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Rice Genomic-Present and Future

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ABSTRACT

The rice genome sequencing project has been pursued as a national project in Japan since 1998. At the same time, a desire to accelerate the sequencing of the entire rice genome led to the formation of the International Rice Genome Sequencing Project (IRGSP), initially comprising five countries. The sequencing strategy is the conventional clone-by-clone shotgun method using P1-derived artificial chromosome/bacterial artificial chromosome (PAC/ BAC) libraries from rice variety Nipponbare as a common template resource. As of September 2000, ten countries from this international collaboration had already contributed about 30 Mb of the rice genome sequence. Analysis of the rice genome should facilitate a better understanding of the concept of inheritance in the rice plant and the development of new research endeavors in physiology and biochemistry. 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. Crucial information from nucleotide sequences will be useful for improving breeding technology as one of the ultimate goals of rice genome research.

KEYWORDS*-Rice Genome; Nipponbare; Climate change and Genetic gain*
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INTRODUCTION

Rice (*Oryza Sativa* L.) is one of the most importantcrops feeding about half of the world's population.With a compact genome, the cultivated rice species*Oryza sativa* represents a model for cereals as wellas other monocot plants [19-21]. Because ofthe availability of the whole genome sequences of both*indica* and *japonica* subspecies and abundant genetic andgenomic resources, including mutants and wild rice species,rice has become a model for comparative genomeanalysis. The completion of the genome sequence of ricein 2005 opens a new and exciting chapter in our quest tofunctionally characterize all of the annotated genes inrice [6]. A systematic approach to characterizingthese genes will allow us to dissect and understandthe regulatory networks and evolutionary selection controllingsuch complex traits as yield, grain quality, bioticand abiotic stresses, reproductive barriers, epigenetic,and flowering time. The next essential steps toward decipheringthe sequenced genome are to develop completeand accurate maps of actively transcribed regions duringrice development, and to generate more and moregenome-wide rice mutant resources. These will facilitatethe identification of all the genes and proteins encodedin the DNA sequence. Such information will allow further analysis of their function and regulation, andhow they cooperate in complex biological processes in asystems manner.

