International Journal of Bio-resource and Stress Management 2011, 2(3):334-337

Identification of molecular marker associated with brown plant hopper (*Nilaparvata lugens* Stal) resistance in rice

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Article History

Manuscript No. 143a
Received in 5th April, 2011
Received in revised form 10th June, 2011
Accepted in final form 3rd September, 2011

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Keywords

Rice, *Nilaparvata lugens*, standard seed box screeing (SSST)

Abstract

In rice, RILs were screened to assess the resistance to brown plant hopper based on the standard seedbox screening test (SSST) in the greenhouse. The parents viz., IR50 and Rathu Heenati had the mean score damage of 5.75 and 3.08, respectively. For the RILs, the leaf damage score ranged from 3.0 to 9.0. As many as 15 SSR primer pairs belonging to chromosome 3 were used to screen the parents for polymorphism. This region was previously reported to be associated with BPH resistance. A total of five polymorphic markers were identified between IR50 and Rathu Heenati. The polymorphic markers were surveyed on 133 RILs to establish their segregation pattern. Out of the five marker loci studied, three marker loci viz., RM520, RM3180 and RM6283 showed the expected segregation ratio of 1:1 based on the χ^2 test at five per cent level. The SSR marker RM2346 was identified to be associated with the BPH resistance based on the damage score from SSST. The SSR marker RM 2346 identified in the present study may be useful in marker-assessed select program after further testing, towards the development of new rice lines with enhanced level of resistance to BPH

1. Introduction

Brown plant hopper (BPH), *Nilaparvata lugens* Stål (Delphacidae; Homoptera), is one of the most destructive insect pests causing significant yield loss in rice every year (Sogawa, 1971). In addition to causing physiological damage to rice plant, BPH also causes indirect damage by acting as a vector for rice grassy stunt virus and ragged stunt virus (Heinrichs, 1979). Researchers have identified more than thirty major genes associated with resistance to BPH. It has long been proposed that moderate and polygenic resistance to insect pests, including BPH, should provide more durable resistance than single major genes. Soundararajan et al. (2004) mapped several QTLs associated with resistance to BPH in rice. The use of molecular marker techniques and QTL analysis has opened up new opportunities for working with quantitative resistance to BPH in rice. The number of resistance QTLs in rice germplasm is expected to be very large and the quantitative resistance to BPH in rice can be further enhanced by pyramiding genes/QTLs of different origin by MAS. In rice, a total of five biotypes of BPH have been identified and Biotype 4 is prevalent in South East Asia (Khush and Brar 1991). In the present study, an attempt was made to locate the genomic region associated with BPH resistance in rice involving RILs of IR50/Rathu Heenati cross.

2. Materials and Methods

2.1. Mass rearing of the BPH

BPH undergoes five instars to reach the adult stage. Male insects are dark brown and winged. Female insects are wingless and light brown to dark brown. The BPH was mass reared on the susceptible rice variety Taichung Native 1 (TN1) by following the method of Heinrichs et al. (1985). Initial BPH population was collected from the rice fields at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore.

2.2. Standard seed box screening test

Damage rating of the test lines was done on individual plant basis when 90 per cent of the plants in the susceptible check (TN1) row were killed. The test lines were graded using 0-9 scale (Heinrichs et al., 1985).

The extent of damage on each plant was examined by visual

PRINT ISSN: 0976-3988; 0NLINE ISSN: 0976-4038

Grade	Criterion
0	No damage
1	Slight yellowing in the first leaf
3	Complete yellowing of the first leaf
5	Pronounced yellowing of half of the plant
7	Wilting of more than half of the plant
9	Whole plant dead

scoring and graded as given below.

2.3. Polymorphism survey

A set of 15 SSR primer pairs pertaining to linkage group 3 (McCouch et al., 2002). that were screened between the parents IR50 and Rathu Heenati. Five polymorphic markers were used for segregation analysis among the RILs. After PCR amplification, the products were resolved in 3% agarose gel. (Panaud et al.1996).

2.4. Segregation analysis

The details of RILs used for segregation analysis are shown in Table 1. The segregation analysis among the RILs was carried out using agarose gel electrophoresis. The segregation pattern for SSR markers in RILs was scored as 1 for the presence of IR50 allele, 3 for the presence of Rathu Heenati allele and 2 for the presence of both the alleles (heterozygotes).

