Current Applicable DNA Markers for Marker Assisted Breeding in Abiotic and Biotic Stress Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT Abiotic and biotic stresses adversely affect rice (*Oryza sativa* L.) growth and yield. Conventional breeding is a very effective method to develop tolerant rice variety; however, it takes a decade long to establish a new rice variety. DNA-based markers have a huge potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). The large number of quantitative trait loci (QTLs) mapping studies for rice has provided an abundance of DNA marker-trait associations. The limitations of conventional breeding such as linkage drag and lengthy time consumption can be overcome by utilizing DNA markers in plant breeding. The major applications of DNA markers such as MAS, QTL mapping and gene pyramiding have been surveyed. In this review, we presented the latest markers available for some of the most important abiotic and biotic stresses in rice breeding programs. Achieving a significant impact on crop improvement by marker assisted breeding (MAB) represents the great challenge for agricultural scientists in the next few decades.

Keywords Marker-assisted selection, Marker assisted breeding, Abiotic stress, Biotic stress, Rice, Resistant, Climate change

INTRODUCTION

Climate change is causing huge effects in all living creatures on earth. Few decades ago, scientists have proven that rising greenhouse gas emissions caused by the use of fossil fuels and industries lead to higher temperatures (Harvey 2015). Last year, the United Nations (U.N.) Climate Change Conference in Paris was able to come up with an agreement for a global climate effort. One of the key outcomes of the conference is to reaffirm the goal of limiting global temperature increase well below 2°C, while urging efforts to limit the increase to 1.5°C (Outcomes of the U.N. Climate Change Conference in Paris 2015). Climate change, increasing world population and food security all together requires rice scientists to address these issues in their respective researches.

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and maintaining stable rice production is

extremely important to feed the constantly growing human population in changing climate (Sasaki and Burr 2000; Maclean *et al.* 2002). Rice has been exposed to many environmental stresses. There are two broad areas of environmental stresses, abiotic (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic (pathogens and herbivore) factors (Gomez 2013). Environmental stresses such as heat, cold, drought, and salinity factors have extremely affected on average yield losses in world agriculture (Wang *et al.* 2003). Among these stresses, the single most common limiting abiotic stress is low water supply simply described as drought (Tester and Bacic 2005). Heat stress is one of the environmental stresses that limits biomass productivity and grain production of crops (Boyer 1982). Other significant abiotic stresses limiting growth of both forage grasses and cereals are salinity and acidity, next to these is low temperature (Tester and Bacic 2005). Furthermore, there

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was a huge impact on plants by biotic stresses such as pests and pathogens. Among biotic stresses, three diseases have been considered to be the most devastating worldwide in rice, bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, blast by *Pyricularia grisea*, and sheath blight by *Rhizoctonia solani*. Similarly, three groups of insects, stemborers (yellow stemborer *Tryporyza incertulas* and striped stemborer *Chilo suppressalis*), leafhoppers (*Marasmia patnalis* and *Cnaphalocrocis medinalis*), and planthoppers (mostly brown planthopper [BPH], *Nilaparvata lugens*), have been the most damaging pests (Jiang *et al.* 2012). In addition, infestation of white-backed planthopper (WBPH), *Sogatella furcifera* (Horvath) which is a phloem sucking insect of rice has affected rice growth and productivity.

Development of the disease-resistant or stress-tolerant plant is an important objective in rice breeding programs, because the production of rice can be constantly interfered by several major abiotic and biotic stresses. Rice and other crops have their own mechanisms to tolerate stress and there are many uncovered defense mechanisms at molecular level. The knowledge of physiology and molecular biology of stress tolerance in rice are helpful for the biotechnological improvement of rice productivity (Gomez 2013). One useful biotechnological tool that was developed throughout the years is DNA-based markers. From conventional breeding that takes place around 10 years to select a stable and desirable phenotype to marker-assisted selection (MAS), genetic functional markers were able to shorten rice varietal development. Another use of DNA-based markers is overcoming the barrier of “linkage drag” which refers to the presence of undesirable genes in the chromosomal region of the target gene thereby making it difficult to avoid such traits when using conventional breeding (Kottarachchi 2013). Also, economic analysis has shown the potential impacts of utilizing marker assisted breeding (MAB) by overcoming drawbacks of conventional breeding in rice that ultimately reduce the cost of production and promote economic growth (Kottarachchi 2013).

For this review, we present the recent DNA-based markers for some of the most significant abiotic and biotic stress tolerant genes and for MAS application in rice breeding programs like screening resistant plants from a

germplasm and genotyping breeding populations. These markers are proven to work from different studies published recently from germplasm screening, results of fine mapping and gene pyramiding methods.

