The 3,000 rice genomes project

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Abstract

Background: Rice, *Oryza sativa* L., is the staple food for half the world’s population. By 2030, the production of rice must increase by at least 25% in order to keep up with global population growth and demand. Accelerated genetic gains in rice improvement are needed to mitigate the effects of climate change and loss of arable land, as well as to ensure a stable global food supply.

Findings: We resequenced a core collection of 3,000 rice accessions from 89 countries. All 3,000 genomes had an average sequencing depth of 14×, with average genome coverages and mapping rates of 94.0% and 92.5%, respectively. From our sequencing efforts, approximately 18.9 million single nucleotide polymorphisms (SNPs) in rice were discovered when aligned to the reference genome of the temperate *japonica* variety, Nipponbare. Phylogenetic analyses based on SNP data confirmed differentiation of the *O. sativa* gene pool into 5 varietal groups – *indica*, *aus/boro*, basmati/sadri, tropical *japonica* and temperate *japonica*.

Conclusions: Here, we report an international resequencing effort of 3,000 rice genomes. This data serves as a foundation for large-scale discovery of novel alleles for important rice phenotypes using various bioinformatics and/or genetic approaches. It also serves to understand the genomic diversity within *O. sativa* at a higher level of detail. With the release of the sequencing data, the project calls for the global rice community to take advantage of this data as a foundation for establishing a global, public rice genetic/genomic database and information platform for advancing rice breeding technology for future rice improvement.

Keywords: *Oryza sativa*, Genetic resources, Genome diversity, Sequence variants, Next generation sequencing
ultimate goal is to establish, through collective efforts by the international scientific community, a public rice database containing genetic and genomic information suitable for advancing rice breeding technology.

**Selection of germplasm**

A total of 3,000 germplasm accessions were chosen for sequencing, including 2,466 accessions from the International Rice Genebank Collection (IRGC) at the International Rice Research Institute (IRRI), and 534 accessions from the China National Crop Gene Bank (CNCGB) in the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS). The 2,466 accessions (in Additional file 1: Table S1A ) contributed by IRRI represent a panel that was randomly selected from a core collection of 12,000 *O. sativa* accessions that was established by a semi-stratified selection scheme from more than 101,000 rice accessions in the IRGC; taking into account factors, such as the country of origin, eco-cultural type and varietal grouping with even coverage of the name space while limiting potential duplicates from each country, and complemented by specific, nominated entries from IRRI and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (Cirad). The 534 accessions (in Additional file 1: Table S1B) contributed by CAAS included a mini-core collection of 246 accessions selected from a core collection of 932 accessions established in the same way from the 61,470 *O. sativa* accessions preserved in the CNCGB [10], plus 288 accessions selected based on their isozyme diversity [1], and used as parental lines in the international rice molecular breeding network [2]. Together, the sampled 3,000 rice accessions came from 89 different countries/regions, 77.1% of which are from the centers of rice genetic diversity -Southeast Asia (33.9%), South Asia (25.6%) and China (17.6%) (Figure 1).

Genetic stocks derived from the *O. sativa* accessions were generated for each of the sampled 3,000 rice accessions by one or more cycles of single-seed descent purification under field or screen-house conditions. New accession numbers were assigned to seeds derived from one or more rounds of multiplication starting from a single plant of each source accession. As of March 2013, new accession numbers have been assigned to 1,958 of the IRRI accessions. Purified seeds of the sequenced accessions are (or will be available) from the IRGC or CNCGB as genetic stocks. Information on obtaining seeds from the IRGC can be found at [11] and from the CNCGB at [12].

