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Marker-assisted selection for biotic stress tolerance in rice: current status and emerging needs

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Introduction

Because of the intensive studies done on host plant resistance and host-pathogen interactions for some rice pathosystems, many useful resistance genes and markers are available in rice. This makes it possible to practice marker-assisted selection (MAS) for disease resistance breeding at a relatively early stage. Beside scientific advances, there are also practical reasons why MAS is particularly relevant for breeding for biotic stress tolerance:

- Most disease assays require the use of pathogens and insects, which are often variable and contribute considerable variation in the phenotypic evaluation. In some cases, the pathogens and insects as screening agents are geographically limited due to quarantine measures.
- Interactions between host and pathogen often exhibit strong epistasis; i.e. resistance tends to mask the phenotypic expression of other genes. Thus, gene pyramiding is possible only through the use of genetic markers.
- In the case of insect-transmitted viruses, phenotypic evaluation is further complicated by the complex interactions of host, vectors, and viruses.

These limitations can be addressed by using genetic markers as a surrogate for the target phenotype. Thus, MAS is used not only as a tool to increase efficiency but an essential approach for achieving a breeding objective. However, no MAS will be successful if the genes/markers do not represent high-quality genes expressing

the appropriate spectrum of resistance. In this brief review, we will discuss the current status of MAS applications for key disease and insect pests of rice, the on-going needs, and emerging challenges.

Bacterial blight resistance: a successful case of MAS in rice breeding

MAS in bacterial blight (BB) is the most advanced in rice breeding program, both in terms of science and in producing commercial products. A number of releases with different *Xa* combinations have been made in the past decade. The pathway to success dated back to over 20 years ago when a concerted effort was made to produce a comprehensive series of near-isogenic lines for all bacterial blight resistance genes (*Xa*). In retrospect, the key features for success may include the following:

- A strong research environment exists for studying host-pathogen interactions of bacterial blight, leading to many mapped and cloned *Xa* genes, hence providing either very tightly linked or gene-based markers. A list of widely used genes is summarized in Leung (2008).
- Most of the discovered *Xa* genes are bred into the indica recurrent parent IR24, a variety with wide adaptability and high agronomic qualities. This breeding-ready background accounts for the extensive use of *Xa* genes in breeding programs of both the public and private sectors. Recurrent parents in japonica (Toyonishiki) and indica-japonica (Milyang 23) backgrounds were also made.
- Extensive testing data on individual *Xa* genes and gene pyramids across locations.
- A capacity building network sustained continuously for 9 years by the Asian Rice Biotechnology Network. It facilitated distribution of NILs and gene pyramids, provided training and capacity building, and on-site technical backstopping to breeding programs.

These positive attributes appear simple, yet establishing such a system requires an understanding of the biology of the pathosystem, systematic development of specialized genetic stocks, and a network to evaluate and disseminate the genes/breeding lines and appropriate diagnostic BB strains.

Yet, even with this successful record, bacterial blight has not disappeared as a problem. There is a continuing need for different combinations of *Xa* genes to cope with new virulences and changing production system. This is particularly evident in India and China. Bacterial blight is a key problem in the fast expanding lowland rice production in western Africa. Because of the generally low level of resistance in many hybrid rice varieties, bacterial blight is expected to become more prominent with the spread of hybrid rice technology. This will require an increased effort to incorporate *Xa* genes into parental lines for both 3-way (CMS line) (Borines et al, 2008; Agarcio et al, 2007) and 2-line hybrids (Perez et al. 2008).

Blast

Just like the *Xa* genes, many blast resistance genes *Pi* have been reported in the literature. However, the use of MAS in blast resistance breeding has lagged behind bacterial blight. There are at least two reasons for this gap. First, breeding for blast resistance cannot follow the simple pyramiding of major R gene as in bacterial blight. The blast fungus appears to have a dynamic genome with great capacity to change in response to R genes deployed in rice varieties. Consequently, breeding for blast resistance requires a diversity of *Pi* genes and QTL. Second, many *Pi* genes have been bred into the genetic backgrounds of Co39 (indica type) and LTH (japonica type) because of their wide-susceptibility to blast (Fukuta et al 2004, Kobayashi et al, 2007; Fukuta et al, 2009). This comprehensive series of NILs and monogenic lines are ideal materials for genetic studies but less so for varietal development because they do not have the wide agronomic adaptability, such as IR24.

