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IDENTIFICATION OF QTLS FOR BROWN PLANTHOPPER (*NILAPARVATA LUGENS* STAL.) RESISTANCE IN RIL MAPPING POPULATION OF RICE (*ORYZA SATIVA* L.)

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Abstract

The brown planthopper (*Nilaparvata lugens* Stal.) is one of the major insect pests of rice (*Oryza sativa* L.). Exploitation of Host Plant Resistance is a major component to manage this pest. The development of rice varieties that are resistant to the brown planthopper is an important objective in current breeding programmes. This study was undertaken to identify QTLs for BPH resistance from an elite local accession.

In the present study, two hundred seventy lines of recombinant inbred lines (RILs) of Danteshwari/Dagad Deshi were evaluated under glass house condition for brown planthopper resistance. Two leaf stage seedlings were artificially infested in the screen house with second and third instar brown planthopper nymphs, using standard seed box technique. Reactions of the seedlings were recorded seven to ten days after infestation when the susceptible check TN1 was completely killed. The lines found resistant for BPH were further tested by probing mark test and feeding test.

The genotypic data of the whole population was developed using SSR and SNP markers. The genotypic data thus obtained was used for QTL analysis using single marker analysis to find out association between markers and trait under study. 10 SSR and 3 SNP markers showed significant association with the trait.

Key words : Rice (*Oryza sativa* L.), BPH (Brown planthopper), Quantitative Trait Loci (QTL), Recombinant Inbred Line (RIL).

Introduction

Rice (*Oryza sativa* L.) is an ancient cereal crop. Rice is probably the most genetically diversified crop among the cereals. Approximately half of the people on earth obtain the majority of their caloric intake from rice. Rice is the host of more than 100 insect pest species. Planthoppers are highly destructive pests in crop production worldwide. Brown planthopper (BPH) causes the most serious damage of the rice crop globally among all rice pests. Growing resistant varieties is the most effective and environment-friendly strategy for protecting the crop from BPH. More than 19 BPH-resistance genes have been reported and used to various extents in rice breeding and production (Du *et al.,* 2009).

The brown planthopper is the most destructive pest in all rice growing areas. At present this insect pest is a serious threat to rice production throughout the Asia. The loss in grain yield due to this insect range from 10% in moderately affected fields to 70% in those severely affected. The damage to the standing crop sometimes reached 100%. Exploitation of Host Plant Resistance (HPR) is a major component to manage this pest. The development of rice varieties (*Oryza sativa* L.) that are resistant to the brown planthopper (*Nilaparvata lugens* Stal.) is an important objective in current breeding programmes (Park *et al.,* 2007).

Breeding resistant rice cultivars with some of the major genes was highly successful (Khush, 1989). However, in some cases, this major gene resistance was short-lived because of the adaptation of the BPH population to the highly resistant varieties, harboring any one of these major genes (Gallagher *et al.*, 1994; Ketipearachchi *et al.*, 1998).

The inheritance of polygenic traits is complex. The basic assumption is that many genes with small and roughly equal effects govern the trait and expression of the trait is strongly influenced by the environment. Therefore, selection becomes inefficient in such condition. In this context identification of Quantitative Trait Loci (QTLs) could help in increasing selection efficiency through Marker Assisted Selection (MAS).

Quantitative trait loci (QTLs) were found to confer more durable BPH resistance in cultivar IR64 (Cohen *et al.*, 1997). Further analysis of recombinant inbred lines (RIL) and double haploid lines (DHL) identified QTLs for BPH resistance on several chromosomes (Alam and Cohen, 1998; Su *et al.*, 2002; Xu *et al.*, 2002), providing valuable information for future map-based cloning of BPH-resistance genes and marker-assisted selection of stably resistant varieties.

Materials and Methods

Planting material

The experimental material consisted of two parents *viz*. Danteshwari and Dagad Deshi and their 270 recombinant inbred lines (RILs) in F_9 generation. These lines were evaluated for their reaction against BPH infestation. Danteshwari is highly susceptible to BPH with average score of 9.0 whereas Dagad Deshi is tolerant with score of 2.5. The mapping population of recombinant inbred lines (RILs) was developed by using modified single seed descent method (SSD) to F_9 generation.

Phenotypic evaluation

Screening of rice genotypes were conducted, under controlled conditions of glass house, as per methodology suggested by Kalode *et al.* (1979). Feeding test was assessed by quantifying the area of honeydew excreted by the insect on the filter paper after 24 hours of confinement on the test genotype and Probing mark test was carried out according to methodology suggested by Naito (1964) on the selected genotypes.