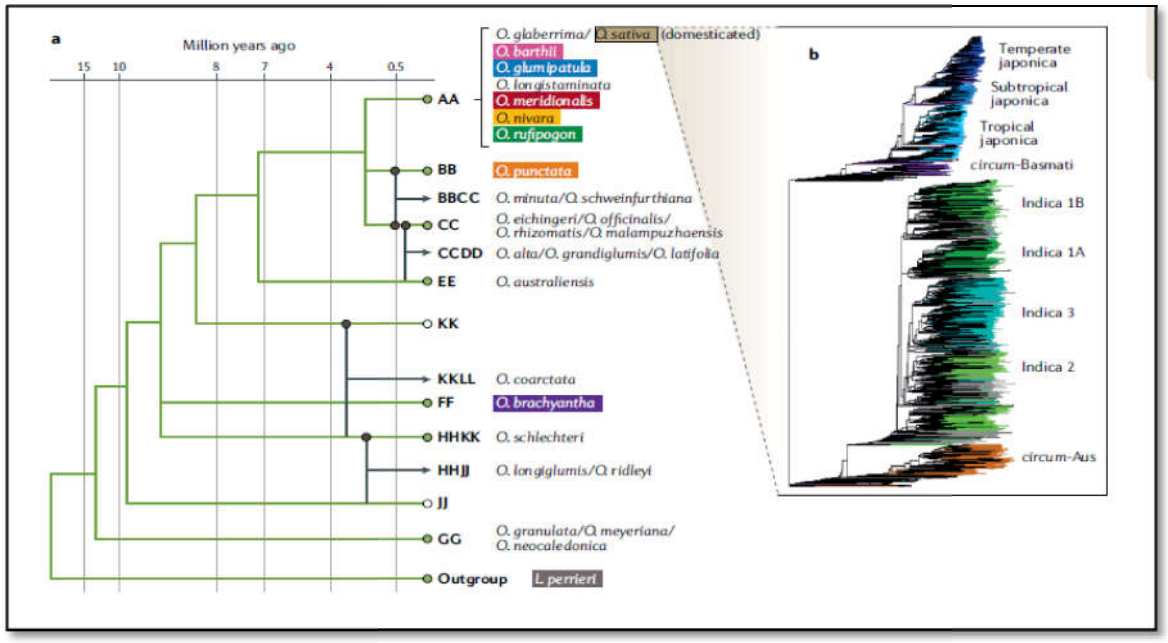

Fig 1 Phylogeny and distribution of the genus *Oryza*

PROGRESS IN SEQUENCINGAND ANNOTATION OF THE GENOME

The International Rice Genome Sequencing Project(IRGSP) has adopted the clone-by-clone approach forsequencing rice *Oryza sativa* ssp. *japonica* Nipponbaregenome sequence, because it allows efficient gap-filling,avoids problems arising from distant repetitive sequencesand results in the early completion of larger contiguoussegments of a genome (Sasaki and Burr, 2000). A mapbased,fi nished quality sequence of rice *japonica* Nipponbarewhich covers 95% of the 389 Mb genome, includingvirtually all of the euchromatin and two complete centromeres,wascompleted in 2005 [6, 2, 3, 4], The Rice Chromosome10 Sequencing Consortium, 2003). At the same time, awhole-genome shotgun sequencing approach was performedfor sequencing an *indica* variety 93–11 genome [25].With the completion of the rice genome sequencing,the Rice Annotation Project Database (RAP-DB) wascreated to provide the genome sequence assembly of theIRGSP release 3, a manually curated annotation of thesequence, and other genomics information that couldbe useful for comprehensive understanding of rice biology [18]. Also, the Rice Genome Annotation Project of The Institute for GenomicResearch (TIGR) continues to improve the quality of theannotation and to update the rice genome sequence withnew data (Ouyang et al., 2007). In the current release4.0 of the annotation, 42,653 non-transposable elementrelatedgenes encoding 49,472 gene models were identified as a result of the detection of alternative splicing.Moreover, transposon is an important content of the ricegenome, which is populated by representatives from allknown transposon superfamilies [11, 7]. Among them, Pack-MULEs can mobilizethousands of gene fragments, which may have an impacton rice genome evolution [12, 13]. The completion and annotation of the ricegenome have afforded an unprecedented opportunityfor systematic studies of plant gene function. The ricegenome sequence provides a complete catalog of genesthat are important for improving not only rice but alsoother cereals, as functionally important sequences are conserved and may be identified by their similarity.

COMPARATIVE GENOMICS IN RICE

The Asian cultivated rice *Oryza sativa* contains twomajor subspecies *indica* and *japonica*, which show distinctdivergence from sequence variations to phenotypicchanges (Oka and Chang, 1962; Cheng and Lu, 1984).*Indica–japonica* genome comparison has been chosenas a model system for understanding the origin, speciation,domestication, and genome evolution of rice. *Indica*and *japonica* cultivars can be classified based on theiragronomic traits and theuse of molecular markers [5, 2]. The sequencing of *indica* and *japonica* ricegenomes provides a powerful resource for comparative analysis, which could help to detect polymorphismsbetween them and also give clues to recent genome variations.With the available genome sequences and comparativeanalysis, DNA polymorphisms across the

entire ricegenome were discerned to develop molecular markers [6-9], which greatly facilitates gene cloningand also molecular-assisted breeding in rice.Comparisons of chloroplast, mitochondrial, andnuclear genomes revealed that the two subspecies diverged~0.44 million years ago [21, 22]. In-depthsequence comparison of genomic DNA sequences hasalso shown a large number of variations in intergenic andgenic regions between *indica* and *japonica* [3, 4, 6]. Among them, transposable elements(TEs) serve as an important evolutionary driving force for intra-specific variation. The genome sizes of both*indica* and *japonica* have increased substantially, mainlybecause of the insertions of TEs [5, 9]. The activities of the mobile elementsare responsible for a series of genetic differences betweentwo subspecies via various ways, including interruptinghost genes, creating different expression forms, drastically changing intron length, and affecting expression levels ofadjacent genes [5].From a comparative standpoint of the genus *Oryza*,a comprehensive set of 12 bacterial artificial chromosome(BAC) libraries that represent the 10 genom types of *Oryza* has been constructed, as a first step toperforming comparative genomic analysis within the genus. Furthermore, rice is alsoan important model species for the Poaceae. Comparativegenome analysis of closely related species in cerealswould be a powerful tool to identify conserved functionalunits and regulatory elements. Through comparisonswith other plant genome sequences and transcriptsequences, structural and functional features of the rice genome itself have been confirmed and improved.To further understand rice adaptation and facilitategenetic improvement of rice a comparative genomicsapproach will be necessary to make a more integratedand detailed map that collects all kinds of genetic variations,which will need to include copy-number variation,gene loss caused by frame-shift or point mutation, and other specific evolutionary events.

