TAGGING OF BLAST RESISTANCE GENE(S) TO DNA MARKERS AND MARKER-ASSISTED SELECTION (MAS) IN RICE IMPROVEMENT

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Abstract

This paper reports progress made on the tagging of blast resistance gene(s) to DNA markers and on the initiation of marker-assisted selection (MAS) for blast resistance in rice improvement.

A pair of near isogenic lines, K80R and K79S, were developed using a Chinese landrace Hong-jiao-zhan as the resistance donor. Ten putatively positive markers were identified by screening 177 mapped DNA markers. Using the F_2 population of 143 plants and the derived F_3 lines, three Restriction Fragment Length Polymorphism (RFLP) markers (RG81, RG869 and RZ397) on chromosome 12 of rice were identified to be closely linked to the blast resistance gene *Pi-12(t)*. The genetic distance between *Pi-12(t)* and the closest marker RG869 was 5.1 cM. By employing the bulk segregant analysis (BSA) procedure, six of 199 arbitrary primers were found to produce positive Randomly Amplifiedl Polymorphic DNA (RAPD) bands. Tight linkage between *Pi-12(t)* and three RAPD bands, each from a different primer, was confirmed after amplification of DNA of all F_2 individuals. Two fragments were cloned and sequenced, and two sequence characterised amplified region (SCAR) markers were established.

In two other F_3 populations, Xian-feng 1/Tetep and Xian-feng 1/Hong-jiao-zhan, the blast resistance was found to be controlled by interactions of two or more genes. One resistance gene was located in the vicinity of RG81 in both populations. Work to identify other gene(s) is currently under way.

Marker assisted selection for blast resistance was initiated. Crosses were made between elite varieties and blast resistance donors to develop populations for DNA marker-assisted selection of blast resistance. In addition, 48 varieties widely used in current rice breeding programs were provided by rice breeders. DNA marker-based polymorphism among these varieties and resistance donors were analysed to produce a database for future MAS program.

1. INTRODUCTION

Rice blast, caused by *Pyricularia oryzae* Cav., is generally considered as the most important disease of rice because of its world-wide distribution and because of the severe losses in yield it may cause. Growing resistant varieties has been the most economical and effective way of controlling this disease. Unfortunately, blast resistance in rice varieties can be lost soon after large scale cultivation due to the development of new races or pathotypes of the pathogen. Breeding varieties with multiple resistance genes or a series of near isogenic lines (NILs) with different resistance genes (*i.e.* multi-lines) has been suggested as a solution to this problem [1, 2]. However, the reaction of different resistance genes to races of the pathogen may be similar, and the identification of blast resistance through inoculation with the fungus can be greatly limited by plant developmental stages and environmental factors. Conventional ways of host resistance identification would not always be helpful for rice breeders to determine whether different resistance genes have been integrated into a given line or a series of multi-lines.

On the other hand, indirect selection based on tightly linked genetic markers seems to be more promising in pyramiding the resistance genes and in the development of multi-lines. This relies on the exploitation of the tightly linked markers and the establishment of convenient and low cost detection procedures. The development of DNA restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) techniques has provided powerful tools for mapping genes of the interest [3, 4]. This paper reports our progress on the identification of DNA markers closely linked to a blast resistance gene, on the

studies of the interaction of different blast resistance genes, and on the initiation of DNA marker-assisted selection (MAS) for blast resistance.

2. MATERIALS AND METHODS

2.1. Tagging of a blast resistance gene to DNA markers

A pair of NILs, K80R (resistant to the rice blast) and K79S (susceptible) were developed [5]. The resistance donor used was a local Chinese *indica* cultivar Hong-jiao-zhan, which is well-known for its wide-spectrum and durable resistance to blast. The recurrent parent is an *indica* variety IR24.

An F_2 population of K80R/K79S consisting of 150 plants was grown at China National Rice Research Institute (CNRRI) in 1992. Blast resistance was evaluated by inoculation of the race ZB1 of *Pyricularia oryzae* Cav to each F_2 individual, and verified by inoculation to 12 plants of each F_3 line. DNAs of each F_2 individual as well as the four parental lines were extracted and subjected to RFLP analysis following methods described previously [6, 7].

Of 177 probes used, probe/enzyme combinations detecting polymorphisms between the donor and recurrent parents were used for the analysis of the NILs. Putatively positive results with a probe were examined further to see if the probe pattern co-segregated with blast resistance in the F_2 population. The computer program MAPMAKER [8] was used for linkage analysis. Distances between markers were presented in centimorgons (cM) derived using the Kosambi function [9].

