Towards molecular breeding and improvement of rice in China

Yonghong Wang, Yongbiao Xue and Jiayang Li

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

China is the largest producer and consumer of rice in the world and a pioneer in applying hybrid rice technology. Although hybrid rice has contributed greatly to Chinese agriculture in the past decades, its potential to improve grain quality further is being questioned. However, to meet the challenges posed by severe crop damage by pests and diseases, the extensive use of pesticides and chemical fertilizers, and a shortage of water and energy, more elite rice cultivars are needed. In recent years, China has seen continued improvements in rice genetics, powered by functional genomics as a way forward to safeguard its rice production. Here, we briefly review the current status of rice breeding in China through strategies integrating hybrid rice technology, molecular marker-assisted breeding, functional genomics and genetically modified rice.

China meets challenges of rice production
Rice (Oryza sativa) is the most important staple crop, feeding more than half of the world's population. Among the main rice producers, which include India, Indonesia, Philippines and Japan, China is the largest producer and consumer of rice in the world and also a pioneer of hybrid rice technology, which has substantially increased China's grain output over the past 30 years. However, with an increasing population and a decrease in land availability, China's agriculture is suffering from severe damage by pests and diseases, extensive use of pesticides and chemical fertilizers, and water and energy shortage. The Chinese government has become concerned about tackling these problems and has actively promoted a sustainable agriculture policy, including the use of non-chemical fertilizers and the production of genetically modified (GM) foods. It is hoped that these challenges can be met by using advanced biotechnologies such as hybrid rice technology and genetic engineering, with the aim of isolating important genes and identifying their functions so that elite rice varieties with a high and stable yield potential, good grain quality and enhanced stress resistance could be bred in the near future. Over the past five years, the Ministry of Science and Technology of China has funded the China Rice Functional Genomics Program (CRFGP) to develop tools and resources for functional genomics and characterization of important genes for rice molecular breeding [1]. The government will continue to fund this program until 2010 to further dissect rice gene function of agronomic importance: scientists will attempt to obtain elite rice cultivars through so-called molecular breeding, which is expected to be an effective strategy to meet the challenge of the rapidly increasing food demand in China. This review will briefly highlight the current status and the future development of improved rice cultivars in China.

Hybrid rice in China
The phenomenon termed heterosis or hybrid vigor in first generation (F1) seeds, by crossing genetically distant breeding lines, is well known in crop breeding [2]. Rice is a strictly self-pollinating crop, the identification of several cytoplasmic male-sterile (CMS) wild rice germplasms in the 1970s, and their subsequent use, allowed China to make significant progress in developing hybrid rice technology based on heterosis. Thereafter, it was widely applied in China and other countries such as India, Indonesia, Myanmar, Philippines and Vietnam, making China a pioneer in hybrid rice technology. At present, more than half of the total rice-growing area in China is planted with hybrid rice cultivars, using both three-line and two-line systems [3].

In 1996, the Chinese Ministry of Agriculture initiated the Super Rice Project to increase rice grain production in China. The Super Rice Project aimed to produce elite rice varieties by combining an ideal plant architecture with heterosis by hybridizing indica and japonica subspecies to achieve ultimately a 'super' high yield [4]. The objective of the super rice project to obtain yields of 12 tonnes per ha over large areas was achieved in 2004; the new objective is to obtain yields of 13.5 tonnes per ha.

However, developing super hybrid rice depends largely on the germplasm resources of the parental lines and the conventional breeding technology. Improving rice varieties and breeding technology is cumbersome and time-consuming, which has led to the slow pace of progress of hybrid rice technology in recent years. Uncovering the molecular genetic control of rice heterosis would contribute to further improvements in hybrid rice technology and, therefore, the objective of obtaining yields of 13.5 tonnes per ha could be realized sooner. With this aim, scientists from the Beijing Genomics Institute and the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences together with the National Hybrid Rice Engineering Research Center have undertaken the Super Hybrid Rice Genome Project (SHGGP).
The aim of this project is to decode the genetic code of hybrid rice. Obtaining a draft genome sequence for 93-11, a cultivar of Oryza sativa ssp. indica and the paternal line of the super-hybrid rice Liang-You-Per-Jiu (LYP9), was an important part of this project [5]. Next, the SAGE (serial analysis of gene expression) technique was performed to dissect transcriptomes of three major tissues (panicle, leaves, roots) of LYP9 and its parental lines 9311 and PA64s (japonica) [6]. Compared with its parents, many up-regulated genes were identified as being involved in photosynthesis in LYP9, which strongly suggests that increased expression of photosynthesis-promoting genes in leaves might be a determinant of heterosis. By contrast, down-regulated genes in LYP9 indicate the importance of the role of the suppressed photosynthesis process in improving the yield of hybrid rice.

