Strategies for developing Green Super Rice

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From a global viewpoint, a number of challenges need to be met for sustainable rice production: (*i*) increasingly severe occurrence of insects and diseases and indiscriminate pesticide applications; (*ii*) high pressure for yield increase and overuse of fertilizers; (*iii*) water shortage and increasingly frequent occurrence of drought; and (*iv*) extensive cultivation in marginal lands. A combination of approaches based on the recent advances in genomic research has been formulated to address these challenges, with the long-term goal to develop rice cultivars referred to as Green Super Rice. 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. Large efforts have been focused on identifying germplasms and discovering genes for resistance to diseases and insects, N- and P-use efficiency, drought resistance, grain quality, and yield. The approaches adopted include screening of germplasm collections and mutant libraries, gene discovery and identification, microarray analysis of differentially regulated genes under stressed conditions, and functional test of candidate genes by transgenic analysis. Genes for almost all of the traits have now been isolated in a global perspective and are gradually incorporated into genetic backgrounds of elite cultivars by molecular marker-assisted selection or transformation. It is anticipated that such strategies and efforts would eventually lead to the development of Green Super Rice.

genomics | rice genetic improvement | sustainable agriculture

Rice is the main staple food for a large segment of the world population. In the last half-century, rice yield has undergone two big leaps, primarily as the result of genetic improvement: increasing harvest index by reducing plant height making use of the semidwarf gene and utilization of heterosis by producing hybrids. Consequently, rice yield has more than doubled in most parts of the world and even tripled in certain countries within a period of four decades from the 1960s to 1990s (http://faostat.fao.org/). However, rapid population growth and economic development have been posing a growing pressure for increased food production. To further increase the yield potential, several major national and international programs that were initiated in the last decade with the goals to develop "super rice" or "super hybrid rice" for breaking the yield ceiling have made significant progress (1, 2).

However, a number of challenges have to be met to achieve the goal of increasing rice production in a sustainable manner. The first challenge is the increasingly severe occurrence of insects and diseases in almost all of the rice-producing areas causing great yield loss (3). Three diseases, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, blast caused by *Pyricularia grisea*, and sheath blight caused by *Rhizoctonia solani*, are considered to be the most devastating diseases in most rice-growing regions. 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 (BPH), *Nilaparvata lugens*], have been the most damaging pests. For a long time, disease and insect control relied heavily on indiscriminate applications of chemical pesticides. Although such intensive use of chemicals creates serious environmental pollutions, causes hazards to the health of producers and consumers alike, and kills natural enemies resulting in pest outbreak, chemical control is not very effective, and heavy crop loss still frequently occurs in many rice-producing areas. For example, stemborers occur in ≈15 million hectacres annually in China (4). Although two to three sprays on average were applied per year in the affected fields with costs totaling approximately $650 million in the country, yield loss caused by residual insects was still estimated to be approximately $840 million, leading to a total cost of approximately $1.49 billion in China.

The second challenge is related to fertilizer application. There has been a dramatic increase worldwide in fertilizer applications in the last 40 years, and this has particularly been the case in China. Statistics show that in the year 2002 China used ≈30% of N- and P-fertilizers produced worldwide, although its arable land accounts only for ≈10% of the world total (http://faostat.fao.org/). Peng *et al.* (5) compared China with other major rice-producing countries for N fertilizer efficiency. Their analysis indicated that the N application per unit area for rice production in China is 75% higher than in other countries and that the N-use efficiency is much lower. Overfertilization has not only greatly reduced the economic return of the fertilizers applied and placed a heavy economic burden on the farmers, but it has also resulted in widespread water eutrophication (6). Moreover, overapplication of N fertilizer often reduces rice grain yield because plants grown under excess N conditions are more susceptible to lodging and pest damage. Overapplication of N fertilizer also partly accounts for the poor eating and cooking quality of the rice grains produced. Thus, developing crops that are less dependent on the heavy application of fertilizers is essential for the sustainability of agriculture.

The third major challenge stems from a global water shortage. Again, taking China as an example, it is estimated that the total water usage in this country is ≈557 billion cubic meters per year. Agriculture uses 392 billion cubic meters, accounting for 70.4% of the total water consumption of the country, of which ≈70% is used for rice production alone (7). However, drought stress is still identified as the single most important constraint in rice production in many rice-producing areas of China (8) for a number of reasons, including variation in the rainfall patterns from one year to another, uneven distribution of rainfall in the rice growing season, and inadequate rainfall in many areas.

As the fourth challenge, rice in many areas is cultivated in marginal lands where productivity is low because of a combination of constraints such as low soil fertility, drought, and other

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Abbreviations: GSR, Green Super Rice; *Bt*, *Bacillus thuringiensis*; MAS, marker-assisted selection; BPH, brown planthopper; NIL, near isogenic line; QTL, quantitative trait locus; DA, drought avoidance; DT, drought tolerance; AC, amylose content; GT, gelatinization temperature; GC, gel consistency.

