II.1 Rice

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1 Introduction

Rice (*Oryza sativa* L.) is one of the most important agricultural staples that feed more than half of the world's population. The demand for rice production is likely to increase in the coming decades, especially in the major rice-consuming countries of Asia, Africa and Latin America, due to the population explosion and cropland reduction. The ideal traits for future rice varieties include high and stable production yields, good grain quality and enhanced resistance to biotic and abiotic stresses. Consequently, it is necessary to renovate current rice biotechnologies, such as hybrid rice development techniques and genetic manipulation of important agricultural traits. This chapter briefly introduces the ideas and principles of rice biotechnology and its applications in developing elite rice varieties.

2 Hybrid Rice

The application of heterosis (hybrid vigor) in the first generation (F_1) progeny has improved crop breeding universally for many decades. Davenport (1908) and East (1908, 1936) hypothesized dominance and overdominance theories in the early twentieth century to grasp the genetic basis of heterosis. However, recent studies suggest that heterosis may result from partial to complete dominance, overdominance, epistasis, or a combination of all of these effects (Comstock and Robinson 1952; Yu et al. 1997; Hua et al. 2003). Although agricultural practitioners have exploited the genetic and molecular basis to improve rice crop yields, the mechanism that underlies heterosis remains poorly understood.

Because rice is a strictly self-pollinating crop, it is not amenable to hybrid varietal production. In fact, hybrid rice emerged only 30 years ago when Chinese scientists successfully identified and transferred a cytoplasmic male sterile (CMS) wild rice trait to cultivated rice. Since then, China has pioneered multiple hybrid rice technological developments and applications. The land on which hybrid rice grows exceeds more than 50% of the total rice-growing area

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in China (Yuan 2004). Rice hybrid technology is now widely applied in most rice-producing countries, such as The Philippines, India, Indonesia, Myanmar and Vietnam.

Hybrid rice emerged in agriculture in 1973 with a three-line system. The three-line system comprises CMS, maintainer and restorer lines. Crossing CMS and fertility restorer lines produces hybrid rice seeds. Cytoplasmic male sterile lines persist by intercrossing with maintainer lines. Both maintainer and restorer lines are preserved through self-pollination. Scientists use heterosis to identify, study and apply various CMS and restorer lines to develop elite cultivars. To date, three main CMS types (wild abortion, WA; Honglian, HL; Baotai, BT) and five fertility-restoring genes (*Rf1* through *Rf5*) have been reported and the *Rf1* gene was cloned recently (Shinjyo 1984; Yao et al. 1997; Zhang et al. 1997, 2002; Huang et al. 2000; Wang et al. 2006). These are expected to facilitate future hybrid rice technological development.

Although the three-line hybrid system has gained wide recognition for increasing rice yields, hybrid seed production remains economically costly and technologically complicated. To overcome these obstacles, a two-line system was developed in 1995 and is currently being utilized. In the two-line system, the CMS line reverts under certain conditions and functions as a maintainer line. Conditions that affect sterility of CMS lines include temperature and/or photoperiod. The photoperiod-sensitive and temperature-sensitive genic male sterile (PGMS and TGMS, respectively) lines developed under long-day or high-temperature conditions, respectively, are sterile and suitable for making hybrids. Researchers can maintain these lines under short-day or low-temperature conditions by self-pollination. In China, two-line hybrid rice techniques are well developed and have been applied widely in agriculture through a super rice project initiated in 1996.

Production of hybrid seeds through three-line and two-line systems is still costly and sophisticated for most farmers, especially for those in developing countries. As a result, rice farmers must purchase new hybrid seeds each growing season from professional hybrid seed production agencies. A one-line system is ideal for producing hybrids. This is because the heterosis of the F_1 hybrid can persist by apomixis (reproduction without fertilization), thus enabling the farmers to produce hybrid seeds themselves as conventional cultivars. Despite great strides in rice apomixis technology over the past few decades, research in this area has not yet achieved a significant advance. However, studies on rice apomixis are still ongoing (Virmani et al. 2003).