To understand the molecular mechanism of CMS in rice, Yaoguang Liu and his colleagues have shown that in BT-cytoplasm CMS rice, an abnormal mitochondrial open reading frame (orf79) that is co-transcribed with a duplicated copy of atp6 (B-atp6) encodes a cytotoxic peptide and confers gametophytic CMS (Yaoguang Liu, personal communication). Two fertility restorer genes, Rf1a and Rf1b, have been identified at the genetic locus Rf-1 as members of a multigene cluster encoding pentatricopeptide repeat (PPR) proteins. Restoration of male fertility occurs by silencing orf79 via Rf1a- or Rf1b-mediated endonucleolytic cleavage or degradation of the dicistronic B-atp6 and orf79 mRNA. These findings have provided an interesting mechanistic link between CMS and its restoration and could be used to improve the efficacy of rice hybrid technology.

Recently, progress in facilitating the production of rice hybrid seeds has been achieved. A recessive ‘tall rice’ mutant that has an elongated uppermost internode (eui) has been successfully applied to produce hybrid seeds by improving the heading performance of male sterile lines as a result of eliminating panicle enclosure [7,8]. The Eui gene was isolated recently and characterized as a new cytochrome P450 monoxygenase, CYP71A1D1, which is involved in catalyzing 16α, 17-epoxidation of non-13-hydroxylated gibberellins (GAs), leading to extremely high levels of GA4 and GA1 accumulating in the eui mutant plants (Zuhua He, personal communication).

Although progress has been made in developing the hybrid rice technology and cloning genes involved in rice heterosis, the molecular mechanism that underlies rice heterosis in hybrid rice production has not yet been elucidated. Nevertheless, these studies have provided valuable information, giving us insight into the genetic regulatory network of rice heterosis.

Molecular breeding is an effective strategy to achieve better rice cultivars

Conventional breeding has played an essential role in rice cultivar innovation over the past decades. However, progress is slow owing to several barriers, such as the time-consuming selection process and difficulties in appropriate genotype selection because of the quantitative nature of most agronomic traits, which has prompted breeders to apply molecular biotechnologies to rice breeding, which is known as molecular breeding.

Marker-assisted selection (MAS), quantitative trait locus (QTL) analysis and genetic transformation techniques are the most useful tools for rice molecular breeding, and have been used to identify new germplasms and elite rice cultivars. Chinese rice geneticists and breeders have made rapid progress in identifying QTLs with important agronomic traits such as grain yield and quality, growth and development, disease and pest resistance and abiotic tolerance [9–15].

MAS is a method that uses molecular markers closely linked to a target gene as a molecular tag that can be used for quick indirect selection of the target gene. In China, MAS is widely used to pyramid functional genes into popular hybrid rice cultivars to improve important agronomic traits of hybrid rice, such as resistance and grain quality [16–18].

Compared with MAS, genetic engineering of rice (transformation of rice plants with desired genes) is a time-saving, efficient and direct way to improve agronomic traits and thus is widely used to achieve rice cultivar improvement in China. Because the damage caused by pests and diseases has resulted in severe losses in rice grain yield in China, Chinese scientists have made great efforts to generate elite rice cultivars with enhanced resistance to pests and diseases through genetic modification. For example, Zhu et al. introduced a modified CpTI (Cowpea Trypsin Inhibitor) into Minghui 86, an elite rice cultivar used for three-line hybrid breeding in China. The transgenic plants showed a greatly increased level of resistance to the rice stem borer without the application of pesticide [19] (Figure 1). Also, Bt rice (which is engineered to express Bacillus thuringiensis toxins) has become an important component of integrated pest control methods. The transgenic CMS restorer line Minghui 63 and its derived hybrid plant expressing the Bt fusion protein derived from CryIA(b) and CryIA(c) exhibited strong protection against yellow stem borer and natural outbreaks of rice leaffolder without reduced grain production [20]. The Xa21 gene is useful for breeding bacterial blight-resistant rice varieties because of its wide-spectrum resistance to Xoo (Xanthomonas oryzae pathovar oryzae) blight. The Xa21 gene was therefore transferred into various rice cultivars mediated by Agrobacterium tumefaciens to develop new cultivars harboring bacterial blight resistance [21–24].

However, many valuable agronomic traits are usually controlled by multiple genes and transformation of a single gene is insufficient to improve a target trait. Conventional plant transformation vectors can transfer only a couple of genes in a transformation event, so Liu and colleagues [25] developed a multi-gene assembly vector system for transferring multiple genes at a time by Agrobacterium-mediated transformation. This system allows several genes to be integrated into a TAC (transformation-competent artificial chromosome)-based vector via a Cre/LoxP site-specific recombination system and homing endonucleases. This polygenic transformation system should contribute significantly towards generating pest and/or disease resistant rice cultivars and in...
the transfer of traits controlled by multiple genes in rice molecular breeding.

