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REVIEW & INTERPRETATION

Molecular Markers and Their Use in Marker-Assisted Selection in Rice

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ABSTRACT

Increasing world population, shrinking cultivable rice (*Oryza sativa* L.) land area, water scarcity and excess, evolution of new biotypes of pests and diseases, and climate change pose serious challenges to rice breeders to increase production and productivity with multiple resistances to biotic and abiotic stresses. Recent advances in rice genomics research and completion of the rice genome sequence have made it possible to identify and map precisely a number of genes through linkage to DNA markers. Noteworthy examples of some of the genes tightly linked to markers are resistance to or tolerance of blast, bacterial blight, virus diseases, brown planthopper (*Nilaparvata lugens*), drought, submergence, salinity, and low temperature and improved agronomic and grain quality traits. Marker-assisted selection (MAS) can be used for monitoring the presence or absence of these genes in breeding populations and can be combined with conventional breeding approaches. Marker-assisted backcross breeding has been used to effectively integrate major genes or quantitative trait loci with large effect into widely grown varieties. Pyramiding different resistance genes using MAS provides opportunities to breeders to develop broad-spectrum resistance for diseases and insects. The use of cost-effective DNA markers derived from the fine mapped position of the genes for important agronomic traits and MAS strategies will provide opportunities for breeders to develop high-yielding, stress-resistant, and better-quality rice cultivars.

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Abbreviations: AC, amylose content; AFLP, amplified fragment length polymorphism; BB, bacterial leaf blight; BPH, brown planthopper; GRLH, green rice leafhopper; GC, gel consistency; GT, gelatinization temperature; IRRI, International Rice Research Institute; MAB, marker-assisted backcross; MAS, marker-assisted selection; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged sites.

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. It is cultivated on all the continents except Antarctica, over an area of more than 150 million ha, but most rice production takes place in Asia. The Green Revolution technology developed at the International Rice Research Institute (IRRI) in the 1960s increased world rice production. However, during the past decade, production potential of modern cultivars has remained stagnant. It is imperative to increase rice production in different rice-growing ecosystems to feed the increasing world population (Khush, 2005).

Advances in cellular and molecular biology have made cultivated rice, a model monocot species because of several landmark achievements (International Rice Genome Sequencing Project, 2005): (i) successful production of transgenic plants and genetic transformation potential in indica and japonica cultivars;

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(ii) cultivar development through anther and pollen culture (iii) Construction of a comprehensive genetic and physical map of the rice genome; (iv) development of the genetic maps of chloroplast and mitochondrial genomes; (v) construction of a high density molecular map for gene mapping and map-based gene cloning; (vi) development of BAC and YAC libraries and development of the *Oryza* Map Alignment Project (OMAP); (vii) small genome size (around 389 Mb) and synteny with other cereals like wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and barley (*Hordeum vulgare* L.); and (viii) complete sequencing of the rice genome in indica and japonica rice cultivars and annotation of gene sequences.

Several biotic and abiotic stresses, as well as narrow genetic diversity in modern cultivars of rice, are the major constraints to further increases in productivity. With the development of a comprehensive molecular genetic map of rice, 1488 genes have been identified corresponding to several traits of economic importance (http://www.gramene.org/; verified 29 May 2008). In addition to several genes of morphological and physiological traits, 28 genes for bacterial blight, 40 for blast, 3 for virus diseases, and about 30 genes for resistance to insects such as brown planthopper (*Nilaparvata lugens*), green rice leafhopper (*Nephotettix cincticeps*), and gall midge (*Orseolia oryzae*) have been identified. Several genes and quantitative trait loci (QTL) have been identified for abiotic stresses such as drought, salinity, submergence, and cold.

The development of gene identification technologies using the tools of biotechnology provides ample opportunities for scientists to further improve modern cultivars. This would help to accelerate the application of marker-assisted selection (MAS) and marker-assisted backcross (MAB) breeding in rice improvement. This review discusses the availability of DNA markers linked to important traits in rice improvement and their potential use in MAS.