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adverse environmental conditions. In addition, the input level in marginal lands is usually low.

There is also a tremendous need for improvement of rice grain quality. Historically, many breeding programs took yield potential as the primary target, particularly in China. Consequently, many popular high-yielding cultivars and hybrids have relatively poor quality. With the increased living standard, the improvement in cooking, eating, and appearance quality of the rice grain has become a priority. Additionally, more than half of the world's population, mostly the poor in developing countries, suffer from the devastating consequences of micronutrient malnutrition. For areas in those countries where rice is the major staple food, there is also a need for improving the nutritional quality of the rice grains to enhance the intake of micronutrients.

Thus, as the main staple, rice production has to meet increasing requirements, in both quantity and quality, and should be in harmony with the environment thus ensuring a proper level of sustainability. This requires a gradual reduction in application of pesticides, fertilizers, and water while still achieving continuous yield increase and quality improvement, a goal we referred to as Green Super Rice (GSR). Clearly, GSR should possess the following characteristics: adequate resistances to major diseases and insects, high efficiency in nutrient uptake and utilization, resistance to drought and other abiotic stresses, good quality, and increased yield potential. The objective of this article is to provide a perspective of the strategies, useful resources, and progress toward the development of GSR.

The Strategies for Developing GSR

Rice is rich in germplasm resources. The cultivated rice consists of two species, *Oryza sativa* L., referred to as Asian cultivated rice, and *Oryza glaberrima* Steud., referred to as African cultivated rice. There are also 20 wild species in the genus *Oryza* (9). The International Rice Genebank holds >105,000 types of Asian and African cultivated rice and ≈5,000 ecotypes of wild relatives. In addition, many major rice-producing countries have established national germplasm banks. Collectively, these germplasm collections contain genes that can be used to address a broad range of research objectives.

With the completion of the rice genome sequencing project, there have been rapid developments in functional genomic resources, including large mutant libraries by T-DNA insertion, transposon tagging, and chemical mutagenesis (10). Whole-genome microarray technique has been developed and applied to profiling expression of all of the genes in the entire life cycle of rice growth and development. Full-length cDNAs for both *indica* and *japonica* rice have been constructed, with a total of >40,000 full-length cDNA clones available (11, 12).

Such germplasm and genomic resources have provided an unprecedented opportunity for rice genetic improvement. For the development of GSR, a combination of strategies has been formulated by integrating germplasms, genomic resources, and molecular technology and breeding with insect and disease resistances, N- and P-nutrient efficiency, drought resistance, quality, and yield as the target traits (Fig. 1). The approaches used for identifying genes and germplasms for the defined traits include screening of germplasm collections, mapping and identification of genes, screening of mutant libraries, microarray analysis of differentially regulated genes, and functional test of candidate genes by transgenic analysis. The genes would then be incorporated into breeding lines either by transformation or molecular marker-assisted selection (MAS), and accumulation of the desired genes would result in progressive improvement of rice cultivars, eventually leading to GSR.

Progresses in Gene Identification and Development of GSR

Resistance to Stemborers and Leaffolders. In the last two decades, considerable research effort has been invested to introduce

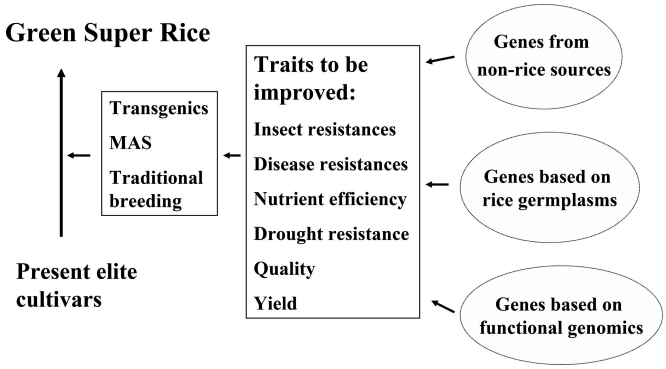

Fig. 1. Schematic representation of combinations of genes and approaches for the development of GSR.

insecticidal crystal protein genes from *Bacillus thuringiensis* (*Bt*) into crops including rice. Utilization of these crops has benefited the growers and the environment by greatly reducing the use of chemical insecticides (13). However, there is also an increasing concern that widespread adoption of *Bt* crops may lead to the development of resistance to the insecticidal genes in the pest populations (14–16). For resistance management, high-dose/refuge and gene stacking have been proposed as two effective strategies to prevent or delay the occurrence of pest resistance to *Bt* toxins (16–18). Nonetheless, the high-dose/refuge strategy does not seem to be applicable in most rice-producing countries because the majority of the rice growers are small-scale farmers, which makes it difficult to designate certain areas of nontransgenic crops for refuge. The gene-stacking strategy suggests that plants containing two or more dissimilar *Bt* toxins have the potential to delay resistance more effectively than those producing only a single toxin, because the insects have to develop resistance to two or more insecticides to survive. A requirement for this strategy to work is that the stacked toxins have different modes of action.

