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Research review paper

DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice

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Abstract

Bacterial leaf blight caused by the bacterial pathogen *Xanthomonas oryzae* pv *oryzae* (Xoo) limits rice yield in all major rice-growing regions of the world, especially in irrigated lowland and rainfed conditions where predisposition factors favor disease development to epidemic proportions. Since bacterial pathogens are difficult to manage, development of host plant resistance is the most effective means of disease management. As many as 24 major genes conferring resistance to various races of the pathogen have been identified and utilized in rice breeding programs. However, large-scale and long-term cultivation of varieties carrying a single gene for resistance resulted in a significant shift in pathogen race frequency with consequent breakdown of resistance in these cultivars. To combat the problem of resistance breakdown, pyramiding of resistance genes into different cultivars is being carried out. Pyramiding of resistance genes is now possible with molecular markers that are developed for individual genes. This review discusses the various bacterial blight resistance genes identified and their corresponding molecular markers developed for breeding durable resistance into modern rice cultivars. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Rice; Bacterial blight; *Xanthomonas oryzae*

1. Introduction

Bacterial blight caused by the pathogen *Xanthomonas oryzae* pv *oryzae* (Xoo) is one of the most destructive diseases of rice throughout the world. In some areas of Asia, Xoo has

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the potential to reduce yield by more than 50%. Eight races of the bacterium have been identified in the Philippines (Finckh and Nelson, 1999) and two major pathotypes in India (Reddy and Reddy, 1992). Each race has specific virulence to varieties with different resistance genes, showing a gene for gene relationship in the host pathogen interaction (Mew, 1987; Veracruz and Mew, 1989). So far, 24 genes conferring resistance to specific races or clusters of races of Xoo have been identified through classical genetic analysis. As of now, 10 major genes have been mapped using RFLP, RAPD and microsatellite markers (McCouch et al., 1991; Ronald et al., 1992; Yoshimura et al., 1992; Borines et al., 2000). The advent of molecular markers tagged to different resistance genes enabled convergence breeding and pyramiding of more than two different genes into an agronomic variety. Marker-assisted breeding (MAS) has been successfully used by Huang et al. (1997) for pyramiding four resistance genes into IR-24 background. Resistance gene deployment in hybrid rice parents is much more complicated and needs a different strategy. Hybrid rice cultivation requires large quantities of inputs of nutrients and water, which predisposes the plant to bacterial blight incidence. Besides this, hybrid rice seed production involves three parental lines. Hence, a variety of resistance genes have to be deployed into different hybrid rice parents. MAS has been successfully used to transfer resistance genes into seed parents (Borines et al., 2000) and restorer lines (Chen et al., 2000). Molecular breeding approach has been successfully utilized in the Philippines and China for the improvement of bacterial blight resistance in modern rice cultivars. In this review, we highlight the markers which can be employed in MAS for breeding varieties and hybrids with durable bacterial blight resistance.

2. Genetic variation in the pathogen

Pathotype studies conducted under the All India Coordinated Rice Improvement Project (AICRIP) have identified two major pathotypes within the *X. oryzae* pv *oryzae* (Xoo) populations in India (Reddy and Reddy, 1992). Rajabhosale et al. (1997) and Yoshitala et al. (1997) studied the extent of diversity in the pathogen. They analyzed 67 Xoo isolates from 18 locations in India by DNA fingerprinting using two separate repeat element probes from the *X. oryzae* pv *oryzae* genomes. The results indicated that isolates from 12 out of 18 locations sampled are related and can be grouped into a single pathogen lineage. Pathotype analysis revealed that isolates in the dominant lineage belong to pathotype Ia and Ib. Similarly, Adhikari et al. (1995) tried to group the pathotypes of Asia. They analyzed 308 strains of Xoo from several rice growing regions in Asia using RFLPs and virulence typing and grouped all the pathotypes into five major clusters. Of the five clusters, three consisted of strains from a single country. However, strains within clusters were found in different countries suggesting the dissemination pattern of the pathogen. The regional differentiation of clusters of Xoo in Asia and the association of some pathotypes of Xoo with single cluster suggest that regional resistance breeding and gene deployment strategies are most feasible. Finckh and Nelson (1999) reported the presence of eight races of Xoo which are widely used to represent the diversity of the

pathogen in IRRI, Philippines. Such studies are useful for resistance breeding against the Philippines isolates.