DNA-BASED MOLECULAR MARKERS

DNA markers are defined as a fragment of DNA revealing changes in sequences, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool (Andersen 2013). DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion, and substitution) between different individuals (Andersen 2013). It is a part of the gene itself to be representative of the phenotypic traits and/or a fragment of flanking regions, which is located very close to the candidate gene, and are also considered useful tools that can be indirectly displaying the variable phenotypic traits. There are two basic methods to detect the polymorphism: southern blotting and polymerase chain reaction (PCR). Mohler and Singrün (2004) gave three key issues for an effective molecular marker in MAS: i) markers should co-segregate or map as close as possible to the target gene (within 2 cM), in order to have low recombination frequency between the target gene and the marker, ii) for unlimited use in MAS, markers should display polymorphism between genotypes with and without the target gene, iii) cost-effective, simple PCR markers are needed to confirm genotyping efficiency for the rapid screening of large populations. Lateef (2015) simplified the different marker systems based on generation development. The first and low-throughput marker system is restriction fragment length polymorphisms (RFLPs), medium-throughput marker systems include random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs) while the latest generation is the high-throughput marker system comprised of single nucleotide polymorphisms (SNPs), KBioscience Competitive Allele-Specific PCR (KASpar), and genotyping by sequencing (GBS). Based on various MAS breeding approaches, RFLPs are the best marker type

for many purposes, but are low throughput technology and have high cost of genotyping. Therefore, PCR-based markers like RAPDs and AFLPs are faster and cheaper, and can also be applied in genetic diversity and gene mapping, however, their limiting factors are lack of reproducibility and specificity (Lateef 2015). With the availability of rice whole genome sequence and re-sequencing data, SNPs are exhorting in construction of high resolution genetic maps, investigation of population evolutionary history and discovery of marker-trait associations. The use of SNPs is further exemplified in KASPar assay, which is the patented SNP genotyping system from KBioscience based on fluorescent resonance energy transfer (FRET) (Robinson and Holme 2011). It allows detection of SNPs without a separation step coupled with the use of competitive allele specific PCR, thus, the KASP system offers a superior system for determination of SNP or insertion/deletion genotypes (Robinson and Holme 2011). As different kinds of markers were described above, each kind can be more advantageous to one breeding design and less valuable to another breeding method.

Conventional plant breeding has depended on phenotypic factors for the selection/confirmation of agronomic traits. MAB approaches to conventional breeding substantially has reduced the plant breeding cycles and increased the precision and efficiency of new cultivar development in plant breeding programs (Ribaut and Hoisington 1998; Morris *et al.* 2003).

ABIOTIC STRESSES

Drought

Water is the carrier of nutrients and minerals from soil through roots to all parts of the plant for distribution, aside from the transport of biomolecules. Drought is one of the most common stresses that impact growth and development of plants under water-limiting environments. The drought tolerance of plants can be divided into following various types including drought avoidance, escape, recovery, and dehydration tolerance (Kramer and Boyer 1995). Drought avoidance includes root depth, which can absorb available water in deep soil and dehydration

tolerance consists of plant's capability such as drought recovery and growing again (Salekdeh *et al.* 2002). As drought mainly occurs in upland, selection for a well-developed root system with long and thick roots should improve the drought tolerance of upland rice because the plant will be able to absorb stored water in the deep soil layers. Phenotypic selection for root morphological traits in conventional breeding programs is impractical. The use of molecular markers could provide a useful tool to support phenotypic selection. Several mapped populations were developed to detect quantitative trait locus (QTLs) influencing root morphology and other drought-related traits that could be used in MAS to improve upland varieties. Uga *et al.* (2011) detected a new major QTL controlling ratio of deep rooting (RDR) on chromosome 9 by using 117 recombinant inbred lines (RILs) between lowland cultivar IR64 and upland cultivar Kinandang Patong (KP). The RDR QTL *Dro1* (deeper rooting 1) was mapped within 1 cM between RM24393 and RM7424. They suggested *Dro1* QTL, which is associated with root growth angles, would be useful for detecting an increased deep rooting trait under limited water conditions. In a report by Lang and Buu (2008), they worked on the mapping and MAS of major genes for drought stress in rice, they found out drought tolerance in the region that indicated the occurrence of recombination between segments derived from 329 plants of BC₂F₂ from OM1490/WAB880-1-38-18-20-P1-HB. Twenty markers were used to genotype the 329 BC₂F₂ plants. SSR markers located at the drought recovery score (DRS) genes between RM201 (0.4 cM) and RM328 (13.8 cM) on chromosome 9 (Lang and Buu 2008). The target segment on chromosome 9 (RM201) significantly related to increase root length and drought tolerance under drought stress treatment (Lang and Buu 2008). Another SSR marker that can be used for MAS is RM8085, it is the only marker that showed complete co-segregation among individual RILs. RM8085 was mapped on chromosome 1 at 139.9 cM and is linked to leaf rolling and leaf drying under drought stress (Salunkhe *et al.* 2011). Shamsudin *et al.* (2016) used three drought yield QTLs, *qDTY*_{2.2}, *qDTY*_{3.1}, and *qDTY*_{12.1} with consistent effect on grain yield under reproductive stage drought stress in gene pyramiding work for an elite Malaysian rice

cultivar. These three QTLs have effectively worked in foreground selection in each of their breeding generation. The markers mentioned above can be applied in QTLs related to DRS, leaf rolling, leaf drying and drought yield.