**Sequencing**

Genomic DNA was prepared from bulk harvested leaves of a single young plant for each sampled accession by a modified CTAB method either at IRRI or at CAAS. Genomic DNA samples were then shipped to BGI-Shenzhen and were used to construct Illumina index libraries...
following the manufacturer’s protocol. Following quality control, at least 3 μg genomic DNA of each sample was randomly fragmented by sonication and size-fractionated by electrophoresis, and DNA fragments of approximately 500 bp were purified. Purified 500 bp DNA fragments from each of the 24 accessions were labeled independently using distinct 6 bp nucleotide multiplex identifiers, followed by pooling prior to library construction for NGS. Each sequencing library was sequenced in six or more lanes on the HiSeq2000 platform and 90 bp paired-end reads were generated. Subsequently, the reads from each sample were extracted based on their unique nucleotide multiplex identifiers as 83 bp reads (90 – 6 – 1, where 1 is the ligation base “T”). To ensure high quality, raw data was filtered by deleting reads having adapter contamination or containing more than 50% low quality bases (quality value ≤ 5).

Data generation and analyses

Read alignment and variant identification

The clean reads were mapped to the temperate japonica Nipponbare reference genome – the unified-build release Os-Nipponbare-Reference-IRGSP-1.0 (IRGSP-1.0) [13], using the BWA software with default parameters except for “aln -m 10000 -o 1 -e 10 -t 4”. The alignment results were then merged and indexed as BAM files [14,15]. SNP calling was based on alignment using the Genome Analysis Toolkit 2.0-35 (GATK) and Picard package V1.71 [16]. To minimize the number of mismatched bases for SNP and InDel calling, all reads from each accession were further cleaned by:

1. deleting the reads that are unmapped to the reference in the alignment result;
2. deleting duplicate reads;
3. conducting alignment by the IndelRealigner package in GATK; and
4. recalibrating realignments using the BaseRecalibrator package in GATK.

SNP and InDel calling for each sample were performed independently using the UnifiedGenotyper package in GATK with a minimum phred-scaled confidence threshold of 50, and a minimum phred-scaled confidence threshold for emitting variants at 10. To ensure the quality of variant calling, the conditions for every site in a genome were set at >20 for mapping quality, >50 for variant quality and >2 for the number of supporting reads for every base.

SNP and InDel calling at the population level (i.e., for all sequenced genomes concurrently) was performed using the UnifiedGenotyper package in the GATK pipeline with 50 for the minimum phred-scaled confidence threshold for variant calling, 30 for the minimum phred-scaled confidence threshold for variant emitting, >20 for the mapping quality, MAF >0.001 for every SNP, and >2 sequence depth for genotypes in every sample. Five independent, randomly selected sets of 200,000 SNPs with minimum missing data were then selected for phylogenetic analysis.

For each of these five sets, distance matrices using the p-distances model were calculated, and NeighborJoining trees were constructed with 1,000 bootstraps using the TreeBeST software [17]. Consensus trees were exported as Newick format and imported into DarWIN v5.0.158 for topology visualization [18]. For each of the five consensus trees, prior information on variety group designation (based on SSR or isozyme classification) was used to define assignment to one of the five groups – indica, aus/boro, basmati/sadri, japonica (tropical or temperate). Groupings assigned for each of the five trees were compared using a majority rule criterion (i.e., a minimum of three trees to support the assignment). Those accessions that failed this test were labeled as intermediate types.

Findings

Using IRGSP-1.0 as the reference, the 3,000 sequenced genomes had an average depth of ~14x, ranging from ~4x to greater than 60x, and yielded a combined total of approximately 17 TB of high quality sequence data. Of the 3,000 entries, 2,322 accessions had >10x sequence depths. When aligned with IRGSP-1.0 using the BWA software, the average genome coverage and mapping rate were 94.0% and 92.5%, respectively. BWA alignment followed by variant calling using GATK identified approximately 18.9 million single nucleotide polymorphisms (SNPs) (Table 1). The distribution of the identified SNPs across different chromosomes varies considerably, with chromosomes 4, 1 and 11 having the highest numbers of SNPs and chromosomes 9, 10 and 5 having the lowest. Most SNPs were detected in intergenic regions and introns, based on comparison with gene annotations provided by MSU v7 [13,19]. Only 18.24% of the detected SNPs occur in exons, of which ~40% are synonymous.