3. Genetic diversity of host plant resistance

A large number of studies conducted in several countries have identified the presence of 24 major genes (Lee et al., 2001) conferring resistance to various races of the pathogen. These genes are designated as *Xa-1* to *Xa-24* (Table 1). Only 10 out of 24 genes have been tagged

Table 1
Genes conferring resistance to different races of bacterial blight pathogen

Gene	Cultivar	Isolate/race	Reference
Xa-1 and *Xa-2*	Kogyoku	Japanese race I and II	Sakaguchi (1967)
Xa-3	Wase Aikoku, Chukogu-45	Japanese race II and III	Ezuka et al. (1975), Ogawa et al. (1986)
Xa-4	IR20, IR22, IR1529-680-3	Philippine race I	Petpisit et al. (1977), Sidhu et al. (1978)
xa-5	IR1545-248, BJ-1, IR291-7, DV85	Japanese races	Petpisit et al. (1977), Sidhu et al. (1978), Singh et al. (1983), Blair and McCouch (1997)
Xa-6	Malaget sunsong, IR994-102, IR1698-241, Zenith	Philippine race I	Sidhu et al. (1978)
Xa-7	DV85, DV87	Philippine race I	Sidhu et al. (1978, 1979)
xa-8	P1231129	Philippine isolates	Sidhu et al. (1978, 1979)
Xa-9	Sateng	Philippine isolates	Singh et al. (1983)
Xa-10	Cas209	Philippine and Japanese isolates	Yoshimura et al. (1983)
xa-11	IR8, RP9-3	Japanese isolates	Ogawa and Yamamoto (1986), Ogawa et al. (1991)
Xa-12	Kogyoku and Java14	Japanese and Indonesian isolates	Ogawa et al. (1978a,b)
Xa-13	Long grain	Philippine isolates	Zhang et al. (1996b)
Xa-14	TN(1)	Japanese isolates	Taura et al. (1989)
xa-15	M41	Japanese isolates	Noda (1989)
Xa-16	Tetep and IR24	Japanese isolates	Noda (1989)
Xa-17	Asominori	Japanese isolates	Ogawa et al. (1989)
Xa-18	Toyonishiki	Burmese isolates	Ogawa and Yamamoto (1986)
Xa-19	XM5	Japanese isolates	Taura et al. (1991)
Xa-20	XM6	Japanese isolates	Taura et al. (1992)
xa-21	*O. longistaminata*	Philippine and Japanese isolates	Khush et al. (1990)
xa-22	Zhachanglong	Chinese isolates	Lin et al. (1996)
Xa-23	*O. nivara*	Indian isolates	Kumar (1999)
Xa-24	DV85, DV86, Aus295	Philippine race 6	Lee et al. (2001)

and mapped to different chromosomes (Table 2). These genes are both dominant and recessive in nature.

4. Major dominant genes — cloned and sequenced

Majority of genes conferring resistance to *X. oryzae* pv *oryzae* are dominant in nature. Among the extensively studied genes are the following.

4.1. Xa-1 gene

The resistance gene *Xa-1* conferring resistance to the Japanese Xoo race I was first reported by Sakaguchi (1967). *Xa-1* gene was extensively studied and tagged with a RFLP marker XNpb235 and mapped to chromosome 4 (Yoshimura et al., 1996). Positional cloning of the gene in the rice genome project in Japan revealed that a 340-kb YAC clone (Y5212) carries the *Xa-1* gene. Later, Yoshimura et al. (1998) found that *Xa-1* gene encodes a nucleotide-binding site leucine-rich repeat (NBS-LRR) type of protein.

4.2. Xa-21 gene

The broad spectrum bacterial blight resistance gene *Xa-21* was introgressed from a wild species *O. longistaminata* into *O. sativa* background (Khush et al., 1989). Ronald et al. (1992) tagged the *Xa-21* gene with RAPD marker RAPD 248. Later, it was found that the marker is located within the gene per se. The RFLP marker RG103 was found to be tightly linked to the gene at a distance of 1.2 cM. Based on these markers, a PCR-based STS marker pTA248 was developed, which can be used efficiently in marker-assisted selection (Ronald et al., 1992). Ronald (1997) adopted a map-based cloning strategy for the first time to clone the disease resistance gene *Xa-21* in rice (monocotyledonous species). The plasmid pC822 was found to contain this gene. Sequencing of the gene revealed that *Xa-21* codes for a receptor kinase domain carrying serine–threonine specificity.