Rice functional genomics contributes to rice improvement

The identification and validation of candidate genes for molecular breeding depends on acquiring data for some, and preferably all, of the following gene features: expression pattern, chromosomal position, perceived biological function, and behavior of alleles under phenotypic selection. For this reason, China initiated the China Rice Functional Genomics Program (CRFGP) to develop tools and resources for functional genomics and to characterize essential genes for rice molecular breeding and genetic improvement.

Rapid progress in rice genome sequencing [5,26] has provided opportunities for the CRFGP to set up a technique platform for rice functional genomics research. To understand the expression profiles of rice genes on a genome scale, a 10K rice cDNA microarray has been developed, which has been instrumental in identifying 253 cDNAs that are regulated by pollination and fertilization [27]. Furthermore, a tiling path microarray has been developed based on PCR-generated genomic DNA fragments to analyze the transcriptional activity of rice chromosome 4 [28]. Using six representative rice organ types with this microarray, they successfully catalogued the transcribed regions and demonstrated organ- and developmental stage-specific transcription patterns of rice chromosome 4. Recently, an oligomer microarray has been constructed based on the presently known and predicted gene models in the rice genome, and 86% of the 41 754 known and predicted gene models were expressed [29]. Interestingly, most of these expressed gene models are organized into chromosomal regions of ~100 kb and exhibit a co-expression pattern.

To elucidate the function of rice genes on a whole genome scale, reverse genetics based on insertional mutagenesis using T-DNA tags has been performed in China over the past several years. To date, funded by the Chinese Ministry of Science and Technology, scientists from Beijing, Shanghai and Wuhan have generated 230 000 T-DNA insertion lines. Of these 230 000 lines, 24 500 have been used for phenotyping, revealing varied phenotypes related to important agronomic traits, such as plant height, tiller number, panicle morphology, fertility and tolerance [30] (Hongwei Xue and Chengcai Chu, personal communications). For example, a new T-DNA tagging vector pCAS04 was developed, which can be used for both promoter trapping and activation tagging of rice (O. sativa) (Figure 2). Using Agrobacterium-mediated transformation, >25 000 T-DNA insertional lines have been generated. About 20 000 lines of mutants were phenotyped in the T1 generation and some valuable mutants have been identified. Analysis of flanking sequences of T-DNA insertion sites showed that about half of the sequences have homology with sequences in rice genome databases (QiFa Zhang, Hongwei Xue and Chengcai Chu, personal communications). The Wuhan group is developing the Rice Mutant Database (RMD) under a joint national program, the National Special Key Program on Rice Functional Genomics of China. The database is now available on line (http://rmd.ncpgr.cn) and contains detailed information of ~129 000 rice T-DNA insertion lines generated by an enhancer trap system [30,31].

Chinese scientists have made great progress in characterizing rice genes that confer important traits by

---

**Figure 1.** Transgenic rice plants harboring a modified CpTI (Cowpea Trypsin Inhibitor) grown in a trial field in the Fujian province of China in 2002. (a) Before pest burst, no apparent difference could be observed between the transgenic plants and the non-transgenic control. (b) After pest burst, the transgenic plants (green) showed a high level of resistance to rice stem borer, whereas the non-transgenic control was seriously damaged (yellow). Photographs courtesy of Zhen Zhu (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences).

**Figure 2.** A T-DNA tagging vector developed to generate rice T-DNA insertion lines. The binary vector contains the promoterless β-glucuronidase (GUS) reporter gene, which is next to the right border (RB), and the rice actin1 promoter, which is next to the left border (LB).
using a map-based cloning strategy. In addition to the *MONOCULM1 (MOC1)* gene, which was first identified as a key regulator controlling rice tiller bud initiation and outgrowth [32], *BRITTLE CULM 1 (BC1)* has also been characterized through a map-based cloning approach. The *BC1* gene encodes a COBRA-like protein that functions in regulating the biosynthesis of secondary cell walls to provide the main mechanical strength for rice plants [33]. Because brittleness is one of the most important agronomic traits, affecting not only grain production but also the usefulness of cereal straws as animal forage, *BC1* should make a significant contribution to the future improvement of rice varieties.

Rice bacterial blight, the most destructive rice disease worldwide, is caused by *X. oryzae pv. oryzae*. As the first step towards improving resistance against rice bacterial blight, we need to understand the corresponding regulatory genes governing the signaling pathways involved in defending the plant against pathogen invasion. In addition to the cloned genes *Xa21* [34] and *Xa1* [35], *Xa26* has also been isolated and characterized recently through a map-based cloning approach [36]. In-depth studies have revealed that *Xa26* encodes a leucine-rich repeat (LRR) receptor kinase-like protein and is constitutively expressed. The rice germplasm carrying this gene is Minghui 63 (*O. sativa ssp. indica*), the restorer line of Shanyou 63, which has been the most widely cultivated hybrid in China for more than two decades. Therefore, this gene has contributed significantly to the success of hybrid rice production. Moreover, transgenic plants carrying *Xa26* show enhanced resistance compared with the donor line of the gene at both the vegetative and reproductive developmental stages [36].