FUNCTIONAL GENOMICS STUDIES

Rice functional genomics is a scientific approach that seeks to identify and define the function and interactionof genes to produce phenotypic traits. Rapid progressin rice genome sequencing has facilitated research in rice functional genomics. The rice functional genomicsresearches include development of technical platforms,and molecular cloning and functional analysis of agronomic genes. The platforms are aimed at enabling highthroughput analyses and effective determination of genefunctions, which consist of three major components

(i)Generation and characterization of a large mutant library

(ii) Expression profiling of the predicted exons andexpressed sequence tags (ESTs) of the entire genome

(iii)Isolation of full length cDNAs.

Development of Technical Platforms After the release of the rice genome sequence, one of thesignificant challenges has been the large-scale identification of gene functions. Various methods and technicalplatforms have been employed to enable high-throughput analyses and effective determination of gene functions.Two major platforms of functional genomics studies aregeneration of a large mutant library and isolation of full-lengthcDNAs. Insertional mutagenesis, an effective strategy tostudy gene function, has been widely applied to constructmutant libraries [5, 6, 15, 19]. For mutant generation, T-DNA is themost frequently used foreign DNA, as it both disrupts thegene function to facilitate gene identification and providestags making gene isolation easier. About 29,482, 47,932,and 13,804 T-DNA tag lines in *japonica* rice were individuallygenerated [18-24]. Mapping T-DNA flanking sequencetags on chromosomes revealed that T-DNA integrationfrequency was generally proportional to chromosomesize; however, T-DNA insertions were non-randomly distributedon each chromosome [24]. The*Tos17* disruption system is another efficient method andwidely used for mutational analysis. More than 50,000 disruptionlines of *japonica* were produced using the endogenousretro-transposon Tos17 [15]. Phenotypes of these lines in the M2 generation were observed and characterized. This combination of phenotypic and flankingsequence data will stimulate the functional analysis ofrice genes. In the rice T-DNA mutant library, large-scalecharacterization of Tos17 insertion sites has also been conducted [16]. Full-length cDNA clones are valuable resources forthe functional analysis of genes not only at transcriptionallevel but also at the translational level (Nishiyamaet al., 2003). Th e promoter sequences can be obtained bycomparing the 5′-end sequences of cDNAs with the ricegenome sequences. Large-scale new gene discovery

wasaccelerated by utilizing these procedures. Over 32,000*japonica* full-length cDNAs have been published [15]. Collections of over 20,000 full-length cDNAs and over 40,000 5′ESTs isolated from various cDNA libraries of two *indica*varieties Guangluai 4 and Minghui 63 have also beendone [13, 23]. In addition, the*indica* cDNA clones are useful for comparative analysisbetween *indica* and *japonica* subspecies, improvementfor genome sequence annotation, and for identification oflineage of specifi c genes. Moreover, about 1888 wild rice*Oryza rufi pogon* W1943 full-length cDNAs have beencollected [13]. All these full-length cDNAclones provided important resources for further functionalstudies and could be broadly utilized in rice biologicalstudies. Moreover, through comparative analysisamong rice varieties, some putative *indica*-specific genesand wild rice specific genes were identified [12, 13].