5. Major recessive genes–mapped and cloned

Out of 24 genes identified, six are recessive in nature. Among the extensively studied genes are the following.

5.1. xa-5 gene

The recessive resistance gene *xa-5* was tagged with RFLP markers RG556 and RZ390 and microsatellite markers RM122 and RM390. It was mapped on chromosome 5 based on the segregation data for 207 F_2 individuals of two populations, IR 24×IRBB5 and Chinsurah BoroII×IR64 (Blair and McCouch, 1997). The RFLP marker RG556 was used

Table 2
Bacterial blight resistance genes tagged and cloned

BB resistance gene	Nature of gene	Donor/cross	Type of marker	Linked marker	Chromosomal location	Linkage distance (cM)	BAC library details	References
Xa-1	Dominant	IRBB1 Kogyoku/IR24	RFLP	XNpb235	4	0.9	Y5212 (340 kb)	Yoshimura et al. (1996)
				XNpb181				
Xa-3	Dominant	IRBB3 (Chugoku45)/IR24	RFLP	XNpb186	11			Yoshimura et al. (1992)
Xa-4	Dominant	IRBB4 (IR1545-339)/IR24	RFLP	XNpb181	11	1.7	RS13 (420 kb)	Yoshimura et al. (1995a), Wang et al. (2001)
				XNpb78		1.7		
				G181				
xa-5	Recessive	IRBB5/IR24 (Chinsurah Boroll)/IR64	RFLP	RG556	5		9E8 (8 kb) and 28N22 (2 kb)	McCouch et al. (1992), Blair and McCouch (1997)
				RZ390		0.4		
			SSR	RM122		0.7		
				RM390		0.4		
Xa-7	Dominant	No information	RFLP	G1091	6	6.0		Borines et al. (2000)
Xa-10	Dominant	Cas209	RFLP	No information	11	No information		Yoshimura et al. (1995a)
xa-13	Recessive	IR66699-55-42/IR24	RAPD	OPAC05	8	5.3	2027 contig 21H14 clone	Zhang et al. (1996a), Sanchez et al. (1999)
			RFLP	RG136		3.7		
			RFLP	R2027		1.1		
				S14003		1.7		
				G1149		1.7		
Xa-14	Dominant	Japonica/Zhengzhuai (indica)	RFLP	RG620	4	20.1		Tan et al. (1999)
				G282		19.1		
Xa-21	Dominant	*O. longistaminata*/IR24	RFLP	RG103	11	1.2	pC822 plasmid	Ronald et al. (1992), Williams et al. (1996)
			RAPD	RAPD248				
				OPAB09				
				OPAE03				
Xa-22(t)	Dominant	Zhachanglong/ Zhonchu Ali	RFLP	R543	11	7.1		Lin et al. (1996)

to develop a PCR-based STS marker for use in marker-assisted selection (Huang et al., 1997). The STS marker was found to give monomorphic banding pattern on PCR amplification between the resistant and susceptible plants. Hence, the PCR product was digested with a restriction enzyme *Dra*I to generate specific amplicon polymorphism (SAP). Sanchez et al. (2000b) have successfully cloned the *xa-5* region on chromosome 5. They identified two bacterial artificial chromosome (BAC) clones 9E8 and 28N22 which contained the *xa-5* locus. The cDNA clone 5P2 was identified by both the BAC clones following colony hybridization. Both the cDNA clone and the BAC clones were used for transformation experiments and sequencing.