To fine map the resistance gene with additional DNA markers, the bulk segregant analysis (BSA) procedure was employed. Equal quantities of DNAs from 10 homozygous resistant F_2 individuals were mixed to construct a resistant pool (R pool), and equal quantities of DNAs from 10 homozygous susceptible F_2 individuals were mixed to construct a susceptible pool (S pool). RAPD analysis of the pools was performed as described previously [5].

Primers generating polymorphic RAPD bands between the two pools were used to analyse their co-segregation with blast resistance in the F_2 population. RAPD fragments which co-segregated with blast resistance were cloned and sequenced. Two pairs of primers were synthesised for specific amplification.

2.2. Studies of gene interactions

Two F_3 populations were constructed, using a susceptible *indica* variety Xian-feng 1 as the common female parent, and blast resistance cultivars Tetep and Hong-jiao-zhan as the male parents, respectively. The three parental lines were inoculated with each of 20 blast races. The race ZC13 was selected to inoculate 161 F_3 lines of Xian-feng 1/Tetep and 175 F_3 lines of Xian-feng 1/Hong-jiao-zhan. Eighteen individuals of each line were inoculated. DNAs were extracted from bulked samples of 12 F_3 individuals of each line. RFLP analysis and specific amplifications were conducted by using DNA markers in the vicinity of mapped resistance genes on chromosome 12. BSA was then employed to identify RAPD markers linked to other resistance genes.

2.3. Initiation of MAS for blast resistance

Crosses were made between elite varieties and blast resistance donors in Hainan Province in the winter of 1995. The F_1 was grown in CNRRI in 1996 and backcrossed with elite varieties. A polymorphism survey was conducted using DNA markers linked to resistance genes.

Forty-eight varieties which were widely used in current rice breeding programs were provided by rice breeders. DNA marker-based polymorphism among the 48 varieties and resistance donors are being surveyed.

3. RESULTS AND DISCUSSION

3.1. Tagging of a blast resistance gene to DNA markers

Of the 177 RFLP probes tested, 75 were polymorphic between the donor and recurrent parents. They were then used to search for polymorphisms between the two NILs. When a probe produced an identical pattern for the donor parent and the resistant NIL, and produced an identical pattern for the recurrent parent and the susceptible NIL, this probe would likely be linked to a resistance gene(s). Ten probes of this class were found and termed putatively positive markers.

Most of the F_2 plants showed extreme responses to inoculation with conidial suspensions. One-hundred and ten plants were scored 0 or 1 (highly resistant) and 33 plants scored 7 or 9 (highly susceptible) to the race ZB1, fitting an expected ratio of 3:1 when the resistance was controlled by a single dominant gene (χ^2=0.33, P=0.50-0.75). Co-segregation of the blast resistance with putatively positive markers was then tested using the 143 plants.

Of the ten putatively positive markers, only three probes, RG81, RG869 and RZ 397, on chromosome 12 of rice, were found to be linked to the blast resistance gene.

The resistance genotypes of the F_2 individual were identified using the F_3 population, but of the 143 total, only 85 were verified. Map distances were estimated based on data from the 85 individuals. RG869 was most closely linked to the resistance gene, with a map distance of 5.1 cM. The gene for resistance to race ZB1 of *Pyricularia oryzae* Cav. was first tentatively named as *Pi-11(t)*, but later revised as *Pi-12(t)* [10].

Of the 199 10-mer arbitrary primers screened, 6 fragments, each generated by a different primer, were found to be polymorphic between the two pools (present in the R pool and absent in the S pool). After amplification of DNA from the two isolines and a subset of F_2 individuals, linkage between the resistance gene and three RAPD bands (1.3 Kb fragment produced by primer P622; 0.56 Kb fragment by primer P265, P265-560; and 0.35 Kb fragment by primer P286, P286-350) were confirmed. Complete co-segregation of the RAPD markers and blast resistance was detected against all the 143 F_2 individuals, indicating tight linkage of these three markers and the blast resistance gene.

The three fragments were cloned and used as probes to analyse RFLPs of the F_2 individuals. The 1.3-Kb and 560-bp fragments were found to contain repeat sequences. The 350-bp fragment was found to contain single copy sequence and detect a null allele in the susceptible individuals. The results of RFLP analysis using the clone of the 350-bp fragment are in agreement with those of RAPD analysis.

