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Invited Review Article

Rice Omics and biotechnology in China

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Abstract

Rice is the major source of food for over half of the world's population. As a model cereal crop, the complete genome sequence of rice has become fundamental for analyzing gene functions and relating them to practical applications in plants. At present, rice researchers devote much effort to generating mutants and tagged lines, or utilizing elite germplasms to clone important genes and identify their functions. Such processes combine different -omic technologies including genomics, transcriptomics and proteomics. In the past decade, Chinese scientists have made great contributions toward integrated analyses of rice omics and biotechnological applications, particularly whole genome shotgun sequencing of *Indica* rice 9311, and sequencing of chromosome 4 of *japonica* rice Nipponbare, cloning and identifying 220 functional genes, and using certain identified genes to improve rice agronomic traits through molecular breeding approaches. This review presents a summary of the advances made in rice omics and biotechnology in China, as well as future prospects.

Keywords: rice; genomics; gene cloning; transcriptomics; proteomics; germplasm; molecular breeding.

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops for over half the world's population. Due to its small genome size of ~430Mb (Harushima et al., 1998), relative ease of *Agrobacterium-mediated* transformation, and high-syntenic relationships with other cereal genomes, rice acts as a model crop for research on both plant development and genomics. Therefore, the sequencing and functional analysis of the rice genome play critical roles in studying other important crops such as wheat, corn, millet, sorghum, soybean; such analysis can help to resolve the world food crisis (Liu and Xue, 2006). As of August 2005, the International Rice Genome Sequencing Project (IRGSP) completed a high-quality sequence of the 389 Mb *japonica* rice *Nipponbare* genome (IRGSP, 2005). Chinese scientists completed the sequence of chromosome 4 using a map-based strategy (Feng et al., 2002; Zhao et al., 2002; IRGSP, 2005). At the same time, a whole-genome shotgun sequencing of an *indica* variety 93-11was completed by Chinese scientists (Yu et al., 2002; Yu et al., 2005). In addition to supporting rice genome sequencing, the Ministry of Science and Technology of China has funded China Rice Functional Genomics Program (CRFGP) comprising twenty research groups under the National Basic Sciences Initiatives in 1999 (Xue and Xu, 2002). The CRFGP has defined roles in gene discovery and for using genes to improve agronomic traits of rice, as well as other crops (Xue et al., 2003). Their platforms are aimed at enabling high throughput analyses and effective determination of gene functions, and consist of three major components: (1) generation and characterization of a large mutant library, (2) expression profiling of predicted exons and expressed sequence tags (ESTs) for the entire genome, and (3) isolation of full-length cDNAs (Han et al., 2007). It was reported that CRFGP has made extensive progress on rice functional genomics in the last several years (Han et al., 2007). Moreover, with advances in rice genome research, a series of molecular markers were developed and considerable progress has been made by Chinese scientists in gene mapping, diversity and evolution of rice genetic resources. Here, we review recent achievements in rice genomics, transcriptomics, proteomics and biotechnology applications in China.

1. Rice Genomics Research

Genomics is the study of genomes, with an emphasis in China on agronomically important plants. Completing the rice genome requires the entire complement of genetic information contained in whole chromosomes of rice. In the last few years, scientists have made intensive efforts to complete the entire DNA sequence of rice, and a great deal of work in exploring functional genes in rice using mutant resources.

1.1 Rice genome sequencing

Because it is a model monocot plant, whole genome sequencing of cultivated rice will facilitate the identification and functional analysis of genes not only in rice but also in other cereals. The International Rice Genome Sequencing Project (IRGSP) has adopted a clone-by-clone shotgun sequencing strategy for obtaining a finished rice genome sequence. The group comprises ten members, including China and Tailand, with the goal of sequencing a standard rice cultivar (*Nipponbare*) (Sasaki and Burr, 2000). Here, we describe the important contributions to rice genome sequencing, both in *indica* and *japonica* subspecies, being made by Chinese scientists.

The complete sequencing of Nipponbare (*japonica*) chromosome 4

As one part of international efforts to sequence the entire genome, the National Center for Gene Research (NCGR) of the Chinese Academy of Sciences completed sequence of rice *O. sativa* ssp. *japonica* cv. Nipponbare chromosome 4, which is one of the first two rice chromosomes to be completely sequenced using a map-based strategy (Feng et al., 2002; Zhao et al., 2002; Sasaki et al., 2002; IRGSP, 2005). A 34.6Mb region representing 97.3% of chromosome 4 has been sequenced from *japonica* Nipponbare. There are 4,658 protein encoding genes and 70 transfer RNA genes, 1,681 of them have been identified as matching available unique rice expressed sequence tags (Feng et al., 2002). In addition, the 124-kb rice chromosome 4 centromere has been sequenced completely, and shown to consist of 18 tracts of 379 tandemly arrayed repeats known as CentO, along with a total of 19 centromeric retroelements (Zhang et al., 2004). The complete sequence of this centromere is very helpful for fully understanding centromere function. To characterize interspecific DNA-sequence variation between *indica-japonica* subspecies, NCGR conducted sequencing of a 22.1 Mb region from the *indica* Guangluai 4 (GLA4) chromosome 4. A sequence comparison of homologous regions from *indica* GLA4 and *japonica* Nipponbare chromosome 4 showed not only the co-linearity of extensive sequence, but also deviations in both intergenic regions and some of the coding regions (Feng et al., 2002).

1.1.2 A draft genome sequence of *indica* variety 93-11

A whole-genome shotgun sequencing strategy was carried out on *indica* variety 93-11 by the Beijing Genomics Institute (BGI) of the Chinese Academy of Sciences (Yu et al., 2002; Yu et al., 2005). This variety is one of the major rice cultivars in China and is the paternal cultivar of super-hybrid rice Liang-You-Pei-Jiu (LYP9), which has 20 to 30% more yield per hectare than other rice crops in cultivation (Yu et al., 2002, Yuan, 1997). The complete sequencing of the 93-11 genome revealed it to be 466Mb in size, with an estimated 46,022 to 55,615 genes. The functional coverage in the assembled sequences of 93-11 was 92.0%. As the maternal cultivar of LYP9, a draft sequence for Peiai 64S (PA64s), which has a major background of *indica* and a minor background of *japonica* and *javanica* also was produced by BGI (Yu et al., 2002). Whole-genome comparisons between rice *indica–japonica* cultivars have greatly accelerated analyses of the origin, speciation, domestication, and evolution of rice (Han et al., 2007). The sequence comparison between 93-11 and Niponbare revealed that at least a quarter of the two sequences could not be aligned; between alignable regions single nucleotide polymorphisms (SNP) varied from as little as 3.0 SNP/kb in the coding regions to 27.6 SNP/kb in transposable elements (Yu et al., 2005). Besides nuclear genomes, the comparisons of chloroplast and mitochondrial genome sequences were also carried out. The chloroplast genome size is 134,496bp for 93-11 and 134,551bp for PA64s (http://www.genomics.org.cn/bgi/rice/main.htm); comparative analyses suggest that the divergence between the *indica* and *japonica* chloroplast genomes occurred approximately 86,000 to 200,000 years ago (Tang et al., 2004). Based on mitochondrial genome sequences of 93-11 (491,515 bp) and PA64s (490,673 bp) (http://www.genomics.org.cn/bgi/rice/main.htm),the divergence was estimated at approximately 45,000-250,000 years ago (Tian et al., 2006).