5.2. xa-13 gene

The recessive gene *xa-13* confers resistance to the Philippines race 6 of Xoo. The gene was tagged with the RAPD marker OPAC05 900 and RFLP marker RG136 and mapped on chromosome 8 using the doubled haploid mapping population of IR64 and Azucena (Zhang et al., 1996b). The RFLP marker RG136 was used to develop a PCR-based STS marker. The STS primer like *xa-5* STS primer did not produce any polymorphism upon PCR amplification. Hence, the PCR product was digested with *Hin*fI restriction enzyme to produce SAP. Four DNA markers RG 136, R2027, S14003 and G1149 in the target region of *xa-13* gene on chromosome 8 were used to identify BAC clones potentially harboring the *xa-13* locus from a rice BAC library (Sanchez et al., 1999). Genetic mapping indicated that the *xa-13* locus was present in the R2027 contig and the 96-kb clone 21H14. The study resulted in the development of a PCR-based marker 21H14F and 21H14R which is more powerful for MAS involving closely related parents because it shows polymorphism even between the *xa-13* lines that are nearly isogenic.

6. Recessive gene in Ajaya (national resistant check variety of India)

Recently, a new recessive gene conferring resistance to Indian isolates of Xoo was identified in the cultivar Ajaya. The resistance gene was tagged with a RAPD marker $OPA12_{1467}$ (Fig. 1) and mapped on chromosome 5 using linked SSR markers (Jena and Kameswara Rao, 2001).

These major genes (*Xa-1* to *Xa-13*) have been used for developing resistant cultivars. However, the resistance lasts only a few years due to evolution of new pathotypes. Therefore, continuous efforts are made to transfer genes from wild relatives. The first broad spectrum resistance gene introgressed from wild species *O. longistaminata* is *Xa-21* (Khush et al., 1989). Jena and Khush (1990) have transferred genes for BPH and bacterial blight from the wild species *O. officinalis* having CC genome. The recurrent parent in this back-crossing program had *Xa-4* gene which confers resistance to BB race 1 and 5, whereas *O. officinalis* is resistant to all six Philippine races. The F_3 progenies of this cross-segregated for susceptibility to race 1, suggesting that the gene for resistant to bacterial blight in *O. officinalis* and *O. sativa* are nonallelic to *Xa-4*. Similarly, Amante-Bordeos et al. (1992) transferred gene

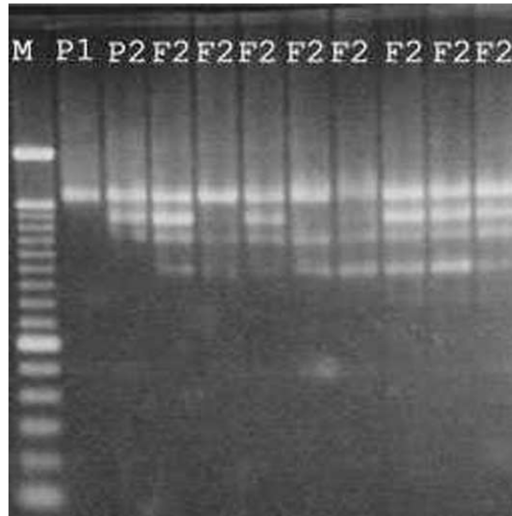

Fig. 1. RAPD marker MRFOP12$_{1467}$ cosegregating with BB disease-resistance gene in Ajaya. M = molecular weight marker, P_1 = susceptible parent, P_2 = resistant parent, F_2 = segregating population.

resistance to bacterial blight from wild species possessing BBCC genome to *O. sativa* background. Recently, three resistant lines were developed by introgression of genes from *O. minuta* (Yang et al., 2000).

Before commencing a breeding program for transferring these resistance genes into modern rice cultivars, a thorough knowledge of the mechanism of bacterial blight resistance is required.

7. Mechanism of bacterial blight resistance

7.1. Biochemical basis

Studies on the multiplication of Xoo indicated that bacteriostasis occurs in resistant cultivars due to incompatible reactions. Bacteriostasis is correlated with early accumulation of bright yellow green fluorescent compound and host cell death in incompatible reactions (Reimers and Leach, 1991). It was also found that during incompatible reactions, lignin-like polymers formed rapidly whereas in compatible reactions these polymers were not deposited. Bacteriostasis and lignin-like polymer deposition were correlated with an increase in the activity of extracellular peroxidases during incompatible reactions (Reimers et al., 1992). Peroxidases are formed in the last enzymatic step of lignin biosynthetic pathway, i.e., oxidation of cinnamyl alcohol into free radical intermediates which are polymerized into lignin. Since Xoo primarily resides in the vascular tissues and does not directly penetrate the host cells, it is unlikely that lignin per se serves as a physical barrier to pathogen spread. Probably, the peroxidase activity, toxic phenolic compounds and free radicals that are formed

during the lignin biosynthetic process, play an important role in the defense against Xoo in resistant cultivars.