MOLECULAR MARKERS FOR RICE

Molecular markers for trait selection have numerous advantages over morphological markers used in conventional plant breeding. The application of molecular markers in rice improvement has been reviewed recently (Collard and Mackill, 2007; Mackill, 2007). These reviews mainly addressed the application of DNA markers in improvement of crop plants in general and emphasized the marker application in heterosis breeding. They also described the limitations of marker use in crop improvement.

The main advantages of molecular markers include the following:

Time saving: Genomic DNA can be isolated from any part of the plant tissue, and target trait information can be obtained with linked DNA markers before pollination, thus allowing breeders to carry out more informed genetic crosses.

Consistency: Phenotypic evaluation of genetic traits is often complicated by environmental factors. However, DNA markers are mostly neutral to environmental variation.

Biosafety: Diagnostic tests for the presence or absence of traits for disease resistance can be conducted by DNA markers tightly linked to the target gene without resorting to pathogen inoculation in the field or greenhouse. Additionally, molecular markers facilitate introgression of genes into elite cultivars in advance of the occurrence of certain races of diseases or biotypes of insects.

Efficiency: Evaluation of breeding lines in early generations of the breeding process with DNA markers can allow breeders to reject progenies from the program and improve the genetic quality of breeding materials.

More accurate selection of complex traits: Polygenic traits are often difficult to select for using conventional breeding approaches. DNA markers linked to QTL allow them to be treated as single Mendelian factors.

Initially, a restriction fragment length polymorphism (RFLP) framework map was developed with 250 markers (McCouch et al., 1988). This map was supplemented with additional markers such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) markers, expressed sequence tags, sequence tagged sites (STS), and amplified fragment length polymorphism (AFLP) markers (McCouch et al., 2002). Many agronomically important genes of rice have been mapped with linked markers (Table 1). Thousands of RFLP, RAPD, AFLP, SSR, and STS markers are reported in the Gramene database (http://www.gramene.org/markers/index.html, verified 29 May 2008) and the number of class I SSR sequences was listed as 18,828 (International Rice Genome Sequencing Project, 2005). Using the saturated rice molecular map and genome sequence information, a number of agronomically important genes have been isolated from the genome using a map-based cloning strategy (Dai et al., 2007). These include bacterial blight resistance genes *Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27* (Chu et al., 2006; Gu et al., 2005; Iyer and McCouch, 2004; Song et al., 1995; Sun et al., 2004; Yoshimura et al., 1998); blast resistance genes *Pib*, *Pita*, *Pi2*, *Pizt*, *Pi9*, *Pid2*, *Pi36*, and *Pi37* (Bryan et al., 2000; Chen et al., 2006; Lin et al., 2007; Wang et al., 1999; Zhou et al., 2006); and submergence tolerance gene *Sub1A* (Xu et al., 2006).

QTL and Polygenic Traits

Most of the important agronomic traits of rice are complex and polygenic in nature, controlled by QTL. Several

parameters, such as heritability of the target trait, population size, and possibility of false QTL detection (type I error), should be taken into consideration for the efficiency of QTL for MAS. A simulation study conducted by Moreau et al. (1998) revealed the following relationships between QTL and MAS:

Table 1. List of some major genes and quantitative trait loci (QTL) for agronomic traits associated with DNA markers.