The phylogenetic analyses revealed clear differentiation of the 3,000 accessions into two major groups – indica and japonica, two small varietal groups – the aus/boro and basmati/sadri types, plus a small group (134) of intermediate (admixed) types (Figure 2). The indica group represented the largest and most diverse group comprising 1,760 (58.2%) accessions in five major subgroups of diverse origins. The japonica group contains 843 (27.9%) accessions, which had two well-differentiated subgroups – 388 temperate japonicas and 455 tropical japonicas. The aus/boro group is composed of 215 accessions and is more closely related to indica, while the aromatic basmati/sadri group is more closely
Table 1 Characteristics of the single nucleotide polymorphisms (SNPs) identified in the 3,000 rice genomes when aligned to the reference *japonica* Nipponbare genome IRGSP-1.0

| Chrom. | Gene | mRNA | 5'-UTR | CDS | Intron | 3'-UTR | Intergenic | Total | Syn | Non-syn | Total | Non-syn/Syn |
|--------|------|------|--------|-----|--------|--------|------------|-------|-----|--------|-------|------------|
| Chr1   | 634,912 | 630,396 | 25,880 | 291,817 | 286,601 | 26,098 | 1,252,989 | 1,887,901 | 118,095 | 173,722 | 291,817 | 1.471 |
| Chr2   | 528,417 | 524,172 | 20,087 | 243,967 | 238,738 | 21,380 | 1,013,475 | 1,541,892 | 97,306 | 146,661 | 243,967 | 1.507 |
| Chr3   | 490,402 | 487,611 | 19,899 | 231,196 | 224,129 | 20,387 | 962,304 | 1,452,706 | 88,477 | 134,719 | 223,196 | 1.523 |
| Chr4   | 730,310 | 727,473 | 19,018 | 388,220 | 301,071 | 19,018 | 1,176,274 | 1,906,584 | 160,101 | 228,115 | 388,220 | 1.425 |
| Chr5   | 489,370 | 485,848 | 13,623 | 223,196 | 224,129 | 20,387 | 867,799 | 1,357,169 | 103,723 | 153,604 | 223,196 | 1.481 |
| Chr6   | 560,506 | 557,361 | 16,943 | 319,401 | 251,103 | 16,730 | 998,651 | 1,580,719 | 124,025 | 178,759 | 319,401 | 1.441 |
| Chr7   | 548,266 | 546,569 | 16,210 | 280,994 | 231,797 | 17,568 | 937,670 | 1,521,936 | 115,332 | 165,662 | 280,994 | 1.436 |
| Chr8   | 582,068 | 580,181 | 16,396 | 302,785 | 244,991 | 16,009 | 998,651 | 1,580,719 | 124,025 | 178,759 | 302,785 | 1.441 |
| Chr9   | 436,037 | 434,440 | 10,692 | 222,916 | 190,025 | 10,807 | 763,771 | 1,199,808 | 90,299 | 132,617 | 222,916 | 1.469 |
| Chr10  | 476,710 | 473,603 | 11,735 | 280,013 | 192,214 | 11,641 | 806,940 | 1,283,650 | 109,451 | 148,561 | 258,013 | 1.357 |
| Chr11  | 684,803 | 681,891 | 16,642 | 354,874 | 291,049 | 19,326 | 1,148,735 | 1,833,538 | 140,772 | 214,101 | 354,874 | 1.521 |
| Chr12  | 607,336 | 603,783 | 16,549 | 319,401 | 251,103 | 16,730 | 1,055,044 | 1,662,380 | 129,296 | 190,105 | 319,401 | 1.470 |
| ChrUn  | 19,706  | 19,706  | 0     | 12,615  | 7,091   | 0     | 26,669   | 46,375   | 5,819   | 6,796   | 12,615  | 1.168 |
| ChrSy  | 11,463  | 11,463  | 0     | 7,913   | 3,550   | 0     | 15,043   | 26,506   | 3,846   | 4,067   | 7,913   | 1.057 |
| Total  | 6,800,306 | 6,764,497 | 203,674 | 3,444,971 | 2,905,301 | 210,551 | 12,084,837 | 18,885,143 | 1,401,167 | 2,043,797 | 3,444,971 | 1.459 |