7.2. Molecular basis

Classical genetic analysis of disease resistance has led to the identification of genes controlling pathogen recognition in both the plant and the pathogen (Flor, 1971). Race-specific interaction in the bacterial blight disease of rice follows the gene for gene model according to which the incompatible interactions are the consequences of the positive function encoded by pathogen avirulence genes and corresponding host resistance genes (Kelemu and Leech, 1990). Gene for gene interactions have been described for numerous plant pathogen pairs (Martin et al., 1993; Song et al., 1995). From a study of such interactions between several pathogens and their corresponding host plants, these workers concluded that a plant disease resistance is expressed by a single resistance (R) gene in the plant, that corresponds to a single avirulence (avr) gene in the pathogen. Plant disease resistance genes enable the host to recognize pathogens producing specific signal molecules. Production of these molecules is, in turn, controlled by avirulence (avr) genes in the pathogen. The recognition events specified by matching pairs of resistance and avr genes trigger the rapid induction of host defense response and ultimately result in phenotypic resistance (Kunnel, 1996). Disease susceptibility is the result of either the plant R gene or the pathogen avirulence gene being absent from the interacting organisms.

Hopkins et al. (1992) have identified three independent clones from Xoo, each of which contains an avirulence gene (avr *xa-5*, avr *Xa-7* and avr *Xa-10*) that controls bacterial elicitation in rice cultivars containing the *xa-5*, *Xa-7* and *Xa-10* resistance genes. Further, it was found that these clones are members of a multigene family and are highly similar to avr Bs3, a gene from *X. campestris* pv *vasicatoria* that specifies disease resistance on pepper cultivars carrying the resistance gene Bs3 (Bonas et al., 1989). The avr Bs3 encodes a 122-kDa protein, the internal portion of which contains a 34-amino acid motif which is repeated 17 times in tandem (Knoop et al., 1991). In the pepper pathogen, deletion derivatives carrying varying numbers of repeats have altered race specificity. In Xoo, avr *Xa-7* and avr *Xa-10* contains 15 and 25 repeats, respectively, and deletion of these repeats results in either a compatible reaction or altered race specificity (White et al., 1995).

Gene cloning is beginning to revolutionize our understanding of the molecular basis of plant–pathogen interactions. With this technique, virtually any viral or prokaryotic gene can be obtained in large quantities, sequenced and analyzed in detail. Bacterial genes controlling avirulence, host range and induction of the host hypersensitivity response have been recently cloned and characterized. These studies helped to unravel molecular and cellular mechanism of pathogenicity and paved the way for using novel strategies for creating disease-resistant plants. Most of the bacterial resistance genes are known by their phenotype and not by their biochemical effects. Hence, traditional cloning strategies cannot be followed. In this context, a new approach to gene cloning which is not based on prior knowledge of the product encoded by the gene is developed. This strategy is known as "map-based cloning," in which

the chromosomal location of the gene/s is used as the basis for cloning. In map-based cloning, first the DNA sequences near the target gene are located. These tightly linked DNA sequences then act as starting point for cloning the region around and including the gene of interest. The target gene is pinpointed within the cloned regions by transformation studies. Subsequently, the gene is sequenced and by homology search, the protein encoded by the gene is identified. Map-based cloning has recently been used to clone genes conferring resistance to a variety of bacterial, fungal and viral diseases of dicotylednous plant species (Ronald, 1997).

