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Research review paper

Rice functional genomics research: Progress and implications for crop genetic improvement

Yunhe Jiang, Zhaoxia Cai, Weibo Xie, Tuan Long, Huihui Yu, Qifa Zhang *

National Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, China

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ABSTRACT

Rice is a staple food crop and has become a reference of monocot plant for functional genomic research. With the availability of high quality rice genome sequence, there has been rapid accumulation of functional genomic resources, including: large mutant libraries by T-DNA insertion, transposon tagging, and chemical mutagenesis; global expression profiles of the genes in the entire life cycle of rice growth and development; full-length cDNAs for both *indica* and *japonica* rice; sequences from resequencing large numbers of diverse germplasm accessions. Such resource development has greatly accelerated gene cloning. By the end of 2010, over 600 genes had been cloned using various methods. Many of the genes control agriculturally useful traits such as yield, grain quality, resistances to biotic and abiotic stresses, and nutrient-use efficiency, thus have potential utility in crop genetic improvement. This review was aimed to provide a comprehensive summary of such progress. We also presented our perspective for future studies.

Contents

1. Introduction

Rice is a staple food crop providing calories for about half of the world's population. Rice has now become a model plant species for functional genomic studies due to a number of biological characteristics

* Corresponding author. Tel.: +86 27 87282429; fax: +86 27 87287092.
E-mail addresses: jyhhzau@webmail.hzau.edu.cn (Y. Jiang), zhaoxiac@126.com (Z. Cai), xwbcn@webmail.hzau.edu.cn (W. Xie), longtuan2001@yahoo.com.cn (T. Long), yingcai@webmail.hzau.edu.cn (H. Yu), qifazh@mail.hzau.edu.cn (Q. Zhang).

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of the plant and research advances. (1) It has one of the smallest genomes in crop plants; (2) The genome shares co-linearity with members of the grass family including major cereal crops like corn and wheat; (3) High-efficiency transformation technology is available, and has gained widespread utility; (4) It is the first genome of crop plants sequenced with high precision; (5) Rice is rich in germplasm resources, and large collections of cultivated rice and its wild relatives have been maintained in germplasm banks; (6) Large amounts of genetic stocks have been developed and made available to the research community; (7) There has been a long history and large scale of genetic research; and (8) There are extensive breeding programs in almost all the rice producing countries.

The last decade has seen rapid advances in rice functional genomic research globally. Most of the efforts can be summarized in three fronts: (1) construction of technological and resource platforms for high throughput gene identification; (2) functional genomic analysis of agronomic traits and biological processes; and (3) identification and isolation of functional genes. In this paper, we will review some of the progress made in recent years in these three areas, presenting examples of the findings that, in our view, have implications for crop genetic improvement. We will also discuss prospects of future studies and possible impacts of these findings on the strategies in crop genetic improvement.

2. The scope of rice functional genomic research

Cultivated rice consists of two species, Asian cultivated rice (*Oryza sativa* L.) that is grown in most parts of the world and African cultivated rice (*O. glaberrima* Steud.) that is grown in certain regions of Africa. The Asian cultivated rice (*O. sativa* L.) consists of two subspecies, *indica* (*O. sativa* L. *ssp. indica*) and *japonica* (*O. sativa* L. *ssp. japonica*).

Rice is the first sequenced crop plant (Goff et al., 2002; International Rice Genome Sequencing Project, 2005; Yu et al., 2002). According to the latest release of the Rice Genome Annotation Project (RGAP 6.1, http://rice.plantbiology.msu.edu/), the rice genome size is ~370 Mb. A total of 56,797 loci are predicted, including 40,577 non-TE (transposable element) loci encoding 50,939 gene models, and 16,220 TE loci encoding 16,454 gene models. These numbers will change from time to time with new knowledge gained from the research community.

The overall goal of rice functional genomic research is to understand how the genome functions to make the plant and produce phenotype, by deciphering the information conserved in the sequences, including genes and regulatory elements at the whole genome level. An equally important goal is the application of the findings to genetic improvement of rice which may also serve as a model for other crops.

3. Development of technical and resource platforms for rice functional genomic research

The overall goal of the platforms is to provide toolkits and resources for high throughput identification of genes and pathways. Efforts in the last decade have been focused on (1) large mutant libraries; (2) full-length cDNAs; (3) global expression profile; and (4) bioinformatics analyzing and mining of datasets produced by diverse research teams.

3.1. Mutant libraries

Changing expression level or pattern of the gene by knocking-out, knocking-down, overexpression and ectopic expression are among the frequently used strategies for deciphering the gene function. Thus construction of saturated mutant libraries is essential for identification of gene function. Currently, mutant libraries, making use of several strategies including T-DNA, transposon and retrotransposon insertions, have been developed in a number of laboratories in different countries (Table 1). And the lines and related data in these libraries have been accumulating rapidly.

In addition to knocking-out or knocking-down the expression of genes, such libraries can be used for monitoring expression patterns of tagged genes. T-DNA and transposon can be modified by adding the functional elements such as activation tag, gene trap tag, promoter trap tag and enhancer trap tag (Eamens et al., 2004; Hsing et al., 2007; Jeon et al., 2000; Kim et al., 2004; Kolesnik et al., 2004; Sallaud et al., 2004; van Enckevort et al., 2005; Wu et al., 2003). For example, the constructs used by Wu et al. (2003) to generate the mutant library adopted three strategies for functional analysis of the rice genome: insertional mutagenesis, enhancer trapping and ectopic expression. Insertional mutagenesis is a common feature of the mutant libraries. Many of the enhancer trapping systems have utilized the GAL4-VP16 element, in which *GAL4-VP16* would be expressed when the T-DNA is inserted nearby an enhancer, and GAL4-VP16 would bind to the UAS leading to transcription of the reporter gene *GUS* or *GFP*. For ectopic expression, target lines need be generated, in which the target gene would also be driven by UAS. When a pattern line is crossed with a target line, GAL4-VP16 can bind to UAS thus directing the expression of the target gene. It was shown that this system worked very well for testing gene function by ectopic expression (Liang et al., 2006). Thus, in addition to loss-of-function mutations by insertions, the enhancer trap mutant libraries provide a set of tissue specific expression lines that can be

Table 1
Current status of global gene tagged mutant resources (Update 07/2010).