3 Marker-Assisted Selection and Quantitative Trait Locus Analysis

Conventional rice breeding approaches have improved rice cultivars for thousands of years. However, progress is slow, due to the time-consuming process, the quantitative nature of most agronomic traits and difficulties in genotype se-

lection. Over the past decade, researchers have developed and applied marker-assisted selection (MAS) and quantitative trait locus (QTL) analysis techniques to rice breeding. These approaches promote new germplasm identification and new elite cultivar establishment.

A large number of molecular markers must be developed for MAS to be effective in rice breeding. They include expressed sequence tags (ESTs), restriction fragment length polymorphisms (RFLPs), cleaved amplified polymorphic sequences (CAPS), simple sequence repeats or microsatellites (SSR or MS), single nucleotide polymorphisms (SNPs), and insertions/deletions (InDels). Rice geneticists and breeders have leveraged the full-scale sequencing of rice genomes to develop molecular markers for nearly every rice gene and to select desired genotypes, even at the seedling stage. The molecular polymorphisms or markers between the *japonica* rice cv. Nipponbare and the *indica* cv. 93-11 are available to the public (Shen et al. 2004; Bertin et al. 2005; Yu et al. 2005; Zhang et al. 2005).

Most important agronomic traits are quantitative in nature, as they are controlled by multiple genes. Each single gene or QTL accounts for only a portion of the genetic variance and the final phenotype. QTL analysis dissects the genetic network regulating important agronomic traits, including grain yield, grain quality, plant architecture, flowering time, disease and pest resistance, and abiotic tolerance. By using QTL analysis, rice geneticists and breeders have identified many QTLs involved in the control of important agronomic traits. Several major QTLs were cloned or tagged. For example, flowering time or heading date of *japonica* rice is affected by 14 QTLs, eight of which have been cloned (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002). Therefore, the cloned QTLs or genes and their linked molecular markers will provide breeders with trait targets for effective MAS, even at the seedling stage. Furthermore, breeders can select desirable genotypes for subsequent investigation. Clearly, MAS, together with QTL analysis and gene cloning, will allow breeders to develop new rice varieties in the near future.

4 Rice Transformation and Genetic Engineering

Compared to conventional or MAS breeding, transformation of rice plants with desired genes is a time-saving, efficient and direct way to improve agronomic traits. In order to modify rice genetically, one must have a suitable gene promoter, a target gene and an efficient transformation system. Unlike dicotyledonous plants such as *Arabidopsis thaliana*, which can be transformed easily, rice transformation is a laborious process. However, in the past two decades, scientists have strived to improve rice transformation. Presently, most rice cultivars are transformation-competent.

Rice transformation can be achieved by polyethylene glycol (PEG)-mediated protoplast regeneration (Datta and Datta 1999), biolistic bombardment (Chris-

tou 1997) or *Agrobacterium*-mediated transformation (Hiei et al. 1994). Of these methods, *Agrobacterium*-mediated rice transformation displays the highest transformation efficiency, promotes minimal rearrangement of the transgene and produces a relatively high percentage of transgenic plants harboring a single transgene copy. Also, *Agrobacterium* transformation yields good transgenic plant fertility (Budar et al. 1986; Feldmann and Marks 1987; Klee et al. 1987; Zambryski 1988; Hamilton et al. 1996).

A. tumefaciens is a soil bacterium that channels a defined piece of DNA (T-DNA) from its tumor-inducing (Ti) plasmid into a receptive plant genome. The first high-efficiency *Agrobacterium*-mediated transformation producing fertile and heritable transgenic rice plants was reported in 1994 by Hiei and coworkers (Hiei et al. 1994). Currently, this method is used routinely by many laboratories worldwide working either in rice genetic engineering or on characterization of rice gene functions. A successful *Agrobacterium*-mediated rice transformation system should provide high transformation efficiency, a desirable expression profile of the transgene and a suitable vector system for delivering multiple genes.

