MOLECULAR MARKER AIDED BREEDING FOR BLAST RESISTANT RICE IN NEPAL

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ABSTRACT

Molecular markers tightly linked to target gene have been identified in different chromosomes to impose the genetic selection. This paper summarizes the progress and achievement made in breeding for blast resistance rice based on DNA markers. At least 40 genes conferring resistance to blast isolates with multiple alleles have been described. Both dominant and recessive resistance alleles have been found in many rice landraces. Highly polymorphic and easily detectable SSR markers are being used in breeding for blast resistance. Bulked segregant analysis (BSA) is the simple method for tagging resistance gene by SSR markers. Quantitative trait loci (QTLs) have also been mapped and most of them are linked to qualitative genes. Simple sequence repeat (SSR) markers linked to the gene are being used to select plants possessing the desired trait and markers throughout the genome are being used to select plants that are genetically similar to recurrent parent. Using SSR markers it may be possible to select blast resistance genotypes at any stage of crop development from any small part of crop, to conduct many round of selection, to select without inoculums, without scoring, and without testing in hot spot or artificial inoculation. Molecular based blast resistance breeding work is necessary to initiate in Nepal focusing on resistance gene tagging in Nepalese rice landraces and utilization.

Key words: Blast resistance, DNA marker, gene tagging, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the most preferred crop of Nepalese farmers and they are growing about 2000 different rice landraces and 67 improved rice cultivars from 60 m to 3050 m altitude (Upadhyay and Joshi, 2003). In all rice growing areas blast disease caused by (*Magnaporthe grisea* (Herbert) Borr. (anamorph *Pyricularia oryza* Cav. = *P. grisea* Cav.) is the most serious fungal disease causing heavy yield losses from 10 to 80% (Manandhar et al., 1992). The fungus produces spots or lesions on leaves, nodes and different parts of panicles and grains. The neck blast makes more significant yield and quality losses than leaf blast (Katsube et al., 1970). While highly positive correlation between resistance to leaf and neck blast has been generally observed, inconsistency between resistance to leaf and neck blast was also evident (Zhuang et al., 2002).

Blast disease is the most destructive disease worldwide. Growing resistant varieties has been the most effective and economic way to control the disease but resistance is often lost in a few years after cultivars released because of the high variability of the rice blast fungus. To breed rice varieties with more durable blast resistance,

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multiple resistance genes utilizing both qualitative and quantitative genes must be incorporated into individual varieties. This necessitates the exploration of more efficient selection and breeding strategies than those currently exist. Recent advancements in DNA marker technology may provide new solutions for selecting and maintaining more durable resistance genotypes in rice. In contrast to the traditional selection based on phenotypic screening, molecular markers are refractory to environmental variation. Upon identification of molecular markers closely linked to desirable trait/s, marker assisted selection (MAS) can be performed for multiple resistance in early segregating generations and at early stages of plant development. This paper summarize the progress and achievement made in breeding for blast resistance based on DNA markers which will help in planning blast resistance gene tagging in Nepalese rice genotypes and developing blast resistant inbred line or near isogenic line (NIL).

GENETICS OF BLAST RESISTANCE IN RICE

The genetics of rice blast have been extensively studied and two types of resistance have been described, complete (true or vertical) and field resistance (horizontal) (Fukuoka and Okuno, 2001). Rice blast is one of the best characterized models for understanding molecular mechanisms of natural defense response. Resistance to infection by this fungus follows a classic gene for gene theory (Silue et al., 1992). The *Pi-b* and *Pi-ta* genes are two major blast resistance (R) genes that have been characterized molecularly (Wang et al., 1999). Genetic analysis of blast resistance studied by several researchers indicated either monogenic dominant, monogenic recessive, two dominant independent genes, two dominant complementary genes and two recessive duplicate genes or resistance controlled by minor genes. Mostly the genes resistance to blast fungus is monogenic dominant. Multiple alleles upto 5 are identified (Imbe et al., 2000; Hittalmani et al., 1995). Mackill and Bonman (1992) developed 22 near isogenic lines (NILs) each having a single complete R gene from CO39 to study the genetics of blast resistance. At least four independent loci, *Pi-1* to *Pi-4* were identified after inheritance study using these NILs. Inukai et al. (1994) showed that *Pi-1* was allelic to *Pi-z*. Structural and functional analyses of many major R genes have shown that they encode proteins with similar structural motifs- nucleotide binding site, kinase domains, leucine-rich repeats- that are responsible for ligand recognition and signal transduction (Wu et al., 2004). Most studies have suggested that field resistance to blast is under complex genetic control and multiple genes are responsible for the expression of field resistance (Higashi and Saito, 1985).