1.1.3 The next-generation sequencing technology (NGS)

Next generation sequencing, coupled with the growing number of genome sequences available, opens the opportunity to redesign genotyping strategies for more effective genetic mapping and genome analysis. Huang et al. (2009c) developed the first high-throughput genotyping method that uses SNPs detected by whole-genome resequencing using NGS. The sequencing-based method was ～20× faster in data collection and 35× more precise in recombination breakpoint determination compared to the genetic map based on rice populations (Huang et al., 2009c). Next generation sequencing also was used to investigate genome diversity of rice germplasms. Six rice accessions including four *indica* strains, one *japonica* strain and one *O. rufipogon* strain, have been sequenced with high coverage using NGS technology involving Illumina and Solexa platforms, and a total of two million SNPs have been identified so far (Han et al., 2010 unpublished data). The continuous advancement of NGS technology will facilitate an understanding of complex phenomena, such as heterosis and epigenetics, which have important implications for crop genetics and breeding (Varshney et al., 2009)

1.2 Construction of Mutant Libraries

With the completion of rice genome sequencing, the next challenge is to uncover a large number of annotated genes predicted by sequence information. In recent decades, mutant resources have been widely utilized in forward and reverse genetic approaches to discover genes and to study gene functions. As on 2009, large scale rice mutant libraries for *japonica* rice varieties, Zhonghua 11, Zhonghua 15 and Nipponbare, and *indica* variety, Kasalath were constructed using insertional, chemical and radiation induced mutagenesis in China (Krishnan et al., 2009). Owing to stable, low copy number and random insertion patterns, transferred DNA (T-DNA) is used as the most powerful tool for insertional mutagenesis to generate mutant libraries (Krysan et al., 1999; Delseny M et al., 2001). The T-DNA insertions are applied in rice genome research with three strategies: insertional mutation, enhancer trapping and ectopic expression (Wu et al., 2003). Actually, three main groups in China (Beijing, Shanghai and Wuhan) have gathered together to construct a T-DNA insertion mutant library. Approximately 270,000 independent transformants were produced and 150,000 seed accessions were stored from 2001 to 2005 in Shanghai (Han et al., 2007; Xiao et al., 2009). Besides normal insertional mutation, an enhancer trap system could detect transcription elements via expression of reporter genes. To date, almost 132,000 accessions of rice T-DNA enhancer trap lines, and over 53,432 accessions of seeds are available in the Rice Mutant Database (RMD) founded in Wuhan (http://rmd. ncpgr.cn). Seven expression profiles of reporter genes were captured in large-scale identifications (over 5,000 mutants) in Wuhan (Xiao et al., 2009). The available mutants have become an important resource for high-throughput gene mining and identification. Root development regulating gene *WOX11* and a flowering date control gene were identified from these mutant lines (Zhao et al., 2009; Dai et al., 2010). Moreover, using T-DNA with gene trap, gene knockout and activation tagging, a mutant population containing 55,000 lines was constructed in Taiwan, and T-DNA copies and retrotransposon were studied correspondingly (Hsing et al., 2007). In addition, a large number of mutants (approximately 30,000) were screened in Wuhan, including ones for stress tolerance, nutrition efficiency, and development (Xiao et al., 2009). Notably, *RID1*, a master gene switch from vegetative to floral development, and a culm mechanical

strength controlling gene, *FC1,* were identified screening these mutants (Wu et al., 2008; Li et al., 2009b). Chemical mutagens and radiation can cause a high density of mutations in the rice genome. Guo et al. (2006) described approximately 30,000 accessions of radiation-induced and 20,000 EMS-induced mutations in China. The classical rice mutant *brittle culm1 (bc1)* was one of these radical mutants and the gene *BC1* controlling mechanical strength was isolated using a map-based cloning approach (Li et al., 2003b). Another library containing 40,000 mutant loci, also caused by radiation and EMS, were produced by the state key laboratory of plant physiology and biochemistry in Zhejiang University (http://www.genomics.zju.edu.cn; Krishnan et al., 2009).

1.3 Gene cloning and functional analysis in rice

Rice genome research now has moved from structure genomics to functional genomics; cloning and identification of important genes were believed to be a main issue to address in functional genomics research. To date, over 8,600 QTLs have been mapped worldwide (www.gramene.org), with 642 cloned genes cataloged at the website <www.ricedata.cn>. Since 1994, a large number of genes have been fine mapped and an increasing number of genes, now a total of 220, have been cloned in China, mainly using *in sillico*, map-based cloning and tagging approaches (ST1; ST2). Among them, 60% were cloned using an *in silico* cloning approach, and 27% were isolated using map-based cloning (Fig 1.A). T-DNA and *tos17* tagging are being applied increasingly for gene cloning due to the construction of large-scale mutant libraries in recent years (Fig 1. C). With respect to expressed traits, 40% of the genes are related to stress tolerance, and were cloned mainly using *in silico* cloning approach (Fig1.B, ST2). In addition, map based cloning of genes that control grain yield and anatomical traits have been a focus in the last few years, indicating that rice functional genomics is being linked to agricultural production in China (ST2). In the following chart, we present data on certain genes controlling important rice traits, such as growth and development, grain yield, adaptation to abiotic and biotic stress and others.

1.3.1 Functional genes for traits of growth and development

Genes for root traits

Rice has a fibrous root system, which consists of one primary root originating from the seed, and a mass of adventitious roots formed from the stem during post-embryonic development (Liu et al., 2005). A growing number of studies have focused on characterization of root development in cereals. Han et al. (2008) suggested that the rice gene *ROOT ARCHITECTURE ASSOCIATED1* (*OsRAA1*) could modulate root development mediated by the ubiquitin-proteasome pathway as a novel regulatory pathway of the cell cycle. Over-expression of *OsAGAP*, which encodes ARF-GTPase-activating protein (ARF-GAP) in rice, interfered with both primary and lateral root development (Zhuang et al., 2006). Yuan et al. (2008) characterized a possible feedback regulation mechanism by *METALLOTHIONEIN2b* (*OsMT2b*) on the level of endogenous cytokinins, which is involved in root development and seed embryo germination.

Genes for traits of floral organ

Rice inflorescence morphology, determined mainly by primary and secondary inflorescence branches, is an important trait in both agronomy and developmental biology. Sun et al. (2008) showed that the JMJ706 protein affects spikelet development, including altered floral morphology and organ number. Gao et al. (2010) analyzed the biological role of one *SEPALLATA* (*SEP*)-like gene, *OsMADS34*, in controlling the development of inflorescences and spikelets in rice. Li et al. (2010b) characterized the function of *Os-MADS6* in specifying rice floral organ identities and determination of floral meristems. Mutation of *OsMADS6* resulted in altered palea identity, extra glume-like or mosaic organs, abnormal carpel development and loss of floral meristem determination.

Genes for Tillering

Tillering in rice is an important agronomic trait for grain production because tiller number per plant determines panicle number, which is a key component of grain yield. Li et al. (2003a) isolated and characterized the *MONOCULM 1* (*MOC1*) gene that is important in controlling rice tillering by functioning in the initiation of axillary buds and promoting their outgrowth. Sun et al. (2010) showed that the protein (MIP1) interacts with MOC1, and the overexpression of *MIP1* results in enhanced tillering and reduced plant height. Gao et al. (2009) characterized a tillering dwarf mutant *d88* with excessively short tillers and smaller panicles and seeds compared to the wild-type. Lin et al. (2009) characterized *d27*, a classic rice mutant exhibiting increased tillers and reduced plant height.