The genetic background of rice plants has a decisive effect on the efficiency of transformation. Special attention is required for the induction of *Vir* genes, in addition to the status of calli, medium composition, the selection of bacterial strains, the plant genotype and selectable markers (Ebinuma et al. 1997; Hiei et al. 1997). In general, transformation of cultivars is much easier than that of wild rice species. In particular, transformation of *japonica* varieties is much more efficient than that of *indica* varieties. For certain rice genotypes, the medium composition must be optimized to generate competent calli for transformation. The choice of a selectable marker is also important for efficient transformation. The commonly used selectable markers for rice transformation include *hpt*, *npt*II or *bar*, which render the transformants resistant either to the antibiotics hygromycin and G418 or to the herbicide Bastar, respectively. Among them, *hpt* is the most commonly used, because it is suitable for most rice genotypes.

An appropriate promoter is always required to ensure correct spatial and temporal expression of the transgene. For stable constitutive transgene expression, the rice actin promoter (*Act1*) and two polyubiquitin promoters (*RUBQ1*, *RUBQ2*) exhibit higher activities than the relatively weak CaMV 35S promoter (McElroy et al. 1990; Wang et al. 2000; Wang and Oard 2003). GUS gene expression driven by the *RUBQ1* or *RUBQ2* promoter was 8- to 35-fold greater than that by the 35S promoter in transgenic plants. Furthermore, rice ubiquitin promoters give rise to transgenics that display less gene-silencing frequencies (Wang and Oard 2003). Thus, rice ubiquitin promoters will enjoy popularity in rice transgenic research. The CaMV 35S promoter is used successfully in dicotyledonous plants, but it does not perform as well in monocotyledonous plants, including rice (Guilley et al. 1982; Peterhans et al. 1990).

Thus far, researchers are able to introduce only one or a small number of genes at any one time per transformation. However, since multiple genes

regulate many valuable agronomic traits, transformation of a single gene or a small number of genes is insufficient to improve a target trait. Nevertheless, multiple gene transfer methods are problematic. Recently, Lin et al. (2003) developed a multigene assembly vector system for transferring different genes simultaneously by *Agrobacterium*-mediated transformation. This system combined many different genes into a TAC-based vector via *Cre/loxP* site-specific recombination system and homing endonucleases. This polygenic transformation system will contribute greatly towards generating transgenic rice varieties carrying multiple transgenes to improve yield, quality and stress resistance.

In current transformation systems, at least one selectable marker gene is co-integrated with the transferred foreign gene, allowing for the identification and separation of transformants from non-transformants. However, recent public concerns regarding transformed antibiotic and herbicide resistance genes limit their use for commercialization (Endo et al. 2002). To meet these challenges, scientists are exploring highly efficient but simple and practical strategies for eliminating selectable marker genes to generate marker-free plants. Several strategies have proven successful, including co-transformation, transposon-mediated gene repositioning, site-specific recombination, intrachromosomal homologous recombination and specialized selection (Komari et al. 1996; Ebinuma et al. 1997; Gleave et al. 1999; Zubko et al. 2000; Zuo et al. 2001).

5 Gene Isolation and Characterization

5.1 Map-Based Cloning

Map-based cloning, also termed positional cloning, is one of the most important gene cloning methods applied in rice. This procedure works by identifying a mutation locus that leads to a mutant phenotype through linkage analysis to DNA markers. Before the advent of whole-genome sequencing, map-based cloning was labor-intensive and time-consuming. High-density rice physical/genetic maps contributed to map-based cloning by providing radioactivity-labeled markers, for example RFLP markers. The availability of genome sequence data, for both *indica* and *japonica* rice, have provided PCR-based markers including CAPS, SSR, InDel and SNP markers that facilitate map-based cloning of important agronomic genes in rice.

Isolation of a rice gene by map-based cloning comprises at least three steps, namely: (1) constructing a large mapping population, (2) screening the mapping population with PCR-based markers to pinpoint the interested gene to a defined region and (3) identifying the candidate gene by sequencing and genetic complementation.