Database/resource/ institution	Database web site	FSTs	Genotype	Mutagen
POSTECH RISD	http://signal.salk.edu/cgi-bin/RiceGE	106,110	Dongjin, Hwayoung	T-DNA (promoter trap, activation-tagging), Tos17
RMD	http://rmd.ncpgr.cn/index.cgi?nickname=	37,887	Zhonghua 11, Zhonghua 15, Nipponbare	Tos17, T-DNA (enhancer trap)
NIAS (RTIM)	http://tos.nias.affrc.go.jp/	17,985	Nipponbare	Tos17
Zhejiang University	http://www.genomics.zju.edu.cn/ricetdna.html	1009	Zhonghua 11, Nipponbare	T-DNA
CIRAD-INRA-IRD-CNRS, Genoplante	http://orygenesdb.cirad.fr/index.html	41,159	Nipponbare	T-DNA (enhancer trap), Tos17
Sundaresan Lab	http://www-plb.ucdavis.edu/Labs/sundar/Rice_Genomics.htm	17,684	Nipponbare	Ac-Ds (gene trap), Spm/dSpm
Gyeongsang National University	http://signal.salk.edu/cgi-bin/RiceGE	1047	Dongjin Byeo	Ac/Ds (gene trap)
CSIRO	http://www.pi.csiro.au/fgrttpub/blast_csn.htm	592	Nipponbare	Ac/Ds (gene trap, enhancer trap)
EU-OSTID	http://signal.salk.edu/cgi-bin/RiceGE	1315	Nipponbare	Ac/Ds (enhancer trap)
Taiwan (TRIM)	http://trim.sinica.edu.tw/	11,794	Tainung 67	T-DNA (activation-tagging)
SHIP	http://ship.plantsignal.cn/index.do	10,281	Zhonghua 11	T-DNA (enhancer trap)

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used for several purposes (Johnson et al., 2005; Liang et al., 2006; Sallaud et al., 2004; Wu et al., 2003).

To saturate the rice genome with T-DNA insertions, it can be calculated that a total of 576,441 insertion events would be needed to obtain at least one insertion for each of the 56,797 annotated loci with a probability of 0.99 using the formula $P = 1 - (1 - x/G)^n$ (Krysan et al., 1999), [in which x is the average size of rice gene that is 2.96 kb and G is the genome size which takes the value of 370,637 kb according to the MSU Rice Genome Annotation, Release 6, n is the number of insertions needed to obtain the probability (P) of genome saturation], assuming random insertions of the T-DNA in the genome.

Huge efforts have been invested to isolate the flanking sequences of the insertion sites to provide tags for the insertions (flanking sequence tag, FST), by means of Thermal Asymmetric Interlaced (TAIL)-PCR, Inverse PCR and Adaptor PCR. As of now, a total of 246,863 flanking sequences have been isolated (Table 1), tagging 61.2% of total genes (T. Long et al. unpublished data). Thus it is clear that although the total number of T-DNA transformed lines is huge, the FST isolation has been slow and there is still a long way to go.

However, the complexity in generating, managing, maintaining and screening of such huge libraries is enormous. Moreover, Zhang et al. (2007) showed that nonrandom distributions of the T-DNA occurred at various levels of the genome hierarchy: by favoring large chromosomes, strongly disfavoring TE-related sequences, preferentially occurring in 5′-upstream and 3′-downstream regions of the genes, and differentially occurring in certain categories of functional genes. Such distribution patterns clearly suggested the difficulty of the T-DNA insertion approach in accessing some of the genomic regions and recovering certain classes of genes. Thus alternative and more efficient approaches have to be explored.

Chemical and physical mutagenesis may offer a supplementary approach to T-DNA insertion for generating saturated mutant libraries, which is now more feasible with the enhanced ability to identify the mutation sites by new sequencing technology. Artificial microRNA may provide a complementary approach by creating mutants only for the targeted genes (Warthmann et al., 2008), which can even be tissue specific or inducible. An additional advantage with the targeted approach is that a much smaller number of mutant lines are needed to obtain saturated mutations of the genome (Schwab et al., 2006).

3.2. DNA microarray and expression profiling

Information of gene expression profile is useful in many ways for functional genomic studies. For example, transcript abundance of a gene in multiple tissues would allow the establishment of temporal and spatial patterns of the gene expression, to recognize whether the gene expression is constitutive or tissue specific. The expression pattern of the gene would provide clue to understanding the gene function. Information about expression of a genome sequence is useful for genome annotation. Furthermore, co-expression analysis may lead to identification of regulatory network involving sets of genes.

Large amounts of data sets of whole genome expression have been generated in rice by various platforms (Table 2), including the BGI/Yale 60 K (Jiao et al., 2009; Ma et al., 2005), NSF 45 K (Jung et al., 2008), Agilent 44 K (Sato et al., 2011; Shimono et al., 2007) and Affymetrix 57 K (Fujita et al., 2010; Li et al., 2007; Wang et al., 2010). The expression databases provide expression profiles of each gene which can also be utilized for building co-expression network (Harbison et al., 2009; Nayak et al., 2009). For example, Yale Virtual Center for Cellular Expression Profiling of Rice database collected transcriptomes of 40 rice cell types from shoot, root and germinating seed at different developmental stages through laser microdissection (Jiao et al., 2009). RiceXPro Database collected gene expression data encompassing the entire life cycle of rice using Agilent 44 K platform (Sato et al., 2011). Datasets of over 1000 arrays and several kinds of analytical tools were also incorporated into Rice Array Database (Jung et al., 2008). Rice Functional Genomics Express Database (RiceGE) is a comprehensive database collecting various genomic resources including both expression data of *indica* and *japonica*. CREP and RAD databases also provide co-expression analysis tools. Although datasets of over 1800 arrays have been deposited in NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/), integration of these datasets is needed to make them more user-friendly.

Here we describe in some detail the dataset reported by Wang et al. (2010) to gain a glimpse of the global expression pattern at the whole genome level. This microarray dataset included gene expression of 39 tissues (organs) covering the whole life cycle of the rice plant from Shanyou 63, the most widely cultivated rice hybrid and its parents Zhenshan 97 and Minghui 63 using the Affymetrix GeneChip Rice Genome Array containing 57,381 probesets. The original data were normalized and deposited in the database CREP (Collections of Rice Expression Profiling, http://crep.ncpgr.cn). Analyses of the global transcriptomes of the two parental lines revealed many interesting features of dynamic patterns of gene expression across the tissues and stages. Totally, 38,793 probesets (68%) were detected as expressed, and 69% of the expressed transcripts showed significantly variable expression levels among tissues/organs. Similarity of transcriptomes among organs corresponded well to their developmental relatedness. About 5.2% of the expressed transcripts showed tissue-specific expression in one or both varieties, and 22.7% of the transcripts exhibited constitutive expression, of which 19 genes showed high and stable expression in all the tissues. This dataset provided rich information resource, which can be used for associating the transcriptomes to the developmental processes, understanding the regulatory network of these processes, tracing the expression profile of individual genes, and identifying reference genes for quantitative expression analyses. Indeed use has been made of this dataset for a range of studies (Chen et al., 2009; Huang et al., 2009a; Yan et al., forthcoming; Zhao et al., 2010b).