Salinity

Soil salinity is an increasing limiting factor in rice growing areas. The precise effect of salinity on the rice harvest is determined by a complex interaction of various factors including the severity, timing, and duration of the stress (Thitisaksakul *et al.* 2015). Salinity tolerance at seedling, vegetative, flowering and ripening stages of rice seems to be managed by independent genes (Linh *et al.* 2012). *Saltol* is a major QTL and was identified in the salt-tolerant cultivar Pokkali. Its location was found on chromosome 1. This QTL confers salinity tolerance at the vegetative stage and explains from 64% to 80% of the phenotypic variance (Linh *et al.* 2012). The SSR marker, RM8094 found in *Saltol* is considered to be superior for analysis of genetic diversity (Nejad *et al.* 2008). Rice genotypes with the Pokkali band type for locus RM8094 marker were either highly tolerant or tolerant to salinity stress at the seedling stage (Nejad *et al.* 2008). Aside from RM8094, some of salt-tolerant genotypes had the Pokkali marker allele for RM10745 as well. Nejad *et al.* (2008) have proven in their results that RM8094 and RM10745 are useful for MAS of *Saltol* QTL. In a more recent study, three sequence tagged site (STS) markers were presented for MAS, from the genome sequences of *SKC1*, *SalT*, and *DST*. STS markers were developed based on Insertion/Deletions (InDels) between Nipponbare and 9,311 genome sequences of the previously mentioned gene names. STS markers, Wn11463 and Wn11466, were designed based on 4 bp and 17 bp InDels downstream of *SKC1* (LOC_Os01g20160); Wn13900 was based on a 4 bp InDel upstream of *SalT* (LOC_Os01g24710); Wn13902 and Wn13903 were based on 7 bp and 8 bp InDels in the *SalT* coding region; Th32637 was based on a 3 bp InDel upstream in *DST* (LOC_Os03g57240); and Th32638 and Th32639 were based on 12 bp and 18 bp InDels in the coding region of *DST* (Emon *et al.* 2015). There were some efforts towards breeding salinity tolerance in plant via MAS (Ashraf *et al.* 2012) for the

development of salt tolerant cultivars. It is expected that the utility of MAS for breeding of salinity tolerance traits will be increased.

Chilling stress

Rice is commonly grown in tropical areas, thus it is more sensitive to low temperature. The optimum temperature for seed germination and early seedling growth is from 25°C to 35°C (de los Reyes *et al.* 2003). Chilling stress affects rice growth throughout its development from germination until harvest. Hence, development of chilling stress tolerant cultivar is a common breeding goal. And of course, to aid fast and effective selection of lines with chilling stress tolerance, MAS is a useful and precise strategy. In a review done by Zhang *et al.* (2014), they summarized various QTLs related to chilling tolerance at different growth stages. Here, we'll simply present the markers that can be directly used for MAB. In one research work, a RIL population derived from a japonica/indica cross was assessed for chilling tolerance at early seedling stage at 10°C. Main-effect QTL for the trait was mapped via composite interval mapping using a linkage map with 198 marker loci. The group was able to identify four putative QTLs for the trait, which were mapped on chromosomes 1, 3, 7, and 11. Among these QTL, *qSCT-11* showed a large additive effect on the trait, explaining 26% to 30% of the phenotypic variation in the treatment of 13 days at 10°C with a LOD of 16-19. This QTL was closely linked to SSR marker RM202 and its positive allele came from the parent Lemont (Chen and Li 2005). This allele would be useful for the improvement of rice chilling tolerance through MAS. There are also molecular markers associated with chilling response related phenotypes, like, membrane integrity, photoinhibition and visual assessment of damage (Bonnecarrère *et al.* 2014). SSR marker RM22034 is associated with membrane integrity, RM6547 and RM14978 with photoinhibition, and RM144 with visual assessment of damage (Bonnecarrère *et al.* 2014). All the markers are currently available for marker assisted selection in rice breeding programs located in temperate regions (Bonnecarrère *et al.* 2014). Three more QTLs, *qPSST-3*, *qPSST-7*, and *qPSST-9* were verified from ten chilling-tolerant lines with spikelet fertility (SF) of 51% to

81% compared to 7% (chilling-sensitive parent) and 73% (chilling-tolerant donor) (Jena *et al.* 2012).