Genes for plant architecture

Rice architecture is an important agronomic trait that affects grain yield. Several genes associated with rice architecture were isolated and characterized by Chinese scientists. The *OsCRY1* gene was implicated in blue-light inhibition of coleoptile and leaf elongation during early seedling development in rice (Zhang et al., 2006). The prostrate growth habit of wild rice from Yuanjiang County in China is controlled by the *PROG1* gene, which determines aspects of wild-rice plant architecture, including tiller angle and number of tillers (Tan et al., 2008). Song et al. (2009) isolated the *OsIAA1* gene and characterized its functions in controlling morphological changes, such as decreasing plant height and a looser plant architecture. Zhang et al. (2009b) isolated and functionally characterized a key gene that controls rice leaf rolling from *shallot-like1* (*sll1*) mutant plants, which exhibit extremely incurved leaf phenotype due to the defective development of sclerenchymatous cells on the abaxial side.

1.3.2 Genes for traits of grain yield

Grain weight, grain number per panicle and panicles per plant are the most important components of grain yield. In recent years, several genes or QTLs controlling important traits related to grain yield were isolated and functionally analyzed in China. Song et al. (2007) cloned and characterized *GW2* that accelerated grain milk filling rate, resulting in enhanced grain width, weight and yield. *Ghd7*, a QTL affecting number of grains per panicle, plant height and heading date, was isolated and characterized to play crucial roles for increasing productivity and adaptability of rice globally (Xue et al., 2008). A major rice grain yield QTL, *DEP1*, also was isolated and shown to enhance meristematic activity resulting in reduced length of the inflorescence internode, increased number of grains per panicle and, consequently, an increase in grain yield (Huang et al., 2009b). Furthermore, several genes identified as QTLs associated with grain yield have been isolated by positional cloning (Wang et al., 2008a; Wang et al., 2008e; Shan et al., 2009; Jiao et al., 2010; Li et al., 2010a).

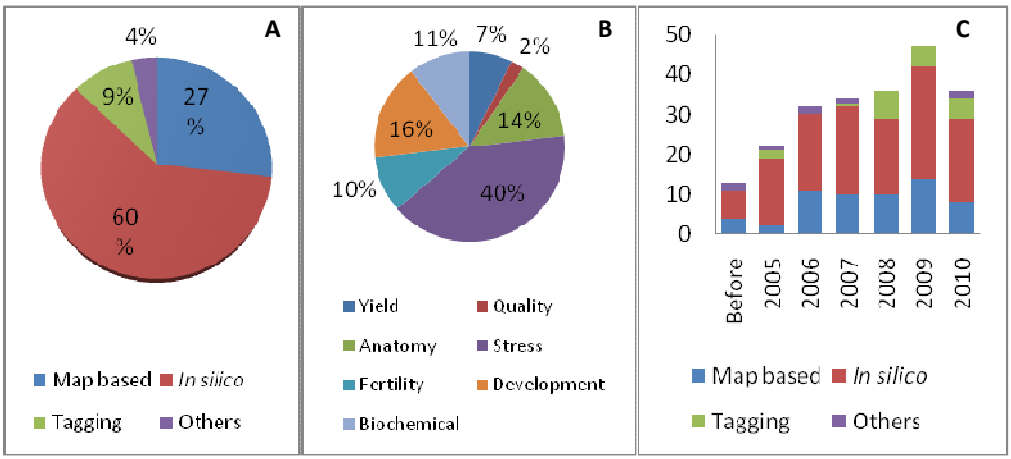

Fig 1. Overview of rice genes isolated in China. (A) Proportion of genes isolated according to cloning approach. (B) Proportion of genes isolated according to plant traits. (C) Number of genes cloned per year. 2010, updated to August 2010.

1.3.3 Genes for traits of abiotic stress

Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. To survive under such conditions, plants have evolved intricate mechanisms to perceive external signals, allowing optimal response to environmental conditions.

Drought and salt stresses

Drought and salinity are major abiotic stresses that cause yield loss in cultivated crops. Mechanisms of plant drought resistance and salinity tolerance are affected by a number of complex quantitative traits controlled by multiple genes. Some of these genes characterized by Chinese scientists are highlighted in the following section. The over-expression of stress response genes *SNAC1* and *SNAC2* (NAC type transcription factor) were shown to improve drought resistance and salt tolerance significantly (Hu et al., 2006; Hu et al., 2008). Huang et al. (2009a) cloned and characterized the *DST* gene, a previously unknown zinc finger transcription factor that negatively regulates stomatal closure by direct modulation of genes related to H_2O_2 homeostasis. *OsbZIP23* and *OsbZIP72* are members of bZIP transcription factor family that confer drought and salinity tolerance, and have great potential utility in genetic improvement of stress tolerance (Xiang et al., 2008; Lu et al., 2009). Ning et al. (2010) identified a *drought hypersensitive mutant* (*dsm1*) gene in rice. *DSM1* could be a novel MAPKKK, functioning as an early signaling component in response to drought stress by regulating the scavenging of ROS in rice. Ouyang et al. (2010) cloned and characterized a putative receptor-like kinase gene, OsSIK1, which plays important roles in salt and drought stress tolerance in rice, through activation of the antioxidative system. Rice OsSKIP is a homolog of human Ski-interacting protein (SKIP). Hou et al. (2009) revealed that OsSKIP involves a specific function in positive modulation of stress resistance through transcriptional regulation of diverse stress-related genes.

Cold stress

Cold stress is a common problem for rice cultivation, and is a crucial factor affecting global food production. Cold tolerance is a complex trait controlled by many QTLs. A QTL designated as *qCTB7* (QTL for cold tolerance at the booting stage on chromosome 7) was analyzed in a cold-tolerant near isogenic rice line, and 12 putative candidate genes were identified (Zhou et al., 2010). Su et al. (2010) reported that transgenic rice constitutively overexpressing *MYBS3* could tolerate 4°C for at least 1 week and exhibited no decline in yield in normal field conditions. Ma et al. (2009) showed that transgenic plants overexpressing *OsMYB3R-2* exhibit enhanced cold tolerance. *OsMYB3R-2* targets *OsCycB1*;1 and regulates progression of the cell cycle during chilling stress.

1.3.4 Genes for traits of biotic stress

Plants face a diversity of pathogens, including bacteria, fungi, oomycetes, nematodes, and viruses throughout their life cycles. They respond to these biotic attacks by activating disease resistance (R) genes, which in turn trigger defense signal transduction cascades that result in to rapid and race-specific disease resistance in host plants. Recently additional R genes have been isolated from rice by Chinese scientists (ST1.). Rice blast, caused by the fungal pathogen *Magnaporthe grisea*, is one of the most devastating diseases in the crop worldwide. The rice *WRKY* gene (*OsWRKY31*), induced by *Magnaporthe grisea* and auxin, was isolated and proved to have transacivation activity in yeast. Transgenic lines with overexpression of *OsWRKY31* not only exhibited reduced lateral root formation and elongation, but also enhanced resistance against infection by *M. grisea*, (Zhang et al., 2008). Brown planthopper (BPH) causes the most serious damage to the global rice crop among all rice pests. Du et al. (2009) cloned the *Bph14* gene, which confers resistance to BPH. In *Bph14*-mediated resistance, the salicylic acid signaling pathway, callose deposition in phloem cells and trypsin inhibitor production are activated and induced after planthopper infestation.