Genotyping using microsatellite markers

Total rice genomic DNA was extracted from young succulent disease free seedlings of parental lines i.e. Danteshwari and Dagad Deshi and from their 270 recombinant inbred lines (RILs) in F_9 generation by Dellaporta method given by Dellaporta *et al*. (1983). PCR analysis was done using a set of known HvSSR, RM, RGNMS and SNP markers to identify the polymorphic loci between the two parental lines, Danteshwari and Dagad Deshi. The markers showing polymorphism with parents were used on the population. A set of 49 markers were found polymorphic and used on the mapping population. These markers consisted of 28 HvSSR, 3 RM, and 18 SNP markers.

Data analysis and QTL mapping

Single market analysis was applied on selected highly resistant lines and highly susceptible lines. The selection was made based on the scoring value obtained during screening. Single marker analysis was used to estimate linkage between marker and trait by using t- test formula given below:

$$t = \frac{\overline{X} - \overline{Y}}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Results and Discussion

The result revealed that on screening the population it was not possible to categorize the population into resistant and susceptible (3:1 ratio). In fact, the lines showed a continuous range of reaction having different degree of resistance for BPH. Hence the inheritance was found to be polygenic. Some 160 tested RIL gave a continuous distribution in their resistance in the tested rice plants to brown plant hopper indicating the polygenic type of resistance.

The probing mark test and the feeding test on the selected lines further confirmed the resistance for brown planthopper (*Nilaparvata lugens* Stal.) and helped in selecting the corresponding 25 resistant lines and 25 susceptible lines for the single marker analysis.

Out of 110 primers exhibiting polymorphism 31 primers were selected. Primers showing polymorphism were further used for PCR amplification on 270 lines along with parents using standardized PCR protocol. The banding pattern of population with different SSR primers has been shown in table 1. The result shows the number of lines in the population following the banding pattern 'A' (Danteshwari like allele) and the number of lines following the banding pattern 'B' (Dagad Deshi like allele). As per the scoring values of screening, Danteshwari is susceptible for brown plant hopper (scoring value = 9.0) and Dagad Deshi is tolerant for brown plant hopper (scoring value = 2.5). The segregation pattern of marker deviated from the normal Mendelian l:1 ratio and exhibited distorted segregation pattern.

Screening of the population

The graphical representation of the reaction of lines to brown planthopper has been represented in the fig. 1. From the graph it is clear that the scoring values obtained is skewed. As majority of the lines of the population (133 lines) are showing score values more than 7, we have obtained a skewed distribution towards susceptibility.

On the other hand 26 lines of the population were highly resistant (score value < 3), 38 lines were moderately resistant (score value between 3 to 5) and 40 lines were moderately susceptible (score value between 5 to 7). The probable reason, for skewed distribution might be that the resistance against brown planthopper in this population

is found to be quantitative in nature which is highly influenced by the environmental conditions and also under such artificial screening the insect population is usually very high which tend to increase the proportion of susceptibility. Such influence of insect population behavior has also been reported by Panda and Khush (1995).

Probing Mark Test

The number of punctures made by the insect in different lines was compared with the number of punctures made in the check varieties (resistant check Ptb 33 and susceptible check TN 1). Lines showing more than about 25 punctures were considered resistant. Line no. 77, 79, 155, 181, 183 and 192 were having values more than 25. Therefore, it can be concluded that 6 lines out of 26 lines used in the probing mark test were found to be resistant. The result of probing mark showed that two recombinant inbred lines *viz.* line no. 77 and 79 exhibited transgressive segregation for reaction to BPH for this test.

Sogawa and Pathak (1970) reported that the probing response of the brown planthopper to the rice varieties and barnyard grass was uniformly high, indicating the absence of mechanical barriers to insertion of stylets. The results of probing behaviour indicated that the resistant varieties received more number of probing punctures than the susceptible ones.

The electronic measurement system greatly facilitates the behavioural study of feeding and probing by piercing and sucking insects on susceptible and resistant plants (Kawabe *et al.,* 1981; Velusamy and Heinrichs, 1986 a).

Feeding Test

Honeydew excreted by brown planthopper, *Nilaparvata lugens* (Stal) had been used as a criterion for determining the amount of sap ingested by the insect on resistant and susceptible rice cultivars. The feeding test was done on 10 selected lines based on their scores of screening. 10 lines having highly resistant scores in the screening were selected for feeding test.