Heat

The year 2016 is the hottest year to date. The temperature recorded in the month of June across global land and ocean surfaces was 1.62°F (0.90°C) higher than the 20th century average of 59.9°F (15.5°C). This was the highest for June in the 1880-2016 record (Climate Central 2016). Heat stress is one of the growing major factors that affect crop productivity negatively. High temperature is detrimental to both the vegetative and reproductive stages of rice (Pareek *et al.* 1995). In rice, once the temperature goes 5°C higher than the threshold level at flowering stage, it can induce floret sterility thereby affecting yield losses with maximum yield loss up to 80% (Ahmad *et al.* 2014). Through molecular breeding strategy, studies have been carried out to create molecular map of heat tolerance QTLs at booting, flowering, grain filling, and ripening stages in rice. In different rice population used by Ye *et al.* (2015), they identified QTL *qHTSF4.1* consistently present across different genetic backgrounds (IR64/Giza178 and IR64/N22 populations; three-way-cross population IR64//Milyang23/Giza178; BC₅F₂ population with clean background of IR64) and solely mentioned that it could be an important source for enhancing heat tolerance in rice at flowering stage. The SF under high temperature was able to increase at 15% by *qHTSF4.1* (Ye *et al.* 2015). In a more recent study, 48 stable lines (17 KMR3/*Oryza rufipogon* introgression lines [KMR3 ILs], 15 Swarna/*Oryza nivara* ILs [Swarna ILs] along with their parents, Nagina 22 [N22], and its 4 ethyl methanesulfonate induced mutants and 7 varieties) were examined for heat tolerance during dry and wet season (Prasanth *et al.* 2016). They found out that 18 of 49 SSR markers linked to SF were valid for five traits related to heat tolerance. RM430 and RM210 in dry season and RM229 in wet season were significantly associated with both SF and its heat susceptibility index (HSI) under heat stress. RM430 was also significantly related in both yield per plant (YP) and its HSI in dry season (Prasanth *et al.* 2016). For an actual application of SSR markers in breeding programs for heat tolerance in rice, a group from Vietnam used RM3735 as one of their six

markers to select heat tolerant lines in backcross population at flowering stage (Buu *et al.* 2013a; Buu *et al.* 2013b; Lang *et al.* 2015). Rice breeders can employ these QTLs in pyramiding genes to increase heat tolerance in rice.

BIOTIC STRESSES

Bacterial blight

Bacterial leaf blight (BLB) is the most destructive disease, which affects greatly the rice yield loss. Enhancing genetic resistance of rice has proven to be the most effective method of controlling BLB disease (Khan *et al.* 2014a). To date, at least 38 BLB resistance genes (R genes) (both dominant and recessive) have been identified (Bhasin *et al.* 2012; Kumar *et al.* 2012) and designated in a series from *Xa1* to *Xa38* (given the prefix *Xa* for *Xanthomonas*) (Khan *et al.* 2014a). There are various DNA markers that have been already developed for many *Xa* genes. Hajira *et al.* (2016) have developed simple PCR-based functional markers for *xa13* and *xa5*. For *xa13*, they designed a functional, PCR-based marker, *xa13-prom* targeting the Indel polymorphism in the promoter of candidate gene, *Os8N3* located on Chr. 8 of rice. For *xa5* a multiplex-PCR based functional marker system, named *xa5FM*, consisting of two sets primer pairs targeting the 2-bp functional nucleotide polymorphism in the exon II of the gene, *TFIIAγ5* (candidate for *xa5*), has been developed (Hajira *et al.* 2016). Both *xa13-prom* and *xa5FM* can differentiate the resistant and susceptible alleles for *xa13* and *xa5*, respectively in a co-dominant fashion. Using these two functional markers along with the already reported functional, PCR-based marker for *Xa21* (pTA248), Hajira *et al.* (2016) designed a single-tube multiplex PCR based assay for simultaneous detection of all the three major resistance genes and demonstrated the utility of the multiplex marker system in a segregating population.

In the study of Hur *et al.* (2013), they cloned and sequenced the *Xa3/xa3* gene in the Korean cultivars Hwayeong, Ilmi, and Goun, conferring resistance or susceptibility to bacterial blight. *Xa3* is involved in the receptor-like kinases, which contain an extracellular leucine-rich repeat (LRR) and an intracellular serine-

threonine kinase domain (Hur *et al.* 2013). The LRR sequence of the LRR-containing R proteins is the major determinant of pathogen recognition (Dangl and Jones 2001) and showed differences between the resistant and susceptible proteins (Xiang *et al.* 2006). Thus, the difference in this region could be the SNP region useful for a functional marker. So Hur *et al.* (2013) designed functional markers (BB3-SR and BB3-RF) in which the 3' terminal nucleotides corresponded to the SNP regions. BB3-SF was located in the upstream promoter region of the *Xa3* cDNA, and the BB3-RR primer was within exon 1 (Hur *et al.* 2013). In addition, molecular marker in *Xa4* was used by Luo *et al.* (2012), as they were developing near-isogenic line carrying both *Xa4* and *Xa21* loci, they used RM224 (Sun *et al.* 2003). Further confirming the effective selection of RM224, two homozygous F₂ individuals were identified with the *Xa4* locus from the 10 F₂ plants that were homozygous at the *Xa21* and *Xa27* loci (Luo *et al.* 2012). Lastly, in introgression of bacterial blight resistance genes *Xa7*, *Xa21*, *Xa22*, and *Xa23* into hybrid rice restorer lines by molecular MAS (Huang *et al.* 2012), they will adopt the marker for *Xa7*. The resistance gene *Xa7* provides dominant resistance against the pathogen with avirulence (*Avr*) gene *AvrXa7* (Chen *et al.* 2008). As by-products of fine mapping done by Chen *et al.* (2008), a number of tightly linked markers for the *Xa7* gene had been developed; eight markers (RM20573, RM20580, RM20582, RM20595, RM20601, RM20603, RM20608, and RM20612) tightly linked to *Xa7* (genetic distance < 2 cM) can be used in MAB programs. As adopted by Huang *et al.* (2012) in their introgression work, they used RM20593 to confirm the presence of *Xa7*. Since there are many studies related to improving bacterial blight resistance in rice, the markers suggested here can be used to further shorten the breeding cycles.