Traits	Gene	Chromosome	Marker	Reference
Submergence tolerance	*Sub1*	9	c1232, RZ698	Xu et al. (2006)
Salt tolerance	*salT*	1	cD0548	Causse et al. (1994)
Salt stress	*qST1,qST3*	1,3	RZ569A, RZ596	Lee et al. (2007)
Salt stress	QTL	2,3,7	C1408	Takehisa et al. (2004)
Salt stress	QTL	1,6,7	C813	Lin et al. (2004)
Drought tolerance	QTL	1,5,9	RG810, RZ556, RG206	Courtois et al. (2000)
Drought tolerance	Root/shoot	2,3,6,7,11	RM208, RM231	Li et al. (2005)
Osmotic adjustment	QTL	8	RG1	Lilley et al. (1996)
P deficiency	QTL	6	RM30	Shimizu et al. (2004)
Fe toxicity	*LB1*	1,2, 4	RM315, RM6, RM252	Wan et al. (2005)
Fe toxicity	*LB1*	3	X279-C25	Wan et al. (2003)
Cold tolerance (seedling)	QTL	2	RM561-RM341	Lou et al. (2007)
Cold tolerance (seedling)	QTL	12, 4, 6,11	RM101-RM292	Andaya and Mackill (2003a)
Cold tolerance (booting)	QTL	2, 3	RM324-RM301, RM156	Andaya and Mackill (2003b)
Shattering resistance	QTL	11	XNpb113	Fukuta et al. (1994)
Fertility restorer	*Rf3*	7	XNpb379	Zhang et al. (1997)
Aroma	*fgr*	8	RG28	Ahn et al. (1992)
Semidwarf	*Sd1*	3	XNpb363	Ashikari et al. (1999)
Grain size	*GS3*	3	GS09-MRG5881	Fan et al. (2006)
Spikelets, panicle	*qSpp8*	8	RM544-RM310	Zhang et al. (2006)
Plant height	*qPh8*	8	RM544-RM310	Zhang et al. (2006)
Grain weight	*qgw3.1*	3	JL107-JL109	Li et al., (2004)
Green leafhopper resistance	QTL	4	RZ262	Sebastian et al. (1996)
Green rice leafhopper resistance	*Grh5*	8	RM3754-RM3761	Fujita et al. (2006)
Brown planthopper resistance	*Bph1*	12	em24G, em32G	Sharma et al. (2003)
	bph2	12	KPM3	Murai et al. (2001)
	Bph15	4	RG1, RG2	Yang et al. (2004)
	Bph18	12	7312.T4A	Jena et al. (2006)
	bph19	3	RM6308, RM3134	Chen et al. (2006)
Gall midge resistance	*Gm2*	4	RG329, RG476	Mohan et al. (1994)
Blast resistance	*Pi1*	11	RZ536	Mew et al. (1994)
	Pi2	6	R2123-RG64	Yu et al. (1991)
	Pi9	6	pB8	Qu et al. (2006)
	Pi36	8	RM5647-CRG2	Liu et al. (2005)
	Pi37	1	RM543-FPSM1	Chen et al. (2005)
	Pi39	12	RM27933-RM27940	Liu et al. (2007)
	Pi40	6	9871.T7E2b	Jeung et al. (2007)
	Pib	2	R2511	Wang et al. (1999)
	Pita	12	SP4B9-Sp9F3	Bryan et al. (2000)
Bacterial blight resistance	*Xa1*	4	XNpb235	Yoshimura et al. (1998)
	Xa4	11	R1506-M196-1	Sun et al. (2003)
	xa5	5	RS7-RM611	Blair et al. (2003)
	xa13	8	E6A, SR6, SR11	Chu et al. (2006)
	Xa21	11	pB18	Song et al. (1995)
	Xa27	6	M964-M1197	Gu et al. (2005)
Rice tungro spherical virus	*RTSV*	4	RZ262	Sebastian et al. (1996)
Rice stripe virus	*Stvb-i*	11	7L-21R	Hayano-Saito et al. (2000)
Rice yellow mottle virus	*Rymv*	4	RM273-RM252	Albar et al. (2003)

- If the heritability is high, the genotypic values are well estimated by the phenotype, and the weight given to markers is equivalent to phenotypic selection.
- MAS is not effective at an α (selection index) of 5% and heritability < 0.15.
- The efficiency of MAS decreases as the number of QTL increases.
- The efficiency of MAS increases when individual QTL explain a large part of the genetic variance.
- The relative efficiency of MAS increases with population size (the population should be larger than 100 or 200 individuals) and if the distance between markers and QTL decreases.

The advantages of using MAS in rice improvement have been well documented (Koebner, 2003; Jena et al., 2003; Mackill and McNally, 2004; Xu et al., 2004; Toojinda et al., 2005; Liu et al., 2006; Dwivedi et al., 2007; Mackill, 2007). The following section provides some examples of how MAS is being used.