A shortcoming of the microarray data is that the expression information is limited to the probesets fixed on the arrays, which is dependent on the genome annotation, while all of the expression platforms mentioned above were based on annotations done quite a few years ago. Moreover, the hybridization signals in the microarrays could not differentiate alternative transcripts. The new sequencing technology enabling RNA sequencing (RNA-seq) which can map and quantify the transcriptomes may provide a solution to this constraint. Lu et al.

Table 2
Rice expression databases.

Database	URL	Description
Collection of rice expression profiles (CREP)	http://crep.ncpgr.cn	*Indica*, Affymetrix 57 K, tissues covering the entire life cycle, 190 samples
Yale virtual center for cellular expression profiling of rice	http://bioinformatics.med.yale.edu/riceatlas/	*Japonica*, BGI/Yale 60 K, diverse cell types, 220 samples
Rice expression profile database (RiceXPro)	http://ricexpro.dna.affrc.go.jp/	*Japonica*, Agilent 44 K, whole developmental tissues, 209 samples
Rice array database (RAD)	http://www.ricearray.org/index.shtml	*Indica* and *japonica*, NSF 45 K, NSF 20 K, Affymetrix 57 K, Agilent 22 K, BGI/Yale 60 K, 1790 samples
RiceGE: gene expression atlas	http://signal.salk.edu/cgi-bin/RiceGE	*Indica* and *japonica*, Affymetrix 57 K, 22 K custom oligoarray, 163 samples

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Table 3
World-wide available full-length cDNA clones.

Species	Genotype	Number of clones	Database website
O. sativa L. *ssp. japonica*	Nipponbare	37,132	KOME: http://cdna01.dna.affrc.go.jp/cDNA/
O. sativa L. *ssp. indica*	Guangluai 4	10,081	RICD: http://www.ncgr.ac.cn/ricd
O. sativa L. *ssp. indica*	Minghui 63	12,727	RICD: http://www.ncgr.ac.cn/ricd
O. rufipogon	W1943	2045	RICD: http://www.ncgr.ac.cn/ricd

(2010) performed RNA-seq using tissues from three rice cultivars 9311 (*indica*), Guangluai 4 (*indica*) and Nipponbare (*japonica*). Their analysis identified 15,708 novel transcriptional active regions, of which 51.7% have no homolog to public protein data and >63% are putative single-exon transcripts, which are highly different from protein-coding genes (<20%). They also found that ~48% of rice genes show alternative splicing patterns, which is considerably higher than previous estimates. On the basis of the available rice gene models, 83.1% (46,472 genes) of the annotated rice gene models were validated by RNA-seq, and 6228 genes were identified to be extended beyond the 5′ and/or 3′ ends by at least 50 bp. Moreover, 3464 genes exhibited differential expression patterns between cultivars. This work demonstrated the usefulness of the new sequencing technology in profiling the transcriptomes.

3.3. Full-length cDNA collections

Information provided by full-length cDNAs can greatly help annotation of the genomic sequences by identifying transcription units, introns and exons as well as likely promoters, and predicting gene products. Moreover, a collection of full-length cDNAs may provide an inventory of gene clones that are frequently needed for gene isolation thus facilitating genomic and proteomic research by saving considerable workload.

Two large efforts have been made to collect full-length cDNA clones (Table 3). Kikuchi et al. (2003) started the work by collecting full-length cDNA clones from libraries made of *japonica cv.* Nipponbare. Currently approximately 38,000 full length cDNA clones have been isolated and made available worldwide by searching the database of Knowledge-based Oryza Molecular Biological Encyclopedia (KOME, http://cdna01.dna.affrc.go.jp/cDNA). For *indica* rice, over 20,000 full-length cDNA clones were isolated from *indica cv.* Guangluai 4 and Minghui 63 (Liu et al., 2007a; Lu et al., 2008; Xie et al., 2005). Recently, 2045 full length cDNAs from *O. rufipogon* were isolated. These clones have also been made available to the research community by searching the Rice *Indica* cDNA Database (RICD, http://www.ncgr.ac.cn/ricd). In addition, these