2. Rice Transcriptomics Research

Transcriptomics is the study of the set of all transcripts in a cell, and their quantities at specific developmental stages or under different physiological conditions. As the link between the genome, the proteome and the cellular phenotype, study of the transcriptome is essential for understanding functional elements of the genome, molecular components of cells and tissues, and also for developmental analyses (Wang et al., 2009). At present, various technologies have been developed for analyzing

structure and expressional levels of RNAs, including hybridization-based approaches (RNA chip, Microarray, Tiling microarray) and sequencing-based approaches (SAGE, CAGE, MPSS, PET, RNA-Seq). Here, we describe some of the achievements in rice transcriptomics in China.

2.1 Full-Length cDNA libraries

Rice full-length cDNAs are essential for gene annotation, determination of gene structure, and gene functional analyses at transcriptional and translational levels. A collection of 28,469 full-length cDNAs from *japonica* Nipponbare provided a detailed description of the rice transcriptome (The Rice Full-Length cDNA Consortium 2003). Because few *indica* rice full-length cDNAs are available, rice functional genomics research in China now mainly focuses on separating FL-cDNA from *indica* rice varieties. A normalized full-life-cycle cDNA library from different pathogen induced tissues and growth stages of Minghui 63, one of the most important *indica* rice in China, was constructed by The National Rice Functional Genomics Project in China (RFGC) (Chu ZH et al., 2003). One normalized full-life-cycle cDNA library and two full-length-enriched libraries were produced from pollen and young panicle of Minghui 63 (Chu ZH et al., 2003; Xie et al., 2005), and details of each clone are shown on the REDB Web site (Rice EST Data-Base, http://redb.ncpgr.cn/modules/ redbtools/). A total of 10,096 full-length cDNAs from *indica* Guangluai 4 were identified (Liu et al., 2007). In addition, collection and comparative analysis of 1888 putative FLcDNA clones from wild rice *Oryza rufipogon Griff.* W1943 showed that it was more similar to *japonica* rice than to *indica* rice, and also identified some wild rice specific genes (Lu et al., 2008b). As far as 2008, over 22,000 putative FL- cDNAs and over 48,000 total ESTs were isolated from various cDNA libraries of two *indica* varieties Guangluai 4 and Minghui 63 (Lu et al., 2008a). The details are available on RICD website (Rice *Indica* cDNA Database, http://www.ncgr.ac.cn/ricd). These identified FL-cDNA clones are a resource for further functional verification and for utilization in rice biological studies.

2.2 cDNA Microarray

cDNA microarrays can be prepared from existing cDNA libraries of known (or partially known) gene loci, and provide a high throughput means of analyzing genome expression in an organism, or changes in expression among plant species in response to stresses (Singh et al., 2006; Zhou et al., 2007). An oligonucleotide microarray covering 41,754 annotated rice gene models, with or without experimental support, was produced and applied to the analysis of transcriptomics in representative rice organs. Compared with *Arabidopsis* transcriptomics, similar proportions of the two genomes were expressed in their corresponding organ types. The expression patterns of rice and *Arabidopsis* best-matched homologous genes in distinct functional groups reveals significant differences in their degree of conservation between the two species (Ma et al., 2005). Additionally, the same microarray was used to compare the light signal response expression profile of *Arabidopsis* and rice; the result showed that the expression of almost 20% of the genomes of both species are regulated by white light, and indicated both similarities and differences between the two expression profiles (Jiao et al., 2005). Two hundred and fifty-three rice cDNAs regulated by pollination/fertilization were identified using a 10K cDNA microarray, and many of them appear to be involved in drought and wounding responses (Lan et al., 2004, 2005). Subsequently, expression profiles of an *indica* rice Minghui 63 at the seedling stage were analyzed or identified using cDNA microarray analysis under low N stress (Lian et al., 2006), upland and lowland rice cultivars under water stress (Wang et al., 2007), rice stigma-specific or preferential gene expression profiles (Li et al., 2007), genome expression changes in rice shoot, flag leaf and panicle under drought or high-salinity conditions (Zhou et al., 2007), a number of salt-responsive genes in rice (Chao et al., 2005), expression profiles of rice root under low P stress treatment (Li et al., 2009a). In addition, transcriptome profiles in developing leaves and panicles of super hybrid rice LYP9, and its parental cultivars 93-11 and PA64s, were investigated using a whole-genome oligonucleotide microarray based on *indica* rice genes. Clustering results indicated that the expression profiles of F_1 hybrid were more like those of its parental lines than that of intermediate between the 2 parental lines (Wei et al., 2009a). Furthermore, transcriptomics analysis of strong seed dormancy *indica* rice N22 and its weak dormancy mutant Q4646 identified candidate genes colocalized with seed dormancy QTLs, which provides important clues for future efforts to clone seed dormancy genes in rice (Qin et al., 2010).

2.3 Tiling microarray

Oligonucleotide tiling arrays are a subtype of microarray chips. In contrast to traditional microarrays, short fragments for probes are designed to cover the entire genome or contiguous regions of the genome. Because of the high resolution and sensitivity, tiling arrays can be used to decipher a variety of information hidden in the genome (Mockler et al., 2004). To study chromosome transcriptional activity during rice development, a tiling microarray consisting of overlapping genomic fragments covering approximately 33 Mb (95.5%) of *japonica* rice chromosome 4 was developed by XingWang Deng's and Bin Han's laboratories (Jiao et al., 2005). They found that genes in euchromatic regions are more actively transcribed in juvenile-stage rice than those in transposon-rich heterochromatic portions of the chromosome, suggested a chromosome-level regulation of transcription. Oligonucleotide tiling microarrays covering chromosome 10 of *japonica* and *indica* subspecies were used to identify the transcriptome and relate its expression to chromosomal architecture (Li et al., 2005). Expression analysis revealed that the heterochromatin region had relatively low transcription activity under normal growth conditions compared to under mineral/nutrient stress conditions.

A genome-wide transcription analysis of *indica* rice was done using high-density oligonucletide tiling microarrays by Li et al. (2006). They detected transcription of 35,970 (81.9%) gene models, and identified 5,464 unique novel transcriptionally active regions in intergenic regions. These results provided a useful whole-genome wide transcription map for further understanding the rice genome.

2.4 Serial Analysis of Gene Expression (SAGE)

SAGE is a recently developed sequence-based approach for gene expression investigation, allowing both qualitative and quantitative evaluation of thousands of genes without any prior information (Velculescu et al., 1995). Owing to high sensitivity and high accuracy, it can successfully detect differentially expressed genes, especially relatively rare ones, new transcripts, genome expression regulation region, and antisense transcriptss. In order to provide new data and insights into the molecular mechanism of heterosis, Jun Yu and his colleges carried out SAGE analyses of a hybrid rice strain (LYP9) and its parental cultivars (Bao et al., 2005). As a result, 465,679 tags were

obtained from the SAGE libraries, which were consolidated into 68,483 unique empirical tags. Comparative analysis identified 595 up-regulated and 25 down-regulated genes in LYP9; most of the up-regulated genes were related to enhancing carbon- and nitrogen-assimilation, while among the down-regulated genes is an essential enzyme in photorespiration (Bao et al., 2005). Subsequently, Jun Yu and his colleagues analyzed the transcriptome based on these SAGE data and candidate genes related to rice heterosis by using an improved strategy of tag-to-gene mapping, and two recently annotated genome assemblies (93-11 and PA64s) (Song et al., 2007). They identified a group of up-regulated genes related to male sterility, and most of the down-regulated genes related to signal transduction and protein processing. These candidates for heterosis-related genes provided a more comprehensive view on heterosis, and new avenues for exploring the molecular mechanisms underlying heterosis.