CLONING AND FUNCTIONAL ANALYSISOF AGRONOMICS RELATED GENES AND QTLS:

The rice genome sequence provides a complete catalog ofgenes that are very important for identification of the ricegenes through map-based cloning strategy. Great progresshas been made by rice researchers in recent years in thecloning and functional analysis of agronomically importanttraits, including plant architecture [11],stress tolerance [17, 3, 23] disease resistance (bacteria blight) [1], grain yielding [3, 7-9, 21], shatteringhabit [11, 9], fertility [1], and nutrition effi ciency.Here, we describe the cloning of a number of ricegenes and quantitative trait loci (QTLs). Tillering in riceis an important agronomic trait for grain production, andalso a model system for the study of branching in monocotyledonousplants. A gene *MONOCULM 1* (*MOC1*) thatcontrols rice tillering has been cloned and characterized [10] Soil salinity is a major abiotic stress incrop productivity worldwide. Salt tolerance is a complextrait controlled by QTLs and is the fi nal manifestation ofseveral components, such as Na+ uptake, K+ uptake, ionsbalance, and ions compartmentation. The *SKC1* gene thatencodes an ion transporter was cloned from a high salttolerance *indica* variety 'Nona Bokra' by using advancedbackcross progeny and map-based cloning [17]. Th e QTL Ghd7, which has played crucial roles inincreasing productivity and adaptability of rice globally,was isolated from an elite rice hybrid and was found toencode a CCT domain protein [23]. *Ghd7* hasmajor eff ects on an array of traits in rice, including numberof grains per panicle, plant height, and heading date.Enhanced expression of *Ghd7* under long-day conditionsdelays heading and increases plant height and panicle size.Natural mutants with reduced function enable rice to becultivated in temperate and cooler regions.

RECENT MILESTONES ACHIEVED IN RICE GENOMES

In the modern era of crop improvement, the hurdle of conventional breeding curtailed with emergence and advancement in modern genome editing technology. Genome editing technology enables us to modify gene at specific location in the genome with the usage of SSNs (engineered site of specific nucleases) [4-8]. The applications of genome editing tools have extended rice research to develop new varieties which have better yield and quality.The limitation of traditional breeding method has replaced by recent genome editing technologies that leads to new era of crop enhancement. The site-specific nucleases (SSNs) like zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) break the targeted DNA and repaired by cells of homologous recombination (HR) or non-homologous end joining (NHEJ) through natural repair mechanism. The NHEJ repair is the error prone pathway while HR pathway is much more precise in the exchange of homologous sequence leading to gene knock in or gene replacement. First time targeted mutagenic reported in rice crop in early 2012 when gene of bacterial blight susceptible Os11N3 (also famous as OsSWEET14) was usually targeted for TALEN-based distraction for making disease resistant plant lines [17]. Then further studies done by using TALENs for targeting multiple susceptible genes against blight disease in rice crop [18-20]. TALEN technology was not only used for targeting disease but also used for enhancement of fragrance by interrupting Oryza sativa betaine aldehyde dehydrogenase 2 (OsBADH2) genes in rice. In a study, it was confirmed that Lig4 plays significant role in the cNHEJ pathway in rice and lack of DNA lig4 knockout rice lines can improve the frequency of TALEN-mediated targeted mutagenesis. During 2013, there were published almost five articles related to effective type of targeted mutagenic by using CRISPR/Cas9 system in rice. A single modified sgRNA was used to induce target mutations in three genes of rice,