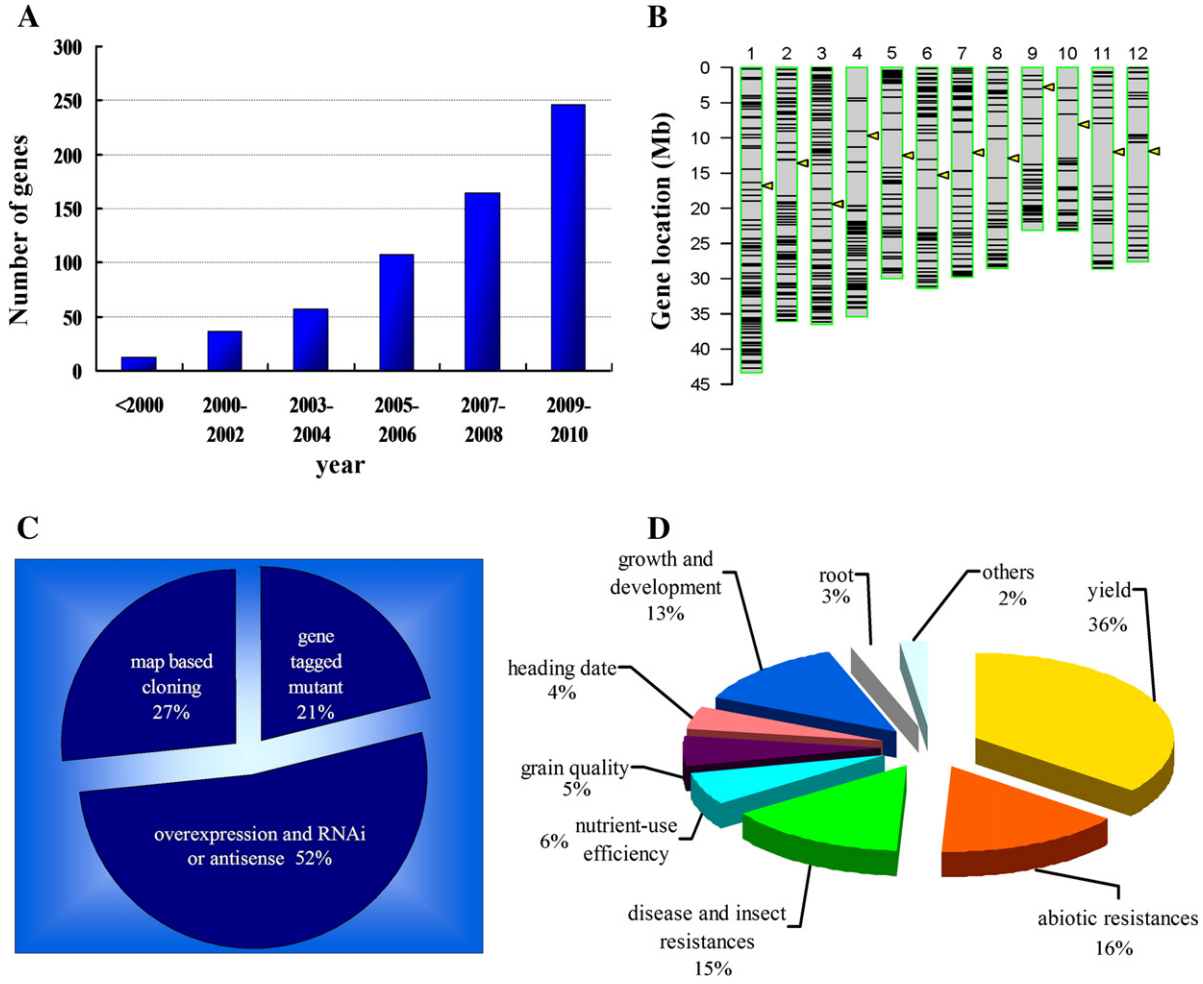

Fig. 1. Current status of isolated genes in rice. (A) Number of genes cloned in different years. (B) Chromosome localizations of 592 isolated genes. Each thin horizontal line represents a gene. The yellow triangles indicate the positions of the centromeres. (C) Numbers of genes isolated by different methods. (D) Functional categories of the isolated genes.

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indica, *japonica* and wild rice full-length cDNA clones also provided a resource for comparative genomics (Liu et al., 2007a).

4. Characterization of genes controlling important agronomic traits

4.1. Approaches for gene cloning

Gene cloning in rice is now progressing at an accelerating rate (Fig. 1A). By the end of 2010, over 600 genes had been cloned, and genomic positions for 592 of the isolated genes are shown in Fig. 1B. Three major classes of methods have been employed in rice gene cloning.

The traditional method for gene isolation is map-based cloning which has been extensively used in rice, mostly for identifying natural mutations of agriculturally useful traits. The availability of high quality genome sequence in recent years has greatly facilitated gene cloning using map-based cloning approach. Recently, new sequencing technology has been successfully applied for constructing SNP-based ultrahigh-density linkage maps (Huang et al., 2009b; Xie et al., 2010), which has considerably improved the resolution and accuracy of mapping for quantitative trait loci (QTLs) (Wang et al., 2011; Yu et al., forthcoming). It is anticipated that the process of map-based cloning will be significantly accelerated by making efficient use of the above described technological and resource platforms.

Recently, insertional mutant libraries have become a major source of gene cloning, using both forward and reverse genetic approaches. By forward genetic approach, the insertion lines are screened by phenotype, cosegregation between the phenotype and T-DNA insertion is analyzed, and usually a complementation test is performed for further confirmation of the phenotypic effect of the gene. In reverse genetic approach, the candidate gene is used to search the FSTs of the mutant databases, and the mutant obtained is then analyzed for phenotype.

Another major approach of cloning genes is functional characterization of genes that show differential expression mostly from microarray analyses (chip-based approach), for example treatment with abiotic stresses. Differentially expressed genes are subjected to overexpression, knocking-down, and/or knocking-out, and the phenotypic effects of such mutants are evaluated.

The number of genes cloned by each of the methods is listed in Fig. 1C. More genes have been identified using the third approach than the other two methods, which also included functional tests of candidates selected by their homology to known genes. We anticipate that the mutant libraries will gradually play a more and more prominent role in gene isolation especially with the rapid accumulation of FSTs.

4.2. A glimpse of the cloned genes

To gain a glimpse of the cloned genes, we will describe them in perspective of agricultural importance divided by agronomic traits, rather than biological processes, such as yield, quality, resistances to biotic and abiotic stresses, and nutrient-use efficiency (Fig. 1D). Features for some of the genes that in our view may have potential utility in crop genetic improvement will be mentioned in particular.

4.2.1. Genes for yield

Grain yield is a complex trait determined by three major components: number of panicles per plant, number of grains per panicle and grain weight. Number of panicles per plant is dependent on tillering ability and is largely determined by numbers of primary and secondary tillers. Similarly number of grains per panicle is largely determined by primary and secondary branches. Panicles and tillers are different forms of branching, resulting from the activity of lateral meristem. Grain weight is determined by grain size, specified by length, width and thickness, and degree of filling. Grain size variation is controlled by a number of biological processes including cell division, cell expansion and division polarity. The genetic and molecular bases of rice yield including the yield component traits and underlying biological processes were comprehensively reviewed recently by Xing and Zhang (2010). We prepared here a list of genes which may have potential utility in breeding for yield improvement (Table 4), and highlight some of the new progress in identification of yield genes.

More and more findings show that microRNAs regulate a wide range of biological processes. Overexpression *miRNA156* in rice greatly increased number of tillers but with decreased panicle size (Xie et al., 2006). *OsSPL14*, a target of *miRNA156*, is responsible for the QTL *IPA1* controlling plant architecture. *OsSPL14* is highly expressed in the shoot apex and primordia of primary and secondary branches, reduced tiller number and promoted panicle branching (Jiao et al., 2010; Miura et al., 2010).

Ghd8/*DTH8* is a QTL conferring pleiotropic effects on grain yield, heading date, and plant height (Wei et al., 2010; Yan et al., forthcoming). *Ghd8*/*DTH8* enhances the expression of *MOC1* which is also involved in

Table 4
Examples of genes controlling grain yield traits.

