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Current Applicable DNA Markers for Marker Assisted Breeding in Rice (*Oryza sativa* L.)

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Abstract

Rice, (*Oryza sativa* L.) account as the second cereal most cultivated in the world. Unfortunately, global rice production is rendered by significant number abiotic and biotic stresses. Breeding for resistant variety through conventional breeding is an economical method; generally, it takes at least 10 years to release a new rice variety. Advance technology in molecular marker had revolutionized and irreversibly changes the disciplines of plant genetic and breeding. Integration of DNA-based markers in selection process enhances the effectiveness and accuracy of conventional plant breeding. It offers a novel tool for discovering and tagging alleles and genes specifically in plant. Ubiquitous of DNA marker-trait associations for diverse crops species are available with the findings of many quantitative trait loci (QTLs) mapping studies. The linkage drags, and time-consuming in conventional breeding can minimize with the application of DNA markers in plant breeding. The utilization of DNA marker in QTL mapping, MAS and gene pyramiding has been investigated. In this chapter, we discussed the recent utilizing markers in rice breeding program against abiotic and biotic stresses. In a few decades, molecular marker assisted breeding (MAB) provide a boundless task for breeders in attaining an important impact on crop development.

Keywords: abiotic stress, biotic stress, marker-assisted selection, rice, resistant

1. Introduction

Oryza sativa is an essential food crop contributing 30% of calories for at least 62.8% of the world population. Presently, rice was cultivated over 167.13 m ha within 114 countries with annual production of 486.62 mt (on milled basis) [1]. Majority of population in Asian consumed rice as a basic staple crop with over than 90% of rice were produced in Asia [2]. Revolution in Green technology played an important role in adoption of major increasing in rice production over last four decades. Approximately 450 million tons to 650 million tons of world rice consumption is projected to surge by 2011 and 2050, respectively [3].

Scientist and plant breeder are facing a new challenge in crop improvement due to climate change issue including limitation of water and cultivated land, new emerging pathogen and pest resulted serious yield losses. Among the major constraints, blast disease, bacterial blight, sheath blight, tungro viruses, brown spot and a number of grain discoloration diseases [4] accounted for serious rice losses. Several

amendments can be implemented to overcome these limitations such as breeding high yielding varieties, durable resistance to diseases and tolerance to abiotic stresses [5]. Rice yield potential can be enhanced by using numerous approaches such as conventional hybridization, ideotype breeding, heterosis breeding, wide hybridization and molecular breeding [6]. In molecular rice breeding, there are two main strategies can be used by applying the biotechnology concept which are MAS and Genetically Engineering. MAS like genetic engineering is a technique used to introgress targeted gene however its offers tools for specific selection for further breeding in existing plant material. The field of plant genetics and breeding has irreversibly changed with the development of DNA (or molecular markers) [7].

Since past two decade, revolutionized in molecular marker to enhance the effectiveness in breeding and to significantly shorten the development time of varieties bring about the concept of molecular marker assisted selection (MAS) as an efficient plant selection [8–10]. A genetic marker is any noticeable character or otherwise assayable phenotype, for which alleles at individual loci segregate in a Mendelian manner. The genetic markers covered include (1) morphological markers (2) biochemical markers (alloenzymes and other protein markers) and (3) molecular markers (based on DNA-DNA hybridization) [11, 12]. In this chapter, we present the latest DNA-based markers in a few studies related to abiotic and biotic stress genes using the MAS application in rice breeding programs. These markers have proven significantly useful from different findings published in screening, fine mapping and gene pyramiding approaches.

2. Molecular marker

Rediscovery Mendelian theory in the early part of 20th century, reveal that inheritance (genes) linked together in chromosome. Molecular marker defines as individual genes flanking within a defined close interval. It can be found at a specific location of the genome and linked with inheritance of a trait or gene [13]. Thottappilly et al. [14] refers molecular marker as polymorphism identified between species including proteins and nucleic acids. Collard et al. [15] had divided molecular marker into 3 major groups which are morphological marker, biochemical marker and DNA marker (**Figure 1**). However, Xu [16] had categorized markers into two