TABLE I. TWO SCAR MARKERS TIGHTLY LINKED TO *Pi-12(t)*

Marker	Sequence
P265-560	CAGCTGTTCAGTCGTTTG
	CAGCTGTTCATACAAGAAAT
P286-350	GCTCCGCATTAACGGGAAG
	AGCCGGCTCCGGAGGTGA

Clones of the 560-bp and 350-bp fragments were sequenced. Specific primers were synthesised for an establishment of (Sequence Characterised Amplification Region) SCAR markers (Table I). Specific amplifications of DNA from the two isolines and all the 143 F_2 individuals were made for P265-560. A 560-bp fragment was observed for all the resistant individuals but none of the susceptible ones, matching the result of RAPD analysis. For P286-350, polymorphsm between K80R and K79S was detected only after digestion of the PCR products with 4 cutters. The 350 bp fragment was observed for all the resistant individuals but none of the susceptible individuals.

3.2. Gene interactions

3.2.1. Gene interactions in the population of Xian-feng 1/Tetep

In both populations, three types of F_3 lines were observed in terms of their resistance segregation patterns, i.e. all individuals resistant to the race ZC13, segregation for resistance, and all individuals susceptible. They were referred as resistant lines, segregating lines and susceptible lines, respectively.

Of the total 161 F_3 lines of Xian-feng 1/Tetep, the number of resistant lines, segregated lines and susceptible lines were 68, 78 and 15, respectively. This fitted the expected ratio of 7:8:1 (P=0.275), when the resistance was controlled by duplicate interaction of two dominance genes (Table II). Of 1402 individuals in the 78 segregated F_3 lines, the numbers of resistant and susceptible plants were 1188 and 214, respectively. This was in good agreement with the expected ratio of 27:5 (P=0.710).

It was already known that a blast resistance gene *Pi-ta* in Tetep was closely linked to RFLP marker RZ397 on chromosome 12 [11]. In this study, RZ397 did not detect polymorphism between Xian-feng 1 and Tetep. Another linked marker RG81 was able to detect polymorphism between the two parents. Preliminary co-segregation analysis of RG81 and blast resistance indicated a gene for the resistance difference between Xian-feng 1 and Tetep was closely linked to RG81 (data not shown).

In order to detect the other resistance gene which was not linked to RG81, equal quantities of DNAs from 10 homozygous resistant F_2 individuals were mixed to construct a resistant pool (R pool), while those from 10 homozygous susceptible F_2 individuals were mixed to construct a susceptible pool (S pool). RAPD analysis of the pools was performed. Primers generating polymorphic RAPD bands between two pools were used to analyse their co-segregation with blast resistance. Thus far, three RAPD markers have been found to co-segregate with blast resistance, of which two were linked to RG81 and one was not linked to RG81. Mapping of the latter RAPD marker is underway using other mapping populations in our laboratory. After the marker is mapped, verification of the location of the two resistance genes will be conducted using F_6 or F_7 recombinant inbred lines of Xian-feng 1/Tetep.

TABLE II. GENETIC MODEL OF DUPLICATE INTERACTION OF TWO DOMINANT GENES FOR THE CONTROL OF BLAST RESISTANCE IN XIAN-FENG 1/ TETEP POPULATION

F_3 Phenotype	F_2 Genotype	Expected ratio
All plants were resistant	$R_1R_1R_2R_2$	1
	$R_1R_1r_2r_2$	1
	$r_1r_1R_2R_2$	1
	$R_1R_1R_2r_2$	2
	$R_1r_1R_2R_2$	2
Segregation	$R_1r_1R_2r_2$	4 (15R:1S) [a]
	$R_1r_1r_2r_2$	2 (3R:1S)
	$r_1r_1R_2r_2$	2 (3R:1S)
All plants were susceptible	$r_1r_1r_2r_2$	1
Total		16

[a]The numbers before ( ) refer to expected ratios for the number of F_3 lines, and those within ( ) refer to that for the number of individuals within each F_3 line, respectively. R= resistant; S= susceptible.

3.2.2. Gene interactions in the population of Xian-feng 1/Hong-jiao-zhan

Of the total 175 F_3 lines of Xian-feng 1/Hong-jiao-zhan, the number of resistant lines, segregating lines and susceptible lines were 31, 113, and 31, respectively. This ratio fitted none of single-gene or two-gene models, indicating the resistance difference between Xian-feng 1 and Hong-jiao-zhan may be controlled by three or more genes.

Supposing that the resistance is controlled by three genes while the joint effect of two homozygous susceptible genes resulted in susceptibility, the expected ratio of the numbers of resistant lines, segregating lines and susceptible lines would be 10:44:10 (Table 3). This ratio was well fitted by the observed data (P=0.491). Following the three gene controlling hypothesis, the expected ratio of the number of resistant and susceptible individuals in the 113 segregated lines was 135:41. This was also fitted by the observed data of 1545:488 (P=0.450), providing additional evidence for the hypothesis.