Trait	Gene	Encoding product	References
Tillering	*OsTB1*/*FC1*	TCP transcription factors	Minakuchi et al., 2010; Takeda et al., 2003
	D3	F-box LRR protein	Ishikawa et al., 2005
	HTD1	A family of CCD proteins	Zou et al., 2006
	DWARF10	Carotenoid cleavage dioxygenase 8	Arite et al., 2007
	TAC1	Unknown protein	Yu et al., 2007
	DWARF27	Iron-containing protein	Lin et al., 2009
	MIP1	Unknown protein	Sun et al., 2010
	MOC1/*SPA*	GRAS family nuclear protein	Li et al., 2003
Grain number (panicle size)	*RCN1*	Rice TFL1/CEN homologs TFL1/CEN homologs	Nakagawa et al., 2002
	RCN2	Rice TFL1/CEN homologs	Nakagawa et al., 2002
	LAX1	bHLH transcription factor	Komatsu et al., 2003
	Gn1a	Cytokinin oxidase	Ashikari et al., 2005
	APO1	F-box protein	Ikeda et al., 2007
	LOG	Cytokinin-activating enzyme	Kurakawa et al., 2007
	Ghd7	A CCT domain protein	Xue et al., 2008
	RFL	LFY homolog	Rao et al., 2008
	SP1	PTR family transporter	Li et al., 2009
	DEP1	PEBP like domain protein	Huang et al., 2009d
	EP3	F-box protein	Piao et al., 2009
	LRK1	LRR receptor-like kinase	Zha et al., 2009
	DTH8/*Ghd8*	OsHAP3 subunit of HAP complex	Wei et al., 2010; Yan et al., forthcoming
	DEP2	Unknown protein	Li et al., 2010
Grain weight	*GIF1*	Cell-wall invertase	Wang et al., 2008a
	RISBZ1	Basic leucine Zipper factor	Kawakatsu et al., 2009
	RPBF	Prolamin box binding factor	Kawakatsu et al., 2009
	GS3	Unknown protein containing OSR	Fan et al., 2006; Mao et al., 2010
	GW2	RING-type E3 ubiquitin ligase	Song et al., 2007
	qSW5/*GW5*	Nuclear protein	Shomura et al., 2008; Weng et al., 2008
Plant architecture	*OsSPL14*	Plant-specific transcription factor	Jiao et al., 2010; Miura et al., 2010

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the process of panicle branching (Li et al., 2003; Yan et al., forthcoming). It was shown that the previously reported *Ghd7* that showed similar pleiotropic effect (Xue et al., 2008) and *Ghd8* encode HAP2 and HAP3 subunits respectively, while in mammals, HAP proteins act as DNA binding proteins usually by building HAP2/HAP3/HAP5 trimeric complex.

From a biological perspective, grain development reflects a coordination of cell division and cell expansion. *GS3* is a major QTL regulating grain length and has been widely used in breeding for grain size (Fan et al., 2006). The GS3 protein consists of four putative domains: organ size regulation (OSR) domain, a transmembrane domain, a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family cysteine-rich domain, and a von willebrand factor type C (VWFC) in the C-terminus. The N-terminal OSR domain negatively regulates cell division and thus reduces organ size, while the C-terminal TNFR/NGFR and VWFC domains show an inhibitory effect on the OSR function (Mao et al., 2010). The N-terminus of DEP1, a gene for panicle erectness (Huang et al., 2009d), shares high homology with the OSR domain. Both genes play the same role in organ size control: inhibiting cell division, although GS3 regulates grain size while DEP1 controls panicle size.

4.2.2. Genes for resistances to biotic stresses

Rice as a crop suffers heavy losses from a number of diseases and insects (Zhang, 2007). Three diseases have been considered to be the most devastating worldwide, bacterial blight caused by *Xanthomonas oryzae pv. oryzae*, blast by *Pyricularia grisea*, and sheath blight by *Rhizoctonia solani*. Similarly, three groups of insects, stemborers (yellow stemborer *Tryporyza incertulas* and striped stemborer *Chilo suppressalis*), leaffolders (*Marasmia patnalis* and *Cnaphalocrocis medinalis*), and planthoppers (mostly brown planthopper, *Nilaparvata lugens*), have been the most damaging pests.

Cloning of disease resistance genes have made tremendous progress in rice (Table 5), 40 genes for bacterial blight resistance and 48 genes for blast resistance have been cloned and functionally characterized. *Xa21* was the first isolated gene conferring resistance to a wide range of races of the bacterial blight pathogen, encoding a receptor-like kinase (Song et al., 1995). Further work on this gene has greatly enhanced our understanding of gene-for-gene interaction in the host-pathogen system (Chen et al., 2010b; Park et al., 2008, 2010; Wang et al., 2006b).

A very interesting example of recessive resistance gene is *xa13*, which has been widely used in rice breeding in south Asian countries, conferring resistance to bacterial blight pathogen strain PXO99. Molecular cloning and analysis of this gene (Chen et al., 2010a; Chu et al., 2006; Yang et al., 2006) showed that the wild-type *Xa13* is required for both bacterial growth and pollen development. A mutation in the promoter resulted in *xa13* which greatly reduces the response to PXO99 infection and thus confers disease resistance. XA13 works in concert with COPT1 and COPT5 to modulate copper redistribution by reducing copper concentration in the xylem to allow the pathogen infection (Yuan et al., 2010). It was also shown that *Xa13* is a member of the NODULIN3 (N3) gene family and acts as a sugar transporter supplying nutrition for pathogen (Chen et al., 2010a). This represents a distinct mechanism of host-pathogen interaction.

Pib is the first isolated blast resistance gene in rice which encodes a protein containing NBS-LRR (nucleotide-binding site and leucine-rich repeat) structure (Wang et al., 1999). It is now known that NBS-LRR represents the largest R gene family and the majority of blast resistant genes have this structure (Table 5).

For a long time, it has been hypothesized that disease resistances can be classified into two categories: qualitative resistance mediated by major genes for resistance that is highly specific to pathogen race, and quantitative resistance conferred by genes with small effects (typically QTLs) that are pathogen non-specific and durable (Kou and Wang, 2010). A noticeable progress is the identification and characterization of several genes for quantitative resistance underlying resistance QTLs (Ding et al., 2008; Fu et al., 2011; Fukuoka et al., 2009; Qiu et al., 2007; Shen et al., 2010; Wang et al., 2006a). This may provide additional and alternative gene resources for disease resistance breeding.

Another important progress is the isolation of *Bph14*, a gene for brown planthopper resistance (Du et al., 2009). Interestingly, *Bph14* encodes a coiled-coiled, nucleotide-binding and leucine-rich repeat (CC-NB-LRR) protein, which is highly similar to a number of disease resistance genes (Table 5). Expression of *Bph14* activates the salicylic acid signaling pathway which is also similar to defense response regulated by disease resistance genes. Thus there is much in common between defense responses mediated by disease resistance genes and insect resistance genes, suggesting a likely generality of biotic stress responses.

Table 5
Examples of genes controlling biotic resistances.