Although a large number of *Bt* toxins are known, only a small fraction of these are currently used in developing transgenic crops to control the Lepidopteran pests. The most commonly used *Bt* genes in transgenic crops including rice are *Cry1Ab*, *Cry1Ac*, and a fusion gene of *Cry1Ac*/*Cry1Ab* or *Cry1Ab*/*c* (19–28). However, binding tests of midgut brush border membrane vesicles showed that *Cry1Aa*, *Cry1Ab*, and *Cry1Ac* toxins share a common binding site (29–31); thus, a mutant that is able to overcome one of the *Cry1A* genes is also likely to be resistant to other *Cry1A* genes as well. Therefore, combinations of *Cry1A* genes with other groups of *Bt* genes should be explored to prevent or delay the emergence of pest resistance. Based on the assay of δ-endotoxin binding to brush border membrane vesicles of rice stemborers, it was proposed that *Cry1A* genes could be combined with *Cry1C*, *Cry2A*, or *Cry9C* for more durable resistance in transgenic plants (32).

A *Cry1Ab*/*c* fusion gene was transformed to Minghui 63 (Fig. 2 *A* and *B*), the restorer line for a number of elite rice hybrids widely cultivated in China (28). Results from large-scale field tests in several provinces of China showed that this line and its hybrid with Zhenshan 97A, the male sterile line of the most popular hybrid Shanyou 63, were highly resistant to leaffolders and stemborers under field conditions, thus promising to significantly reduce insecticides, labor, and related costs while at the same time increase the yield and benefit the health of the farmers and consumers (33). This line and the hybrid have now completed the production demonstration stage according to the regulatory procedures of the Chinese Government.

To address the issue of resistance management, transgenic rice

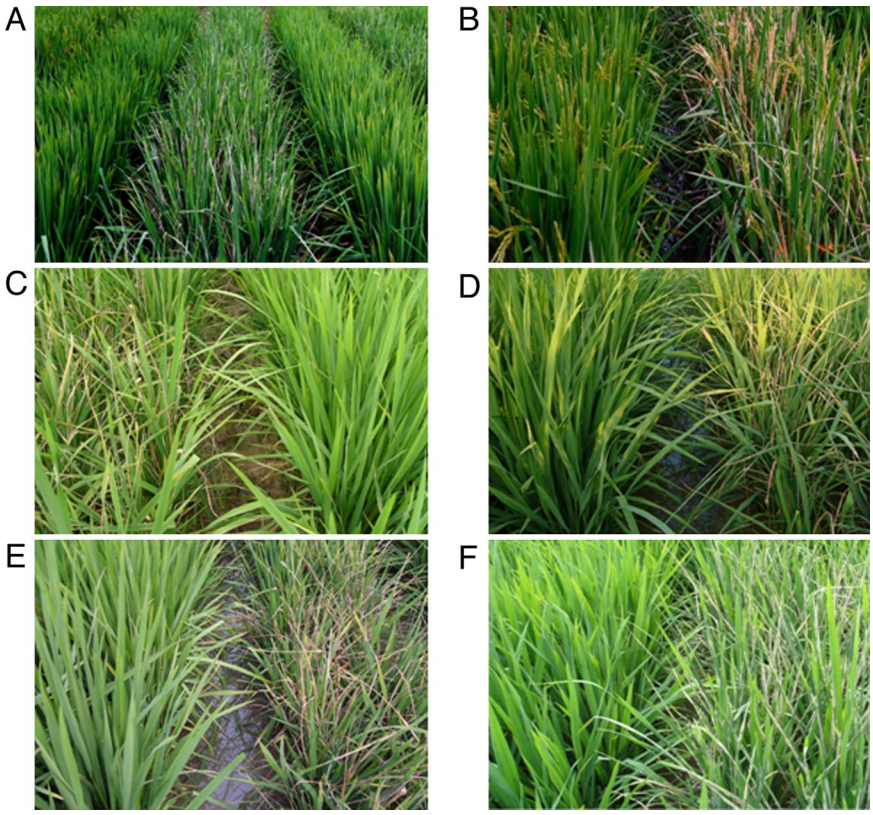

Fig. 2. Pest resistance of Minghui 63 individually harboring five different *Bt* genes. (*A*) Minghui 63 carrying Cry1Ab/c against natural infestation of leaffolders (28). The middle row is the control, and flanking rows are transgenic. (*B*) Minghui 63 carrying Cry1Ab/c against heavy artificial infestation of yellow stemborer (28). The left row is transgenic, and the right is the control. (*C*) Minghui 63 carrying Cry1Ab against natural infestation of leaffolders and stemborers (Y. Lin and Q.Z., unpublished data). The left row is the control, and the right is transgenic. (*D*) Minghui 63 carrying Cry1Ac against natural infestation of leaffolders and stemborers (Y. Lin and Q.Z., unpublished data). The left row is transgenic, and the right is the control. (*E*) Minghui 63 carrying Cry1C against natural infestation of leaffolders. The left row is transgenic, and the right is the control. (*F*) Minghui 63 carrying Cry2A against natural infestation of leaffolders. The left row is transgenic, and the right is the control.