2.5 Massively Parallel Signature Sequencing (MPSS)

MPSS has been developed as an improvement to SAGE. The advantage of MPSS technology is the large number of distinct signatures (more than 1,000,000) that can be identified in a single analysis. Thus MPSS potentially provides greater coverage of the transcriptome than SAGE (Chen et al., 2007). MPSS was used to analyze expression profiles of small RNAs during rice seed development and 79739922-nt sequence signatures, including 111161 distinct ones, and 26 novel miRNAs and 12 miRNA candidates were identified (Xue et al., 2009). miRNAs are an extensive class of small regulatory RNAs, which play an important role in many biological and metabolic processes (Zhang et al., 2006, 2007). At present, MPSS has not been applied widely to study rice transcriptomics because of the expensive hardware and software required.

2.6 RNA sequencing (RNA-Seq)

Based on next-generation DNA sequencing technology, RNA-Seq provides a cost-effective approach for mapping and quantifying the transcriptome. It allows researchers to analyze complex RNA mixtures, the extent of alternative splicing, discover new transcripts that are expressed at very low levels, find potentially functional non-coding RNAs, detect untranslated regions (UTRs) and identify gene fusions. Transcriptome analysis at single base resolution is rare for modern plants. The first transcriptome atlas for eight organs of cultivated rice was first reported by Beijing Genomics Institute using high-throughput RNA-seq (Zhang et al., 2010). An extensive number of novel transcripts, exons, and untranslated regions were detected, as well as some transcripts expressed at extremely low levels, and 234 putative chimeric transcripts that appeared to be produced by trans-splicing. These results indicate that transcription regulation in rice is vastly more complex than previously believed. To resolve whole-genome transcription profiles, Bin Han and his colleagues applied RNA-seq to global sample transcripts of rice *indica* and *japonica* subspecies (Lu et al., 2010). A total of 15,708 novel transcriptional active regions (nTARs) were obtained; 48% of genes showed alternative splicing patterns, 83.1% of the current rice gene models were validated by RNA-seq, and 6,228 genes were extended at the 5' and/or 3' ends by at least 50 bp. A total of 3,464 genes exhibited differential expression patterns between the two subspecies. In total, through interrogating and comparing transcriptomes of the two rice subspecies, these researchers revealed an overall transcriptional landscape at extremely high resolution.

3. Rice proteomics research

The combined advancements in molecular technologies have resulted in the availability of genome-wide DNA data and cDNA-based microarrays to understand the regulation of biological systems (Rakwal and Agrawal, 2003). However, observed phenotypes directly reflect the functions of proteins rather than the genome and transcriptome themselves (Singh and Nagaraj, 2006). Over the past decade, rapid progress has been made in plant proteomic research. The field of proteomics mainly focuses on studying the overall protein population in cells, and proteins expressed specifically in subcellular compartments or tissues of the whole organisms (Van, 2001). These proteins can be identified by powerful proteomic techniques including two-dimensional polyacrylamide gel electrophoresis (2-DE), mass spectrometry (MS) and protein microarrays, that are central proteomics approaches (Rakwal and Agrawal, 2003). With the advancement of these technologies, protein expression profiling of various tissues, organs, stress response pathways and hormone induced responses are being studied extensively in China.

3.1 The proteomes in rice tissues

Progress in functional analyses of rice specific-tissues, such as embryo, seed, leaf, internode and pollen have been rapidly accelerated by proteomic studies. A proteomic analysis of mature embryos from the *LYP9* triad was performed to elucidate molecular mechanisms of heterosis. Fifty-four differentially expressed proteins involved in major biological processes including establishing nutrient reservoirs, response to stress, and various metabolic pathways were identified using systematic proteomic approaches (Wang et al., 2008d). Yang et al. (2007a) analyzed and identified 148 proteins expressed differentially during seed germination process in rice (*Oryza sativa indica* cv. 9311).

Among the proteins with altered expression, down-regulated genes mainly encoded storage, seed maturation and desiccation related proteins, while up-regulated genes mainly were involved in glycolysis. Proteomic studies of rice leaves also were performed to identify the changed protein expression at different leaf developmental stages. Initially, 49 proteins were identified to be differentially expressed in rice leaves collected from the vegetative to ripening stages (Zhao et al., 2005). Later, leaves of 2- to 5- leaf hybrid rice (Shanyou 63) seedlings were collected to identify functions of differentially expression proteins; 41 differently expressed protein spots were detected by 2-DE, and expression changes were deduced to be caused by the emergence of tillers after 3-leaf rice seedling stage (Shao et al., 2008).

Yang et al. (2006) analyzed total soluble proteins from the uppermost internodes of rice at the milky stage because of the critical importance of these internodes in determining seed quality and yield. Among 132 abundant proteins, theycould identify 98 proteins involved in metabolism, signaling, and stress resistance, which suggested the uppermost internodes of rice have a high physiological and stress-resistant activity. In addition, to understand the specialized functions of pollen, Dai et al., (2006, 2007) identified 322 unique proteins from mature pollen of *Oryza sativa L. ssp japonica*, of which are associated with germination and tube growth. These results provide useful data for understanding the mechanisms underlying pollen germination and tube growth, as well as pollen function specialization.

3.2 The proteomes in response to abiotic and biotic stresses

In last few years, in an effort to understand plant environmental responses at the protein level, Chinese research groups have followed up tissue-specific proteome analyses with examinations of proteomes from plants responding to various abiotic and biotic stresses.

3.2.1 Drought and salt response proteomes

Drought is a major factor limiting rice production. The most sensitive yield feature is spikelet fertility, and the spikelet sterility is caused mainly by a deficiency in anther dehiscence (Liu and Bennett, 2010). To understand the effect of drought on the anther proteome, Liu et al. (2010) compared anthers of IR64 and Moroberekan from well watered and drought-stressed/re-watered plants. They identified 93 protein spots with expression were affected reproducibly by drought across two rice genotypes; the result indicated that Moroberekan possesses better recovery capability following drought and re-watering at the anther proteome level than does IR64. Salt stress also is a major abiotic stress in rice agriculture worldwide. The proteomes of rice roots and apoplasts responding to salt stress were investigated using systematic proteomic approaches by two research groups. Two-DE analysis revealed 54 differentially accumulated protein spots, and MS analysis detected 10 different proteins including six novel salt stress-responsive proteins in rice root (Yan et al., 2005). Beyond this, six salt response apoplastic proteins have been identified; among them, OsRMC shows dramatically increased abundance during the initial stages of salt stress. Collective results indicate that plant apoplastic proteins could have important role in plant salt stress response signal pathways (Guo et al., 2009).