Recently, several genes controlling abiotic stresses have also been characterized. A novel transcription factor, OePTF1, has been identified, which has a basic helix–loop–helix domain for tolerance to inorganic phosphate (P_i) starvation in rice [37]. Its overexpression increases tiller number, panicle weight and phosphorus content by >20% compared with wild-type plants at low-P_i levels, in both soil pot and field experiments, indicating the possibility of generating rice cultivars with a high P_i utilization efficiency. Furthermore, a QTL termed *SKC1* has been cloned using a map-based approach. *SKC1* encodes a member of the HKT-type transporters, is preferentially expressed in the parenchyma cells surrounding the xylem vessels, maintains K^+^ homeostasis in a salt-tolerant variety under salt stress and confers salt tolerance in rice. This suggests that *SKC1* has a role in regulating K^+^ and Na^+^ homeostasis under salt stress [38,39]. This finding indicates a potential tool that could be used to improve salt-tolerance in rice as well as in other crops.

**GM rice is important to China’s sustainable agriculture**

Increased urbanization, industrialization and environmental degradation are gradually decreasing farm land and giving rise to a polluted food supply. The Chinese government is seriously concerned about these problems and has actively promoted the use of non-chemical fertilizers as well as GM crops. As a result, GM biotechnology is playing an important role in China’s sustainable agriculture program. China’s bio-safety procedures require transgenic crops to pass through three phases of trials before being commercialized: field, environmental release and pre-production trials.

In China, scientific research on transgenic crops focuses on the control of important pests and diseases because of the excessive use of chemical fertilizers and pesticides. In terms of rice, stem borer, planthopper and bacterial blight are the most serious pests and bacterial disease, respectively. GM rice transformed with *Bt* and/or *CpTI* genes, which confer resistance to one or more lepidopteran pests of rice, and the disease resistance gene *X21*, which confers resistance to *Xoo*, are thus the most important recent developments. These three types of GM rice cultivars have passed field and environmental release trials and applications are now being made for the safety certification for commercialization under strict examination by the Transgenic Bio-safety Committee, directed by the Chinese Ministry of Agriculture.

Unlike GM cotton and maize, which are mainly used for industrial purposes and for animal feed, GM rice, if commercialized, would be the first GM commercial staple food crop in China and in the world. Public concerns about human health and the environmental impact of GM rice are therefore being taken into consideration. Recently, Huang *et al.* [19] assessed the productivity and health effects of two insect-resistant GM rice varieties that are now in farm-level production trials in China. Their study showed that small and poor farm households benefited from adopting GM rice because of the higher crop yields and reduced use of pesticides, which also helps to improve farmers’ health. Although no final decision has been reached as to whether GM rice can be commercialized, GM rice is widely believed to be an important approach to help China meet the challenges of developing a sustainable agriculture.

**Conclusions**

Hybrid rice has made a great contribution to safeguarding the food supply in China and is still a major source of elite rice cultivars. However, hybrid rice production is rather time-consuming and the limited available genetic resources leave little room for the continued improvement of rice. With the completion of the rice genome sequence, scientists are better equipped to unravel rice gene functions on a genome-wide scale, providing breeders with abundant genetic resources for continued generation of elite rice varieties to maintain a sustainable food supply in China. We expect that the successful implementation of a combinatorial approach using hybrid rice technology, functional genomics and GM rice will play a crucial role in our effort to improve rice cultivars in China. The immediate goal is to breed varieties with a further improved yield potential, enhanced stress resistance and good grain quality by using molecular and genomic information to break the rice yield plateau in the near future.

**Acknowledgements**

We thank our colleagues for allowing us to communicate their unpublished work. This work was supported by grants from the Ministry
of Science and Technology of China (2005CB120800), the Chinese Academy of Sciences and the National Science Foundation of China (30221002).

References
21 Zhao, B. et al. (2000) Introduction of wide spectrum rice bacterial blight resistance gene Xo2 into two-line genic male sterile rice variety Pei'ai 64S. Sheng Wu Gong Cheng Xue Bao 16, 137–141
32 Li, Y. et al. (2003) BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. Plant Cell 15, 2020–2031
37 Li, Y. et al. (2003) BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. Plant Cell 15, 2020–2031
38 Bao, J. et al. (2005) A draft sequence of the rice genome. Science 296, 1804–1806
42 Li, Y. et al. (2003) BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. Plant Cell 15, 2020–2031