Blast

Rice blast is another devastating disease causing major yield losses globally. Singh *et al.* (2015) reported that there are more than 100 major blast resistance genes from *japonica* (45%), *indica* (51%), and other (4%) genotypes that have been identified and documented, and many blast resistant varieties have been developed; however, stronger

virulent isolates of rice blast fungus have emerged. So up to now, rice cultivar with broad-spectrum of resistance to highly adaptive virulent isolates/races remains a challenge. In a MAS breeding for blast resistance, F₃ population derived from the cross of Pongsu seribu 2 (resistant) and Mahsuri (susceptible) allowed the association of four SSR markers RM413, RM1233, RM8225, and RM5961 to blast resistant genes (Ashkani *et al.* 2012). Because these markers had high selection accuracy for resistant plant sources, they were suggested to be used in MAS for the resistant gene. Lei *et al.* (2013) identified and mapped two blast resistance genes, *Pi60(t)* and *Pi61(t)*, in Chinese rice cultivar 93-11 using F₂ and F₃ populations derived from a cross between the susceptible cv. Lijiangxintuanheigu and resistant cv. 93-11. *Pi60(t)* and *Pi61(t)* are both embedded in recombination-suppressed regions with several clustered NBS-LRR genes. In addition to these SSR markers, Khan *et al.* (2014b) suggested gene-based molecular markers for MAS in identifying blast resistance gene in fragrant rice. In their study they found out that the reaction pattern of single-spore isolate of *Magnaporthe oryzae* to differential varieties showed that *Pish*, *Pi9*, *Pita-2*, and *Pita* are the effective blast-resistant genes against the tested blast isolates in Bangladesh. After using the four gene-based molecular markers, among the 16 germplasms selected, they found BRRI dhan50, Chinigura, and Bawaibhog that contain more than one target gene and the remaining germplasm contained only one target gene, either *Pish* or *Pita*. Recently, Singh *et al.* (2015) applied 10 SSR markers linked to blast resistance genes (*Pi-9*, *Pi-1*, *Pi5-(t)*, *Piz-5*, *Pi-b*, *Pi-ta*, *Pi33*, *Pi-27(t)*, *Pitp(t)*, and *Pik^h*) in 192 rice accessions to identify which accession contain the most number of resistance genes. They have identified as much as eight blast resistant genes in more than one accession. They also concluded that the linked markers used in their study are well established and effective, thus making them a marker of choice for molecular screening of rice blast resistance genes. We have enlisted blast marker resistant genes for MAS in Table 1 (Jia *et al.* 2002; Fjellstrom *et al.* 2004; Hayashi *et al.* 2006; Qu *et al.* 2006; Takahashi *et al.* 2010; Ashkani *et al.* 2012)

Table 1. Markers used in MAS for abiotic and biotic stresses.

	Marker	QTL/gene	Forward (5'-3')	Reverse (5'-3')	Reference
Abiotic stress					
	Drought				
	Salinity				
	RM201		CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCATA	Lang and Buu 2008
	Wn11463	<i>SKC1</i>	TCCTCCTTCTCTCGGAAC	GATCCACTCGTCACAGG	Emon <i>et al.</i> 2015
	Wn11466		GCTTCCCAATAATTTCGACCT	CCCACCAATACTAAAGATCCTG	
	Wn13900	<i>SalT</i>	GTACGGGTTACATCCTC	ACCTCTATTAAATTCACATCCA	
	Wn13902		CACCAGCGTCATACTCT	CAAAACTGAGTAGGAATACCGTGA	
	Wn13903		CTGTATCAACTGCATTCGTGT	GCTTGGTCAAACCTCGT	
	Th32637	<i>DST</i>	TCGTATAGTAGGCTTTCATGGC	TTTCACAGGTGCGAGAGCTT	
Cold	Th32638		AGAGAAAGCCAAAGAAATCGAC	TCCAAGCTCCACCTACTCC	
	Th32639		CTATTGGCTTCGCAAGGACA	CGCCCACTTTAATCATATATCCCT	
	RM231	<i>qPSS7-3</i>	CCAGATTATTCTCTGAGGTC	CAC TTGCATAGTTGTGCATTG	Jena <i>et al.</i> 2012
	RM1377	<i>qPSS7-7</i>	ATTAGATAGATGAGGGGGG	GTGCTGTACGATGTGATCG	
	RM24545	<i>qPSS7-9</i>	AGAGGAGAGGAGGGGGAAGG	CGAGCAAGAGGAAGGGGATAAGG	
	M4	<i>qHTSF4.1</i>	CATGTGTTTCTTGTAACCTTTAG	TCAAGACTATTTTATCAATCGTTAC	Ye <i>et al.</i> 2015
			CTCTAGGTTTCAACTGAGCA	AAGAGATCGCATGTGGAC	
	RM 229		CACTCACAGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	Prasanth <i>et al.</i> 2016
	RM430		AAACAACGACGTCCTGATC	GTGCTCCGTGGTTATGAAC	
	RM210		TCACATTGGTGGCATTG	CGAGGATGGTTGTTCACTTG	
Biotic stress	RM3735		GCGACCGATCAGCTAGCTAG	ATAACTCTCTCCCTTGCTGCC	Buu <i>et al.</i> 2013a
Blast					