Stress	Gene	Encoding product	References
Bacterial blight	*Xa1*	NBS-LRR	Yoshimura et al., 1998
	Xa3/Xa26	LRR-TM-kinase	Sun et al., 2004
	xa5	γ-subunit of transcription factor IIA	Iyer and McCouch, 2004
	xa13/Os-8N3	Sugar transporter	Chu et al., 2006; Yang et al., 2006
	Xa21	LRR-TM-kinase	Song et al., 1995
	Xa27	Unknown protein	Gu et al., 2005
Blast	*Pib*	NBS-LRR	Wang et al., 1999
	Pi-ta	NBS-LRR	Bryan et al., 2000
	Pi-k (h)	NBS-LRR	Sharma et al., 2005
	Pi9/Piz-t/Pi2	NBS-LRR	Qu et al., 2006; Zhou et al., 2006
	Pi36	CC-NBS-LRR	Liu et al., 2007b
	Pi37	NBS-LRR	Lin et al., 2007
	Pikm2-TS	NBS-LRR	Ashikawa et al., 2008
	Pikm-TS	NBS-LRR	Ashikawa et al., 2008
	Pi5-1	CC-NBS-LRR	Lee et al., 2009
	Pi5-2	CC-NBS-LRR	Lee et al., 2009
	Pid3	NBS-LRR	Shang et al., 2009
	Pb1	CC-NBS-LRR	Hayashi et al., 2010
	Pi-d2/Pi-d(t)2	B-lectin receptor kinase	Chen et al., 2006
	Pi21	Proline-rich protein	Fukuoka et al., 2009
Brown planthopper	*OsHI-LOX*	13-lipoxygenase	Zhou et al., 2009a
	OsLOX1	Lipoxygenase	Wang et al., 2008c
	Bph14	CC-NB-LRR	Du et al., 2009

4.2.3. Genes for abiotic resistances

Frequent occurring abiotic stresses in rice production include drought, salinity and cold, and in certain areas heat may also be a major limiting factor. Progress has been made in the last decade by many groups in identifying genes that may be useful for improving resistance to abiotic stresses. Currently more than 100 rice genes including five QTLs have been identified as showing various degrees of resistance to abiotic stresses (Table 6).

Kinases appeared to be a major group of proteins identified in regulating stress responses including calcium-dependent protein kinase (CDPK), mitogen-activated protein kinase (MAPK), calcineurin B-like protein-interacting protein kinase (CIPK), and receptor-like kinase. For example, overexpression of *OsCDPK7* and *OsCDPK13* significantly improved tolerance to cold, and *OsCDPK7* overexpressors also showed enhanced tolerance to salt and drought (Abbasi et al., 2004; Saijo et al., 2000). Overexpressing *OsCIPK03*, *OsCIPK12*, and *OsCIPK15* could significantly improve tolerance to cold, drought and salt stresses respectively (Xiang et al., 2007). A drought-hypersensitive mutant1 (*DSM1*) encodes a putative MAPK kinase kinase and was induced by salt, drought, and ABA. Analysis of T-DNA insertion mutant *dsm1* and *DSM1* overexpressor showed that DSM1 has a positive effect on stress tolerance (Ning et al., 2010). OsSIK1 is a putative receptor-like kinase and acts as a positive regulator in tolerance to

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Table 6
Examples of genes controlling abiotic resistances.

Stress	Gene	Encoding product	References
Drought	*OsSKIPa*	Homolog of human ski-interacting protein	Hou et al., 2009
	DSM1	Raf-like MAPK kinase kinase	Ning et al., 2010
	DSM2	β-carotene hydroxylase	Du et al., 2010a
	OsCIPK12	Calcineurin B-like protein-interacting protein kinase	Xiang et al., 2007
	OsGH3.13/TLD1	Indole-3-acetic acid (IAA)-amido synthetase	Zhang et al., 2009
Salt	*SKC1*	HKT-type transporter	Ren et al., 2005
	OsNAC6/SNAC2	NAC transcription factor	Nakashima et al., 2007
	OsKAT1	Potassium channel	Obata et al., 2007
	OsCIPK15	Calcineurin B-like protein-interacting protein kinase	Xiang et al., 2007
Cold	*OsCIPK03*	Calcineurin B-like protein-interacting protein kinase	Xiang et al., 2007
	qLTG3-1	Unknown protein	Fujino et al., 2008
	OsMYB3R-2	MYB3R transcription factor	Ma et al., 2009
	MYBS3	MYB transcription factor	Su et al., 2010
	Ctb1	F-box protein	Saito et al., 2010
Drought and salt	*SNAC1*	NAC transcription factor	Hu et al., 2006
	OsbZIP23	Basic leucine zipper (bZIP) transcription factor	Xiang et al., 2008
	DST	Zinc finger transcription factor	Huang et al., 2009c
	AP59	APETELA2 transcription factor	Oh et al., 2009
	OsSIK1	Receptor-like kinase	Ouyang et al., 2010
	OsNAC10	NAC transcription factor	Jeong et al., 2010
Submergence	*Sub1A*	Ethylene response factor	Xu et al., 2006
	SNORKEL1	Ethylene response factor	Hattori et al., 2009
	SNORKEL2	Ethylene response factor	Hattori et al., 2009

salt and drought stresses (Ouyang et al., 2010). In addition, Ca^{2+} known as a second messenger is also involved in stress signaling.

A number of transcriptional factors have also been identified in stress response. cDNA microarray data showed that expression of *SNAC1* was greatly induced in an upland rice cultivar IRAT109 after drought stress, compared to Zhenshan 97, an irrigated rice variety. Overexpression of *SNAC1* significantly improved drought resistance of rice plants in the field under drought stress conditions at the reproductive stage without significant affecting agronomic performance (Hu et al., 2006). Constitutive overexpression of *OsNAC6/SNAC2* improved tolerance of rice to dehydration, salt and cold stresses, but reduced plant growth (Hu et al., 2008; Nakashima et al., 2007). Root-specific overexpression of *OsNAC10* enlarged roots, improved tolerance to drought stress and increased grain yield of rice under field drought conditions (Jeong et al., 2010). Overexpressing *OsbZIP23* in rice significantly improved tolerance to drought and salt stresses and sensitivity to ABA (Xiang et al., 2008). Members of the APETELA2 (AP2), zinc finger, and MYB families have also been shown to play regulatory roles in stress responses (Chen et al., 2008a, 2008b; Dubouzet et al., 2003; Huang et al., 2009a, 2009b, 2009c, 2009d; Ito et al., 2006; Ma et al., 2009; Mallikarjuna et al., 2011; Oh et al., 2009; Su et al., 2010; Wang et al., 2008b; Yamaji et al., 2009; Zhao et al., 2010a).