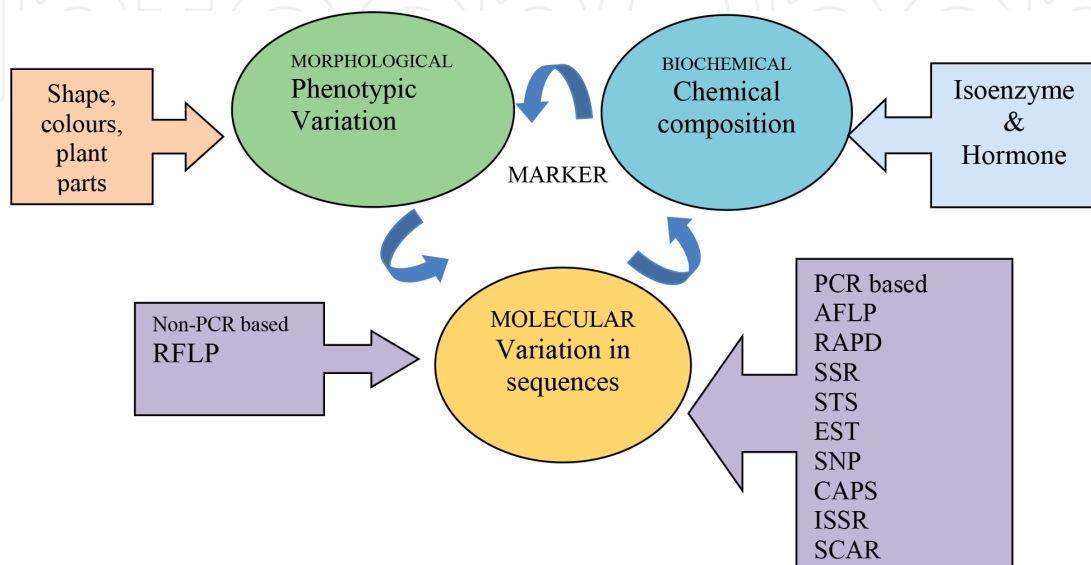


Figure 1.
Different types of molecular marker available.

broad groups based on detecting method; (i) classical marker and (ii) DNA marker. Classical marker was initiated with the mutations in the genomic loci controlling plant morphology [17] (**Table 1**). Advent in polymorphism detecting methods such as Polymerase Chain Reaction, Southern Blotting and Sequencing had invented the development of DNA markers (**Table 2**). Variation were detected at DNA level such as nucleotides changes ie; substitution (point) mutation, rearrangement (insertion and deletions) or errors in replication of tandemly repeated DNA [18].

Types of marker	Morphological marker	Biochemical marker
Advantages	Readily available	Highly reproducible
	Usually require only simple equipment	Applicable for measure a wide range of population genetic parameter
	The most direct measure of phenotype	Inexpensive
Disadvantages	Requires expertise on crop or species	Limited biochemical assay for detection
	Subjected to environmental influences	Phenotype-based analysis
	Limited in number	
Application	Conventional plant breeding program	Genetic diversity Map construction

Table 1.
 Comparison between classical markers.

PCR based markers	Advantages	Disadvantages	Application
RFLP	Reliable and rigid	Harmful and tedious	Map construction Hybrid fixation Genetic diversity
	Easy to conduct across laboratory	Required a larger amount of DNA	
RAPD	A small amount of DNA required	Not transferable across laboratory	Genetic diversity Saturation mapping
	Simple, rapid and lowest cost	Irreproducibility	
	Single primer generates multiple loci		
SSR	Reliable, powerful and easy	Tedious and costly for initial establishment	Linkage and QTL mapping Marker-assisted selection Hybrids fixation
	Transferable between populations	Required polyacrylamide gel electrophoresis	
AFLP	Multiple loci	Required several DNA Complicated	Saturation mapping Genetic diversity
SNP	Multiplexing large number of markers	Costly to assay	Fine mapping Map-based cloning
	Degraded DNA can be used	Marker has less allele	

Table 2.
 Comparison of different PCR based marker.

Although there are many characteristics determine the suitable markers to be considered in MAS development; there are five main characteristics need to be considered [13, [19–22].

1. **Reliability:** Attribute of the desirable marker need to close proximity with the target loci, preferably less than 5 cm genetic distance or one marker every 10 cm [23]
2. **Repeatability and reproducible:** In plant breeding, a lot number of plants are usually screened for desirable marker pattern and result is required instantly. Therefore, level of simplicity in terms of time required and quick detection method are highly desirable for rapid selection. Statistical and bioinformatics software are significant for this purpose.
3. **Cost effective:** Marker assay should be not expensive feasible for selection process.
4. **Level of polymorphism:** Marker should discriminate between two similar accessions of species especially in parent breeding material.
5. **DNA quality and quantity:** A few markers require a complex DNA extraction technique and it too laboratories. In addition, it adds cost to the process.