Table 1. Continued.

	Marker	QTL/gene	Forward (5'-3')	Reverse (5'-3')	Reference
Blast	YL155/87	<i>Pita-Re</i>	AGCAGGTTATAAGCTAGGCC	CTACCAACAAGTTTCATCAAA	Jia <i>et al.</i> 2002
	YL183/87	<i>Pita-Su</i>	AGCAGGTTATAAGCTAGCTAT	CTACCAACAAGTTTCATCAAA	
	pBA14	<i>Pit9</i>	GCAGTGTCATCTTGTCTCC	TGGTGCACTCAGAAAGAA	
	NBS-O/U		TACAACCACTACCATCCCAT	CTCAGAACCTGCAAGTCTCG	
Sheath blight	k6441	<i>Pik-m</i>	TGTAAAATACTTTCTATGCGCAGGC	GTTTATGGAGAGATGTCGCTG	Hayashi <i>et al.</i> 2006
	k39575	<i>Pik-p</i>	GGTGTGGGAACCTGAACCTG	GGTGTGGGAACCTGAACCTA	
	t256	<i>Pit</i>	GGATAGCAGAAGAACTTGAGACTA	CATGCTTTCAACATAAAGAGTTCTC	
		<i>qSb-9TQ</i>	ACCTGTACTTGTCTCCTT	CTTTGACCGTTCTGTTATT	
BPH	RM205	<i>qshb9.2</i>	CTGTTCTGTATGGGAGCAG	CTGGCCCTTACCGTTTCAGTG	Zuo <i>et al.</i> 2014
	RM336	<i>qshb7.3</i>	TCTGTGCTTGTGCTCTTG	AGTCATTTGGCTCAACAGCC	Yadav <i>et al.</i> 2015
	c3-14	<i>QBph3</i>	GGCAAAATAGACGGCAG	GAATATGCATTTTGTGTTGGAG	Yadav <i>et al.</i> 2015
	IN76-2	<i>Bph14</i>	CTGCTGCTGCTCTCGTATTG	CAGGGAAGCTCCCAAGAACAG	Hu <i>et al.</i> 2016
WBPH Yellow stemborer	RM261	<i>Bph15, QBph4.1</i>	CTACTTCTCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	Du <i>et al.</i> 2009
	g12140-2	<i>Bph15</i>	ACCAAAACACGGTGGATGAGA	AATGGAAGAGAGGAGCAACAG	Hu <i>et al.</i> 2016
	xe4-27	<i>Bph15, QBph4.1</i>	GCATAAGCGCCCTAGGC	GCTAGTTGCAGGCACGC	Lv <i>et al.</i> 2014
	20 M14	<i>Bph15</i>	ATGCTGACGGTGTAGGAGT	CAGTCATCCACACAACCTTGA	Hu <i>et al.</i> 2016
	RH7	<i>Bph17</i>	CTTGGTTCCTAGGAGAAAG	TGAGGTAAACCCGAAGTGGC	Lv <i>et al.</i> 2014
	RHC10	<i>Bph17</i>	CAATACGGGAGATTGGAGT	TTGGGAAGCATACGAGTGA	Liu <i>et al.</i> 2010
	IN156	<i>Bph15, Bph17</i>	AGGTGAAGCTGATGCTTG	CGATACTTATTGCAACACAC	Liu <i>et al.</i> 2010
	B43	<i>Bph20</i>	ACTCCAATTGGTTCCTGTGG	TGGACTAAAAGCCGATGAGC	Hu <i>et al.</i> 2016
	RM119	<i>Bph6</i>	CATCCCCCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG	Rahman <i>et al.</i> 2009
	S00310	<i>Bph25</i>	CAACAAGATGGACGGCAAGG	TTGGAAGAAAAGGCAGGCAC	Qiu <i>et al.</i> 2010
	RM589	<i>Bph3</i>	ATCATGTGCGGTGGCTTAAC	CAGGTTCCAACCCAGACACTG	Myint <i>et al.</i> 2012
	RM260	<i>Bph10</i>	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	Jairin <i>et al.</i> 2009
	RM313	<i>Bph10</i>	TGCTACAAAGTGTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC	Ishii <i>et al.</i> 1994
	RM463	<i>Bph2</i>	TTCCCTCTCTTTTATGCTGC	TGTTCTCTCAGTCACTGCG	Ishii <i>et al.</i> 1994
	RM6869	<i>Bph2</i>	GAGCTCCTTGTAGTGACCCG	ATCAGCTCGCCAGCTTC	Sun <i>et al.</i> 2006
	RM6217	<i>Bph9</i>	CGCAGATGGAGATTCTGAAGG	ACAGCAGCAAGAGCAAGAAATCC	Su <i>et al.</i> 2010
	IN187	<i>Bph18, Bph9</i>	GACCCCTTCGAGTCTAAGAAC	CTTCTTTGAATCATAGACAG	Hu <i>et al.</i> 2016
	7312.T4	<i>Bph18</i>	ACGGCGGTGAGCATTTG	TACAGCGAAAAGCATAAAGAGTC	Jena <i>et al.</i> 2006
	RM3331	<i>Bph18</i>	CCTCTCCATGAGCTAATGC	AGGAGGAGCGGATTCTCTC	Suh <i>et al.</i> 2011
	RM5479	<i>Bph21, Bph26</i>	AACTCTGATGCTCCTCTAAG	TCCATAGAAAACAATTGTCG	Myint <i>et al.</i> 2012
	B121	<i>Bph21</i>	CGTCGTACATTCTGAAATGGAG	GGACATGGAGATGGTGGAGA	Rahman <i>et al.</i> 2009
	RM11669		AAACCGTTCAGGGAGACTGACC	TCGCTGTATCCATCCATCCATCC	Yi <i>et al.</i> 2014
	RM241		GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG	Selvi <i>et al.</i> 2003