Recently, several important rice genes were identified with map-based cloning strategies. These genes involve in various developmental processes,

including plant architecture establishment (Komatsu et al. 2003a, b; Li et al. 2003b; Miyoshi et al. 2004; Ashikari et al., 2005; Luo et al., 2006; Sakamoto et al., 2006; Zhu et al., 2006), hormone signaling pathway (Ashikari et al. 1999; Ueguchi-Tanaka et al., 2005), male sterility restoration (Komori et al. 2004; Wang et al. 2006), cell wall biosynthesis (Li et al. 2003c) and stress resistance (Yamanouchi et al. 2002; Bohnert et al. 2004; Sun et al. 2004; Zeng et al. 2004; Ren et al. 2005; Chu et al. 2006).

5.2 RNA Interference

Cellular RNA interference (RNAi) is a powerful post-transcriptional gene-silencing phenomenon caused by double-stranded RNA introduction. In the past few years, RNAi has been used as an efficient and highly specific gene knockdown/knockout technology to study gene functions in a variety of organisms, including animals and plants (Kusaba 2004). In plants, RNAi is usually introduced by a transgene that produces hairpin RNA (hpRNA) with a double-stranded RNA (dsRNA) region. The hairpin structure is highly efficient for target gene silencing (Di Serio et al. 2001; Waterhouse and Helliwell 2003). Briefly, the hpRNA-producing vector is introduced into the rice genome by *Agrobacterium*-mediated transformation. The target gene is cloned into the hpRNA-producing vector as an inverted repeat driven by the maize ubiquitin promoter and the reverse repeat is spaced with an intron.

In rice, RNAi is gaining popularity among geneticists due to its advantages over other established gene-suppression (for example, RNA antisense) systems (Miyoshi et al. 2003; Jan et al. 2004; Kusaba 2004; Lee et al. 2004; Miki and Shimamoto 2004; Teerawanichpan et al. 2004; Wong et al. 2004). One important challenge for rice functional genomics by the reverse-genetic method is gene redundancy resulting from multigene families in the genome. RNAi is an effective tool for circumventing this problem (Lawrence and Pikaard 2003) and offers a promising strategy to suppress target genes, using various homologies to modulate important agronomic traits appropriately.

5.3 Gene Targeting by Homologous Recombination

Gene targeting (GT) by homologous recombination (HR) in higher plants is not as successful as it is in mice, *Escherichia coli*, yeast, or *Physcomytrella patens*. However, recent advances in *Arabidopsis* and rice stem from an HR-modified *AGL5* MAD-box regulatory gene, the first successful endogenous plant gene knockout using the GT strategy (Kempin et al. 1997). Through this revolutionary methodology, the *Arabidopsis PPO* gene involved in heme and chlorophyll biosynthesis was inhibited through HR (Hanin et al. 2001).

Integration events by HR often accompany non-homologous end-joining, giving rise to low frequency transgenic progeny (approximately 6.5×10^{-4}). To overcome this constraint, Terada et al. (2002) established a large-scale *Agrobac-*

terium-mediated transformation procedure with a strong positive–negative selection for gene targeting in rice. In this system, *hpt* is a positive selection marker for small group identification of rice calli harboring the targeted *waxy* gene from a larger number of transformed calli. Two diphtheria toxin A fragment genes provided strong negative markers to remove random integration. This study underscores the importance of GT and HR technologies to offset economic and scientific problems associated with genetic recombination studies in rice (Terada et al. 2002; Hohn and Puchta 2003).

5.4 Microarray

All cells in an organism usually contain an identical gene set, but gene activity depends on many factors, including tissue types, developmental stages and responses to environmental cues. Thus, the same genes are not active in every cell at all times. Studying which and when genes are active in different cell types under different conditions allows us to understand how these genes function normally and in concert. Before the advent of microarrays, scientists relied on rudimentary techniques to examine only a few genes at a time by traditional RNA differential expression methods, such as DDRT-PCR, RFDD-PCR and Northern blot analyses. Microarray technology and full-scale genome sequencing provide scientists with a way to examine the expression profiles of thousands of genes simultaneously under highly controlled conditions. This allows biologists to examine cell-, time- and treatment-specific regulatory pathway constituents.