The segregation distortion analysis was carried out through MAPDISTO^R computer programme using the segregation data for the five marker loci. Segregation ratios for marker classes were tested for the expected 1:1 ratio for SSR loci, based on *chi*-square (χ^2) test. Then single marker linkage analysis was performed to establish the linkage between the putative SSR markers and the respective phenotype for BPH resistance (Sheba, 2006)

3. Results and Discussion

In the present study, RILs of IR50/Rathu Heenati were assessed for the polymorphism among a set of five SSR markers in linkage group 3 associated of BPH resistance. A set of 268 RILs were screened to assess the level of resistance to BPH based on the standard seed box screening test (SSST) in the greenhouse. Under greenhouse condition, the level of resistance to BPH was assessed based on the severity of the symptoms caused by the insects at the day on which TN1, the susceptible control was completely killed by the insects. The parents' viz., IR50 and Rathu Heenati had the mean score of 5.75 and 3.08 respectively. Out of the 268 RILs screened, ten lines were found to be resistant with a damage score between 1 and 3.9, Eighty six lines were moderately resistant with a damage score between 4 and 6.9. One hundred and seventy two lines were scored as susceptible with a damage score between 7 and 9 (Table 1). As many as 15 SSR primer pairs belonging to chromosome 3 were used to screen the parents for polymorphism (Plate 1 and Plate 2). In the present study, a total of five polymorphic markers were identified between IR50 and Rathu Heenati covering linkage group of 3 and were surveyed on the RILs for BPH resistance. The markers RM520, RM3180 and RM6283 showed the expected segregation ratio of 1:1 based on the χ^2 test at 5% level of significance (table 2) and the remaining two marker loci RM473 and RM2346 exhibited (40.00%) segregation distortion across the RILs analysed. The frequency of IR50 allele was found to be more

Table 1: Number of RILs showing different levels of resistance to BPH based on the average damage score recorded in Standard Seed box Screening Test

Damage score	Level of resistance	Number of RILs showing respective scale of damage
1.0-3.9	Resistant	10
4.0-6.9	Moderately resistant	86
7.0-9.0	Susceptible	172

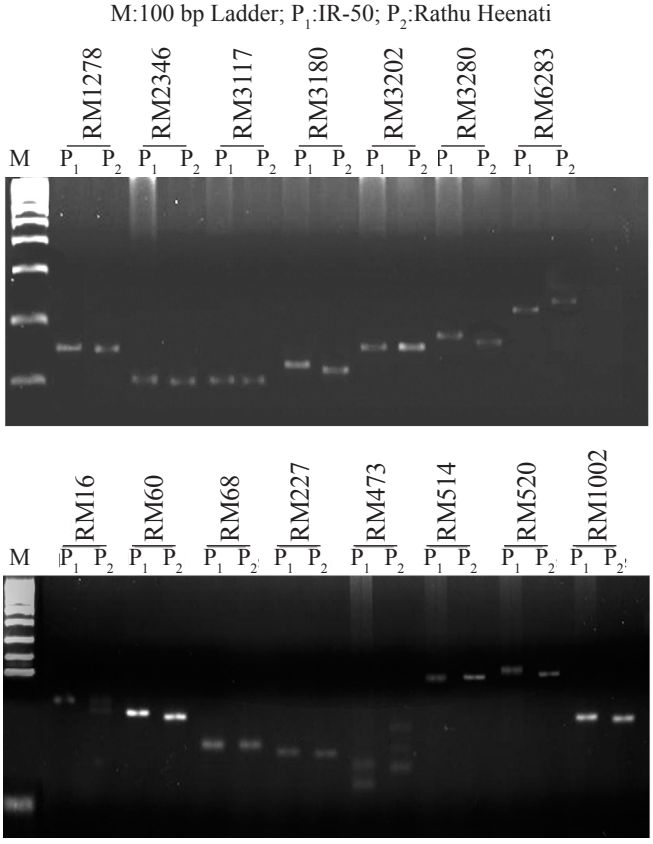

Plate 1: Parental Polymorphism observed for a set of seven SSR primers; Plate 2: Parental Polymorphism observed for a set of eight SSR primers

for these two markers. Then the markers ware analysed for the association with phenotype using single marker analysis (SMA) (table 3). Only one SSR marker RM2346 was identified to be associated with the BPH resistance based on the damage score from SSST.