²⁾Resistant. ³⁾Susceptible.

MAS: marker-assisted selection, QTL: quantitative trait locus, BLB: bacterial leaf blight, BPH: brown planthopper, WBPH: white-backed planthopper.

Sheath blight

In a comprehensive review done by Yellareddygar *et al.* (2014) on disease and pathogen management approaches in rice sheath blight (ShB), ShB is caused by *R. solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* [Frank] Donk), and is a destructive disease worldwide that causes significant yield loss and quality degradation. Aside from rice, it can also infect other crops like lettuce, tomato and maize. This fungus is a universal soil saprotrophic and facultative plant parasite. The sclerotia of *R. solani* can survive even in unfavorable conditions. Once the field is set up with plants, the sclerotia will attached to the soaked organ of rice and starts the disease development. To combat almost all kinds of rice diseases, integrated disease management in the field is still highly recommended.

There are about 50 ShB resistance QTLs that have been mapped to all the 12 rice chromosomes and pyramiding of diverse ShB resistance QTLs could help achieve higher levels of resistance to ShB (Yadav *et al.* 2015). One study showed pyramiding of ShB resistance, *qSB-9TQ* and tiller angle *TACITQ* resulted to NILs carrying both QTLs showing more resistance than the NILs containing only one of them (Zuo *et al.* 2014). Pyramiding major SB resistance QTLs like *qShB9-2* (Liu *et al.* 2013) can give rise to an efficient and effective host resistance. The markers RM215 and RM245 were closely linked to *qShB9-2* and can be used as reliable markers for improving ShB resistance in rice breeding programs using MAS, for fine mapping, and for positional cloning (Liu *et al.* 2013). In addition, two SSR markers namely RM336 and RM205 were found to be closely associated with the major QTLs *qshb7.3* and *qshb9.2* respectively and were attested as well in BC₁F₂ population by bulk segregant analysis approach (Yadav *et al.* 2015). Lastly, Zeng *et al.* (2014) constructed the first physical map of previously reported ShB-QTLs, plant height-QTLs, and heading date-QTLs in which this map can be more conveniently used in MAB programs.

Planthoppers

From the top three major diseases in rice, we go to two of the most serious pests in rice, planthoppers and stem borer. There are two planthoppers that infest rice, BPH and

WBPH. In many areas, BPH frequently occur in numbers large enough to cause complete drying of the crop, but even small populations reduce rice yields. In addition to direct feeding damage, planthoppers are also vectors of most of the currently known rice virus diseases (Pathak and Khan 1994). Just like in abiotic and biotic stresses, the most economical and environment-friendly strategy to control insect pests is to grow genetically resistant rice varieties.