3. Successful application of molecular marker in rice

Recently, MAB has become a well-known approach develop a superior genotype in rice. The aims of the breeding programs were to develop a rice variety with high yield potential, resistant to diseases and insects, tolerance to adverse environments, and acceptable grain quality. Since the first successful experiment stated for rice by Chen et al. [24] with introducing resistance to bacterial blight (BB) disease into Chinese hybrid parents. Till date, many varieties had been developed thru the application of molecular marker with backcrossing method. Varieties developed were improved from the wild type by incorporate desirable gene depending for biotic and abiotic stresses. Recently, in a few years there is a trend in producing more rice varieties that resistant in abiotic stress such as drought tolerance, submergence tolerance and salt tolerance. This demand comes from the periodic natural disaster in many regions of the rice producing country specifically in Asian country [25]. Resistant varieties against pathogenic disease were continuously developed. This scenario can be seen in blast disease where there are increasing number of resistant varieties were developed due to instability of the fungus pathogen.

Numerous molecular markers have been utilized in marker-assisted breeding however microsatellites are the preferable markers for plant breeding applications. The characteristic as codominant markers which inherited in a Mendelian fashion have emerged as a best choice compare to other markers. Additionally, microsatellite identify as preferable markers used in plant breeding programs due to ubiquitous, high polymorphism rates and wide range of distribution throughout the genome [26, 27]. Other markers possess major drawbacks including Restriction fragment length polymorphism (RFLP) markers which are not integrated with high-throughput technique; Random amplification of polymorphic DNA (RAPD) assays which are often not reproducible or immobile. AFLP analysis is not straightforward and commonly produces multiple fragments with the use of large genomic templates [28]. Application of molecular marker in rice breeding was demonstrated in **Table 3** to develop superior varieties in rice against abiotic and biotic stresses.

Stress	Traits	Gene/QTLs	Foreground and background selection		Reference	
Abiotic	Deep roots	QTLs on chromosomes 1, 2, 7 and 9	RFLP and SSR	SSR	[29]	
	Quality	waxy	RFLP	AFLP	[30]	
	Root traits and aroma	QTLs on chromosomes 2, 7, 8, 9 and 11	RFLP and SSR	RFLP and SSR	[31]	
	Submergence tolerance	<i>Sub1</i> QTL		Phenotyping and SSR	SSR	[32]
		<i>Sub1</i> QTL		SSR	SSR	[33]
		<i>Sub1</i> QTL		SSR	STS	[34]
		<i>Sub1</i> QTL		SSR	SSR	[35]
		<i>Sub1</i>		SSR	SSR	[36]
		<i>Sub1</i>		SSR	SSR	[37]
		<i>Sub1</i>		SSR	SSR	[38]
	Salt tolerance	<i>Sub1</i>		SSR	SSR	[39]
		<i>Saltol</i>		SSR	SSR	[40]
		<i>Saltol</i> QTL		SSR	SSR	[41]
		<i>Saltol</i> QTL		SSR	SSR	[42]
	Early maturation	<i>Saltol</i> QTL		SNP	SSR	[43]
		<i>Hd2</i>		SSR	SSR	[44]
	High yield and drought tolerance	<i>qDTY1.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY6.1, and qDTY12</i>		SSR	SSR	[45]
		<i>qDTY1.1 + qDTY2.1 + qDTY3.1 + qDTY11.1 and two QTLs qDTY1.1 + qDTY11.1</i>		SSR	SSR	[46]
	Heading time	QTL (<i>Hd1, Hd4, Hd5 and Hd6</i>)		RFLP, STS, SSR, CAPS, dCAPS	RFLP, STS, SSR, CAPS, dCAPS	[47]
	Phosphorus tolerance	<i>Pup1</i>		SSR	SSR	[48]
Drought tolerance	QTL		SSR	SSR	[42]	
	MQTL1.1		SSR	SSR	[49]	
	QTL		SSR	SSR	[50]	