QUALITATIVE AND QUANTITATIVE GENES FOR BLAST RESISTANCE

True resistance is governed by qualitative gene also called major gene and field resistance by quantitative genes called minor genes. More than 40 qualitative resistance genes for blast fungus have been identified (Annex 1). Most of them are dominant and located in chromosome 6, 11 and 12. In some loci multiple resistance alleles are identified. The wide genetic variation available in blast fungus may be the main factor to evolve many resistance genes in rice. These two resistance types, complete and field resistance are under monogenic and polygenic systems respectively. *Pi-k* has 5 alleles, *Pi-2* locus has 2 and *Pi-ta* has 2 alleles (Bryan et al., 2000; Fjellstorm et al., 2006; Hittalmani et al., 1995). Four R genes have been

identified in Tetep and 3 R genes in Pai-Kan-Tai. Wang et al. (1994) has described 10 QTLs, of which two QTLs are located in chromosome 4. Fukuoka and Okuno (2001), Liu et al. (2004) had also detected QTLs. Majority of the QTLs are associated with qualitative genes.

ALLELES MINING AND MOLECULAR TAGGING

Rice genome is completely sequenced and it is available publicly (www.ncbi.nlm.nih.gov). This helps to discover the genes in rice and other related plant species. Discovery of genes controlling economically important traits can be used for application in MAS and discovery of new beneficial alleles in the germplasm (allele mining). Rice genome has 430 million bp (about 1/10 of human) with estimated 50,000 genes (Fjellstrom et al., 2006). This publicly available DNA sequences have been used to scan for SSR region and to design primers. Fjellstrom et al. (2006) developed markers closely linked to *Pi-z* locus from the analysis of publicly available genome sequence of Nipponbare rice variety.

Molecular mapping and tagging of agronomically important traits are prerequisites for MAS and marker assisted backcrossing (MAB). Different methods are available to tag the genes by DNA markers. These include bulked segregant analysis (BSA), pre-selection using NILs, preparative pulsed field gel electrophoresis and chromosome walking and jumping. BSA provides a rapid, technically simple alternative for identifying markers linked to specific genes. The only prerequisite is the existence of a population resulting from a cross that segregates for the gene of interest (Michelmore et al., 1991). The success of the approach will depend on the genetic divergence between the parents in the target region.

MARKERS LINKED TO BLAST RESISTANCE GENES AND MOLECULAR SCREENING

Dense linkage map of rice is available for restriction fragment length polymorphism (RFLP) and SSR markers. More than 15 RFLP markers tightly linked to blast R genes are identified. Due to some limitation of RFLP markers for examples complex laboratory procedure, research is moving forward in SSR and single nucleotide polymorphism (SNP) markers. About 10 SSR markers closely linked to blast R genes are available. Upon the identification of DNA markers, screening of large germplasm can be done at any time and any growth stages. In case of screening the disease resistance, genotypes can be screened at early stage of segregating generation without inoculating and scoring. For phenotypic screening, genes must be expressed. SSR markers due to very large in number are considered very useful in screening program.

MOLECULAR AIDED BREEDING

Breeding work utilizing both phenotypic and genotypic markers are more reliable and fast. Conventional breeding are based on gene expression however there are many limitations, For example epistatic effect is the one. Molecular breeding on the other hand has an opportunity to monitor and manipulate traits without gene expression. MAS and MAB are the two major approaches to accelerate breeding works. Molecular approach is more suitable to pyramid genes and to dissect the

complex traits such as grain yield, plant height, etc. Hittalmani et al. (2000) developed three genes pyramid rice using MAS. These genes are *Pi-1, Piz-5* and *Pi-ta*. Three varieties, CO39, A57-115-8 and Moroberekan are 3 genes pyramid varieties resistance to blast fungus. Combination of *Pi-18, Pi-21* and *Pi-22* or *Pi-1* and *Piz-5* in a variety is reported effective for durable resistance. Main advantages of molecular breeding are selection at any stage of crop development, at early segregating generation, many round of selection, selection without gene expression. In addition to this, only small part is enough for screening multiple characters.

LEAF AND NECK BLAST RESISTANCE

Neck blast is the major problem in Nepal and yield loss from this is reported higher than leaf blast. Relatively other methods for controlling this disease are available for leaf blast fungus and research is more focused on leaf blast. Positive correlation between leaf and neck blast resistance is observed but inconsistent also exist. Site specific donor for leaf and neck blast resistance should be identified and their genetics should be studied in local germplasm. Neck blast is a major problem in many countries. *Pi-k* in chromosome 11 has been reported effective against neck blast. However, there is reported a cluster of genes in this region. If the gene functions at all growth stages, the gene would be useful, but not all genes function to be effective at all stages, thus, it is possible that resistance at seedling or leaf blast stage may not be useful for neck blast.