There are several types of microarrays, including cDNA and oligonucleotide, and one- or two-color hybridization. Briefly, the single-stranded DNA substrate representing individual genes (cDNAs or oligonucleotides) are spotted (in the case of cDNAs) or chemically "grown" (in the case of oligonucleotides) by robot on a single square inch microscope slide termed a Gene Chip. Next, messenger (mRNA) is isolated and purified from cells or tissues of interest. The mRNA molecules are "labeled" by attaching a fluorescent dye (Cy3-dUTP or Cy5-dUTP, in the case of two-color hybridizations) and are hybridized to the Gene Chip. After hybridization, the arrays are scanned with a fluorometer. The resulting signal intensities represent the number of mRNA molecules present in the starting material and are converted into values for analysis.

Microarray technology is an invaluable tool in rice functional genomic research. Scientists are using microarrays to ask many different questions of the developing plant. For example, which genes are over- or under-expressed in mutant and wild-type plants, in response to abiotic or biotic stress. This methodology, coupled with focused and hypothesis-driven experiments, can reveal candidate genes of interest. Subsequent validation techniques are recommended, such as Northern hybridization, real-time PCR or RT-PCR. These experiments can provide a second tier of hypothesis-driven tests to define gene regulation networks and coordinated expression in rice.

Since the rice microarray project started in 1999 in Japan, various rice Gene Chips for microarray analysis have been developed, such as EST chips, full-length cDNAs and oligonucleotide-based microarray chips. Recently, Affymetrix (Santa Clara, USA) produced a high-density rice Gene Chip that contains probes to query 51,279 transcripts representing two rice cultivars, with approximately 48,564 *japonica* transcripts and 1,260 *indica* transcripts.

5.5 High-Throughput Insertion Mutagenesis

Approximately 50,000 genes are predicted in the rice genome, 50% of which share homologies with those in *Arabidopsis thaliana*, according to the comparative analysis between the two model species (Goff et al. 2002; Yu et al. 2002). In addition, most rice genes annotate with unknown or hypothetical functions. As a result, the primary challenge for future rice genomic research is to identify the functions of these genes by using high-throughput strategies.

Generally speaking, methods such as transposon (*Ac/Ds*) or retrotransposon (*TOS17*) tagging and T-DNA insertions are powerful mutant generators for gene function identification. The characteristic of each method is shown in Table 1. These *Ac/Ds*, *TOS17* and T-DNA insertion rice mutant databases are available online.

Table 1. Methods for high-throughput insertion mutagenesis

Method	Characteristics	References
Ac/Ds tagging system	Use of the maize autonomous transposable *Ac/Ds* element; High somatic and germinal transposition frequencies in rice; *Ds* insertions distribute randomly throughout the rice genome with bias toward chromosomes 4 and 7; *Ds* transposes preferentially into coding regions; Suitable for generating large-scale stable, unlinked and single-copy *Ds* transposon mutagenesis in rice.	Shimamoto et al. (1993), Enoki et al. (1999), Greco et al. (2001a, b), Kolesnik et al. (2004)
Tos17 retrotransposon system	Use of the endogenous retrotransposon element *Tos17*; *Tos17* is activated by tissue culture but becomes inactivated after plant regeneration; *Tos17* integration distributes throughout the rice genome; *Tos17* has a low copy number compared with other plant retrotransposons; *Tos17* prefers gene-rich regions; Suitable for large-scale gene function analysis.	Hirochika (2001), Yamazaki et al. (2001), Miyao et al. (2003)
T-DNA insertion system	T-DNA prefers gene-rich regions; Low frequency insertion in repetitive regions; Equal chance to insert in genic versus intergenic regions.	Jeong et al. (2002), An et al. (2003), Chen et al. (2003), Wu et al. (2003), Sallaud et al. (2004)

6 Application of Rice Biotechnology

The ongoing population increases in the main rice-consuming countries necessitate the rapid development of elite rice varieties. Since the Green Revolution era in the 1960s, scientists have attempted to improve rice production in these areas on the basis of advanced molecular biotechnology and conventional rice breeding, focusing on the following issues.