MARKER-ASSISTED SELECTION FOR BIOTIC STRESSES

Biotic stresses, such as diseases (blast [caused by the fungus *Magnaporthe grisea*], bacterial leaf blight [caused by *Xanthomonas oryzae* pv. *oryzae*], and rice tungro virus) and insects (gall midge, brown planthopper, green leafhopper [*Nephotettix virescens*], and green rice leafhopper), account for significant yield losses annually. Resistance to these diseases and insects is controlled either by dominant or recessive major genes (Table 1) or by QTL (Alam and Cohen, 1998). The DNA markers have been used effectively to identify resistance genes, and MAS has been applied for integrating different resistance genes into rice cultivars lacking the desired traits. For examples, see International Rice Research Institute (2005).

Blast Resistance

Blast disease is a serious disease of rice in both the tropical and temperate regions. Molecular genetics of blast resistance have been extensively studied, and many useful DNA markers corresponding to major genes conferring race-specific resistance have been identified (Fjellstrom et al., 2004). Reliance on major gene resistance is risky because new races can evolve or rapidly increase, resulting in the breakdown of resistance of these varieties. Partial resistance, usually under the control of multiple QTL of relatively small effect, may be more useful for this reason (Wisser et al., 2005; Wu et al., 2004).

Of the 40 major blast resistance genes identified so far, about 30 genes have been mapped on different rice chromosomes, and tightly linked DNA markers have been developed. The PCR-based allele-specific and InDel marker sets are available for nine blast resistance genes, and they provide an efficient marker system for MAS for blast resistance breeding (Hayashi et al., 2006). Eight blast resistance genes have been cloned and the genes have been used for their selective introgression into susceptible rice cultivars (Lin et al., 2007). Recently, a novel resistance gene, *Pi40* derived from the EE genome wild *Oryza* species (*O. australiensis*), has been localized on chromosome 6 and fine mapped using the *e*-landing approach (Jeung et al., 2007). The DNA marker (9871. T7E2b) linked to the blast resistance phenotype in the presence of the *Pi40* gene in a 70-kb chromosomal region was obtained from NBS-LRR disease resistance motif sequences (Jeung et al., 2007). The *Pi40* gene shows promise for broad-spectrum durable blast resistance in rice (Jena, unpublished data). It is imperative to use DNA markers identified within the gene or from the flanking region of the gene as a tool for an efficient MAS strategy in rice improvement (Fjellstrom et al., 2004). Additionally, several blast resistance genes could be combined using MAS in a single genetic background to develop rice cultivars with broad-spectrum durable resistance to blast.

Bacterial Leaf Blight Resistance

Twenty-eight genes conferring resistance to bacterial leaf blight (BB) have been reported in rice (Nino-Liu et al., 2006). Several genes have been associated with tightly linked DNA markers, and some of them have been cloned (*Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26*, *Xa27*) and used for breeding BB-resistant rice cultivars. With the exception of *xa5* and *xa13*, the BB resistance genes are dominant in nature and the markers are developed from the sequencing information of these genes, which are widely used in MAS (Chu et al., 2006; Gu et al., 2005; Song et al., 1995; Yoshimura et al., 1998). Because of the availability of DNA markers derived from the resistance genes, it is now possible to pyramid several resistance genes into susceptible elite rice cultivars. Using the gene pyramiding approach, improved indica rice cultivars with broad-spectrum durable BB resistance have been developed by combining *Xa4* and *Xa21*. The pyramided BB resistance genes, *Xa4+xa5+Xa21*, expressed strong resistance to virulent BB isolates of Korea compared with individual resistance genes that are moderately to completely susceptible (Jeung et al., 2006). The resistance genes *xa5*, *xa13*, and *Xa21* have been pyramided into an indica rice cultivar (PR106) using MAS that expressed strong resistance to BB races of India (Singh et al., 2001). Two commercially cultivated rice cultivars (Angke and Conde) were released in 2002 for cultivation in Indonesia. They possess gene pyramids *Xa4+xa5* and *Xa4+Xa7*, respectively. In the Philippines, two rice cultivars (NSIC Rc142 and NSIC Rc154) have the gene combination *Xa4+xa5+Xa21*. These genes have been integrated into the susceptible cultivar IR64 genetic background using MAS (Toenniessen et al., 2003).