Stress	Traits	Gene/QTLs	Foreground and background selection		Reference
		(qDTY1.1, qDTY2.1)	SSR	SSR	[51]
		QTL	SSR	SSR	[52]
Biotic	Bacterial blight	<i>xa21</i>	STS	RFLP	[24]
		<i>xa21</i>	STS	AFLP	[53]
		<i>xa5</i> , <i>xa13</i> and <i>xa21</i>	STS, CAPS	Not performed	[54]
		<i>xa5</i> , <i>xa13</i> and <i>xa21</i>	STS	Not performed	[55]
		<i>xa33</i>	SSR	SSR	[56]
		<i>xa34(t)</i>	SSR	SSR	[57]
		<i>xa35(t)</i>	SSR	SSR	[58]
		<i>xa38</i>	SSR	SSR	[59]
		<i>xa21</i>	STS	STS	[60]
		<i>xa5</i>	CAPS	CAPS	[61]
		<i>xa13</i> , <i>xa21</i>	STS	SSR	[62]
		<i>xa5</i> and <i>xa13</i>	CAPS	STS	[63]
		<i>xa13</i> and <i>xa21</i>	CAPS	STS	[62, 64]
		<i>xa13</i> + <i>xa21</i> and <i>xa5</i> + <i>xa21</i>	SSR	SSR	[65]
		<i>xa13</i> and <i>xa21</i>	SSR	SSR	[66]
	Blast resistance	<i>Pi1</i> , <i>Pi2</i> , <i>Pi33</i> and <i>Pi54</i>	SSR	SSR	[67]
		<i>Pi</i> genes, <i>xa5</i>	SSR	SSR	[68]
		<i>Pi54</i> and <i>Pi5</i>	SSR	SSR	[69]
		<i>Pi1</i> and <i>Pi2</i>	SSR	SSR	[70]
		<i>Pi1</i>	SSR	ISSR	[71]
		<i>Pikh</i> and <i>pi7(t)</i>	SSR	SSR	[9]
		QTLs on chromosomes 1, 2, 11 and 12	SSR	SSR	[72]

Stress	Traits	Gene/QTLs	Foreground and background selection		Reference
	Submergence tolerance, disease resistance, quality	<i>Subchr9</i> QTL, <i>xa21</i> , <i>Bph</i> and blast QTLs and quality loci	SSR and STS	Not performed	[34]
	Thermo-sensitive genic male sterile (TGMS) and blast resistance	TGMS and <i>Pi</i> gene	SNP	SNP	[73]
	Brown planthopper resistance	<i>Bph14</i> and <i>Bph15</i>	InDEL	InDEL	[74]
		<i>Bph18</i>	SSR	SSR	[75]
	Gall midge resistance	<i>Gm8</i>	SSR	SSR	[76]
	Rice stripe resistance	<i>Stv-bi</i>	SSR	SSR	[77]

Table 3.
 Application of molecular marker in MAB in rice.

Planthopper

4. Abiotic stresses

4.1 Deep root

Four QTL (chromosome 1, 2, 7 and 9) was introgressed to NIL developed from crossed between Azucena (donor parent) and IR64 (recipient parent) [29]. Twenty-nine NIL successfully developed in which three NIL carrying target 1 demonstrated significantly increased trait over IR64 while 3 out of 8 NIL consist combination target 1 and 7 exhibited higher root mass and depth. Four NIL carrying targets 9 showed increased maximum number of root length and 2 NIL showed no significant difference on root phenotypic compare to IR64. Results suggest the introgression of genes linked to root improvement is an ideal system compared to change the morphological characters of plant.

4.2 Quality

Waxy locus (*wx*-MH) from Minghui 63 was successfully introgressed into Zhenshan 97 which having poor agronomically quality including amylose content (AC), gel consistency (GC), gelatinization temperature (GT) and endosperm [30]. MABC scheme had been used to develop 3 backcross and 1 selfing generation. Improved variety of Minghui 63 (Shanyou 63) carrying *wx*-MH gene at 6.1 cm length have similar agronomy characteristic as Minghui 63 with low AC, high GC and GT with low grain opacity. Result indicated that waxy region gives significant effect on quality trait.