As described previously, a blast resistance gene *Pi-12(t)*, derived from Hong-jiao-zhan, was tightly linked to SCAR markers P265-560 and P286-350. By using P265-560, a 560-bp fragment was detected for Hong-jiao-zhan, and no fragment was detected for Xian-feng 1. After amplification of a subset of F_2 DNAs from bulked F_3 samples, the observed segregation of the 560-bp fragment was tested against the expectation (Table 4). The result (P=0.719) supported the hypothesis.

As the genetic control of the blast resistance in the population of Xian-feng 1/Hong-jiao-zhan seems to be rather complicated, identification of resistance genes other than *Pi-12(t)* will not be conducted until the two resistance genes in the population of Xian-feng 1/Tetep are mapped more precisely.

TABLE III. HYPOTHESIS OF THREE-GENE INTERACTION FOR THE BLAST RESISTANCE IN XIAN-FENG 1/HONG-JIAO-ZHAN POPULATION

F_3 Phenotype	Condition	F_2 Genotype			Expected ratio[a]
All plants resistant	Homozygosity for resistance alleles at two or all three loci	R_1R_1	R_2R_2	R_3R_3	1
		R_1R_1	R_2R_2	$--r_3$	3
		R_1R_1	$--r_2$	R_3R_3	3
		$--r_1$	R_2R_2	R_3R_3	3
All plants susceptible	Homozygosity for susceptible alleles at two or all three loci		r_1r_1	r_2r_2	r_3r_3 1
		r_1r_1	r_2r_2	r_3--	3
		r_1r_1	r_2--	r_3r_3	3
		R_1--	r_2r_2	r_3r_3	3
Segregation	All the others	R_1r_1	R_2r_2	R_3r_3	8 (27R:5S)[a]
		R_1r_1	R_2r_2	R_3R_3	4 (15R:1S)
		R_1r_1	R_2R_2	R_3r_3	4 (15R:1S)
		R_1R_1	R_2r_2	R_3r_3	4 (15R:1S)
		R_1r_1	R_2r_2	r_3r_3	4 (9R:7S)
		R_1r_1	r_2r_2	R_3r_3	4 (9R:7S)
		r_1r_1	R_2r_2	R_3r_3	4 (9R:7S)
		r_1r_1	R_2r_2	R_3R_3	4 (3R:1S)
		r_1r_1	R_2R_2	R_3r_3	4 (3R:1S)
		R_1R_1	r_2r_2	R_3r_3	4 (3R:1S)
Total					64

[a]The number before ( ) refer to expected ratios for the number of F_3 lines, and those within ( ) refer to that for the number of individuals within each F_3 line, respectively. R= resistance; S= susceptible.

TABLE IV. THE NUMBER OF F_2 INDIVIDUAL WITH PRESENCE OR ABSENCE OF THE 560-BP FRAGMENT IN XIAN-FENG 1/HONG-JIAO-ZHAN

Resistance pattern	Observed number		Expected ratio	$P\chi^2$
	presence	absence	(presence : absence)	
Resistance	5	0	9 : 1	0.456
Segregation	20	7	32 : 12	0.875
Susceptible	5	10	3 : 7	0.778
Total	30	17		0.719

From the above results, it can be seen that the mode of gene interactions for blast resistance may vary greatly across populations. Using classical approaches, the mode of gene interactions can only be deduced statistically. When tightly linked markers become available, it should be possible to verify the genetic mode of action.

2.3. Initiation of DNA marker-assisted selection

Crosses were made between elite varieties and blast resistance donors in Hainan Province in the winter of 1995. Elite varieties used included an early *indica* variety Zhong 156 and several parental lines of commercial F_1 hybrids, and blast resistance donors used including Hong-jiao-zhan, C101A51 (*Pi-2*) and C101PKT (*Pi-4*). The F_1s was grown in CNRRI in 1996 and backcrossed with elite varieties. A polymorphism survey was conducted using DNA markers linked to resistance genes. In the following years, DNA marker-assisted selection for blast resistance will be employed.

Forty-eight varieties which were widely used in current rice breeding programs were provided by rice breeders. Polymorphism among the 48 varieties and resistance donors are being surveyed by using STS (Sequence Tagged Site) and RFLP markers. More than 40 STS markers have been used, and data are being collected. Based on five STSs linked to disease resistances (rice blast or bacterial leaf blight), pair-wise comparisons indicated that the polymorphism frequency was 13-52%. After digestion of the PCR products with 4 cutters, the frequency was 28-66%. When the data are all collected, they will be distributed to the rice breeders who provided the varieties. It is expected the database will be helpful to breeders for making appropriate decisions whenever they are interested in transferring resistance genes to their materials.

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