Virus Resistance

Tungro virus is one of the most devastating virus diseases of rice in the tropics. Of the two types of viruses (spherical and bacilliform) that cause tungro disease, DNA markers associated with the spherical virus resistance gene have been mapped on chromosome 4 (Sebastian et al., 1996). A new source of resistance to spherical tungro virus has been identified in an Indonesian rice cultivar, Utri Merah, and a recessive gene (*stv1*) confers resistance to tungro virus (I.R. Choi, personal communication, 2007). The gene has been mapped to a 100-kb region on chromosome 7 flanked by markers RM6152 and RM6403. Two QTL on chromosomes 7 and 12 expressing complementary epistasis with yellow mottle virus resistance have been reported (Ahmadi et al., 2001). In a recent study, *rymv*, a recessive resistance gene conferring resistance to rice yellow mottle virus, was localized on chromosome 4 between the SSR markers RM273 and RM252 (Albar et al., 2003). Rice stripe disease caused by rice stripe virus is serious in temperate rice-growing areas in Asia. A resistance gene derived from an indica rice cultivar, Modan, designated as *Stvb-i*, has been fine mapped to a 33-kb genomic region on chromosome 11 with flanking STS markers 7L and 21R (Hayano-Saito et al., 2000). The markers linked to different virus resistance genes could be effectively used in MAS for virus resistance breeding.

Gall Midge Resistance

Resistance to gall midge is under the control of at least 10 resistance genes, 8 of which have been tagged and mapped (Kumar et al., 2005; Himabindu et al., 2007). Flanking markers have been used to identify the resistance genes *Gm1* and *Gm2* in various rice cultivars (Himabindu et al., 2007). The usefulness of resistant cultivars for protection against gall midge infestation suggests that MAS will be a highly useful tool for breeders in areas where the pest is prevalent.

Brown Planthopper Resistance

Brown planthopper (BPH) is a major insect pest of rice that causes serious hopper burn in conducive environmental conditions and damage to rice production in the tropics and subtropics. It also transmits several viral diseases, including grassy stunt and ragged stunt. Advances in biotechnology and genetic research have enabled scientists to identify 19 resistance genes and associate 10 of them with tightly linked DNA markers. Six of these genes (*Bph1*, *bph2*, *Bph14*, *Bph15*, *Bph18*, and *bph19*) have been fine mapped (Chen et al., 2006; Jena et al., 2006; Sharma et al., 2003; Zhang, 2007), and the genes *Bph1*, *bph2*, and *Bph18* have been used for MAS of BPH resistance in temperate japonica and tropical indica rice cultivars (Jena et al., 2006). The presence of *Bph18* in breeding lines with a japonica genetic background provides enhanced resistance to new biotypes of BPH in Korea (Jena et al., 2006). *Bph1* and *bph2* have been introduced into BPH-susceptible japonica cultivars, and progenies with both gene combinations confer strong resistance to the new biotype of BPH in Japan (Sharma et al., 2004). Resistance to BPH is also under the control of QTL, and it has been speculated that the moderate polygenic resistance of varieties such as IR64 may exhibit more durability than the resistance of varieties that rely on major genes (Alam and Cohen, 1998).

Green Leafhopper and Green Rice Leafhopper Resistance

Resistance to green leafhopper (*Glh1*) and to tungro spherical virus is linked to a DNA marker that has been useful for the selection of tungro virus resistance in rice (Sebastian et al., 1996). Green rice leafhopper (GRLH) is a destructive insect pest of rice in temperate regions of East Asia. The six GRLH resistance genes, identified as *Grh1*, *Grh2*, *Grh3*, *Grh4*, *Grh5*, and *Grh6,* have been mapped on chromosomes 5, 11, 6, 3, 8, and 4, respectively (Fujita et al., 2006). These resistance genes have been linked with SSR markers and will be useful for MAS for GRLH resistance in rice.