6.1 Modulation of Rice Plant Architecture

Among the agronomic traits affecting rice grain production, rice plant architecture is one of the most important factors that determine rice yield (Khush 2003). In the 1960s, semi-dwarf wheat and rice cultivars gave rise to a vastly improved crop production known as the Green Revolution. To further increase the production of the existing semi-dwarf rice varieties, scientists from the International Rice Research Institute (IRRI) proposed a model of the ideal rice plant architecture, which should have a low tiller number (9–10 tillers for transplanted conditions), a high number of productive tillers, 200–250 grains per panicle, dark green thick and erect leaves, and vigorous and deep root systems.

Understanding the molecular mechanisms involved in rice plant architecture is essential to rice plant modification. The green revolution rice *semi-dwarf 1* (*sd1*) cultivar was first produced in the 1960s and characterized recently as a GA biosynthesis mutant through map-based cloning (Monna et al. 2002; Sasaki et al. 2002; Spielmeyer et al. 2002). This revelation suggested a modulating hypothesis for plant height manipulation via biotechnology on a molecular level (Sakamoto et al. 2003).

Several other genes, such as *MOC1*, *LAX*, *FRIZZY PANICLE*, *GID1* and *CKX2*, involved in controlling rice plant architecture, were map-based cloned and characterized (Komatsu et al. 2003a, b; Li et al. 2003a, b; Ueguchi-Tanaka et al., 2005; Sakamoto et al., 2006). These genes may play crucial roles in the development of rice tillers and panicles, which are regarded as the main contributors to plant architecture. Genetic dissection and molecular manipulation of these genes have the potential to modify the rice plant architecture and thus generate new rice varieties.

6.2 Improvement of Rice Plant Resistance

Rice yield stability has been attained through the improvement of rice plant resistance, including insect and disease resistance, and drought and salinity tolerance.

6.2.1 Insect Resistance

Rice stem borers are serious rice pests. They infest plants from the seedling stage to maturity and are particularly destructive in Asia, the Middle East and the Mediterranean regions. Stem borer damage results in a severe reduction of rice grain production. In China, stem borer brings an annual 6.45×10^{12} Renminbi (RMB) loss, in addition to a 2.85×10^{12} RMB cost for their chemical control (Sheng 2003).

Bacillus thuringiensis (*Bt*) genes produce toxins in the bacterium that are detrimental to its host insects. Genes encoding various *Bt* crystal (Cry) proteins have been transferred into cotton and maize, and benefit farmers greatly. *Bt* genes, driven by an ubiquitin, inducible or tissue-specific promoter, have also been transferred into rice to produce *Bt* transgenic rice cultivars which show resistance to lepidopteran pests, including stem borer and several species of leaf folders. This resistance is achieved without causing the occurrence of other rice pests, such as the brown plant hopper (Ye et al. 2001; High et al. 2004). The *Bt* rice has been under evaluation in several countries since the beginning of its field trials in China in 1998, but not commercialized yet because of public biosafety concerns (High et al. 2004). However, the *Bt* biotechnology has already become an important component of integrated pest control methods and is compatible with the applications of pest-resistant varieties, cultural practices, insecticides and biological control. Therefore, the *Bt* rice has the potential for acceptance by several countries in Asia (Lei 2004; Huang et al. 2005).

Additionally, proteinase inhibitors (PIs) are widely used to engineer insect-resistant transgenic rice plants. These inhibitors work by disrupting the digestive systems of insects. The cowpea *CpTI* gene was the first *PI* gene to produce enhanced insect-resistant transgenic plants. Constitutive expression of the *CpTI* gene in transgenic rice plants increases the plant's resistance to two species of stem borers (Ussuf et al. 2001). Recently, a modified *CpTI* gene was introduced into Minghui 86, a rice cultivar widely used for three-line hybrid breeding in China; and the transgenic plants showed highly increased resistance to rice stem borer (Huang et al. 2005).