BREEDING FOR BLAST RESISTANCE IN NEPAL

Every year NARC screen more than 1000 rice genotypes using conventional breeding approach and more than 50% are found resistance to blast fungus. Most of the screening materials are breeding lineinsertes generated from national breeders and line received from IRRI. Every year international materials are tested in Nepal and IRRI summarized the results (Table 1). Internationally resistance lines are the good sources of resistance genes. Screening sites are mainly Khumaltar (located in the mid hill), Hardinath (located in Tarai), and Lumle (located in mid hill). Hot spots where pathologists and breeders screen the germplasm for blast disease are Bijayanagar-Jumla, Gokarna-Lalitpur, Chaling-Lalitpur, Dharmasthali-Kathmandu, Kavrasthali-Kathmandu, Bhawasi-Mahottary and Bharatpur-Dhanusha. Bhawasi-Mahottary is the hot pocket for neck blast screening. A number of rice genotypes have been identified (list of genotypes not shown) either resistance or susceptible to blast fungus in different locations (Adhikari and Shrestha, 1986; Chaudhary and San, 1997; Chaudhary and Sah, 1998; Chaudhary et al., 1994; Chaudhary, 1995; Manandhar et al., 1992; Manandhar, 1984; Manandhar, 1987). Regarding blast pathogenic races, 15 pathotypes are reported to date (Chaudhary et al., 2005). It may increase if more isolates are tested. There are many local as well introduced genotypes that are resistant and susceptible to blast in Nepal (Thapa and Manadhar, 1985; Pradhanang, 1988; Chaudhary, 1995; Manandhar et al., 1992; Manandhar, 1984; Manandhar, 1987). However, Laxmi, Sabitri, Janaki, Radha-12, Khumal-11 are some rice varieties found resistant to blast. Laxmi is resistant to the most virulent isolate ever tested (that isolate can produce disease on rice genotype with 3 gene-pyramid). Therefore, Laxmi may be the nationally resistance variety. Masuli or

Sankharika may be nationally susceptible genotypes and CO39 or LTH are international susceptible lines. Tetep may be considered universally resistance genotype (Chaudhary, 1995; Manandhar et al., 1992).

Resistance is often lost in a few years after cultivars being released and undergone mass cultivation. For example Khumal-4 is a fine variety. It is in general observed in plants that interaction between resistant varieties and locations are found significant. Similarly interactions between resistant varieties and years have also been found significant.

Table 1. Best varieties for resistance against blast disease screened in International Network for Genetic Evaluation of Rice (INGER) nurseries during 1975-1995

SN	Variety	Origin
1.	Carreib, Takudan	Philippines
2.	CIATICA-5	Colombia
3.	IR1905-81-3-1, IR1416-128-5-8, IR2793-80-1, IR116-1-42-2-3-3, IR1905-PP11-29-4-61, IR457-6-3-2, IR5533-PP850-1, IR32429-47-3-2-2, WHD-IS-75-1, IR59606-119-3	IRRI
4.	IRAT13	Cote d'lvoire
5.	IRAT144	Burkino Faso
6.	IRI387	Korea
7.	MG3	China
8.	Ta-poo-cho-z, Huan-sen-goo	Taiwan
9.	Tetep	Vietnam
10.	Tres Naruas	Brazil

Source: Chaudhary, 1996

DONORS OF RESISTANCE GENE TO NEPALESE CULTIVARS

Pedigree analysis of Nepalese rice cultivars indicates that 14 ancestors of these cultivars are resistance to blast fungus (Table 2). The ancestor, Sigadis was used in 13 cultivars. These ancestors may not be resistance to all race of blast fungus in Nepal. Suitable resistance gene donor for particular area should be identified so that breeder can use them as parent in hybridization program.

BREEDING APPROACH FOR BLAST FUNGUS MANAGEMENT

Breeding the resistant variety is considered best way for controlling the disease. For that both qualitative and quantitative genes should be utilized. Cultivars mixture, multilines, near isogenic lines or resistance inbred lines are reported effective approaches for disease management. It is also suggested that crop rotation can help to reduce the disease intensity. Gene and individual QTL pyramiding should be considered for durable resistance to blast fungus.