Because of the development of molecular markers (SSR, InDel, SNPs) and functional genomics, the genetic studies of BPH resistance in rice have increased. Currently, more than 30 BPH resistance loci have been reported (Fujita *et al.* 2013) and four BPH resistance genes (*Bph14*, *Bph26*, *Bph17*, and *bph29*) have been cloned (Du *et al.* 2009; Tamura *et al.* 2014; Liu *et al.* 2015; Wang *et al.* 2015). MAB and conventional breeding have enabled resistance genes to be combined in elite rice varieties to improve BPH resistance and its durability. Early this year, a review of recent progress on the genetics and molecular breeding of brown plant hopper resistance in rice (Hu *et al.* 2016) was written and it presents the positional information of BPH-resistance genes and several markers available for MAB (Table 1). In addition, there are reported SSR markers that are specific for BPH biotypes. Shabanimofoad *et al.* (2015) have concluded that their chi-square analysis showed a good fit to a ratio of 3:1 for the segregation of resistance and susceptibility for biotypes 2 and 3 of BPH. SSR markers RM545, RM401, RM22, RM5953, RM210, RM242, RM217, RM224 and RM1103 were significantly associated with BPH resistance to biotypes 2 and 3 of BPH in rice ($P \leq 0.01$). These markers showed high selection accuracy for resistant plant sources with confirmation of resistance effect of about 17% to 20% for phenotypic variation and can be used in MAS for the resistant gene (Shabanimofoad *et al.* 2015).

Relative to BPH, WBPH is less concerned in research, resistance genes *Wbph7* and *Wbph8* happens to work as well in resistance to BPH (Tan *et al.* 2004). In addition to these two genes, an ovicidal gene (*Ovc*) shows a general response to WBPH and BPH (Fujita *et al.* 2013). According to Fujita *et al.* (2013), all other WBPH genes have been identified by segregation analysis but have undergone no further genetic analyses and have no iden-

tified DNA markers. But there is a recent study that applied SSR markers in MAS for WBPH, that study showed 64.9% of coincidence rate of bioassay and MAB efficiency after using SSR marker RM11669 for WBPH resistance when used in double haploid lines derived from the cross of JSNDH13 with CNDH32 (Yi *et al.* 2014).

Stem borer

There are two common stem borers in Asia, yellow stemborer (*Scirpophaga incertulas*) and white stemborer (*S. innotata*). In general, stem borers are polyvoltine, meaning they produce several broods in a year, but the number of generations in a year depends on environmental factors, primarily temperature, rainfall, and crop availability (Pathak and Khan 1994). In places having distinct generations, the first generation usually appears when the plants are in seedling stage; the population increases in subsequent broods and the later generations are often the ones that cause serious damage. This is why the borers are more destructive to the late-planted crops, or the second crop where double cropping is observed (Pathak and Khan 1994). The damage of stem borer becomes evident only as deadheart and whitehead, significant losses are also inflicted by larvae that feed within the stem without severing the growing plant parts at the base. Such damage results in reduced plant vigor, fewer tillers, and many unfilled spikelets (Pathak and Khan 1994). Selvi *et al.* (2002) reported the identification of RAPD markers associated with yellow stemborer resistance and susceptibility, the markers C₁₃₂₀ and K₆₆₉₅ were linked with resistance and AH₅₆₆₀ and C₄₁₃₀₀ with susceptibility. The markers K₆₆₉₅ and AH₅₆₆₀ were linked to the genes at distances of 12.8 cM and 14.9 cM, respectively, their sufficiently closer linkage to the genes regulating the trait and their unambiguous scorability in the resistant and susceptible cultivars were tested. Also, they investigated and described the possibility of screening for yellow stemborer resistance using micro satellite markers. This marker, RM241, located on chromosome 4, was also found to be associated with the trait and is closed to the genes for yellow stemborer resistance (Selvi *et al.* 2003).

Fig. 1. Summary of QTLs/genes presented here as DNA markers in MAB for abiotic and biotic stress tolerance.

BLB: bacterial leaf blight, BPH: brown planthopper.

CONCLUSION

In this review, we have summarized some of the recently used DNA-based markers for MAS in abiotic and biotic stress tolerance in rice as shown in Fig. 1. The efficiency of MAS largely depends on the distance between molecular markers and genes/QTLs associated with target traits. The development of useful markers tightly linked to abiotic and biotic stress resistance traits is accomplished by QTL mapping experiments. Generally, the markers are validated in fine mapping studies. MAB greatly increase the efficiency and effectiveness of breeding. By determining and developing DNA markers for target genomic regions, desired individuals possessing particular genes or QTLs can be identified in germplasm collections based on genotyping rather than phenotyping only. Based on the readings made, in any abiotic and biotic stress tolerance, stronger resistance can be attained by pyramiding validated tolerance genes and QTLs. MAS refers to selection by DNA markers linked to QTLs associated target genes. DNA-based genetic markers are recently expected to play an important role in the future of MAB and molecular genetics analysis for development of stress-tolerance and

disease-resistance in plants through molecular linkage maps. The information of markers for abiotic and biotic tolerant traits gathered here will provide a one-stop read for practical rice breeders and the like to aid in their MAB.

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