The predicted products of the disease resistance (R) genes can be classified into five groups: detoxifying enzymes, kinases, NBS/LRR proteins, extracellular receptors and receptor kinases. The maize *HM* I gene conferring race-specific resistance to the fungal pathogen *Cochliobolus cerbonum* race I represents the first group (Johal and Briggs, 1991). This gene was isolated using a transposon tagging method and was found to encode a NADPH-dependent HC toxin reductase which inactivates the HC toxin produced by the fungus. The second group includes the tomato, *Pto* gene conferring resistance to *Pseudomonas syringae* pv *tomato* containing the avr *Pto* gene (Martin et al., 1993; Ronald et al., 1992). *Pto* belongs to the multigene family encoding serine–threonine protein kinase (STK), suggesting a role for *Pto* in cellular signaling via protein phosphorylation. The third and largest group contains disease resistance genes such as Arabidopsis *RPS*2 and *Rpm*I, tobacco *Prf* and flax *L*6 (Ronald, 1997). Sequencing and analysis of these genes indicate the presence of LRR domains and NBS. The fourth class includes the *cf* genes, which encodes a membrane-anchored extra-cytoplasmic glycoprotein that shows homology to the receptor domain of several receptor-like protein kinases and to members of the LRR family of proteins (Dixon et al., 1996). The fifth class of disease resistance genes encodes a putative receptor kinase. The classical example of such a gene is *Xa-21* resistance gene. The *Xa-21* receptor kinase-like protein carries serine–threonine specificity in the kinase domain. The action of *Xa-21* resistance gene product is depicted in Fig. 2 (Ronald, 1997). Upon ligand binding (pathogen produced peptide) to the receptor kinase domain, a signal is transduced across the transmembrane domain activating the intracellular kinase domain. Upon activation, a phosphorylation cascade transduces the signal to defense molecules

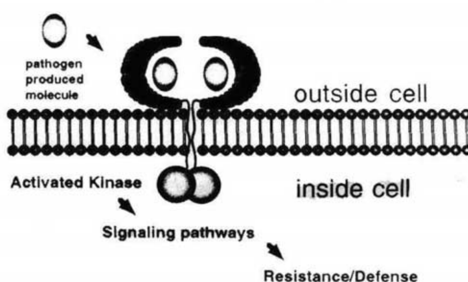

Fig. 2. Proposed model for the action of the *Xa-21* resistance gene product. Adapted from Ronald (1997).

which restrict pathogen growth. Similar results were obtained in case of bacterial blight resistance gene *Xa-1* conferring resistance to Xoo race I. The gene encodes the LRR protein (Yoshimura et al., 1996).

8. Breeding for bacterial blight disease resistance

8.1. Conventional breeding

The most effective approach to combating bacterial blight is the use of resistance varieties (Khush et al., 1989). So far, 24 genes conferring resistance to different Xoo races have been identified and some of them have been incorporated into modern rice varieties. The exploitation of resistant gene *Xa-4* by conventional back cross-breeding resulted in the development of many resistant varieties that have played an important role in protecting rice from Xoo (Khush et al., 1989). In India, TKM6 was used as a donor of bacterial blight resistance and several varieties (e.g. ABT 32, Govind, UR 20, IR 36, Karjat, Radha, Ramakrishna) were developed. The genotype Sigadis was used for developing varieties Ratnagiri and 68-1. BJI was used as a donor for BB genes and varieties PR 4141 and IET 8585 (Ajaya) were developed. However, large-scale and long-term cultivation of varieties carrying only *Xa-4* has resulted in evolution of newer races of the pathogen leading to breakdown of resistance in rice varieties across several Asian countries. One way to delay such a breakdown of BB resistance is to pyramid/converge multiple resistance genes into rice cultivars. The convergence breeding technique was adopted by Xu et al. (1996) in China to combine the early Japonica line G38 carrying the gene *xa-5* with the recurrent parent Xiushui 11, which has adult plant resistance conferred by *Xa-3* gene. As a result of successive back crosses, three late Japonica lines were bred with resistance at all growth stages. Similarly, Yoshimura et al. (1995a,b) combined resistance genes in pairs *Xa-4*/*xa-5* and *xa-5*/*Xa-10* and showed that plants with two genes can have a higher level of resistance to Xoo than would be expected from the sum of the parental levels.

This approach of convergence breeding however can be very difficult or impossible when there is epistasis or masking effect of genes, particularly when a breeding line already harbors a gene like *Xa-21* which shows resistance to all known Xoo races. With the conventional approach, breeding lines with *Xa-21* alone cannot the distinguished from breeding lines with *Xa-21* plus other genes. However, if DNA markers are available for each resistance gene, the identification of plants with multiple genes would become easy.