Phytohormones such as ABA, ethylene, and auxin play important roles in stress response. Drought-hypersensitive mutant2 (*dsm2*) encodes a putative β-carotene hydroxylase (BCH) which is predicted to be involved in biosynthesis of zeaxanthin, a carotenoid precursor of ABA. Overexpression of *DSM2* resulted in significantly enhanced resistance to drought and oxidative stresses. The amount of ABA was significantly reduced in *dsm2* mutant after drought stress compared to the wild-type (Du et al., 2010a). *Sub1A*, *SNORKEL1* and *SNORKEL2* encode ethylene response factors (ERF) conferring submergence tolerance in rice (Hattori et al., 2009; Xu et al., 2006). Comparative expression analysis identified that a set of AP2/ERF family transcriptional regulators was associated with the *Sub1A-1*-mediated submergence response (Jung et al., 2010). *TLD1* encodes an indole-3-acetic acid (IAA)-amido synthetase which conjugates IAA with amino acids. Gain-of-function mutant *tld1-D* shows enhanced tolerance to drought (Zhang et al., 2009).

QTLs for stress tolerance have also been cloned and characterized. A QTL regulating salt tolerance named *SKC1* was shown to encode a member of high affinity K^+ transporter (HKT)-type transporters (Ren et al., 2005). *qLTG3-1* encoding an unknown protein controls low-temperature germinability (Fujino et al., 2008). *qLTG3-1* is expressed in the embryo during seed germination and may function in weakening tissues covering the embryo to help germination. *Ctb1*, a cold tolerance QTL, encodes an F-box protein which interacts with Skp1 *in vitro*, indicating involvement of the ubiquitin-proteasome pathway in cold tolerance (Saito et al., 2010).

An effort has been made to test previously identified genes in a common genetic background under field conditions using a well defined protocol (Xiao et al., 2009). In this test, seven genes selected from the literature, each of which was under the control of a constitutive promoter or a drought inducible promoter, and the 14 constructs were transformed to Zhonghua 11. Transgenic lines were tested in the field and tubes under a rain-off shelter with drought stress at the reproductive stage. Indeed transgenic lines for five of the seven genes showed significant improvement as measured by relative yield compared with the control. More experiments like this should be conducted to identify genes that may have practical utility.

4.2.4. Genes for nutrient-use efficiency

Efficient use of nitrogen and phosphorus nutrients has been among the most important goals in rice genetic improvement (Zhang, 2007). There have also been efforts in identifying genes regulating nutrient uptake and utilization efficiency in rice.

Nitrogen is an essential and growth-limiting macronutrient for plant growth and development. Two enzymes, glutamine synthetase (GS; EC6.3.1.2) and glutamate synthase (GOGAT; EC 1.4.1.14 and EC 1.4.7.1) assimilate inorganic nitrogen into organic molecules. GS shows high affinity for NH_4^+ and plays an important role for rice nitrogen assimilation, especially under anaerobic conditions. Overexpression of *GS1;1*, *GS1;2*, and *glnA* increased soluble protein concentrations in leaves and total amino acid and total nitrogen contents in the plant, but with decreased grain yield (Cai et al., 2009). Aspartate aminotransferase (EC 2.6.1.1; AAT) is a key enzyme involved in carbon and nitrogen metabolism. Overexpression of *OsAAT1*, *OsAAT2*, and *EcAAT* significantly increased amino acid and protein contents in seeds (Zhou et al., 2009b). Osppc4 is a plant-type phosphoenolpyruvate carboxylase (PEPC) and is targeted to the chloroplast. *Osppc4* RNAi plants showed stunting at the vegetative stage, which was much more obviously when grown with ammonium as the nitrogen source than with nitrate (Masumoto et al., 2010). Leaf metabolome analysis indicated that ammonium assimilation and subsequent amino acid synthesis were suppressed in *Osppc4* RNAi plants. *OsENOD93-1* is an N-responsive gene identified from whole-genome GeneChip analysis. Overexpression of *OsENOD93-1* significantly increased shoot dry mass and seed yield (Bi et al., 2009).

Phosphate (Pi) is absorbed via Pi transporters in plant roots. Manipulating the activity of Pi transporters may be a way to regulate the Pi status of the plant. *OsPht1;2* (*OsPT2*) is a low affinity Pi transporter and expressed preferentially in the root stele. Suppressing *OsPT2* expression decreased both the uptake and transport of Pi from roots to shoots, while overexpression of *OsPT2* leads to excessive

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amount of Pi in shoot (Ai et al., 2009). Genetic and molecular analysis identified that *OsPHR2* positively regulates *OsPT2* by physical interaction and upstream regulation of *OsPHO2* in roots. *OsSPX1* inhibits the function of *OsPHR2* by regulating *OsPT2* expression in roots (Liu et al., 2010).

4.2.5. Genes for grain quality

Rice grain quality is composed of a number of attributes: eating quality, cooking quality, appearance quality, milling quality, and nutritional quality (Zhang, 2007). The attributes may evolve with time to meet diverse needs of consumers and industry.

Cooking and eating quality are largely determined by amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) of the grain starch. *Wx* encodes granule-bound starch synthase I (GBSS I) required for the synthesis of amylose in endosperm (Wang et al., 1990, 1995). *ALK* controls the gelatinization temperature (GT) by encoding the soluble starch synthase II (SSSII) (Gao et al., 2003). *Du1* encodes a pre-mRNA processing (Prp1) protein regulating the splicing efficiency of *Wx* (Zeng et al., 2007). *GIF1* encodes a cell-wall invertase and is expressed in the ovular vascular trace, which regulates carbon partitioning during early grain-filling (Wang et al., 2008a). Using a candidate-gene association analysis approach, Tian et al. (2009) systematically studied the coordination of 18 starch synthesis-related genes (SSRGs) controlling rice eating and cooking quality, and constructed a regulatory network underlying these traits. Fu and Xue (2010) used coexpression strategy to identify genes controlling starch biosynthesis. Rice Starch Regulator1 (*RSR1*) encodes an APETALA2/ethylene-responsive element binding protein family transcription factor that is negatively correlated with type I starch synthesis genes. The amylose content was increased in *rsr1* mutants, while the expression of starch synthesis genes was suppressed in *RSR1* overexpressors. Moreover, *RSR1* regulates seed size and yield. In addition, genes controlling rice fragrance, pericarp color, storage time and nutritional quality were also isolated and characterized (Chen et al., 2008a, 2008b; Du et al., 2010b; Furukawa et al., 2007; Kawakatsu et al., 2010; Yu et al., 2008).

5. Genome-wide association studies (GWAS) in rice

Rice is rich in germplasm resources. In addition to the two cultivated rice species, there are also 20 wild species in the genus *Oryza* (Vaughan, 1994). The International Rice Genebank holds >110,000 accessions of the two cultivated rice species and their wild relatives (http://irri.org/our-science/genetic-diversity-conservation). And many major rice producing countries also have national germplasm programs and stored tens of thousands of accessions of cultivated rice and wild relatives (Rai, 1999; Ying, 1999). Theoretically genes conserved in these germplasm resources may be able to provide solutions to a diverse range of problems in rice genetic improvement, and will to a large extent determine the limit of breeding goals. However, it has been hard to make efficient use of the collections because of the difficulty in managing, maintaining and screening the entire collections to meet the needs of genetic analysis or breeding applications.