lines were developed by using Minghui 63 as the recipient to individually harbor the codon-optimized *Cry1C* and *Cry2A* genes (34, 35). Transgenic plants with single-copy transgene were crossed with Zhenshan 97A. Field tests showed that both of the transgenic lines and their hybrids with Zhenshan 97A exhibited strong resistance to natural infestation of leaffolders and stemborers. Together with the lines individually harboring *Cry1Ab/c*, *Cry1Ab*, and *Cry1Ac* in the Minghui 63 background (Fig. 2 *C–F*), the transgenic lines with the new *Bt* genes have provided the critical materials and flexibility for developing rice lines with various combinations of multiple resistances by gene stacking.

Resistance to BPH. BPH often occurs as the most destructive pest of rice in many countries. A total of 19 BPH-resistant genes have been identified from cultivated and wild rice species (Table 1). Molecular mapping of these genes has facilitated MAS of the BPH-resistant genes.

Sharma *et al.* (56) performed a molecular marker-assisted pyramiding of two BPH-resistant genes, *Bph1* and *Bph2*, into a *japonica* line. BPH bioassay showed that the resistance level of the pyramided line was equivalent to that of the *Bph1*-single introgression line, which showed a higher level of resistance than the *Bph2*-single introgression line.

Making use of a molecular marker linkage map, Huang *et al.* (50) identified two genes, *Qbh1* and *Qbh2* (renamed *Bph14* and *Bph15*), for BPH resistance from B5, a highly resistant line that derived its resistant genes from the wild rice *Oryza officinalis*. Both of the genes had large effects on BPH resistance, and the two loci acted essentially independent of each other in conferring the resistance. These two genes were subsequently incorporated by MAS into a number of important parental lines in hybrid rice breeding programs in China. Lines containing either or both of the genes showed enhanced resistance to BPH with artificial infestation (Y. Q. He and Q.Z., unpublished data).

Although the large number of genes identified so far has provided a rich source for developing BPH-resistant cultivars, knowledge about the biotypes of the insects in various rice-producing areas is still lacking. Thus, detailed characterization of the resistant genes against the insect populations in the fields is essential for efficient deployment of these genes.

Identification of Genes for Disease Resistance and Development of Disease-Resistant Rice. Dozens of genes have been identified for resistance to bacterial blight and fungal blast (www.gramene.org), of which 13 have been cloned by various groups, including six genes for bacterial blight resistance and seven for blast resistance (Table 2). In addition, a number of genes with strong resistance have been fine-mapped. This development has provided markers either based on DNA sequences of the genes (frequently referred to as functional markers) or closely linked to the genes for pyramiding by MAS.

For targeted improvement of an elite rice hybrid, MAS was conducted to introgress *Xa21* into Minghui 63 (70). Field examination of a number of agronomic traits showed that the improved versions of Minghui 63(*Xa21*) and Shanyou 63(*Xa21*) were identical to Minghui 63 and Shanyou 63 when there was no

Table 1. BPH-resistant genes reported in the literature

Germplasm	Gene	Chromosome	Reaction to biotype 1	2	3	4	Ref(s).
Mudgo	*Bph1*	12	R	S	R	S	36–38
ASD7	*bph2*	12	R	R	S	S	36, 39
Rathu Heenati	*Bph3*	6	R	R	R	R	40
Babawee	*bph4*	6	R	R	R	R	41, 42
ARC 10550	*bph5*		S	S	S	R	43
Swarnalata	*Bph6*		S	S	S	R	43
T12	*bph7*		S	S	S	R	43
Chin Saba	*bph8*		R	R	R	—	44
Balamawee	*Bph9*	12	R	R	R	—	44, 45
Oryza australiensis	*Bph10*	12	R	R	R	—	46
O. officinalis	*Bph11*	9	—	—	—	—	47
O. officinalis	*bph12*	3	—	—	—	—	48
Oryza eichinger	*Bph13*	2	R	R	—	—	49
B5	*Bph14*	3	R	R	—	—	50
B5	*Bph15*	4	R	R	—	—	50
O. officinalis	*bph16*	4	—	—	—	—	51
B14	*Bph17*	4	R	R	—	—	52
O. officinalis	*Bph17*	3	—	—	—	R	53
O. australiensis	*Bph18*	12	BPH pop on Taebaekbyeo				54
AS20-1	*bph19*	3	—	R	—	—	55

disease pressure. Under heavy disease pressure, Minghui 63(*Xa21*) showed significantly higher grain weight and spikelet fertility than Minghui 63, and Shanyou 63(*Xa21*) was significantly higher than Shanyou 63 in number of grains per panicle, grain weight, and yield. The improved hybrid has been used in commercial rice production in China. MAS has also been successfully applied for pyramiding four genes for bacterial blight resistance (*Xa4*, *xa5*, *xa13*, and *Xa21*) in various combinations (71). The resulting lines showed enhanced resistance in both resistance spectra and disease level, demonstrating the usefulness of marker-assisted pyramiding for developing cultivars with multiple resistance.