8.2. MAS

Abenes et al. (1993) used MAS for gene pyramiding for bacterial blight resistance for the first time. Zhang et al. (1996b) identified *xa-13* gene via linkage to RFLP markers in three F4 populations in which the *xa-13* gene would otherwise have been masked by *Xa-21* gene. PCR-based marker-assisted selection for *Xa-21* gene using the STS primer specific to the *Xa-21* gene was employed by Reddy et al. (1997) in rice improvement program. Huang et al.

(1997) successfully used MAS for pyramiding four BB resistance genes, *Xa-4*, *xa-5*, *xa-13* and *Xa-21* using RFLP and PCR-based markers. The pyramid lines showed wider spectrum and a higher level of resistance than lines with only a single gene. This study resulted in the development of PCR primers for resistance genes *xa-5* and *xa-13*.

MAS-based on STS markers was used by Sanchez et al. (2000a) to transfer three bacterial blight resistance genes *xa-5*, *xa-13* and *Xa-21* into three promising new plant types. They demonstrated that BC_3F_3 population NILs having more than one BB resistance gene showed a wider resistance spectrum and manifested increased levels of resistance to different Xoo races as compared with those having single BB resistance gene. Their studies indicated that MAS reached an accuracy of 95% and 96% in identifying homozygous resistant plants for *xa-5* and *xa-13* genes, respectively.

Deployment of resistance genes into rice hybrids needs altogether a different strategy, as the virulent pathogen is exposed in the hybrid to a host population that is heterogeneous and heterozygous for allele at the resistance loci targeted by MAS.

8.3. Resistance gene deployment strategies in hybrid rice breeding

Marker-assisted selection can be successfully used for resistance gene deployment in hybrid rice parents (Witcombe and Hash, 2000). Molecular markers available for various resistance genes can be used to combine these genes into one of the parents in the most popular three-line breeding program. At this point, the question arises as to the choice of parent: should efforts be concentrated on deployment of resistance genes in seed parent or restorer line? Though the MAS program is technically easier in a restorer line, than in a seed parent that consists of an A/B pair, economic considerations make MAS more attractive in seed parents. Moreover, restorer lines are often used to produce only a single hybrid, whereas male-sterile lines are used to produce many hybrids over an extended period. But the male-sterile lines are difficult to breed.

Producing single cross-hybrids with a single inbred B line is the simplest way of deploying several resistance genes, but all of the R genes have to be employed in a single pyramid. This carries the risk involved with exposing the pathogen to a complete pyramid of R genes in both the hybrid seed production plots and in farmers' fields. Further, durability of a full pyramid of R genes cannot be predicted. Hence, it is always desirable to have two B lines with smaller pyramids, which can be crossed simultaneously to the A line. The resulting A line with all the R genes can be crossed with the restorer line to develop a commercial F_1 hybrid.

Chen et al. (2000) successfully incorporated *Xa-21* gene into Minghui 63 an elite restorer line of hybrid rice by molecular marker-assisted selection. In their study, they regenerated the Minghui 63 plant type using 128 RFLP markers distributed through out the 12 chromosomes. Recently, Borines et al. (2000) attempted to incorporate three BB resistance genes *Xa-4*, *Xa-7* and *Xa-21* into four maintainer lines (IR 58025B, IR 62829 B, lion B and Bob) which are susceptible to Xoo. They successfully incorporated *Xa-7* and *Xa-21* genes into the maintainer line. To distinguish the maintainer line with *Xa-21* gene alone and with both the genes, they tagged the *Xa-7* gene with a RFLP marker G1091.

Assuming that a pyramid hybrid is sufficiently large for the pathogen populations to lack any genotype with all the corresponding virulence alleles, and also assuming that genetic recombination does not take place among different strains of the pathogen, there is a good chance that a virulent pathogen will not evolve. However, this is an ideal situation that cannot exist in nature. If all of the deployed genes are overcome at once, then more resistance loci will have to be found and deployed. At the same time, one cannot rule out the possibility of evolution of new pathotypes. Hence, to combat the problem of breakdown of resistance in hybrids and high-yielding cultivars, efforts have to be made to pyramid the known major genes conferring resistance to the isolates of that specific region.

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