MARKER-ASSISTED SELECTION FOR ABIOTIC STRESSES

Abiotic stresses, including drought, submergence, low temperature, and the effects of several types of adverse soils, are a frequent constraint to rice production. Genetic sources of tolerance of these stresses are available in traditional rice landraces and in some improved cultivars, but the complexity of the traits has hindered transfer of the tolerance genes into elite rice cultivars. However, QTL for these traits have been identified, and MAS has been used for specific QTL introgression into sensitive cultivars (Collard and Mackill, 2007; Steele et al., 2006).

Drought Tolerance

The genetics and physiology of drought tolerance are quite complex, and it is thought that the MAS approach can accelerate breeding efforts. A large number of genes are presumed to be involved in drought tolerance. These include genes involved in signal transduction, osmotic adjustment, and transcriptional regulation. Several QTL associated with drought tolerance are now reported to have a large effect for drought tolerance. The QTL on chromosome 9 is associated with spikelet fertility under stress and root and shoot traits (Courtois et al., 2000; Li et al., 2005; Yue et al., 2006). Two QTL for grain yield under drought were also detected on chromosome 2 (Zou et al., 2005). In a study using 'Teqing' as an indica recurrent parent and 'Lemont' as a tropical japonica donor, a number of alleles from Lemont imparted improved tolerance of drought

(Xu et al., 2005). Bernier et al. (2007) reported the detection of a large effect QTL for drought tolerance (*qtl12.1*) on chromosome 12 under field conditions. This QTL has been localized to a 10.2-cM region between SSR markers RM28048 and RM511, accounting for 51% of the genetic variance (Bernier et al., 2007). A QTL on chromosome 1 near the *sd1* gene accounted for 32% of the genetic variation for yield under drought stress (Kumar et al., 2007). Selecting parents with a higher level of drought tolerance shows promise in identifying more effective QTL and their integration into elite cultivars by MAS. Although the identification of these QTL for drought tolerance provides encouragement for improving this trait, practical applications of MAS for drought tolerance breeding have been still difficult because some markers associated with drought tolerance are either not tightly linked or exhibit small effect QTL (Steele et al., 2006).

Tolerance of Salinity and Adverse Soils

Rice cultivars grown in saline environments are sensitive at both the vegetative and reproductive stages. However, salinity tolerance at different growth stages seems to be controlled by independent genes. A major QTL (*Saltol*) derived from the salt-tolerant cultivar Pokkali has been located on chromosome 1. This QTL confers salinity tolerance at the vegetative stage and explains 64 to 80% of the phenotypic variance (Bonilla et al., 2002); it has also been detected in other varieties (Takehisa et al., 2004). A gene for salt tolerance at the vegetative stage has been identified in a similar position in the cultivar Nona Bokra and positionally cloned. The QTL for salt tolerance, *SKC1*, maintains K^+ homoeostatis in the salt-tolerant cultivar under salt stress, and the *SKC1* gene encodes a member of HKT-type transporters (Ren et al., 2005). The QTL for reproductive-stage salt tolerance have not been reported.

The QTL for Fe toxicity, P deficiency, and Al toxicity have been identified in different cultivars (Mackill, 2006; Nguyen et al., 2003; Wissuwa et al., 2002). A *Pup1* gene responsible for P uptake under low-P conditions derived from cultivar Kasalath has been mapped to chromosome 12 (Wissuwa et al., 1998). Marker-assisted selection is being applied for these reliable QTL at IRRI (Ismail et al., 2007). The QTL, for their ability to tolerate low N level and yield stability, have been identified even though the trait is complex in nature (Lian et al., 2005).