In recent years, there were efforts for constructing core collections in rice (e.g., Yan et al., 2007; Zhang et al., 2011), which by definition would capture the largest amount of genetic diversity with a minimum number of accessions (Brown, 1989). For example, using a selection scheme based on 36 SSR markers and 50 traits, Zhang et al. (2011) constructed a core collection of 932 accessions comprising 1.7% of the 55,908 accessions of the *O. sativa* germplasm in China, which retained more than 85% of variations by both SSR markers and phenotypic traits. Furthermore a mini-core collection, comprising 0.3% (189) of the accessions in the basic collection, retained 70.65% of the SSR variation and 76.97% of the phenotypic variation of the total collection. Such core and mini-core collections can greatly enhance the accessibility of the germplasm, allowing for intensive surveys of natural variation thus facilitating utilization of the variations.

With the development of new sequencing technology and availability of such core collections, it is now feasible to perform association analysis between agronomically important traits and genotype by resequencing using genetically highly diverse germplasm. Huang et al. (2010) performed a genome-wide association study (GWAS) using 517 landraces selected from ~50,000 rice accessions originating in China. With approximately one-fold sequence coverage per accession which allowed generation of haplotype map for each of the accessions, they performed GWAS on 14 agronomic traits. The resolution of the resulting maps was illustrated by localizing six previously cloned genes to regions less than 26 kb in length. This study demonstrated the power of genotyping by resequencing in GWAS, which provided tremendous potential for discovering genes controlling natural variations of complex traits. This may lead to a paradigm shift in gene discovery, especially if the phenotyping capacity can be enhanced.

6. Future directions of rice functional genomic research

Zhang et al. (2008) proposed a call for an international coordinated effort in rice functional genomics, which they referred to as RICE 2020. In this proposal, they suggested seven objectives to be accomplished: (1) Development of enabling tools and genetic resources for the international community of scientists to conduct functional genomics research in rice, including: insertion mutant collections, full length cDNA collections, and artificial micro-RNA (amiRNA) collections. (2) Assignment of biological functions to every annotated gene. They proposed to follow a systematic approach to achieving this goal by the year 2020, including systematic phenotyping and characterization of the mutants and systematic characterization of gene families. (3) Systems-wide epigenomes, gene expression profiles and regulatory networks. They also proposed three aims: comprehensive cell- or tissue-specific epigenomes and transcriptomes for selected developmental stages, abiotic and/or biotic conditions; identification of regulatory elements based on the epigenetic profiles and transcriptomes; and systematic characterization of regulatory hierarchy of genome expression, its relationship to epigenomes during development and responses to various environmental changes, and their effects on growth and development. (4) Global analyses of the proteome and protein–protein interactions. Two main aims were proposed for this objective: tissue specific proteomes of selected developmental stages and under selected defense and stress conditions; and experimentally defining comprehensive protein–protein interaction network. (5) Natural variation of *O. sativa* and its relatives. Two main aims were proposed for this objective: sequencing a core set of *O. sativa* strains and its AA-genome relatives; and developing a comprehensive platform for SNP association study to determine the relationship between phenotype and genotype and to identify functional diversity of agriculturally useful genes. (6) Bioinformatics, data management, and exchange and sharing of information. (7) Establishment of the toolkit for high throughput knowledge-based rice breeding.

In recent years, the international community has made good progress in a number of fronts as reviewed in this paper. New developments especially those associated with the new sequencing technology may greatly accelerate the processes, thus should also be incorporated into the perspective. Comprehensive genome comparison between different rice groups (e.g. *indica* vs *japonica*, cultivated vs wild) may provide an effective way for mining of agriculturally useful genes. Combinatory approaches of GWAS with genetic recombination populations like recombinant inbred lines and doubled haploid lines are now providing a means for efficient dissection of the genetic basis of complex traits and for precise mapping of the underpinning genes. More emphases should be paid to research on strategies making best use of information and resources not only in rice but also other organisms. Moreover, targets of studies should be gradually shifted from individual genes which is the

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case for most of the projects at present to agronomic traits and biological processes, and then to the whole biological systems. At the international scale, it is essential that the efforts are coordinated to avoid redundancy and unnecessary competition in some of the areas, while no efforts in other areas.

7. Implications for rice genetic improvement

Rice yield has undergone two big leaps in the past six decades. The first resulted from the widespread utilization of semidwarf varieties in most parts of the world, and the second was due to the exploitation of heterosis by developing hybrid rice in China and several Asian countries. However, the progress of yield increase has slowed down in the last decade. Zhang (2007) scrutinized the reasons for the yield plateau and found that some of the agricultural systems depend too heavily on high input of chemical fertilizers, pesticides and water use, and the economical return from the high input has diminished over time. Instead, over-applications of pesticides and fertilizers have caused heavy environmental problems and outbreak of diseases and insects, in addition to being highly costly to the farmers. Based on this analysis, Zhang (2007) proposed Green Super Rice as a new goal for rice breeding. On the premise of continued yield increase and quality improvement, Green Super Rice should possess resistances to multiple insects and diseases, high nutrient efficiency, and drought resistance, promising to greatly reduce the consumption of pesticides, chemical fertilizers, and water. In this paper, we reviewed the progress on the efforts on identifying germplasms and discovering genes for resistances to diseases and insects, N- and P-use efficiency, drought resistance, grain quality, and yield. We anticipate that more genes for these traits will be available for the development of Green Super Rice.

The ideal situation of varietal development is breeding according to designed blue prints to breed for cultivars to meet the diverse needs of global rice production for high yield, superior quality, multiple resistances and high nutrient-use efficiency. Such design should be composed of five different levels: (1) the population structure that can make maximum use of the solar energy in given ecological conditions; (2) the plant architecture to realize the population structure; (3) the traits to make up the plant architecture and to achieve high quality, resistances to multiple biotic and abiotic stresses, and high nutrient use efficiency; (4) the genes to produce the traits; and (5) the genomic technology to assemble the genes.

However, compared to other crops, especially corn, with major commercial breeding programs, breeding application of genomic technology has been rather limited in rice, despite more advances made in sequencing and functional genomic research in rice than in any other crops. At present, high throughput and low-cost technologies based on the massive sequence information should be developed for breeding applications, in the forms of multiple sets of oligo-nucleotide chips to meet the needs of rice breeding programs, such as *indica* vs. *japonica*, and inbreds vs. hybrids, in different countries and regions. With the tremendous efforts and rapid progress in functional genomic research, rice should be expected to be the first crop for practicing breeding by design.

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