3.2.2 Cold and high temperature response proteomes

Both of cold and high-temperature are important environmental factors that affect productivity and grain quality, as well as the geographical distribution of rice. To understand the molecular mechanisms involved in adaptation to cold and high-temperature stresses, proteomic analyses were carried out in rice under these conditions. Cui et al. (2005) applied a progressively lower temperature stress treatment to rice seedlings and identified 41 cold stress proteins. A large fraction of these proteins were predicted to be localized to the chloroplasts, implying that chloroplasts are among the organelles mostly affected by cold stress. Yan et al. (2006) carried out a comparative analysis of total proteins in rice (*Oryza sativa* L. cv. Nipponbare) leaves after chilling treatment and recovery. They identified 85 differentially expressed proteins, including many novel cold-responsive proteins. Lin et al. (2005) detected more than 70 differentially expressed polypeptides in response to high temperature treatments, or between varieties during caryopsis development. Among them, 21 were involved in carbohydrate metabolism, 14 in protein synthesis and sorting, and 9 in stress responses. In addition, seven-day-old rice seedlings were exposed to different high temperature conditions, and proteomic analysis indicated that, the higher temperature, the more protection metabolic machineries were involved (Han et al., 2009).

3.2.3 Heavy metal response proteomes

Excess heavy metal is toxic to most plants, causing a wide range of deleterious effects. Rice is one of the most Al-tolerant crops; however, the mechanisms underlying Al tolerance is less well understood. To identify Al-responsive proteins , Yang et al. (2007b) performed proteomic analysis and identified 12 up- and 5 down-regulated proteins involved in signaling transduction and defensive reaction from rice roots exposed to Al stress. In an investigation of the response of rice to Cu stress, 13 up-regulated proteins and 3 down-regulated proteins were identified in germinating rice seed embryos treated with and without Cu (Zhang et al., 2009c). The proteins identified by these studies provide novel insights for understanding plant molecular responses to toxic metal exposure.

3.2.4 Disease and insect response proteomes

Bacterial blight is one of the most serious diseases of worldwide rice. Proteome analysis of *Xa21*-transgenic rice inoculated with compatible and incompatible *Xoo* races was carried out to detect plasma memberane (PM) proteins involved in the early defensive response to bacterial blight. A total of 20 PM protein spots were shown to be differentially regulated in the rice-*Xoo* interactions (Chen et al., 2007). To understand the mechanism of rice resistance to BPH at a molecular level, proteins were separated from rice leaf sheaths responding to infestation by BPH; a number of proteins involved in multiple pathways were identified, and shown to have significant changes in expression levels. Finally, functional analysis of the proteins identified indicated that there was an efficient and specific defense mechanism in rice (Wei et al., 2009b).

3.3 Hormone response proteome

Hormones like abscisic acid (ABA) and gibberellins (GAs) are among the most essential endogenous regulators of plant growth and development, and hormone-related signal transduction is important in plant defense responses (Rakwal and Agrawal, 2003). Abscisic acid (ABA) pretreatment of rice seedlings was observed to induce enhanced salt tolerance (Bohra et al.,1995; Kishor, 1989). Proteomic analyses showed that enzymes involved in energy metabolism, defense, and primary metabolism were up-regulated uniquely in ABA-pretreated rice seedlings (Li et al., 2010c). Wen et al. (2010) reported that gibberellic acid (GA3) appears to exert a beneficial effect on salt-stressed rice. Proteins involved in biochemical pathways such as photosynthesis and glycolysis, as well as some novel proteins, were differently regulated in combined salt and GA3 treatment, indicating GA3 had a significant influence on the abundance of some salt regulated proteins.

4. Genetic diversity, structure and core collection of rice germplasms

As one of the centers of origin of Asian cultivated rice (*Oryza sativa L.*) (termed cultivated rice hereafter) (Oka, 1988; Londo *et al*, 2006), China possesses a very large gene pool of rice germplasm resources, with 77,960 accessions of cultivated rice and its ancestral wild relative common wild rice (*Oryza ruffpongon* Griff.) (termed wild rice hereafter). Cultivated rice in China comprises 50,526 accessions of landrace or local rice varieties and 5,382 accessions of commercial rice varieties (ICGR CAAS, 1996). Investigations on genetic diversity and genetic structure can reveal information about the evolution and domestication of rice. The extent of accessibility to genetic variation present in a large germplasm collection, like what is

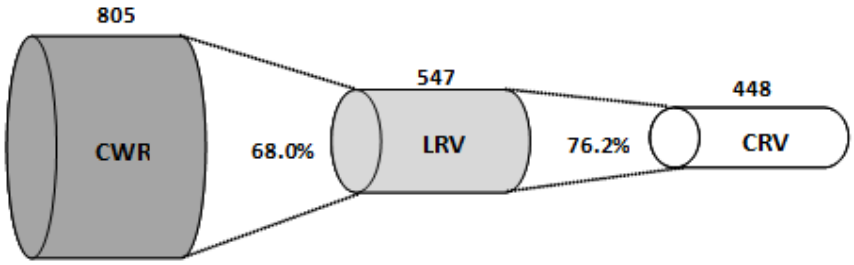

Fig 2. Gene flow revealed by the number of SSR alleles among different variety types of Chinese rice germplasm resources. Here, CWR = Common wild rice, LRV = Local rice varieties, CRV = Commercial rice varieties. 805, 547 and 448 indicate allele numbers for CWR, LRV and CRV, respectively.

present in China, is of interest to both biologists and breeders. Construction of core collections (CC) is a favored approach to efficient exploration and conservation of novel variation in genetic resources.

4.1 Genetic diversity

It was reported that the center of diversity of wild rice in is in southern China (Wang et al, 2008b), while that of cultivated rice locates to China's southwestern area (Zhang et al, 2007a; Zhang et al, 2007b). Wild rice has played an important role in the breeding of rice. The cytoplasmic male sterile gene, which was found in one of the germplasms of Chinese wild rice in the early 1970s, facilitates the utilization of heterosis in rice. Wild rice is genetically more diverse than cultivated rice (Sun et al, 2002; Lu et al, 2002; Song et al, 2005; Gao et al, 2006; Zhu et al, 2007b), and wild rice from China shows greater levels of polymorphism than do those from other countries (Ge et al, 1999). Some studies have shown that the variation present , in cultivated rice, in terms of SSR (Zhu et al, 2002), RFLP (Sun et al, 2001a) and isozyme (Shahi et al, 1967) markers, account for about 60% of the total found in wild rice. However, investigations of core collections of Chinese rice germplasm resources indicated that, in terms of phenotype and SSR genotype, the variation found in cultivated rice accounts for approximately three quarters of that present in wild rice. During the domestication process (from wild rice to cultivated rice) about one third of the variation (32%) present in wild rice was lost, and just 7.6% of the variation found in cultivated rice was newly generated, Moreover, nearly one quarter of the variation (23.8%) in local rice varieties has not been used in commercial rice varieties (Fig.2, unpublished). These results imply that wild rice and local rice varieties, especially the former, are very important potential sources of genetic variation for improving the performance of cultivated rice, and that there is great potential to broaden the genetic background of commercial rice varieties through exploiting novel genes related to high yield, good quality, resistance to various stresses and adaptation to a wide range of environmental factors.