It was observed that the feeding activity on resistant lines was significantly less as compared to susceptible lines. 4 replications of the test genotypes and check varieties was used in the feeding test. The feeding rate of brown planthopper in case of susceptible parent i.e. Danteshwari was found to be 80.5 mm^2 (relatively more) and 16.5 mm^2 (relatively less) in case of resistant parent Dagad Deshi. Feeding rate was 21.5 mm^2 on resistant check Ptb 33 and 89.75 mm^2 on susceptible check TN1.

Therefore, it can be concluded that the line no. 10, 46, 77, 102, 155, 163 and 176 were found to show values < 25 mm^2 in the feeding test which was desirable and line no. 104, 105 and 181 were found to show values > 25 mm^2. Thus, 7 resistant lines were obtained as per the result of feeding test.

Antixenosis, antibiosis and tolerance have all been observed as mechanisms of resistance against *N. lugens* in various rice cultivars. No morphological or anatomical features have been found to be associated with *N. lugens* resistance, but surface and phloem chemistry have been linked to antixenosis and antibiosis. Antixenosis of rice varieties IR36 and IR62, manifest as reduced probing and settling, and movement from the stem to the leaves, has been associated with the chemical composition of surface wax (Woodhead and Padgham, 1988). Probing and sucking stimulants and inhibitors have been identified in various cultivars (Sogawa, 1982; Heinrichs, 1994). Tolerance to *N. lugens* feeding has been identified in the rice cultivars Mudgo, IR46, Triveni and Utri Rajapan (Velusamy and Heinrichs, 1986 b).

Pophaly and Rana (1993) reported the BPH feeding test on resistant cultivars Hinga, Nappe, Dhauri 1043, Dhauri 1163, Jaybay Rang, Khatia Pati, Kanak, Ganjakali, Hiaranakahi and EB 17. Feeding rate was 0.17-65 mm^2 per female in 24 hours, which was much lower than the 173-235 mm^2 per female feeding on TN 1.

Alagar *et al.* (2008) studied the feeding behaviour of brown planthopper, *Nilaparvata lugens* Stal. on some selected rice genotypes under glass house conditions. Feeding marks and feeding rate used as a reliable parameters to evaluate the resistance nature of the genotypes against insect pests. Low honeydew excretion and higher feeding marks was related to resistance of rice genotypes against BPH. The maximum number of feeding marks were observed on ARC 10550 (43.80 mm^2) which was 4.52 times higher than TN 1 and it was followed by ARC 6650 (24.80 mm^2) and KAU 1661 (24.00 mm^2). The feeding rate was assessed in terms of the amount of honeydew excreted. The amount of honeydew excreted is directly proportional to the amount of sap sucked by BPH. W 1263 recorded the lowest feeding rate of 84.62 mm^2 followed by ARC 6650 (129.70 mm^2), IR 72 (144.17 mm^2) and KAU 1661 (177.48 mm^2).

QTL analysis

Single marker analysis was applied on selected highly resistant lines and highly susceptible lines. The selection was made based on the scoring value obtained during screening. Since the observation obtained from the screening of the population was showing skewed distribution towards susceptibility and as the majority of the lines in the population were showing intermediate

Table 1 : Banding pattern of the RILs with different primers.

S. No.	Primer	Number of lines with 'A' banding pattern	Number of lines with 'B' banding pattern	Number of lines with 'H' (both 'A' and 'B') banding pattern
1.	HvSSR 01-31	151	86	33
2.	HvSSR 01-46	168	66	36
3.	HvSSR 01-52	116	125	29
4.	HvSSR 02-01	179	66	25
5.	HvSSR 02-10	117	115	38
6.	HvSSR 02-42	122	105	43
7.	HvSSR 03-06	134	94	42
8.	HvSSR 03-35	122	105	43
9.	HvSSR 03-54	95	107	68
10.	HvSSR 04-25	137	119	14
11.	HvSSR 05-11	81	160	29
12.	HvSSR 06-29	125	116	29
13.	HvSSR 06-43	173	80	17
14.	HvSSR 06-54	139	111	20
15.	HvSSR 08-23	143	90	37
16.	HvSSR 08-28	137	103	30
17.	HvSSR 09-19	127	132	11
18.	HvSSR 09-24	140	98	32
19.	HvSSR 09-26	146	92	32
20.	HvSSR 09-29	84	172	14
21.	HvSSR 10-05	118	127	25
22.	HvSSR 10-17	121	112	37
23.	HvSSR 11-01	156	89	25
24.	HvSSR 11-02	155	105	10
25.	HvSSR 11-03	96	156	18
26.	HvSSR 11-13	147	91	32
27.	HvSSR 12-35	108	141	21
28.	HvSSR 12-39	117	132	21
29.	RM 243	140	88	42
30.	RM 572	130	122	18
31.	RM 7	144	112	14