4.3 Root trait and aroma

MABC strategy had adopted by high yielding India upland rice variety, Kalinga III to insert 5 segments of QTL including 4 QTL carrying root length and thickness and 1 QTL control recessive aroma trait from Azucena donor variety which come from Philippines japonica rice [31]. This experiment was performed until 8 generation which using 3000 SSR markers for almost 8 years to develop NIL. Twenty-two NIL successfully develop which carrying QTL on root length at chromosome number 9 linked with RM242-RM201 and RM248 on chromosome 7 control for delayed flowering.

4.4 Submergence tolerance

Ten improves lines from OM 1490/IR 64 -*Sub1* was developed from Marker-assisted backcrossing and having 90–99% survival under field submergence treatment [78]. Foreground selection was carried out using microsatellite, RM23805 marker which identified allele as 240 and 230 bp bands, respectively from OM1490, susceptible parent and IR64-*Sub1*, tolerant parent. Entire lines of OM1490/IR64-*Sub1* introgression possess an increase rate of survival from original parent. In India, high yielding mega variety, Swarna had been incorporated with *Sub1* gene by using three selections namely foreground, recombinant, and background selection, respectively. Findings indicated that superior variety Swarna successfully transformed into submergence tolerant variety within 2–3 years with three backcross generations [33]. Popular rice variety, AS996 successful incorporated *sub1* gene from rice variety IR64 as a donor parent through MABC. Insertion of *sub1* was confirmed with foreground SSR marker, ART5 and SC3 [36]. Background analysis was screened with 53 polymorphic SSR markers to assess BC₂F₁ and BC₃F₁ generations. The highest recurrent background was achieved 100% donor parent. Results suggest the development a new submergence tolerant rice variety

ASS996-SUB1 to stand with climate change. Superior varieties from India (Samba Mahsuri and CR1009), IRRI, Philippines (IR64), Laos (Thadokkham 1) and Bangladesh (BR11) successfully introgress *sub1* gene exhibited an improve survival rate compare to parental variety by applying MABC approach. Septiningsih et al. [79] studied the expression level of sub genes revealed that *sub1C-1* was dominant over the *sub1A* allele however no reduction of expression observed when intolerant *sub1C* allele merged with the tolerant *sub1A-1* allele. Survival rates of heterozygous plant with *sub1A* expression higher compared to homozygous tolerant parent. Existence of *sub1A-2* will not significantly function in intolerant plant with turn off expression of *sub1C-1*. Study by Ahmad et al. [38] developed 30 newly lines of BC₂F₃ population from backcross of MR219 and Swarna-Sub1 with 95.37% recurrent parent genome (RPG) recovery. Rahman et al. [39] stated a marker-assisted backcross breeding (MABB) approach to incorporate the *Sub1* locus into popular rice varieties of southern India, CO 43, from tolerant FR13A variety. An elite NILs of CO 43 harboring *Sub1* locus possessing 95.78% of the recurrent parental CO 43 genome produced from genotyping and phenotyping analysis.

4.5 Salt tolerance

ASS996 a high yielding and widely grown cultivar in Vietnam had been cross with FL478, a highly salt tolerant rice variety using MABC strategy [40]. Four *Saltol*-AS996 introgression lines, QF3-1, QF3-2, QF 4-3-3, QF6-4 was developed with 3 foreground SSR markers, AP3206, RM3412 and RM10793 and 63 polymorphic markers were applied across rice genome for background analysis. The promising lines showed significant salt tolerance or similar compare to recurrent parent, FL478. MABC approach had been applied for introgressed *Saltol* QTL from donor parent, FL478 into Bacthom 7 recipient rice cultivar. Eighty-nine polymorphic SSR markers including 8 foreground markers to screen heterozygous plant carrying *Saltol* locus in each backcrossed generation. Background analysis demonstrated the restoration of recipient genome up to 96.8–100% in the best plant of BC₃F₁ generation. Finding concluded that improve version of salt tolerance-Bacthom 7 is relevant for cultivation in coastal areas of the Vietnamese Deltas [41]. De leon et al. [43] reported the utilization of SSR and SNP markers to characterize the ILs (Pokkali x 'Bengal) and identify 14 QTLs traits related to salinity tolerance.

4.6 Early maturation

In Indonesia, *Hd2* gene located at chromosome 7 from Nipponbare was transferred to Code variety [44]. Two markers linked with early maturation trait, RM1362 and RM7601 was used to identify heterozygous carrying this gene in 2 backcross and 1 selfing generation. Improved lines showed a similar agronomic characteristic with code variety except for heading time which 73–85 days in improved version compared to recipient parents, 89 days. Maturation days for improved version of Code carrying *Hd2* gene were 103–104 day which is at considerable maturation days. Result concluded the availability to use these improved lines in Indonesia for increasing rice production.