Submergence Tolerance

In south and southeast Asia, rice cultivation is severely affected by submergence in fields because of heavy monsoon rains and poor drainage. Rice plants are sometimes submerged for several days to several weeks, which can result in major yield loss. The *Sub1* QTL on chromosome 9 accounts for 70% of the phenotypic variation for survival under submergence and has been fine mapped on chromosome 9 (Xu et al., 2000). Three ethylene-response-factor–like genes at this locus have been identified, two of which are induced by submergence. The *Sub1A* gene was found to be responsible for submergence tolerance (Xu et al., 2006). The *Sub1A* gene has been successfully integrated into a popular indica cultivar (Swarna) by MAB strategy (Neeraja et al., 2007). This result clearly demonstrates that major QTL controlling tolerance of abiotic stresses can be used to improve varieties that are widely popular among farmers in the target regions (Mackill 2006). These varieties are usually popular because of their yield and quality traits but are sensitive to stresses. The single nucleotide polymorphism (SNP) markers are abundant in the rice genome (Hayashi et al., 2006). The CAPS marker, GnS2, has been designed based on an SNP in the *Sub1A* gene, and this marker amplified the specific band linked to submergence tolerance (Neeraja et al., 2007). Hence, SNP-derived CAPS markers for *Sub1A* gene can be effectively used in MAS for submergence tolerance breeding in rice.

Cold Tolerance

Tolerance of low temperature at both the vegetative and the reproductive stage is an important breeding objective for improving rice cultivars in the temperate and high-altitude areas of the tropics and subtropics. Major QTL on chromosomes 4 (*Ctb1*) and 8 (*qCTB8*) for cold tolerance at the booting stage were identified in a tropical japonica cultivar, Silewah, and markers have been used for introducing the tolerance gene (*Ctb1*) into japonica cultivars (Saito et al., 2004; Kuroki et al., 2007). Andaya and Mackill (2003b) identified eight QTL using a recombinant inbred line population derived from a japonica and indica cross and located the two strongest QTL on chromosomes 2 and 3 for booting-stage cold tolerance. A strong QTL for cold-induced necrosis and wilting tolerance has been identified on chromosome 12 and fine mapped (Andaya and Mackill, 2003a; Andaya and Tai, 2006). Another major QTL (*qCTS4*) associated with tolerance to yellowing and stunting of rice seedlings under cold stress accounts for 40% of the phenotypic variation. The *qCTS4* locus has been fine mapped to a 128-kb region on chromosome 4 and flanked by the markers RM6770 and RM7200 (Andaya and Tai 2007). Two major effect QTL for vegetative-stage cold tolerance have also been identified on chromosomes 1 and 2 (Han et al., 2004; Lou et al., 2007). The QTL conferring major effects on cold tolerance at vegetative and booting stages may be used in MAS for cold-tolerance breeding.

MARKER-ASSISTED SELECTION FOR GRAIN QUALITY

In recent years, consumers' preferences have shifted to higher-quality rice. Rice starch is composed of amylose and amylopectin, and the percentage of amylose is

an important characteristic of cooking quality. The *waxy* (*wx*) gene on rice chromosome 6 codes for granule-bound starch synthase and is largely responsible for amylose content (Wang et al., 1995). An SSR marker linked to *wx*, containing a variable number of cytosine-thymine (CT) repeats, is closely associated with amylose content and grain quality. Two SNPs in exons 6 and 10 were associated with differences in apparent amylose content and viscosity properties of rice cultivars (Larkin and Park, 2003).

Gelatinization temperature (GT) is another important grain quality trait. It is controlled largely by the starch synthase II gene (*Alk* on chromosome 6), and SNPs in this gene affect the activity of SSIIa (Umemoto and Aoki, 2005). A large-effect QTL was identified in the *Wx* locus (Fan et al., 2005). Improvement of four quality traits (amylose content [AC], gel consistency [GC], GT, and translucency) of indica hybrid rice was achieved using MAS (Zhou et al., 2003).

Grain aroma is an important trait of premium quality rice. A major gene on chromosome 8 has been cloned and is associated with the amount of 2-acetyl-1-pyrroline, the major aromatic component. Aroma is associated with loss of function of this gene, betaine aldehyde dehydrogenase, caused by an 8-bp deletion (Bradbury et al., 2005a). A perfect DNA marker has been developed from the *badh2* gene for MAS application (Bradbury et al., 2005b). In an extensive QTL analysis of the premium quality aromatic basmati cultivar Pusa 1121, several QTL for grain length and breadth, cooked kernel elongation ratio, AC, and aroma have been identified (Amarawathi et al., 2008). These QTL may be useful for improving high-quality aromatic rice breeding using MAS. A QTL study of cooking quality traits such as AC, GC, and GT was conducted in Chinese indica rice cultivars, and seven QTL were associated with the traits with high phenotypic variation suggesting improvement of cooking quality of rice (Zheng et al., 2008). Grain chalkiness is a complex but important trait of milling quality of rice. A major QTL for chalkiness has been identified on chromosome 8 and could be effectively used for MAS (Wan et al., 2005).