4.2 Genetic structure

Recently, the analysis of genetic structure using DNA markers and new statistical methods has become a popular topic (Zhou et al, 2003a; Cai et al, 2004; Garris et al, 2005; Zhang et al, 2007a, 2009a; Zhang et al, 2007b; Wang et al, 2008c). Investigation of the genetic structure and diversity of rice in China revealed a hierarchical genetic structure and differentiation dynamics in both wild rice and cultivated rice, and provided information about the crop's origin, evolution and domestication. Two levels of distinct divergence were detected in wild rice (Wang et al, 2008b). Under different climates, two ecotypic populations (an *indica*-like type and a *japonica*-like type) were differentiated; due to isolation by distance and water systems, these two ecotypes further diverged into seven geographical populations. Three levels of distinct divergence were detected in cultivated rice, that is, subspecies, ecotypes and geographical-ecotypic populations (Zhang et al, 2009a). It confirmed the primary differentiation between *indica* and *japonica*, but revealed an ecotypic differentiation that is different from the traditional interpretation (Ting, 1957). *Japonica* showed more distinct differentiation among soil-watery ecotypes, whereas *indica* was more clearly subdivided by seasonal ecotypes. Moreover, within soil-watery ecotypes and seasonal ecotypes, there were different numbers of geographical-ecotypic populations (unpublished). Two subspecies evidently resulted from adaptations to different environments, whereas different cropping systems and isolation by distance imposed on the subspecies led to the differentiation of ecotypes. The results were consistent with our other results using Chinese rice landraces from Guizhou province and Yunnan province of China (Zhang et al, 2007a; Zhang et al, 2007b).

4.3 Core collection

Developing core collections (CC) has been a major area in plant germplasm research in the last 20 years throughouts the world. Chinese scientists began to study CCs from the1990s in the major crops like rice, wheat, soybean. How large a core collection should be, and how both diversity and practicality are taken into account in establishing a core collection, have been under debate since the concept of establishing core collections was put forward. The heritably hierarchical core collection system of Chinese rice germplasm resources can offer a solution to these controversies (Zhang et al., 2010). In the system, the primary core collections (PCC) were established (Li et al, 2003c; Yu et al, 2003) first. Then different core sets with different population scales and genetic diversities were developed, such as C2000, C1500, CC, C500 and mini core collection (MCC), thereby allowing a more flexible use of genetic resources (Fig. 3, Zhang et al., 2010). The CC comprises 1.7% (932) of the accessions in the basic collection, and retains more than 85% of both SSR and phenotypic variation; the MCC comprises 0.3% (189) of the accessions in the basic collection, and retains 70.65% of SSR variation and 76.97% of phenotypic variation. Similarly, a core collection and a mini core collection of *Oryza rufipogon* Griff. were developed, comprising 11.3% (628) and 2.2% (122) of the accessions in the basic collection, retaining about 90% and 70% of the SSR variation respectively (unpublished).

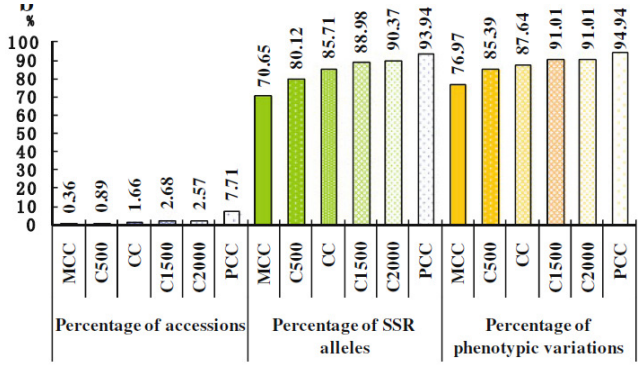

Fig 3. Percentage of accessions, SSR allelic and phenotypic variation in the hierarchical core collections. PA predominant alleles (P[0.1), CA common alleles (0.1 C P[0.01), RA rare alleles (0.01 C P[0.001), IA inferior alleles (P B 0.001), MCC mini core collection, CC core collection, C500 core set with 500 accessions and similar for C1000, C1500 and C2000

4.4 Construction of directional selected ILs and NILs for gene discovery based on germplasms

Because it is abundant in diversity and practical in scale, MCC is an ideal resource for gene discovery. Because they possess similar genetic backgrounds, except one or a few specific DNA fragments from known donors, applying directional selection on introgression lines (ILs) and near-isogenic lines (NILs) is a powerful tool to explore target genes controlling important traits. Using 21 elite varieties as recurrent parents, and 188 donor parents originating from 24 countries or regions, approximately 60,000 Ils and over 17,000 NILs based on the MCC have been developed systemically (Long et al., 2009; Zhang et al., in press). Using 300 genome-wide SSR markers, Zichao Li and his colleagues (2010) conducted association mapping of more than 50 traits characterized at three locations (Beijing, Hangzhou, and Hainan) over 2 years (unpublished). As described above, Zhou et al. (2010) used a cold-tolerant NIL derived from a cold tolerant cultivar, Kunming Xiaobaigu ,as donor parent and a common cultivar, Shihetian, as recurrent parent; they located a QTL *qCTB7* to a 92-kb interval on chromosome 7. Moreover, *GS3*, *PROG1*, *IPA1*, *DTH8*, and others, were cloned successfully using ILs or NILs based on germplasm collections (Fan et al., 2006; Tan et al., 2008; Jin et al., 2008; Jiao et al., 2010; Wei et al., 2010). Double haploid lines and linkage analysis also are applied for utilization of these resources, and improvement of the methods have been prominent achievements in the past few years (Mu et al., 2005; Wen et al., 2009; Jin et al., 2010).

5. Rice Molecular Breeding

Conventional breeding mainly relies on hybridization and phenotypic selection, which is usually time-consuming and difficult to evaluate effectively for the quantitative phenotypes of agronomic traits, particularly with interference environmental effects. With advances in biotechnology from both omics and bioinformatics, molecular breeding is gradually becoming an actuality, including marker-assisted, transgenic, and molecular-designed breeding.

5.1 Marker-assisted breeding

Marker-assisted selection (MAS) is a method that uses molecular markers closely linked to a target gene as a molecular tag, to precisely trace the target genotype of the hybrid progeny and improve the efficiency of genotypic selection in cross breeding. In order to screen suitable molecular markers, tremendous efforts have been made by Chinese rice geneticists and breeders to identify genes and QTLs relevant to important agronomic traits. MAS is widely used to pyramid functional genes into popular hybrid rice cultivars to improve important agronomic traits, such as yield, grain quality, disease resistance and tolerance abiotic stresses. In order to improve the eating and cooking quality of Zhenshan 97, the female parent of a number of widely used rice hybrids in China, the genome region containing the *Waxy* gene was introgressed from Minghui 63 (wx-MH) by Zhou et al. (2003b) via MAS. Using a cleaved amplified polymorphic sequence marker, PCR-Acc □, Liu et al. (2006) introduced the *Wx* gene into the elite variety Teqing , resulting in three elite lines with improved cooking and eating quality; these are Teqing TT-1, Teqing TT-2 and Teqing TT-3. Chen et al. (2000) developed an elite Minghui 63 line (*Xa21*) by introgressing *Xa21*, a broad-spectrum bacterial blight resistance gene. Field examination showed that Minghui 63 (*Xa21*) had higher grain weight and spikelet fertility than Minghui 63 under heavy contaminant with the pathogen of bacterial blight. A similar result was obtained when *Xa21* was introgressed into 6078, an elite restorer line of hybrid rice with high yield potential (Chen et al., 2001). Furthermore, gene pyramiding combined with MAS has been successfully applied in rice breeding programs, and many varieties and lines have been produced. Deng et al. (2005) developed a promising family of Q611, in which two yield-enhancing QTL alleles, *yld 1.1* and *yld 2.1* from *Oryza rufipogon* Griff. were introgressed into Ce64, one of best restorers used in Chinese hybrid rice. All Q611 family individuals showed very strong heterosis and great potential for increasing the yield of hybrid rice. The dominant bacterial blight resistance gene *Xa21* and a fused *Bt* gene, *cry1Ab/cry1Ac*, conferring resistance to lepidopteran insects, were introduced into the same target line 'Minghui 63'through MAS. Field trials demonstrated that the pyramided line and its derived hybrids showed high levels of resistance to both insect and disease (Jiang et al., 2004). Chen et al. (2008) introgressed three rice blast resistance genes (*Pi-1*, *Pi-2* and *Pi-33*) into an elite variety Jin 23B by crossing, backcrossing and multi-crossing through MAS, and obtained six near isogenic lines of Jin 23B carrying the three resistance genes.

