglucans in primary wall formation³¹. The CSLA proteins synthesized $β$ -(1-4)-linked mannan in the cell wall³². The recent study found that some member of both *CslA* and *CslC* subfamily were involved in hemicellulose predominantly in Golgi membranes³³. Correspondingly, the *CslA* and *CslC* subfamilies were observed to have the highest copies among the *Csls*. So potentially, evolution of *CslC* and *CesA* families were important to formation of bamboo-specific characters in shoot development.

Supplementary Table 16 Gene expression of selected loci with significantly increased transcription level (>2- fold increase, Q-value < 0.001) in the floral tissues. The involved pathways referenced the function information of the Arabidopsis/rice homologs (TAIR10 and [MSU RGAP 6.1\)](http://rice.plantbiology.msu.edu/) and conserved domains identified by Interpro. The 7 sequenced tissues were shown as S20 (tip of 20-cm shoot), S50 (tip of 50-cm shoot), RH (rhizome), RT (root), LF (leaf), P1 (panicle at the early stage), and P2 (panicle at the flowering stage). Only the loci with higher expression in two or more copies were listed in the table. Table listed the genes carrying clustered conserved function domains (Interpro domains).

Note: description of the abbreviation was listed at the follows: ERF, ethylene-responsive transcriptional factor ; bZIP, Basic-leucine zipper (bZIP) transcription factor; CCT/B-box, CCT/B-box zinc finger protein; F-box, F-box domain containing protein; HTH myb-type, Helix-turn-helix transcriptional regulator, Myb-type; Homeobox, Homeobox domain containing protein; MADS-box, Transcription factor, MADS-box; NAC, NAC domain transcription factor; WD-40, WD-40 repeat family protein; YABBY, YABBY domain containing protein; zf-Dof, dof zinc finger domain containing protein. HSP20, Heat shock protein Hsp20; HSP70, Heat shock protein Hsp70; HSP DnaJ, Heat shock protein DnaJ; HSF, Heat shock factor (HSF)-type; Peroxidase, Plant peroxidase; Dehydrin, Dehydrin domain containing proteins; Thaumatin, Thaumatin domain containing proteins; HM, Heavy metal transport/detoxification protein); MT, Plant metallothionein, family 15; BURP, BURP domain containing proteins; MIP, Major intrinsic protein.

Supplementary Table 17 Insertion of TEs in homologs of *CO* **and FPI genes.**

† Abbreviations of gene description: *CONSTANS (CO), Heading date 1 (Hd-1), FLOWERING LOCUS T (FT), TWIN SISTER OF FT (TSF), BROTHER OF FT AND TFL2 (TFL1), Reduced Culm Number 2 (RCN2), Heading date-3a (Hd3a), Arabidopsis thaliana CENTRORADIALIS (ATC), BROTHER OF FT AND TFL1 (BFT), MOTHER OF FT AND TFL1 (MFT), BROTHER OF FT AND TFL2 (TFL2), Early heading date 1 (End1), rice LFY homolog (RFL),* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1 (OsSOC1)*.

‡ For the position of repeat insertion, the 3'-UTR (3'-untranslated region), 5'-UTR, and promoter regions were estimated according to the analysis of the bamboo full-length cDNAs when the end of the gene model was not supported by the full-length cDNA. The estimated 3-UTR length was set at less than 500 bp from stop-codon, 5'-UTR less 250 bp from start-codon, and promoter region at over 250bp upstream of start-codon.

¶ Very low meant RPKM less than 5.

Supplementary Table 18 Quantified transcription levels of bamboo genes with homologs of Arabidopsis floral genes.

† Abbreviations of the genes: *AGAMOUS-LIKE 24 (AGL24), SHORT VEGETATIVE PHASE (SVP), Arabidopsis thaliana CENTRORADIALIS (ATC), BROTHER OF FT AND TFL1 (BFT), TERMINAL FLOWER 1 (TFL1), MYB DOMAIN PROTEIN 33 (ATMYB33), CRYPTOCHROME 1 (CRY1), CRYPTOCHROME 2 (CRY2), EARLY BOLTING IN SHORT DAYS (EBS), EARLY IN SHORT DAYS 4 (ESD4), FLAVIN-BINDING KELCH DOMAIN F BOX PROTEIN 1 (FKF1), FLOWERING LOCUS KH DOMAIN (FLK), FLOWERING PROMOTING FACTOR 1 (FPF1), FRIGIDA (FRI), FLOWERING LOCUS T (FT), FVE (FVE), GA REQUIRING 1 (GA1), GA INSENSITIVE (GAI), REPRESSOR OF GA1-3 (RGA), RGA-LIKE (RGL), GAI AN REVERTANT (Gar), HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), LUMINIDEPENDENS (LD), PHYTOCHROME AND FLOWERING TIME 1 (PFT1), PHYTOCHROME (PHY), PHOTOPERIOD-INDEPENDENT EARLY FLOWERING (PIE), SLEEPY 1 (SLY1), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), SPINDLY (SPY), VERNALIZATION INSENSITIVE 3 (VIN3),* and *VERNALIZATION INSENSITIVE 3-LIKE 1 (VIN3-L)*.

Supplementary Table 19 Bamboo floral genes sharing high identities (> 50%) with known rice genes.

OsClpB-cyt; *HSP100*⁴⁷ 0.87 Heat stress tolerance 0sGRX17¹⁰⁰ 0.57 O sHsfB2b 97 0.54 $OsHsfA2c⁴⁷$ 0.83 **OsOSC11; OsIAS1** 0.78 $OsClpB-c^{107}$ 0.73

† Abbreviation of the pathways: Floral pathways of FMI or FPI (Flowering), abscisic acid pathway (ABA), Gibberellin pathway (GA), ethylene-responsive pathway (ETH), jasmonic acid pathway (JA).