Table 1. *Pi*-genes used in breeding program of NARES partners.

Pi-gene	Designation	Donor	Chro	Class of gene	Near-isogenic line			Being used in breeding
					LTH	CO-39	US-2	
Piz	IRBLz-Fu	Fukunishiki ML: IRBLz-Fu	6	-	BC3F1 -	- -	- BC4F1	-
Pish	IRBLsh-S	Shin 2 MlL: IRBLsh-S	1	-	- BC3F1	BC6F14 BC4F1	- BC3F1	Philippines
Pi1	IRBL1-CL (C101LAC)	C101LAC ML:IRBL1-CL (C101LAC)	11	-	BC6F16 -	BC6F14 -	- BC6F7	Indonesia; India
Pi3	IRBL3-CP4 (C104PKT)	Pai-kan-tao ML: IRBL3-CP4 (C104PKT) Taebeg	9	-	BC6F16 - BC6F15	- BC4F1 -	- BC4F1 -	Vietnam
Piz-5 = Pi2	IRBLz-CA (C101A51)	C101A51 ML: IRBLz-CA	6	NBS-LRR (*Nbs4-Pi2*)	BC6F16 -	BC6F14 -	- BC4F1	Indonesia
Pi5(t)	IRBL5-M	RIL249 (Moroberekan) ML:IRBL5-M (RIL249)	9	-	BC6F16 -	BC6F14 -	- BC6F8	-
Pi9(t)a	IRBL9-W	Oryza minuta WHD-IS-75-1-127 ML:IRBL9-W (WHD-IS-75-1-127)	6	NBS-LRR (*Nbs2-Pi9*)	BC6F16 -	- BC3F1	- BC6F7	India
Pik-p	IRBLkp-K60	HR 22 K60 ML:IRBLkp-K60 (K60)	11	-	- BC3F1	BC6F14 -	- BC6F7	-
Pik-h	IRBLkh-K3	HR 22 K3 ML: IRBLkh-K3 (K3)	11	-	BC6F14 -	BC6F14 -	- BC6F7	Bangladesh
pi21	-	Owarihatamochi		Proline-rich protein	-	-	-	Japan
Pi36	-	Kasalath	8	Rice coiled-coil-NBS-LRR, resembles *Mla1* and *Mla6* than *Pita, Pib, Pi9*, and *Piz-t*.	-	-	None	None; evaluated against Chinese isolates
Pi40(t)	IR65482-4-136-2-2b	*O. australiensis* (Acc. 100882)	6	NBS-LRR motifs	-	-	-	-

Recent developments in blast research provide new opportunities to implement MAS in blast resistance breeding:

1. Increasing amount of experimental data has enabled us to distinguish high-quality *Pi* genes (Table 1). Some of these genes have been cloned, hence providing gene-based markers for use (Liu et al. 2009).
2. Blast resistance QTL are available that can provide broad-spectrum resistance—e.g. germin and germin-like protein, complex gene loci together give QTL effect (Manosalva et al, 2009; Carrillo et al, 2009).
3. Novel genes conferring broad-spectrum disease resistance have been identified. One such example is *pi21*, a resistance gene that provides stable blast resistance in upland rice grown in Japan. (Fukuoka et al, 2009). Unlike the "traditional" R genes with NBS-LRR domains, the wild type *Pi21* gene encodes a proline-containing protein such that a recessive mutation of the gene confers partial resistance. It would be interesting to introduce *pi21* into multiple genetic backgrounds and agronomic settings.

Because gene-based markers are available, production of gene pyramids can be accelerated by marker-assisted selection and without total dependency on phenotypic evaluation. It means that genotypes can be constructed with combinations of multiple R genes and QTL.