6.2.2 Disease Resistance

Rice bacterial blight (BB) is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This blight is the most destructive rice disease worldwide, especially in Asian rice-growing countries. As the first step towards improving resistance to rice BB, we need to understand the corresponding regulatory genes governing signaling pathways leading to defense against pathogen invasion. At present, more than 20 BB-resistant genes have been identified, but only a few, such as *Xa21*, *Xa1*, *Xa26* and *Xa13*, have been cloned. In-depth studies revealed that *Xa21* and *Xa26* both encode a protein family that contains both leucine-

rich repeat motifs and a serine-threonine kinase-like domain, suggesting their roles in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response (Song et al. 1995; Sun et al. 2004). Unlike *Xa21* and *Xa26* BB-resistant genes, the deduced amino acid sequence of the *Xa1* gene product contains nucleotide binding sites (NBS) and a new type of leucine-rich repeats, suggesting that *Xa1* is a member of the NBS-LRR class of plant disease-resistant genes (Yoshimura et al. 1998). Promoter mutations in *Xa13* result in down-regulation of gene expression during host–pathogen interaction, leading to the fully recessive *xa13* that confers race-specific resistance. Among these genes, the *Xa21* gene has proved a useful tool for breeding BB-resistant rice varieties by genetic engineering due to its wide-spectrum resistance to the *Xoo* blight. It has been transferred into various rice cultivars mediated by *A. tumefaciens* to develop new varieties resistant to BB (Zhao et al. 2000; Li et al. 2001; Wang et al. 2004; Zhai et al. 2004).

However, large-scale and long-term cultivation of varieties containing a single resistance gene are most likely to make the pathogen overcome BB resistance. Singh et al. (2001) made an effort to delay this process through pyramiding three BB genes (*xa5*, *xa13*, *Xa21*) into *indica* rice by marker-assisted selection. Their work pyramided these three genes in a high-yielding but BB-susceptible cultivar, *PR106*, to generate enhanced and wide-spectrum BB resistance. Moreover, the *Xa21* gene was also pyramided with the *Bt* and chitinase genes (for tolerance of sheath blight) to produce stable elite rice lines resistant to disease and insect pests through conventional crossing of two transgenic parental lines transformed independently. The progeny carrying these three transgenes showed resistance to bacterial blight, yellow stem borer and sheath blight disease (Datta et al. 2002).

In addition to bacterial blight, blast caused by the fungus *Magnaporthe grisea* is another serious and widespread rice disease. *M. grisea* can attack the aerial parts of rice plants during all developmental stages. Infection is characterized by lesions on the leaves, nodes and panicles. *M. grisea* infects rice leaves through a series of steps. First, airborne spores attach to the rice plants and sense the waxy aerial surface. Second, the fungal conidia germinate on the leaf surface and the germ tubes differentiate into a dome-shaped penetration unit called an appressorium. Finally, the rapid reproduction of appressoria generates turgor pressure to penetrate the leaf surface and in this manner infect rice tissues (Howard and Valent 1996). The latest relevant study showed that *M. grisea* also infects plant roots (Sesma and Osbourn 2004). So far, more than 30 rice blast resistance genetic loci, denoted *Pi* genes, have been identified (Song and Goodman 2001; Talbot 2003). The availability of both host rice and pathogen *M. grisea* genome sequence data provides the opportunity to study the host–pathogen interaction and uncover effective ways to control this disease.

6.2.3 *Abiotic Tolerance*

As the world population continues to increase, farmers must employ inhospitable lands to grow crops. As a result, new crop varieties that can withstand long periods of drought or high-salt environments are needed.

Rice is an aquatic crop that is sensitive to water deficit (Courtois et al. 2003). Over the past decade, a severe bottleneck in rice grain production occurred in response to unusual drought circumstances. To tackle this problem, the Rockefeller Foundation is mapping and identifying traits and genes associated with rice drought tolerance in order to improve the grain yield performance of rice cultivars in the rain-fed lowland rice areas in north and northeast Thailand.