reaction for brown planthopper resistance, therefore, 25 highly resistant and 25 highly susceptible lines were selected and used for t-test. The selection of these lines was based on the results obtained in the screening of the population for brown planthopper resistance. The reaction of the resistant lines was further confirmed by feeding test and probing mark test.

The t-value was highly significant for the SNP marker linked to chromosome 1 followed by the SNP marker linked to chromosome 3 (table 2). The single marker analysis showed 13 linked markers which consisted 7 highly variable SSR markers, 3 RM markers and 3 SNP markers. The QTLs were identified on the chromosome 1, 2, 3, 5, 11 and 12. Four QTLs were present on chromosome 1 and 3 each, two on chromosome 2 and 1 QTL on chromosome 5, 11 and 12 each. The linked SSR markers obtained were HvSSR01-46, HvSSR02-10, HvSSR02-42, HvSSR03-06, HvSSR03-35, HvSSR11-03, HvSSR12-35, RM 243, RM 572 and RM 7.

Seven main-effect QTLs and many epistatic QTL pairs had been identified and mapped on the 12 rice chromosomes (Xu *et al.,* 2002). QTL analysis detected six QTLs on chromosomes 1, 2, 6, and 7 associated with resistance to BPH in a doubled haploid (DH) population derived from the cross IR64/ Azucena (Soundararajan *et al.,* 2004). Sun *et al.* (2005) reported the presence of

Table 2 : t-test analysis of 13 primers.

S. No.	Primer	t- Value	Chromosome No.
1.	HvSSR 1-46	2.016	1
2.	HvSSR 2-10	3.035	2
3.	HvSSR 2-42	2.134	2
4.	HvSSR 3-06	2.448	3
5.	HvSSR 3-35	2.465	3
6.	HvSSR 11-03	2.032	11
7.	HvSSR 12-35	2.046	12
8.	RM 243	2.232	1
9.	RM 572	2.996	1
10.	RM 7	2.600	3
11.	01-608-4_C_375.fasta	5.508	1
12.	03-3478-1_C_206.fasta	3.841	3
13.	05-48-1_C_279.fasta	2.416	5

three loci on chromosomes 3, 4 and 10. Liu *et al.* (2009) mapped four quantitative trait loci (QTLs) on chromosome 2, 4, 7 and 9 respectively. Single-marker analysis through one way analysis of variance showed that the markers RM 3766, RM 14687, RM 251 and RM 7 on chromosome 3 were linked to the resistance locus (Santhanalakshmi *et al.,* 2010). The result obtained in the single marker analysis of the given RIL population also showed the linkage of RM 7 marker to chromosome 3 which has been reported earlier.

A collection of 515 rice landraces originating from Vietnam and China were screened for the reaction to brown planthopper (BPH) infestation and four quantitative trait loci (QTLs) contributing to BPH resistance were mapped on chromosomes 2, 4, 7 and 9, respectively with the help of an F_2 mapping population created from the cross between a BPH resistant Vietnamese landrace Yagyaw and the susceptible cultivar Cpslo17 (Liu *et al.,* 2009). QTLs for resistance to BPH were detected on chromosomes 3, 4, 12 using ninety-four recombinant inbred lines (RILs) from the cross between Hinohikari (susceptible japonica variety) and IR54742-1-11-17 (resistant *indica* line introgressed from *O. officinalis*) (Hirabiyashi *et al.,* 2003).

Conclusion

The screening of the population indicated that there was variation among the RILs for resistance to brown plant hopper. It also showed quantitative inheritance of resistance against brown planthopper. Single marker analysis revealed that there were 10 SSR and 3 SNP markers showing linkage. QTLs were identified on chromosome 1, 2, 3, 5, 11 and 12 distributed as such that 4 QTLs were present on chromosome 1 and 3 each, 2 QTLs on chromosome 2 and 1 QTL on chromosome 5, 11 and 12 each.

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