MARKER-ASSISTED SELECTION FOR AGRONOMIC TRAITS

The major challenge to rice breeders for increasing yield potential of cultivars is to improve the agronomic traits contributing to rice yield. It is difficult to achieve this goal by using conventional breeding technologies because of the epistatic interaction of different yield-contributing genes (Mei et al., 2006). Promising technologies such as the use of DNA markers for MAS of agronomic traits for yield and the use of a physiologically based crop simulation model to define the best combination of yield component traits for a range of agroecosystems are essential to increasing yield. The QTL are identified at one or two loci that explain a substantial portion of the increase in grain yield for the genes (*yld1.1* and *yld2.1*) derived from the wild rice progenitor, *O. rufipogon* (Xiao et al., 1996a). However, the QTL for grain yield under various environments explain a small portion of variability for grain yield and have little correspondence between QTL for yield components and actual grain yield (Septiningsih et al., 2003; Xiao et al., 1996b). In another study, SSR markers for yield-enhancing QTL have been used for improving yield of variety '9311' through MAS, and many backcross lines were obtained with high yield potential (Liang et al., 2004). Major and minor QTL for several yield components, such as plant height, panicle length, spikelets per panicle, grain weight, grain length, harvest index, and grain yield, have been reported (Bernier et al., 2007; Septiningsih et al., 2003). Among the several genes linked to QTL for yield, the QTL for grain length (*qGL3*), grain length and weight (*qGS3*), grain weight (*qgw3*), grain width and weight (*qGW2*), grain number (*qGn1*), and plant height (*Ph1*) have been dissected as simple Mendelian factors and fine mapped (Ashikari et al., 2007; Fan et al., 2006; Li et al., 2004; Song et al., 2007; Wan et al., 2006). For example, the cloned *GW2* gene on chromosome 2 is a RING-type protein that controls grain width and weight (Song et al., 2007). *GS3* from chromosome 3 encodes a putative transmembrane protein and controls grain length and width (Fan et al., 2006). These potential genes and alleles associated with DNA markers could be used in MAS to improve yield in rice.

FUTURE PROSPECTS

Plant breeding plays a key role in increasing rice production and productivity and maintaining food security. However, plant breeders encounter immense challenges to increasing production because of global warming and climate change, the development of new biotypes of diseases and insects, and several abiotic stresses that often reduce rice yield. Advances in rice biotechnology and genomics have paved the way to meeting the challenges and new genes for resistance to biotic and abiotic stresses, and major agronomic traits have been systematically identified using DNA markers. Following the completion of the rice genome sequence, full genome microarrays have been developed and applied to profiling expression of genes controlling the entire life cycle of rice. For some genes, functional molecular markers derived from fully characterized sequence motifs have been identified showing complete linkage with the target traits (Andersen and Lubberstedt, 2003). Agronomically valuable genes can be efficiently incorporated into the genetic background of elite indica and japonica cultivars without linkage drag through an appropriate breeding strategy combined with biotechnology and bioinformatics tools by MAS.

It is still difficult to accurately identify reliable QTL for complex traits like yield and tolerance to abiotic stresses. Through partitioning of total variation, it will be possible to associate QTL for each of the component traits of yield and tolerance to abiotic stresses. Fine mapping of component trait QTL and background selection for the recurrent parent genotype of advance backcross progenies would be useful for marker-assisted breeding of complex traits. Integration of desired genes or gene combinations in different elite cultivar backgrounds will eventually lead to a widening of the gene pool of rice as well as the development of improved cultivars suitable for various agroclimatic conditions. An efficient cost-effective MAS technology must be developed that will allow breeders to assess the genotype across the full genome to recombine genes of agronomic importance from diverse sources.

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