It is important to understand the pathogenicity spectrum of the pathogen populations to have targeted development in breeding for disease resistance. Chen *et al.* (72) assayed pathotypes of 792 single-spore isolates of *P. grisea* using samples collected from 13 major rice-growing provinces of central and southern China. These isolates were tested by inoculation with 13 host differentials consisting of six *indica* and seven *japonica* near isogenic lines (NILs), collectively carrying most of the genes for blast resistance identified previously. The results showed a large difference in the frequencies of the isolates producing compatible reactions on the NILs. For example, very small proportions (10% or less) of the isolates could cause compatible reactions on NILs carrying *Pi1* or *Pi2*, but a large proportion (41.5%) of the isolates could overcome the resistance of the NIL carrying *Pi3*. Moreover, a combination of *Pi1* and *Pi2* would be susceptible to only 2.0% of the isolates, and adding *Pi4* would make this proportion even smaller. The data provided very useful information for formulating strategies for improving blast resistance in rice breeding programs, which have now been implemented.

Table 2. Disease resistance genes isolated from rice

Gene	Encoding product	Ref(s).
Xa1	NBS-LRR	57
Xa3/*Xa26**	LRR-TM-kinase	58, 59
xa5	γ-Subunit of transcription factor IIA	60
xa13	Unknown plasma membrane protein	61
Xa21	LRR-TM-kinase	62
Xa27	Unknown protein	63
Pi2	NBS-LRR	64
Pi9	NBS-LRR	65
Pi36	NBS-LRR	66
Pib	NBS-LRR	67
Pita	NBS-LRR	68
Pid2	B-lectin-TM-kinase	69
Piz-t	NBS-LRR	64

LRR, leucine-rich repeat; TM, transmembrane region; NBS, nucleotide-binding site.
**Xa3* and *Xa26* are the same gene.

Although MAS is effective for pyramiding disease resistance genes, a transgenic approach may still have a role in using the cloned genes. For example, *Xa26* was isolated from the *indica* cultivar Minghui 63 (58), where it confers moderate resistance against bacterial blight at seedling and adult stages. When this gene was introduced into several *japonica* cultivars with its native promoter, transgenic plants showed enhanced resistance, featuring broadened resistance spectrum and increased resistance level in extended growth stages (73). It was shown that the enhanced resistance is closely associated with the elevated expression of the gene. Overexpressing *Xa26* with a constitutive strong promoter further enhanced the resistance in both *indica* and *japonica* backgrounds, whereas regulating the gene expression by a pathogen-inducible weak promoter impaired the resistance. These results suggest that optimizing expression level of the resistant genes by a transgenic approach could be combined with MAS for developing resistant cultivars.

With the increasing availability of cloned disease-resistant genes, new strategies are now feasible for developing cultivars with multiple resistances. For example, cloning of multiple genes with different resistance spectra into a single construct to transform rice may be used for developing cultivars with broad spectrum and durable resistance. Compared with pyramiding of multiple genes using MAS, transformation of a single construct containing multiple genes not only takes less effort to develop, it also has the advantage of being transferred and transmitted as a single unit to different genetic backgrounds and from one generation to another, given that the efficacy of the genes can be individually tested. This may be expanded to include in the same

cassette genes for insect resistance as well as those for other traits, in addition to the disease-resistant genes. If properly optimized, this strategy may hold great promise for efficient development of cultivars with multiple resistances.

The difficulty now is the improvement of sheath blight resistance, a major disease for a number of cereals in addition to rice. So far, no major gene for sheath blight resistance has been identified, although a few quantitative trait loci (QTL) have been reported (74–76). Thus, intensive efforts based on advances in the genomic researches in both the host and pathogen systems are needed to develop innovative technology to combat this disease.

Identification of Genes for Nutrient-Use Efficiency. The reduction of fertilizer application in fertile soils and increased productivity in poor soils both require improvement of nutrient-use efficiency, including both uptake and utilization efficiency. Unlike the traits described above, not many genes are currently available to be directly useful for developing nutrient-efficient cultivars. Thus, most of the work is still at the stage of discovering genes and QTL for nutrient efficiency.