Enhancement of plant drought tolerance via transgenics is underway. Several candidate genes corresponding to abiotic stress tolerance have been incorporated into rice, leading to the accumulation of biomass under drought stress (Cheng et al. 2001, 2002). Garg et al. (2002) engineered trehalose overproduction in rice through stress-inducible or tissue-specific expression of a bifunctional trehalose-6-phosphate synthase/phosphatase (TPSP) fusion enzyme without any negative effects on rice plant growth or grain yield. During abiotic stress, transgenic plants accumulated increased amounts of trehalose and exhibited high-salt, drought and low-temperature stress tolerances. This research suggested the potential use of a transgenic approach in developing new rice cultivars with increased abiotic stress tolerance and enhanced rice productivity. In addition, cDNA microarrays were used to identify stress-inducible genes in rice (Rabbani et al. 2003). Although most of these gene functions are currently unclear, they can be utilized to investigate molecular mechanisms of drought tolerance and for applications of gene manipulation. Very recently, a novel rice QTL *SKC1* was cloned and characterized as a sodium transporter that confers salt tolerance, providing a potential tool to improve salt tolerance of rice (Ren et al. 2005).

6.3 Improvement of Rice Grain Nutrition and Quality

In many developing countries, rice is the main staple for impoverished people. However, the edible part of the rice grain lacks several essential nutrients, such as vitamins and minerals, leading to serious malnutrition. For example, rice lacks beta-carotene, which can be converted to vitamin A when digested and represents a vital dietary nutrient. As a result, in excess of 180 million children and women suffer from vitamin A deficiency in Asia and other developing countries. Furthermore, about half a million children worldwide are permanently blind due to vitamin A deficiency (Chong 2003). Fortunately, achievement of both rice biotechnology and molecular understanding of carotenoid biosynthetic pathways allows us to create elite rice varieties. Researchers at the Swiss Federal Institute of Technology created a strain of “golden” rice contain-

ing high beta-carotene concentrations (Beyer et al. 2002; Paine et al. 2005). They introduced a *de novo* biosynthetic pathway into rice endosperm with *Agrobacterium*-mediated transformation to increase the pro-vitamin A content.

A latent iron deficiency occurs in developing and some developed countries (Heath and Fairweather-Tait 2002). The ferritin genes from *Phaseolus vulgaris* and *P. limensis* have been expressed in rice, resulting in increased concentration of iron in transgenic rice grains (Lucca et al. 2002; Vasconcelos et al. 2003; Liu et al. 2004).

Amylose, one of the components of starch, determines rice eating and cooking quality. Reduction of amylose is an efficient way to improve rice starch quality (Liu et al. 2003). The *Waxy* gene encodes the granule-bound starch synthase. The *japonica* and *indica* rices differ in their abundance of mature *Waxy* RNA, as a result of a natural nucleotide substitution that confers different splicing efficiencies, leading to a varied ratio of amylose to total starch content (Isshiki et al. 1998). Liu et al. (2003) generated amylose reduction transgenic lines via *Agrobacterium*-mediated technology in both elite *japonica* and *indica* rice cultivars. In these cultivars, the starch quality was variably improved. Recently, Terada et al. (2002) also took advantage of the *Waxy* gene to carry out a GT strategy in rice (mentioned in sect. 4.3), thus providing a good example of how new biotechnology can be applied to solve economic and scientific problems.

7 Conclusions

Traditional rice breeding strives to produce elite varieties. However, this process is time-consuming and limited genetic resources leave little room for continued improvement. Rice has a small genome and was the first monocotyledonous plant to be sequenced. Through the available rice sequence data, scientists can unravel their functionality, providing researchers with abundant genetic resources for fruitful manipulation. Noticeably, increasing public concerns regarding transgenic biosafety will challenge rice-producing efforts. The goal remains to create varieties with improved production, stress resistance and grain quality and nutrition, using newly developed biotechnological approaches.

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