4.7 Heading time

Koshihikari and Kasalath were crossed by using MABC strategy to introgress 3 QTL segment which code for *Qdth3*, *Qdth6* and *Qdth8* [47]. Genotyping analysis was performed using five different types of DNA marker including RFLP, STS, SSR, CAPS and dCAPs. Three NIL successfully produced namely Wakei 367, Wakei 371 and Kanto IL1 which carrying *Hd2* gene coded for heading time. All NIL exhibited

same morphological trait with Koshihikari with early heading time for Kanto IL1 (12 days), Wakei 367 (10 days) and Wakei 371 (11 days) compare to 95 days on Koshihikari.

4.8 Phosphorus tolerance

MABC approach had been successfully adapted to introgress a major quantitative trait locus (QTL), *Phosphorus uptake1 (Pup1)*, positioned on rice (*Oryza sativa*) chromosome 12 into two types of Indonesian rice varieties, irrigated and upland. SNP and CAPs had been used as foreground marker to identify desirable traits and background selection had been conducted using 47 to 61 polymorphic SSRs markers and restored 89–93% of the recipient genome in BC₂F₃ population. Phenotyping finding suggests that *Pup1* is effective to enhance grain yield under field treatment [48].

4.9 Drought tolerance

Crossing by hand emasculation and artificial pollination was performed between IR20 (*japonica*) and lowland (*indica*) cultivar to develop near isogenic lines (NILs) [42]. Foreground and background selection were conducted using 100 SSR markers that distributed entire genome. Five NILs exhibited high yield performance compare to IR20 in rain-fed and irrigated conditions. NIL-212 and 297 successfully incorporated with three and two root QTLs with NIL-297 demonstrated maximum value of nodal root and surface area whereas increase number of nodal roots than recurrent parent, IR20 observed in NIL 212. Backcrossed population (BC₂F₃) was developed through Marker Assisted Selection (MAS) to transfer QTLs for drought tolerant characters into Thai famous variety KMDL105 using 3 different donor parents, DH212, DH103 and DH126, respectively. Hundred and three KMDL 105 introgression lines were developed carrying selected target [80]. Genome analysis claimed that donor segment was found in non-carrier chromosomes and minimum proportion of recurrent genome was determined in carrier chromosome. Results suggest that background analysis is required to restore the recurrent genome segments. Sandhu et al. [46] enhance the grain yield of Samba Mahsuri under reproductive-stage drought (RS) through marker-assisted selection (MAS) and marker-assisted recurrent selection (MARS). Findings recorded four *qDTYs* (*qDTY_{1.1}* + *qDTY_{2.1}* + *qDTY_{3.1}* + *qDTY_{11.1}*) and two QTLs (*qDTY_{1.1}* + *qDTY_{11.1}*). Presence of gene MQTL1.1 responsible for the drought tolerance was identified from the cross (Sarjoo52 × Nagina- 22) in the study reported by Awasthi and Lal [49]. Ramchander et al. [50] study 101 Backcross Inbred Lines (BILs) of rice (BC₁F₅) for drought tolerance by develop a cross between Norungan//TKM9/Norungan. Marker aided selection used in this study efficiently pyramiding favorable QTL alleles to enhance drought tolerance in elite background of famous rice varieties using 15 polymorphic SSR markers. Muthu et al. [51] successfully developed abiotic stress-tolerant rice genotypes of the famous rice variety, Improved White Ponni (IWP) through a marker assisted backcross breeding approach by introgression major effect quantitative trait loci (QTLs) conferring tolerance against drought (*qDTY11.1*, *qDTY2.1*), salinity (*Saltol*), and submergence (*Sub1*). Backcrossed inbred lines (BILs) developed in this study showed minor effect caused by drought, salinity, and submergence. Root trait genes were introgressed through marker-assisted backcross breeding (MABC) between aerobic rice variety, AERON1 and MRQ74, a high yielding rice variety of Malaysia, [52]. Six best rice lines in BC₂F₁ with highest recovery percentage and resemble similar phenotypic appearance were identified with RM242 and RM263 foreground and 57 background markers.