Identifying genes for N-use efficiency. N uptake and assimilation pathways in higher plants have been well documented. They involve a variety of transporters functioning to absorb the nutrients from the soil (77–81) and a number of enzymes for assimilation and transfer of the absorbed N into amino acids and other compounds (82–84). However, little is known regarding how these elements and the processes are regulated, especially under low-N conditions.

Using a high-density linkage map, Lian *et al.* (85) analyzed the genetic components associated with low-N tolerance in rice at the seedling stage using a population of 239 recombinant inbred lines from a cross between Zhenshan 97 and Minghui 63. Seedlings were cultivated in both low- and normal-N solutions. Root, shoot, and plant weight in the two N treatments were measured, and the relative weight of the two treatments for each trait was considered as measurements for low-N tolerance. Four to eight QTL with main effects were detected for each of the nine traits. Very few QTL were detected in both low- and normal-N conditions, and most QTL for the relative measurements were different from those for traits under the two N treatments, indicating very little commonality in the genetic basis of the traits and their relative performance under low- and normal-N conditions. Although additional studies are necessary for fully understanding the biological mechanisms, these QTL may provide the starting point for identifying and exploiting the genes for improving low-N tolerance in rice breeding programs.

To characterize the genes and processes involved in early response to low-N stress, Lian *et al.* (86) analyzed the expression profiles of Minghui 63 at the seedling stage at 20 min, 1 h, and 2 h after low-N stress with the normal N as the control using a microarray of 11,494 rice expressed sequence tags representing 10,422 unique genes. Although no significant difference was detected in the leaf tissue, a total of 471 expressed sequence tags were detected as responsive to low-N stress in the root tissue with 115 showing up-regulation and 358 showing down-regulation. The analysis of expression profiles after low-N stress identified the following patterns: (*i*) the genes involved in photosynthesis and energy metabolism were down-regulated rapidly; (*ii*) many of the genes involved in early responses to biotic and abiotic stresses were up-regulated while many other stress-responsive genes were down-regulated; (*iii*) regulatory genes including transcription factors and those involved in signal transduction were both up- and down-regulated; and (*iv*) the genes known to be involved in N uptake and assimilation showed little response to the low-N stress. These results provided a set of candidate genes for functional analysis by transformation to assess their usefulness in improving N efficiency of rice.

Although combining genetic analysis with gene expression profiles can provide an effective strategy for identifying genes and pathways involved in specific physiological processes, it is obvious that more efforts are needed to identify genes to be practically useful for improving N-use efficiency in rice breeding programs.

Identifying genes for P-use efficiency. The overwhelming majority of soils in the rice-producing areas are P-deficient with a high P-fixing capacity (87). Most of the arable soils are either acidic (tropics and subtropics) or calcareous (temperate regions). In acidic soils free iron and aluminum oxides bind native and applied P into forms unavailable to plants, whereas in calcareous soils the abundant calcium and magnesium compounds bind inorganic phosphates into forms highly unavailable to plants. The high P-fixing capacity in both types of soils results in very low P-availability and thus low rates of uptake by the plants. Moreover, it is highly alarming that the global P resources will be exhausted before the end of this century (88).

Thus, improving the uptake efficiency of the rice plant in P-fixing soils has been a major research target. From a cDNA library constructed by the suppression subtractive hybridization method, Yi *et al.* (89) identified *OsPTF1*, a P-deficiency responsive transcription factor containing a basic helix–loop–helix domain. The gene was cloned from Kasalath, a P-efficient *indica* landrace. Transgenic plants of the low-P sensitive rice variety Nipponbare overexpressing *OsPTF1* showed enhanced P efficiency in both solution and soil cultures. Tillering ability, root and shoot biomass, and phosphorus content of the transgenic plants were ≈30% higher than the wild-type plants in P-deficient culture solution. In soil pot and field experiments at low-P levels, tiller number, panicle weight, and phosphorus content increased >20% in transgenic plants compared with wild-type plants.

Wissuwa and Ae (90) analyzed P uptake of 30 rice varieties representing a wide diversity of the cultivated rice germplasm on normal and P-deficient soils. The analysis revealed very wide variation among the genotypes in low-P tolerance, as measured by P uptake on P-deficient soil relative to that on normal soil, indicating a tremendous potential of using natural variation for improving P efficiency of rice cultivars. They (91) further developed NILs for two QTL, a major one on chromosome 12 and a minor one on chromosome 6, by introgressing the alleles from Kasalath, a P-efficient variety, to Nipponbare, a P-inefficient variety. P uptake of the NIL carrying the Kasalath allele of the QTL on chromosome 12 on a P-deficient upland soil was three to four times that of Nipponbare, whereas the advantage of NIL carrying the Kasalath allele of the QTL on chromosome 6 was in the range of 60–90%.

These genes hold promise for improving P uptake efficiency of the rice crop, although further study is needed to evaluate their effectiveness in the genetic backgrounds of elite cultivars under diverse field conditions.