Brown Planthoppers (BPH)

Application of MAS for selection of insect resistance is most advanced for gall midge because of the strong interaction phenotypes between gall midge and rice. The observations so far suggest a gene-for-gene interaction similar to that found in highly specialized host-pathogen interactions. For tropical Asia, the key insect problem amenable to a MAS breeding approach is brown planthopper because the number of genes identified for BPH is high (Yencho et al, 2000; Jena et al, 2006; Rahman et al, 2009). Recent study has evaluated the virulence of laboratory strains of the BPH (*Nilaparvata lugens*) and the whiteback planthoppers

(*Sogatella furcifera*) collected between 1966 and 2005 using rice differential varieties carrying different planthopper resistance genes. The study suggested that long-term mass rearing in the laboratory has not affected virulence status, thus will be useful in analyzing resistance genes against BPH and WBPH (Myint et al, 2009).

Resistance from wild rices has become the potential for exploring novel genes. Of 21 BPH resistance loci, 11 have been identified from wild rice species; they are *Bph10* (*O. australiensis*), *Bph12(t)* (*O. latifolia*), *Bph13(t)* in chromosome 2 (*O. eichingeri*) and another *Bph13(t)* in chromosome 3 (*O. officinalis*), *Bph14* and *Bph15* (*O. officinalis*), *Bph18(t)* (*O. australiensis*), *bph11(t)* and *bph12(t)* (*O. officinalis*), *Bph20(t)* and *Bph21(t)* (*O. minuta*). Major genes conferring BPH resistance in several cultivated rice and wild species have been mapped with markers that will facilitate MAS for BPH resistance (Jena et al, 2006). Also, there is an on-going effort to produce series of NILs carrying important *Bph* resistance genes.

We foresee a growing need for MAS for insect resistance breeding. For example, the donor cultivar Rathu Heenati carrying *Bph3* was combined with KDML105 essential grain quality traits. The linkage drag between *Bph3* and Wx^a alleles was separated by phenotypic and MAS (Jairin et al, 2009). All introgression lines from this population showed broad spectrum resistance against BPH populations in Thailand and had grain quality similar to KDML105.

Tungro viruses

Since the 1990s, a number of tungro resistant varieties has been developed by direct phenotypic screening. Initially, most selected varieties were resistant to the green leafhopper (GLH). Emphasis was later shifted to resistance to tungro viruses (e.g., the development of Matatag 1). However, there is difficulty in conducting screening because the phenotypic assay is often based on the percent of plants showing symptom expression within a line. The complexity of the

interactions involving three biological agents: GLH harboring two different viruses—RTSV and RTBV makes tungro virus resistance an excellent target for MAS application.

To identify gene(s) involved in RTSV resistance, the association of genotypic and phenotypic variations for RTSV resistance was examined in backcross populations derived from Utri Merah and rice germplasm with known RTSV resistance. Resistance in Utri Merah was found to be under the control of a recessive gene (*tsv1*). The gene was mapped to a 200-kb region (22.0 to 22.2Mb) of chromosome 7. Within this region, Lee et al (2009) identified SNP variants in a gene encoding initiation elongation factor (IEF) on chromosome 7 that is strongly associated with RSTV resistance. Donors and germplasm showing resistance all have the same SNP variants affecting the Val variants in the IEF gene. Thus, the evidence so far suggest that mutations in the IEF could be responsible for resistance to RTSV, and the SNP variants could serve as markers for selection for RTSV resistance.

Sheath blight

Although there have been increasing number of reports on the mapping of QTL for sheath blight resistance, most of them are coarsely mapped, spanning a large genetic distance. Furthermore, the phenotypic effects of these QTL are relatively small. This level of phenotypic effects does not inspire the use of the QTL by breeders due to the inherent difficulty in assessing the components of resistance associated with disease progression. In a recent study, 12 QTLs were identified for sheath blight resistance on the HP2216/Tetep recombinant inbred population from a cross between HP2216 and Tetep. These QTLs on chromosomes 1, 3, 7, 8, 9 and 11 and the respective alleles explain 8.13-26.05% of the total phenotypic variation (Channamallikarjuna et al, 2009). Progress has also been made in identifying good sources of genetic resistance using standard evaluation

procedure for sheath blight resistance (IRRI SES, 1996). We evaluated over 300 accessions of *Oryza rufipogon.* Of these, three accessions were selected and backcrossed to elite lines and varieties. Advanced F_4 population derived from the cross *O. rufipogon* (Acc 105757) x PSBRc80 have been selected for further backcrossing and intermating to develop populations for resistance breeding.