4.10 Grain fragrance

Ahn et al. [81] was first discover the molecular marker linked with grain aroma. Lorieux [82] was used RFLP marker, RG28, which linked with aromatic located at chromosome 8 with genetic distance of 4.5 cm. The uniqueness of RG28 was interchangeable into STS marker and can be used to distinguish between fragrance and non-fragrance rice cultivars [83–85]. Improved version Pusa Bashmati and improved versions of PRR78 was successfully introgressed with quality trait from Basmati 370 as a donor with Manawthukha cultivar as a recipient using MABC scheme [86, 87].

4.11 Semi-dwarfing

Sd1 gene coded for semi-dwarf trait creates an interest among breeder as it enhances crop production. Advent in MAB had made it possible to track the introgression of *sd1* gene by using SSR markers in *two traditional japonica varieties*, 'IR36' and 'Allorio' (IRGC284) [88].

5. Biotic stresses

5.1 Bacterial blight resistance

Marker assisted backcrossing coupled with stringent phenotypic screening was adapted to transfer BB resistance gene, *Xa23* into Minghui63, YR293 and Y1671 which famous recipient lines. Foreground selection was screened with RM206 located at 1.9 cm near to the *Xa23* locus [89]. The improved version of BB resistant was identical with original recipient for agronomic traits [30]. NSIC Rc142 and NSIC Rc154 donor cultivar for *Xa4* + *Xa5* + *Xa21* genes were incorporated into susceptible cultivar IR64 in Philippines [61]. Currently, *Xa13* and *Xa21* had been successfully inserted into recurrent line PRR78 through MABC [90]. In Thailand, three backcrossed generation required to insert *Xa21* (BB resistance gene) from IR1188 variety into famous KDML105 variety using MABC approach together with phenotypic selection. Similar agronomic characteristic was observed between developed introgressed lines having BB resistant gene (*Xa21*) with local variety KDML105 [91]. Foreground selection was screened with six SSR markers namely RM302, RM212, RM210, RM149, RM287, RM224 at different chromosome while 66 rice SSR markers was applied for background genotype. In China, popular restorer line, Yihui1577 was incorporated with *Xa7* and *Xa21* from Huahui20 thru MABC strategy [92]. Foreground marker were applied using RM20582 in existence of *Xa7* to map a 0.14 cM interval at chromosome 6 between RM20582 and RM20593 [93]. Huang et al. [40] in his study successfully employed of *Xa7*, *Xa21*, *Xa22* and *Xa23* from Jinke 1 a cytoplasmic male sterile line into Huahui 1035 line using MABC. High yielding F₁ hybrid was used to develop more hybrid rice in China. Samba Mahsuri a mega high-yielding, fine grain-type variety was selected as donor for *Xa21* and *Xa13* to incorporate into susceptible varieties, Taraori Basmati and Basmati 386 through MABC scheme [94]. Molecular backcross breeding was used to pyramided *Xa21* + *xa13* and *Xa21* + *xa5* combinations genes in a background of lowland cultivar, Jalmagna [65]. Twenty pyramided lines with targeted gene combinations showed resistant against BB pathogen. Balachiranjeevi et al. [66] reported the development of durable BB resistance through marker-assisted backcross breeding (MABB) into background of elite rice, DRR17B by introgress *Xa21* and *Xa33* dominant genes.

Six promising pyramiding lines of DRR17B with durable resistance and similar or superior agro-morphological attributes to DRR17B have been identified.