5.2 Transgenic breeding

Compared with MAS, transgenic breeding is a time-saving, efficient and direct way to improve agronomic traits. Transgenic breeding introduces functional genes, using recombinant DNA technology, into a receptor line's genome to

produce a desired trait. Transgenic technology can break the barrier of gene flow between species, which is difficult to overcome using conventional breeding. Thus, the candidate gene used in transgenic breeding can be derived from an alternative species.

5.2.1 Transgenic insect-resistant rice

Insect damage is one of the major factors leading to yield loss in crop plants. The Bt toxin gene derived from *Bacillus thuringiesis* (Bt) is one of the most broadly-used insecticidal genes worldwide. A transgenic elite rice line expressing two Bt fusion genes *cryIA(b)* and *cryIA(c)* was developed and showed strong protection against leaf folder and yellow stem borer without reducing yield (Tu et al., 2000). Besides the BT gene, some plant-derived insect-resistant genes also have been used to improve insect resistance of rice. Among them, plant lectin genes, such as the *Galanthus nivalis* agglutinin (GNA) gene, have been widely applied. Sun et al. (2001b) obtained homogenous transgenic GNA rice lines via particle bombardment. Experimental data indicated that the brown planthopper feeding on homogeneous transgenic lines suffered significantly lower survival rate and fecundity, retarded development and reduced feeding. In addition to plant lectin genes, protease inhibitor genes like the cowpea trypsin inhibitor *CpTI* are another group of plant-derived insect-resistant genes. A modified *CpTI* gene was introduced into Minghui 86, and the transgenic offsprings showed stronger resistance to the rice stem borer than the parent line (Huang et al., 2005).

5.2.2 Transgenic disease-resistant rice

Bacterial blight (BB), fungal diseases blast, and sheath blight are three major diseases in rice production. With the progress in identification and cloning of disease-resistant genes, introducing corresponding genes into the desired rice varieties has proved to be direct and convenient for transgenic breeding. Zhang et al. (1998) introduced the broad-spectrum BB resistance gene *Xa21* into Minghui 63. Transgenic Minghui 63 showed significantly enhanced resistance to *Xanthomonas oryzae* pv. *Oryzae* (Xoo), which is the most devastating rice bacterial disease worldwide. Wu et al. (2001) obtained BB-resistant transgenic Minghui 63 and Wan B (a rice maintainer line) by introducing *Xa21*, and hybrids also exhibited significantly enhanced BB-resistance. Zhu et al.(2007b) integrated four antifungal genes, *RCH10*, *RAC22*, *β-Glu* and *B-RIP*, into the genome of 9311, and then developed transgenic F_1 plants from the cross of R_2 homozygous lines with high resistance to rice blast and Peiai 64S. Field experiments revealed that transgenic F_1 plants not only have high resistance to *M. grisea* but also enhanced resistance to rice false smut (a disease caused by *Ustilaginoidea virens*) and rice kernel smut (another disease caused by *Tilletia barclayana*).

5.2.3 Transgenic herbicide-tolerant rice

A herbicide-tolerant gene now is wildly used to screen the progeny in transgenic breeding. Transgenic rice containing the herbicide-tolerant gene is expected to enhance herbicide-tolerance in rice production and to be used to remove false hybrid seeds to increase the seed purity in rice hybrid production. The *bar* gene from *Streptomyces hygroscopicus* was the first and remains the most common herbicide-resistant gene used in transgenic rice. Using transgenic Minghui 86B with *bar* gene, novel hybrids IIyou 86B and Teyou 86B were developed by the South China Botanical Garden, Chinese Academy of Sciences (Wu et al., 2006). Currently, only two transgenic herbicide-tolerant lines have been allowed to enter field testing in China due to questions about the safety of genetically modified crops and products.

5.3 molecular design breeding

The advances in rice omics and the enrichment of large-scale marker data sets provide us with tools to determine the genetic basis of all important agronomic traits. Based on the genes uncovered for these traits, breeders can design and combine all the most favourable alleles into one variety, and then use molecular markers closely linked to these loci to develop the desired varieties. Synthetic biology is a concept related to molecular design breeding but includes much more. Synthetic biology includes the design and construction of new biological parts, devices, and systems, or the re-design of existing, natural biological systems for useful purposes. The introduction of the *Bt* gene into rice belongs to one kind of synthetic biology. Chen et al. (2005) and Tang et al. (2006) developed transgenic rice with synthetic cry2A* and cry1C*, respectively. Both transgenic Cry2A* rice and Cry1C* rice showed high resistance against lepidopteran rice pests in field experiments. With the quick development of rice omics, synthetic biology approaches will become important in rice improvement and germplasm innovation. To sustainably increase the yield potential of rice varieties and satisfy needs of a rapidly growing population, the Ministry of Agriculture in China set up a super hybrid rice program in 1996. In the last decade, ‘super hybrid rice' has made significant progress and rice breeders have successfully developed some super hybrids with harmonious plant types and adaptations to different ecological regions. So far, the Ministry of Agriculture in China has released more than 80 super hybrids, such as the representative three-line hybrids Xieyou 9308, and the representative two-line hybrids Liang-You-Pei 9. In all, these super hybrids covered a cumulative total area of 1333 million hm^2 by 2009. To tackle new challenges of protection against insects and diseases, water shortages, extensive cultivation and others, Zhang (2007c) advocated a new idea – development of a Green Super Rice (GSR) to achieve the goal of increasing yield and improving quality. The targets of Green Super Rice Breeding program is to reduce the amount of pesticides, fertilizers, and water required in rice agriculture and, at the same time increasing and improving rice yield and quality. Fulfillment of this idea depends largely on advances in rice omics, including characterization of large numbers of genes and QTLs related to insect resistance, disease resistance, cold resistance, drought tolerance, large grain size, quality and ideal plant type, and obtaining information about gene-by-gene and gene-by-environment interactions at different omics levels. On April 8th 2010, this program was launched officially in Huazhong Agricultural University. Green Super Rice may exert important influence on food security in China and throughout the world.

6. Prospects

Rice will become a model species for plant omics research in the near future. The advance of the rapid, high throughput and cheaper technologies about whole genome resequencing and single molecular sequencing permit us to study the genome and transcriptome of a population rather than individual. The advances in rice omics have provided us with more detailed DNA variation patterns, large amount of functional genes, transcription patterns and protein expression profiling, which have threw highlight on the genetic basis of the development of important agronomic traits. The core collection of rice

germplasm and their ILs or NILs, being genetically representatives, could be extensively used as the uniform panel of genetic materials for omics research. The achievement of several transgenic crops and discovery of large-scale marker data sets indicates that breeders can design to pyramid the most favourable alleles into one variety. With more and more genes controlling the important agronomic traits cloned and functionally annotated, the molecular design breeding or synthetic biology will make more progress. Consequently, the more desired varieties will be developed by these high-tech approaches.

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