OsMPK2, Os02g23823, and OsBADH2 resulted in high frequency of mutation in CRISPR/ Cas9 construct designed for OsSWEET14 and OsSWEET11, which caused deletion of nine and seven nucleotides from the promoter of the OsSWEET14 and OsSWEET11 genes. In the similar year, a CRISPR/Cas9 concept was used for immediate targeting of three rice genes, young seedling albino (YSA), stromal processing peptidase (SPP) and rice outmost cell-specific gene 5 (ROC5) resultant in homozygous or bi-allelic mutants with the high mutation frequency up to 84 percent in the T0 and T1 rice lines. Furthermore, four sugar efflux transporter genes also targeted by CRISPR/Cas9 named (OsSWEET11, OsSWEET12, OsSWEET13, and OsSWEET14) resultant in large chromosomal deletions between two nucleasetargeted loci. As such, the concepts were suggested for knockout screening of whole rice genome with sgRNA libraries for mutant rice populations with greater heritable variability and precision. These studies evidently showed that CRISPR/Cas9 system can be used as real tool for chromosomal engineering, production of insertion, deletion, substitution, and translocation lines which showing greater efficiency for the advancement of new cultivars with better novel traits. In rice targeted mutation was successfully used to knockout the multi paralogous gene with the help of CRISPER/Cas9 system. Targeted mutation of three rice genes, namely, OsMPK2, Os02g23823 and phytoene desaturase (OsPDS) exposed a high comutation rate with the range of mutation frequency between 66.4 and 81 percent. In second study, a high-efficiency multiplex genome editing was tried in rice by producing multiple sgRNA cassettes. In rice genome up to 46 target sites were edited with an average 85.4% mutation frequency. As many as 46 target sites were edited in the rice genome with an average mutation frequency of 85.4%. Multiplex genome editing was also testified with the help of endogenous tRNA processing system in rice, wherever each sgRNA was flanked by tRNA and processed into single sgRNAs which caused of large deletions in genomic sequences of T0 generation. Likewise, it was reported a new strategy in rice for CRISPR/Cas9-sgRNA multiplex editing system where 21 sgRNAs were designed and the equivalent Cas9/sgRNAs expression vectors were created. The successfully edition of transformed rice plant were significantly edited and up to 82 percent of the desired target sites represented deletion, insertion, substitution, and inversion, thus exhibiting high editing efficiency. All these reports clearly show that the CRISPR/Cas9 system is highly effective to create multiple gene mutations by using conventional strategy that could be subsequently used for the rice breeding in near future.

CONCLUSION AND FUTURE RESEARCH

Although tremendous progress has been made in ricegenomics, there is still a huge gap of knowledge betweenthe genotype and phenotype; a gap which must bebridged in order to breed elite varieties suitable for sustainableagriculture. A highly coordinated effort thatbrings together scientists and resources worldwide is adesirable step and perhaps the only practical and efficientone. Zhang *et al.* [24] proposed an International RiceFunctional Genomics Project (IRFGP), with the ultimategoals of determining the function of every gene inthe rice genome by the year 2020, to identify functionaldiversity of alleles for agriculturally useful genes from the primary gene pool, and to apply the findings of functional genomics research to rice crop genetic improvement and beyond. The following objectives for this international effort, with elaboration of specific aims tobe achieved, were proposed: (i) development of enablingtools and genetic resources for an international communityof scientists to conduct functional genomics researchin rice; (ii) assignment of biological functions to everyannotated gene; (iii) global analyses of the proteome andprotein-protein interactions; (iv) natural variation of *O.sativa* and its relatives; (v) bioinformatics, data management,and exchange of information; (vi) establishmentof the toolkit for high throughput knowledge-based rice breeding. These provide vision for future research directionsand resource-building in rice genomics studies.In the application aspect, Zhang (2007) outlinedstrategies for the development of what he referred to asGreen Super Rice based on advances in genome research.On the premise of continued yield increase and qualityimprovement, Green Super Rice should possess resistancesto insects and diseases, high nutrient efficiency,and drought resistance; all of which promises to greatlyreduce the consumption of pesticides, chemical fertilizers, and water. This would ensure that rice productionis in harmony with the environment, thus safeguardinga proper level of sustainability. As rice is a major worldcrop, rice functional genomics research will have animmense global impact on sustainable agriculture.CRISPR/Cas9 and other tools improve

the rice genome through revolutionary change which meets the curial requirement and demand of rice for future generations. The further research is requiring to optimized refine the CRISPR/Cas9 protocol in rice and need more effort for making freely accessible and user friendly in the practical application. The efficient transformation technology would facilitate the development biotic and abiotic stress crop. However, the multidisciplinary and integrated approaches allow the complete characterization of rice function genome, inferring the single gene function and talking full benefits. The advance in rice genome will integrated with developing high throughput technology, information of proteomics, transcriptomics, bioinformatics, epigenomics and genomics in the future breeding program.

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