5.2 Blast resistance

C101A51 and Tetep contribute for *Piz-5* and *Pi54* blast gene was introgressed into PRR78 variety [69]. Two improved lines were developed, Pusa1602 (*Piz5*) and Pusa1603 (*Piz54*) through MABC breeding strategy by two backcross series. Foreground selection was performed by using AP5930 and RM206 while background analysis using SSR polymorphic markers revealed 89.01 and 87.88% in Pusa1602 and Pusa1603 lines recovery of RPG, respectively. Hybrid develop carrying blast resistance gene produced exhibited similar performance with parental Pusa RH10 in terms of yield, grain and cooking quality traits. Multiple resistance gene against leaf blast (*Pi1*), neck blast (*Pi2*) (donor BL122) and bacterial (*Xa23*) (donor CBB23) was successfully introgressed into Ronfeng B, a mega variety with early maturing line thru MABC scheme [70]. Improved lines D521, D524 was developed using three foreground markers (MRG4766, AP22 and RM206) and 131 polymorphic background markers. Proportional recipient genome for D521 and D524 were 96.18 and 96.56%, respectively after 4 backcrossed generations. The size of lesions exhibited from 0.77 to 1.18 cm with 96.7–100% resistance frequencies values. Rongfeng 3A was developed as an improve version of cytoplasmic male sterile line from Rongfeng by a series of conducive backcross breeding with *Pi1*, *Pi2* and *Xa23* gene. A series of superior lines with resistance to bacterial blight and blast diseases were developed by using MAB [30, 90, 95]. Hybrid Pusa RH10 is likely susceptible to BB and blast therefore by employing the bacterial and blast resistance gene, it will be improved adjustability to disease endemic parts and also retain the rice production. In Iranian, two crosses were made between susceptible, Tarom Mahali (TAM) and resistance cultivar, Khazar (KHZ) to increase blast resistance. Screening with 74 polymorphic markers on 192 F_{2,3} families demonstrated both parental exhibited maximum genetic variation between the two varieties on entire chromosomes except chromosome 6, 7, 8 and 12 [96]. In Malaysia, improved version of MR219 was developed by introgression of *Pi-z* and *Pikh* and *Pi-b* by using two different donor, Pongsu Seribu 1 and 2 respectively [10, 97]. Hasan et al. [98] had successfully introgressed *Pi7(t)* and *Pikh* in MR264 from Pongsu Seribu 2 by using MABC approach. Thailand rice, the popular Sakon Nakhon (SKN) rice had been introgressed with QTLs on chromosomes 1, 2, 11 and 12 by using marker-assisted backcrossing. Srichant et al. [72] in his study presented 2 lines (SKN 39-10-19-29-12 and SKN 39-10-19-29-13) with high resistance to leaf and neck blast; and having similar agronomic traits as the SKN.

5.3 Brown plant hopper resistance

Bph3, gene coded for resistance against brown plant hopper had been introgressed into the famous Thai variety 'Khao Dawk Mali 105' [99]. Backcrossed population had been screened with RM589 and RM190, highly linked to *Bph3* and *Wx*-RH loci. Fifty NIL were developed from background selection using 75 polymorphic SSR markers disperse 12 rice chromosomes. Cross had been made between Junambyeo, an elite japonica cultivar with IR65482-7-216-1-2 a donor indica rice by a series of backcrossing through MAB [75]. *Bph18* gene was transferred and 7312T4A, the most closely polymorphic STS marker was applied to screen the existence of the *Bph18* gene in BPH-introgress backcrossed lines [100]. Two hundred and sixty SSR marker were used to screen the RPG percentage in the four NIL breeding lines.

5.4 Gall midge resistance

Gm8 resistance gene code for rice gall midge had been employed from donor parent Aganni into Samba Mashuri having gene *Xa21* using MABC. Four plants identified in selfing population (BC2F2) carrying *Gm8* and *Xa21* genes and further tested for phenotypic selection against BB and gall midge [76]. Exploration of gm gene had been a great achievement in modern crop however resistant variety carrying 1 major gene easily lost the resistancy [101]. Till date, 11 Gm genes in the plant [102] and 7 pathogens have been identified [103].

6. Conclusion

Advent in marker technology has offer a remarkable progress in crop improvement specifically in rice. Present paper reviews the application of DNA markers in abiotic and biotic stresses in rice using MAS. Interval between molecular markers and gene/QTLs linked with target traits play a significant role in efficiency of MAS technique. Generally, marker will be validated in fine mapping study. Thus, the development of important marker associated with abiotic and biotic stresses resistance is proficient by QTL mapping experiments. MAB greatly increase the effectiveness and efficiency of breeding. Desirable individual in germplasm collection with genes or QTLs can be characterized in genotyping analysis by using the DNA markers for target genomic regions rather than phenotyping analysis. Pyramiding tolerance genes and QTLs will develop a resistant cultivar against abiotic and biotic stresses. MAS refers to selection by DNA markers linked to QTLs or target genes. Nowadays, DNA-based genetic markers play a significant part in the forthcoming of MAB and molecular genetics analysis for establishment of stress-tolerance and resistance in plants through molecular linkage maps. Knowledge of markers for abiotic and biotic tolerant traits presented here will serve a fundamental guide and help rice breeders in their MAB.

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