Table 2. Ancestors of 48 improved rice cultivars released in Nepal (1965-2002) and their reaction to blast fungus

SN	Ancestor	Origin	Group	Reaction to blast fungus	Contributed cultivars, n
1.	AKIYUDAKA	Korea	Japonica	Resistance	1
2.	ANNAPURNA	?	?	Susceptible	1
3.	B531B-TK39	?	?	-	1
4.	BPI 76	Philippines	Indica	Susceptible	1
5.	C4-63-GB	?	?	-	1
6.	CENTURY PATNA	USA	Indica	Susceptible	13
7.	CHINA 1039	India	Indica	Susceptible	1
8.	CHINA 971	China	?	-	1
9.	CHINA-1039-DWF-MUT	China	Indica	Susceptible	1
10.	CHINA-45	China	?	Susceptible	1
11.	CINA	China	?	-	28
12.	CO-18	India	Indica	Susceptible	15
13.	CO-29	India	Indica	Susceptible	1
14.	DEE GEO WOO GEN	Taiwan	Indica	Susceptible	23
15.	DUNGHAN SHALIL	?	INDICA	Susceptible	1
16.	FUJI-102	Japan	Japonica	Resistance	2
17.	GEB 24	India	Indica	Susceptible	15
18.	GHANDRUK LOCAL	Nepal	Japonica	Resistance	2
19.	GP-15	?	?	-	8
20.	H4	Ceylon	Indica	Resistance	1
21.	H501	Ceylon	Indica	Resistance	3
22.	HR 21	India	Indica	Susceptible	1
23.	HSINCHU-4	Taiwan	Japonica	Susceptible	1
24.	JARNELI	Nepal	Indica	-	1
25.	JERAK	?	?	-	4
26.	JINLILNG-78-102	China	Japonica	-	1
27.	JUMLI MARSHI	Nepal	Japonica	Susceptible	1
28.	K-28-76-B-1	India	Japonica	-	1
29.	KN-1B-214-1-4-3	Indonesia	Indica	-	1
30.	KULU	Australia	Indica	-	1
31.	LALNAKANDA	India	Indica	Susceptible	1
32.	LATISAIL	Pakistan	Indica	Susceptible	28
33.	LD-66	?	?	-	1
34.	MAS	Indonesia	Indica	Susceptible	3
35.	MAYANG EBOS-80	Malaysia	Indica	Resistance	3
36.	MCVA	?	?	-	1
37.	MTU15	India	Indica	Susceptible	1
38.	MUDGO	India	Indica	Susceptible	1
39.	MUTANT-65	?	?	-	1
40.	O. NIVARA	?	?	-	11
41.	OB678	Srilanka	?	-	1
42.	O-LUAMCHU	?	?	-	1
43.	POKHRELI MASINO	Nepal	Indica	Susceptible	3
44.	PP	?	?	-	3

SN	Ancestor	Origin	Group	Reaction to blast fungus	Contributed cultivars, n
45.	PTB 18	India	Indica	Resistance	5
46.	PTB 21	India	Indica	Resistance	5
47.	R. HEENATI	?	?	-	1
48.	REMADJA	Indonesia	Indica	Resistance	3
49.	RP72	India	Indica	-	1
50.	SHANKARA	Nepal	Indica	-	1
51.	SHINEI	Japan	?	Susceptible	1
52.	SHINIRI AIKOKU	?	?	-	1
53.	SIGADIS	Indonesia	Indica	Resistance	13
54.	SLO	India	Indica	Susceptible	13
55.	T141	India	Indica	Susceptible	3
56.	TADUKAN	Philippines	Indica	Resistance	11
57.	TAICHUNG NATIVE1	Taiwan	Indica	Susceptible	22
58.	TAICHUNG-150	Taiwan	Japonica	Resistance	1
59.	TAICHUNG-45	Taiwan	Japonica	-	1
60.	TAICHUNG-65	Taiwan	Japonica	Resistance	4
61.	TAIPEI-7	Taiwan	?	-	1
62.	TCA-80-4	India	Indica	-	1
63.	TETEP	Vietnam	?	Resistance	2
64.	TSAI YUAN CHUNG	Taiwan	Indica	Susceptible	2
65.	WAIKAKKU	?	?	-	1
66.	YUNLEN-1	China	Japonica	Susceptible	1

? Not known. Source: Joshi, 2004; Joshi, 2005.

WAY FORWARD

For effective management of blast disease, breeding work should be focused on utilizing the broad spectrum of resistance genes (*Pi-2, Pi-9 (t), Pi-5 (t), Pi-z, Piz-5)* and pyramiding genes and QTLs. Positive correlation between leaf and neck blast resistance are reported but, this is not applicable in all cases. Breeding for neck blast resistance is therefore necessary in Nepal. Introduction of exotic genes for resistance induced the occurrence of new races of blast fungus, therefore breeding work should be concentrated in local resistance genes using molecular markers. Mapping of resistance genes in rice landraces are necessary to speed up the marker assisted blast resistance rice breeding in Nepal. DNA sequences of rice can be considered to design the primers linked to blast resistance.

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