In rice, several genetic maps using SSR markers have been constructed based on the wealth of information available (McCouch et al., 1997; Chen et al., 1997; Temnykh et al., 2000; Temnykh et al., 2001 and McCouch et al., 2002). Mapping with high resolution requires a high density of genetic markers covering the entire genome having normal segregation pattern. Considering the previous work, an attempt was made to explore the chromosome 3 for BPH resistence. In the present study, a total of 5 polymorphic markers out of 15 marker loci surveyed were utilized for segregation analysis.

According to Lakshminarayana and Khush (1977), the Sri Lankan cultivar Rathu Heenati has a dominant gene *Bph3* for resistance. Gomathi (2002) identified two SSR markers viz., RM168 and RM186 associated with BPH resistance using the F_3 population of IR50/Rathu Heenati.

Among the five polymorphic markers surveyed across the RILs, three markers RM520, RM6283 and RM3180 segregated in the normal 1:1 ratio and remaining two markers (RM2346 and RM473) showed segregation distortion (Table 2). Both the markers showed segregation distortion towards IR50. Segregation distortion of markers may be caused by genetic, physiological and environmental factors (Xu et al., 1997).

Xu et al. (1997) analysed the segregation distortion in six different mapping populations involving marker data and found the range of segregation distortion was 6.8–31.8 per cent. In the present study, segregation distortion might have occurred due to the small population size and sampling errors. Segregation distortion could be reduced by taking a larger population size for the study.

Higher order of segregation distortion for the marker loci has a negative impact on genetic map construction by unwanted pseudo linkages between marker loci (Wang et al., 1994). Establishing linkage maps showing segregation distortion may not be ideal considering the utility of genetic maps for QTL mapping

In the present study, marker- phenotype association analysis was carried out by a method called single marker analysis (SMA) using one way ANOVA. The SMA is a good start for practical data analysis. One way ANOVA was made to identify the markers having the association with targeted phenotypic trait by involving the segregation data from five marker loci and the phenotypic data. The SMA by one way ANOVA resulted in one marker-phenotype association. The strong association of a marker RM2346 with BPH resistance was evident from the significant F value (28.74) (Table 3). This marker was found to be associated with BPH resistance.

Several QTLs associated with resistance to BPH have been reported by various workers. A total of seven QTLs associated with resistance to BPH were identified by Alam and Cohen (1998) in the doubled haploid population derived from IR 64 (*O. indica*) and Azucena (*O. japonica*). The QTLs were located on six of the 12 rice chromosomes viz., chromosomes 1, 2, 3, 4, 6 and 8. Using the same population, a total of six significant QTLs for BPH resistance was detected.

Table 2: Segregation pattern of SSR markers across the RILs

No.	Marker	Observed values			χ^2Value
		Score 1	Score 2	Score 3	
1	RM520	58	8	67	1.08
2	RM3180	63	13	57	1.53
3	RM2346	74	0	52	4.00
4	RM6283	70	3	48	1.80
5	RM473	54		51	5.96

Table 3: SSR loci on linkage group 3 showing significant association with BPH resistance based on single marker analysis

No.	SSR marker	No. of RILs	Threshold value based on single ANOVA		
			F cal.	*p* value	F critical
1	RM520	133	3.06	0.08	3.91
2	RM3180	133	28.74	3.62	3.91
3	RM2346	133	2.11	0.14	3.92
4	RM6283	133	1.15	0.28	3.91
5	RM473	133	2.03	0.15	3.90

4. Conclusion

In the present study, one SSR marker RM2346 was identified in linkage group 3 indicating a possible genetic locus conferring resistance to BPH in rice. Fine mapping of this region with more markers and other *in silico* approaches are expected to give closer markers to the gene of interest facilitating marker assisted breeding programme for BPH resistance. It also offers a great opportunity to understand the relationship between resistance genes, their origins and mechanism of resistance.

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