Identification of Genes for Drought Resistance and Development of Drought-Resistant Rice. The mechanisms of drought resistance include drought escape via a short life cycle or developmental plasticity, drought avoidance (DA) via enhanced water uptake and reduced water loss, drought tolerance (DT) via osmotic adjustment, antioxidant capacity, and desiccation tolerance. It is thus important to understand the genetic basis of the individual components, especially DA and DT, to formulate strategies for developing drought-resistant cultivars. Yue *et al.* (92) analyzed the genetic bases of DT and DA at reproductive stage in rice using a recombinant inbred line population from a cross between Zhenshan 97 (irrigated rice) and a drought-resistant upland cultivar, IRAT109. The plants were grown individually in polyvinyl chloride pipes, and two cycles of drought stress were applied at the reproductive stage, with unstressed plants as the control. A total of 21 traits measuring fitness, yield, and the root

system were investigated. Negligible correlation was detected between relative yield traits with potential yield, plant size, and root traits, suggesting that DT and DA were well separated in this experiment. A total of 27 QTL were resolved for seven traits of relative performance of fitness and yield, 36 QTL for five root traits under control, and 38 for seven root traits under drought stress conditions, suggesting the complexity of the genetic basis of both DT and DA. Only a small portion of QTL for fitness and yield-related traits overlapped with QTL for root traits, indicating that DT and DA had distinct genetic bases.

MAS has been applied to pyramid QTL for several root traits (93, 94), which resulted in positive effects for increasing root length and root mass. However, the effects of the change in root traits on drought resistance at the field level remained to be determined.

Large efforts were also made to identify genes for the development of drought-resistant rice. Trehalose is a nonreducing disaccharide of glucose that functions as a compatible solute in the stabilization of biological structures under abiotic stress in bacteria, fungi, and invertebrates. Garg *et al.* (95) overexpressed a fusion gene made of two *Escherichia coli* trehalose biosynthetic genes (*otsA* and *otsB*) in rice. Depending on growth conditions, the transgenic rice plants accumulate trehalose at levels 3–10 times higher than that of the nontransgenic controls. Compared with nontransgenic rice, several independent transgenic lines exhibited sustained plant growth, less photooxidative damage, and more favorable mineral balance under salt, drought, and low-temperature stress conditions.

In another study, Hu *et al.* (96) identified the transcription factor gene *SNAC1* as showing elevated expression by drought stress, based on data from a cDNA chip analysis. Overexpression of *SNAC1* in rice significantly enhanced drought resistance in transgenic rice (22–34% higher seed setting than the control) in the field under severe drought stress conditions at the reproductive stage while showing no phenotypic changes or yield penalty. The transgenic rice also showed significantly improved drought resistance and salt tolerance at the vegetative stage. Compared with the wild type, the transgenic rice was more sensitive to abscisic acid and lost water more slowly by closing more stomatal pores yet displayed no significant difference in the rate of photosynthesis.

The challenge ahead is to incorporate these genes into the genetic backgrounds of elite cultivars and hybrids and to evaluate their performance under real agricultural field conditions. Gene discovery and innovative strategies based on germplasm screening and functional genomic research are also needed for developing drought-resistant rice cultivars.

Identification of Genes for Quality Improvement. Grain quality of rice consists of several components: cooking quality, eating quality, appearance quality, milling quality, and nutritional quality. Cooking, eating, and appearance qualities of the rice grain represent a major problem of rice production in many rice-producing areas of the world. Currently, there is a strong emphasis in China on improving eating, cooking, and appearance qualities of hybrid rice, especially the quality of *indica* hybrids.

Cooking and eating qualities are mostly determined by amylose content (AC), gelatinization temperature (GT), and gel consistency (GC) of the grain starch. Appearance quality is mainly specified by grain shape as defined by grain length, grain width, the length–width ratio, and the translucency or chalkiness of the endosperm.

Molecular marker-based genetic analysis in the last decade established that each of the quality traits is mainly conditioned by a major locus (Table 3). For example, the *Wx* locus on chromosome 6 plays major roles in specifying AC and GC plus a minor role in GT (97, 98), and the *Alk* locus, tightly linked to *Wx*, has a major effect on GT (98, 99). For appearance quality traits, grain length is mostly controlled by the *GS3* locus on chromosome 3, and grain width is largely conditioned by *GS5* on chromosome 5 (100). A major locus for chalkiness (*Chk5*) was also identified on chromosome 5 (100). Several genes for these traits have been cloned (101–104).