The MSU V7.0 rice gene annotation for 55,986 genes and 66,338 mRNA [13] as a raw gff3 file type was downloaded from the Rice Genome Project Annotation ftp site [19]. Prior to categorization of SNP types, the raw gff3 file was processed 1) to remove all but the primary mRNA transcript and 2) to select the gene models with the highest support in cases where there are overlapping gene models. Hence, SNP characteristics are reported here for 55,107 of the 55,986 gene models. Characteristics of SNPs in pseudogenes or where the reference base is N (unknown or missing) are not reported. Syn = synonymous; Non-syn = non-synonymous.

Figure 2 Classification of 3,000 rice accessions into five distinct varietal groups based on 5 sets of 200,000 random sets from the 18.9 million discovered SNP variants.
related to *japonica* and consists of 68 accessions primarily from South Asia.

**Availability and requirements**

**Data availability**
The sequencing data of the 3,000 rice genomes project (3K RGP) is now deposited in the *GigaScience* database (*GigaDB*) and has a citable digital object identifier (DOI) [20]. The dataset consists of separate directories for sequences from each of the 3,000 rice genomes. These directories are named by the DNA UNIQUE IDs given in Additional file 1: Tables S1A and S1B. If the DNA_UNIQUE_ID contains a space, the space is replaced by an underscore. Each directory contains from 12 to 40 Fastq (fq) files of trimmed, filtered reads that are compressed using GNU gzip (gzip, .gz). The dataset consists of about 15.4 terabytes (Tb) of files. Individual data files can be downloaded using tools such as File Transfer Protocol (FTP). In order to obtain the complete dataset, use of FTP is not possible due to the time required for file transfer and bandwidth consumed; other tools will be needed.

**Dataset name:** The 3,000 rice genomes project data

**Operating system:** Platform-independent, UNIX/Linux preferred

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**Data requirements**
After download or acquiring, depending on the task, from 8 Gb (reference-guided alignment and variant calling) to 16 Gb (*de novo* genome assembly) or more main memory is needed and from 16 to 64 Gb or more swap space allocated for each pipeline; computation will require from 7 hours (alignment and calling) to 3 days (assembly) per core per pipeline.

**Discussion**
This 3,000 rice genomes dataset provides an unprecedented resource for rice genomic research. With access to the genome sequences of the 3,000 accessions representing various varietal types of diverse origins and availability of additional high-quality rice reference genomes, further comparisons can be made among the 3,000 genomes and reference genomes of different rice types. These analyses are expected to uncover the within-species diversity and genome-level population structure of *O. sativa* in great detail. Thus, we hope that this data note will be the beginning of a new round of accelerated discoveries in rice science. Here, we would like to call for an international effort to analyze and mine the dataset. The expected information explosion from follow-up studies of the project will provide a foundation to revolutionize rice genetics and breeding research.

Ultimately, this could lead to a more thorough understanding of the molecular, cellular and physiological machineries/networks responsible for the growth and development of rice plants and their responses to various abiotic and biotic stresses.

This data note is accompanied by a ‘Commentary’ article, where the intent and plans for the projected uses of the 3,000 rice genomes dataset are further expanded [21]. Through the public release of this dataset, we encourage the global science community to analyze the data and to contribute in building a public rice genetic/genomic database and information platform that will accelerate rice breeding.

**Availability of supporting data**
The data set supporting the results of this article is available in the *GigaScience* GigaDB Database [20]. Information on SNP variants will be available on analysis of the population-level genome diversity of the 3,000 rice genomes. Raw sequence data is also available from the SRA at PRJEB6180.