Effort is also underway to refine the phenotyping procedures. Until we have confidence in the assay of sheath blight resistance at different levels (tillers, single hill, to plant population), it will be difficult to develop molecular markers with high predictive power on the phenotype.

Brown spot

Relatively little is known about the genetic control of resistance to brown spot. Significant differences in resistance were observed in a diverse set of germplasm tested against isolates of *B. oryzae*. Among the germplasm tested, N22, MAAL 6, IR36 and restorer line IR69726-29-1-2-2-2 showed variation in resistance to different isolates of the pathogen.

Of special interest is Dinorado (IRTP 12568) which shows resistance to multiple virulent isolates of *B. oryzae*. To determine the resistance in this tall traditional japonica variety, a mapping population was developed by crossing Dinorado with IR36, a susceptible indica variety. Based on segregation of F_2 and F_3 progenies, two pairs of recessive genes appeared to be responsible for the resistance to brown spot. Based on inheritance study, the two genes are tentatively designated as *bs1* and *bs2*.

A QTL was defined by markers RM277 and RM1261 on chromosome 12, which accounts for 48% of total phenotypic variation. The second QTL was mapped on chromosome 3 with lesser effect. Further fine mapping of these two QTL may yield markers useful for MAS.

Conclusions

For diseases such as bacterial blight and blast where resistance genes have been identified and their quality assessed, incorporation in improved high yielding varieties have been made and released for commercial production. An intensive knowledge of host-pathogen interaction for diseases with specific resistance contributes to identification of good quality genes that confer broad spectrum resistance. The mapping and cloning of well-studied genes allows identification of tightly linked markers as well as design of gene-based markers for the breeding program. For quantitative traits, testing in multi-location sites for studying GxExM interactions allows for precise phenotyping of the target traits in response to the diverse population of pathogen and insect pests. Despite these challenges, genetic resources and tools have been developed for biotic stress tolerance in rice (Table 2) and genetic stocks with value-added traits are available for some traits, and for other traits are still in progress (Table 3). Efficiency in utilizing these genetic resources to develop and sustain durable resistance in agricultural system is needed.

Under an environment influenced by drivers of global changes in rice production (climate, labor, water scarcity, etc), impacts on production technologies become a concern. Diseases such as false smut and those causing grain discolorations are emerging problems in rice production that need to be addressed in the future. These are concerns closely associated with long term sustainability and environmental consequences of intensification of agricultural systems.

Table 2. Genetic resources and tools for biotic stress tolerance.

Biotic stress	Conventional breeding	MAS Component			Genes/QTL transferred into new backgrounds	MAS products (commercial)	Trans genes or GM
		Genes/QTL mapping	Candidate genes	Gene discovery			
BB	+++	+++	-	-	+++	+++ (released)	+
Blast	+++	+++	+++	++	+++	-	-
Tungro	+++	+++	+	++	+++	+	+
Sheath blight	+	+++	-	-	-	-	++
Brown spot	+	+	-	-	-	-	-
False smut (emerging)	-	-	-	-	-	-	-
BPH	++	+++	-	-	+	-	-
WBPH	+	+	-	-	-	-	-
Stemborer	+	+	-	-	++	-	+++

Table 3. Genetic stocks with value-added traits for biotic stress tolerance.

Trait	Genetic stocks
Bacterial blight	Near-isogenic lines (IR24, Toyonishiki, Milyang 23) MAS pyramided lines (IR24) Alien introgression lines
Blast	Near-isogenic lines (*Pi* genes & candidate genes in 2-3 backgrounds, in progress) Alien introgression lines
Tungro	Elite tungro resistant lines Alien introgression lines
Sheath blight	Genetic donor Mapping population (in progress)
Brown spot	Genetic donor Mapping population (in progress)
BPH	Elite breeding lines Alien introgression lines
WBPH	Genetic donor Elite WBPH resistant lines
Stemborer	Donors Elite stemborer tolerant lines

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