Table 3. Traits and gene loci for cooking, eating, and appearance quality of the rice grain

Quality	Trait	Gene	Chromosome	Ref(s).
Cooking and eating quality	AC	*Wx*	6	97, 98
	GC	*Wx*	6	97, 98
	GT	*Alk*	6	98, 99
Appearance quality	Grain length	*GS3*	3	100
	Grain width	*GS5*	5	100
	Chalkiness	*Chlk5*	5	100

The single-locus inheritance clearly indicated that MAS can play a major role in quality improvement. Indeed, Zhou *et al.* (105) were able to simultaneously improve the quality of Zhenshan 97, the female parent of a number of widely used hybrids in China with poor quality because of a high AC, low GC, and a low GT, together with a chalky endosperm. MAS was applied to introgress the *Wx* gene region from Minghui 63 that has medium AC, soft GC, and high GT to Zhenshan 97, then from Zhenshan 97B to Zhenshan 97A. The selected lines and their hybrids with Minghui 63, or Shanyou 63(wx-MH), showed a reduced AC and an increased GC and GT, coupled with a reduced grain chalkiness, representing a significant improvement in cooking, eating, and appearance quality.

A transgenic approach has been successfully applied to enhance micronutrients of rice cultivars. The most successful example is perhaps the development of golden rice with engineered pathway for provitamin A biosynthesis (106, 107). Currently, there is a coordinated international initiative for biofortification of the rice grain for provitamin A, iron, and zinc (www.harvestplus.org/pdfs/rice.pdf). Such effort will provide rice cultivars to improve the nutritional status of people in the target areas.

Identification of Genes for Yield Traits. In rice, grain yield is multiplicatively determined by three component traits: number of panicles per unit surface, number of grains per panicle, and grain weight. Hundreds of QTL for yield and yield component traits have been identified during the last decade thanks to high-density molecular marker linkage maps (www.gramene.org). For a long time, yield has been generally regarded as a complex trait that is controlled by multiple genes of small effects. However, development of QTL-based NILs revealed that many QTL have major effects in homogeneous genetic backgrounds, which has enabled QTL cloning following the map-based cloning approach (108). Currently, several QTL for yield components have been cloned, including ones for number of tillers per plant (109), number of grains per panicle (110), and grain size (103, 104).

The major effects observed between the NILs and the cloning of QTL have fundamental implications for yield improvement, suggesting that yield, like other traits, can also be improved by individually manipulating the component traits using both MAS and transformation. A limiting factor may be the biological productivity of the rice plants to provide sufficient carbohydrates to achieve the grain yield set by the component traits of yield so that the gain in one component trait is not compensated by loss in the other traits. Progress in expressing C_4 photosynthetic enzymes in rice to increase the photosynthetic rate was reported in recent years (111), which may be a promising line of research.

Genetic variation in photosynthesis-related traits including photosynthetic rate, canopy development, plant architecture, and ability to maintain green leaves in the rice germplasm may also be explored.

Prospects

Development of GSR, with improved insect and disease resistances, N- and P-use efficiency, drought resistance, high grain yield, and superior quality, is critical for a more sustainable rice production. In this article we summarized the progress in identification of genes for the target traits in rice and outlined the strategies for the development of GSR. In a global perspective, genes for most of the traits have now been isolated and become available for breeding applications, although further evaluations of genes for some of the traits are still necessary, especially those for N-use efficiency and drought resistance. Moreover, molecular marker-based germplasm evaluation and genetic studies have identified a large variety of genes and tightly linked markers for the target traits. With rapid advances in the international efforts on rice functional genomic studies, hundreds of genes for various traits will be isolated in the next few years. Combinations of high-throughput genomic tools and phenotyping platforms are also becoming available or are under development. The availability of the genes and the genomic tools would greatly facilitate rice cultivar improvement. Such development will eventually lead to crop breeding according to designed blueprints, an ideal situation that may be particularly feasible in rice.

The goals of GSR can be achieved as a two-stage process. In the first stage, which has been partly realized, elite lines carrying single genes of interest are developed and thoroughly evaluated, which by themselves are useful for varietal release. In the second stage, the genes introduced into these lines will be combined in various ways to develop cultivars with the desired traits for GSR. Utilization of these cultivars will result in increased rice productivity with much reduced inputs to ensure a greater sustainability of rice production and agriculture in general.

A big challenge is the assembling process to combine all of the favorable alleles into a single cultivar and ensure their proper functioning. In this regard, it may be more advantageous to breed for hybrids than conventional pure line cultivars, because it may take less effort to have two complementary sets of genes in two parental lines than stacking all of the genes in a single genetic background.

It should also be noted that new challenge will emerge with the global climate change. Indeed, Peng *et al.* (112) reported that the increase in temperature, especially the night temperature, has a negative effect on rice grain yield. Global warming is expected to have a profound impact on the climate such that drought and other natural disasters would occur more frequently. Presently, not much research has been conducted to address this issue; a large effort will be required to understand the possible impact of the climate change on rice genetic improvement, which will then be translated as new target traits for GSR.

A final comment is that both field management and improved cultural practices are also extremely important for tailoring GSR. Integration of pest, nutrient, and water managements in accordance to the biological characteristics of the cultivars is no smaller challenge than genetically improving the cultivars *per se*, which requires large and collaborative efforts of scientists working in different disciplines.

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