**The 3,000 rice genomes project: participants and affiliations**

**Participants by institute**

**CAAS**
- Zhihong Li Email: zlhi2153@126.com or lizhihong@caas.cn
- Bin-Ying Fu Email: fubinfu@caas.cn
- Yong-Ming Gao Email: gaoyongming@caas.cn
- Wen-Sheng Wang Email: wangwensheng2@caas.cn
- Jian-Long Xu Email: xujianlong@caas.cn
- Fan Zhang Email: zhangfan03@caas.cn
- Xiu-Qing Zhao Email: zhaoxiuqing@caas.cn
- Tian-Qing Zheng Email: zhentaiqing@caas.cn
- Yong-Li Zhou Email: zhoulongli@caas.cn

**BGI**
- Gengyun Zhang Email: zhanggengyun@genomics.cn
- Shuaishuai Tai Email: taishuaishuai@genomics.org.cn
- Jiabao Xu Email: xujiaobao@genomics.org.cn
- Wushu Hu Email: huwushu@genomics.org.cn
- Ming Yang Email: yangming@genomics.org.cn
- Yongchao Niu Email: niuyongchao@genomics.org.cn
- Miao Wang Email: wangmiao@genomics.org.cn
- Yanhong Li Email: liyanhong@genomics.org.cn
- Lianle Bian Email: bianlianle@genomics.org.cn
- Xuelian Han Email: hanxuelian@genomics.org.cn
- Jun Li Email: lijun3@genomics.org.cn
- Xin Liu Email: liuxin@genomics.org.cn
- Bo Wang Email: wangbo@genomics.org.cn

**IRRI**
- Kenneth L. McNally Email: k.mcnally@irri.org
- Ma. Elizabeth B. Naredo Email: e.naredo@irri.org
- Sheila Mae Q. Mercado Email: s.mercado@irri.org
Myla Christy Rellosa Email: m.rellosa@irri.org
Renato A. Reaño Email: r.reano@irri.org
Grace Lee S. Capilit Email: g.capilit@irri.org
Flora C. de Guzman Email: f.deguzman@irri.org
Jauhar Ali Email: j.ali@irri.org
N. Ruaraidh Sackville Hamilton Email: r.hamilton@irri.org
Ramíl P. Mauleon Email: r.mauleon@irri.org
Nickolai N. Alexandrov Email: n.alexandrov@irri.org
Hei Leung Email: h.leung@irri.org

Additional file

Additional file 1: Table S1A. Information for the 2,466 rice accessions from the International Rice Genebank Collection at the International Rice Research Institute. Table S1B. Information for the 534 rice accessions from the China National Crop Genebank and the CAAS working collections.

Abbreviations
3K RGP: 3,000 rice genomes project; BGI: Beijing Genomics Institute; IRRI: International Rice Research Institute; NCSB: National Crop Seed Bank; NGS: Next generation sequencing; GRiSP: Global Rice Strategic Program; GD1393: Genomic Data 1393; IRGC: International Rice Genebank Collection; IRGC: International Rice Genebank Collection; CI: Chinese Academy of Agricultural Sciences; Cirad: Centre de Coopération Internationale en Recherche Agronomique pour le Développement; CNCGB: China National Crop Gene Bank; GATK: Genome Analysis Toolkit; IRGC: International Rice Genebank Collection; IRRI: International Rice Research Institute; NGS: Next generation sequencing.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ZKL, GZ, KLM, and HL designed the project; KLM and NRSH selected the IRRI materials; KLM, MEBN, SMQJ, RAR, MR, GLC, and FCG prepared and curated the IRRI materials; ZKL, WSW, YMG, TQZ, JLX, XQZ, FZ, YLZ and JA selected and prepared the CAAS materials; MEBN, SMQJ and MCR prepared the IRRI DNAAs; WSW, YLZ, BYF, TQZ prepared the CAAS DNAAs; WH, MY, YN, MW, YH, LB, XL, BW, JL, JX, ST and XL performed the sequencing and NGS data analyses; SS and KLM performed the phylogenetic analyses; ZKL, GZ, KLM, NNA, RPM, HL and JA wrote the manuscript. All authors have read and approved the final manuscript.

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