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RESEARCH ARTICLE

Fine mapping of QTLS for *brown planthopper* (BPH), *Nilaparvata lugens* (Stal.) resistance in rice

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ABSTRACT

The development of resistant varieties requires to in depth studies on interaction between insect pests and genetics of plant mapping Quantitative Ttait Loci (QTL) for BPH resistance therefore, study was conducted for fifty recombinant inbred lines (RILs) of Danteshwari/Dagad Deshi. These lines were first screened in glasshouse condition against BPH insect by using standard seed box technique. The genotyping of BPH resistant and susceptible plant was carried out by using 49 SSR primers but, only ten SSR markers showed parental polymorphism and also the result showed that the segregation pattern of marker deviated from the normal Mendelian 1:1 ratio and exhibited distorted segregation pattern. However, no clear cut linkage/ association was noticed between markers and phenotypes.

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INTRODUCTION

Among the sucking pests, brown planthopper (BPH) *Nilaparvata lugens* (Stal.) causes the most serious damage to the rice crop directly by feeding on its tissue and acting as a vector for plant pathogen. Attempts to control this pest with chemical method have given rise to many problems and it is not an effective and ecofriendly measures. Exploitation of host plant resistance (HPR) is a major component to manage this pest.

Researchers are seeking to identify BPH-resistance genes and use them to breed resistant cultivars that guard against the negative impacts of BPH infestation since it gained importance of major pest. Mapping and tagging of pest resistance genes have been greatly facilitated by a wide array of molecular markers in crop plants. Marker assisted selection facilitates the transfer of genomic region of interest followed by accelerated recovery of recurrent parent genome. About 22 major genes associated with BPH resistance have been identified and mapped on rice chromosomes (Renganayaki *et al*., 2002 and Chen *et al*., 2006). Keeping this in view, the present investigation was conducted based on fine mapping of QTL's using molecular markers by using two parent *viz*., Danteshwari and Dagad Deshi, and their 50 recombinant inbred lines (RILs) in F_{10} generation.

MATERIAL AND METHODS

Plant material :

The experimental material consisted of two parent *viz*., Danteshwari and Dagad Deshi, and their 50 recombinant inbred lines (RILs) in F_{10} generation. The mapping plant population of recombinant inbred lines (RILs) was developed by using modified single seed descent method (SSD) to F_{10} generation. These lines along with their parent *viz*., Danteshwari and Dagad Deshi and the standard resistant check PTB33 and susceptible check TN1 were evaluated for their reaction against BPH infestation as per the methodology suggested by Kalode *et al.* (1979).

Table A : Characteristic features of parent

Sr. No.	Parent	Pedigree	Plant damage score	Reaction to BPH
1.	Danteshwari	Shamridhi x IR 8608-298	9.0	Highly susceptible
2.	Dagad Deshi	Land race	2.5	Resistant

Total rice genomic DNA was extracted from young succulent seedlings infested with reaction to BPH of parental lines *i.e.* Danteshwari and Dagad Deshi and a set of 10 resistant and 10 susceptible lines from their recombinant inbred lines (RILs) in F_{10} generation by Dellaporta method (Dellaporta *et al*., 1983). The set of RIL population were sorted based on damage rating obtained by BPH screening. The extracted DNA samples were dissolved in TE buffer (1M Tris HCl, 0.5M EDTA). For quantification, 5ìl undiluted DNA isolated from each line, along with standards of known quantity of DNA was loaded on 0.8% agarose gel stained with ethidium bromide (3.0µl ethidium brodmide dye per 100ml of agarose gel). The electrophoresis was performed at 70 volts for 60 minutes and the gel was observed under UV transilluminator. The crude DNA after quantification was diluted suitably for amplification. DNA was diluted in such a way that the diluted samples contained about 40 ng/µl of crude DNA and subsequently used for PCR amplification.

PCR analysis was done using a set of random SSR (single sequence repeat) markers to identify the polymorphic loci between the two parental lines, Danteshwari and Dagad Deshi. Amplification reactions were carried out in a 20µl mixture containing 14.5µl Sterile and nanopure H_2O, 2.0µl PCR buffer with 15mM $MgCl_2$ (Bangalore Genei), 1.0µl dNTPs (Mix) of 2.5µM concentration, 0.5µl of each primers (forward and reverse) having 5Pmol concentration, 0.5µl Taq polymerase 1 unit / µl and 40ηg concentration of 1.0µl DNA template. Reaction was performed using 30 cycles of Verti 96 well thermal cycler of Applied Biosystems (USA) programmed are as follows: 94°C for 5 min. initial denaturation; followed by 30

Table 1 : SSR primer showing parental polymorphism with their band size

Sl. No.	Primer	Forward/ Reverse	Sequence 5'----------> 3'	Band size (bp) P1	P2
1.	RM242	Forward	GGCCAACGTGTGTATGTCC	150	175
		Reverse	TATATGCCAAGACGGATGG		
2.	RM17	Forward	TGCCCTGTTATTTTCTTCTCTC	160	175
		Reverse	GGTGATCCTTTCCCATTTCA		
3.	RM517	Forward	GGCTTACTGGCTTCGATTTG	220	210
		Reverse	CGTCTCCTTTGGTTAGTGCC		
4.	RM251	Forward	GAATGGCAATGGCGCTAG	175	185
		Reverse	ATGCGGTTCAAGATTCGATC		
5.	RM566	Forward	ACCCAACTACGATCAGCTCG	190	225
		Reverse	CTCCAGGAACACGCTCTTTC		
6.	RM553	Forward	AACTCCACATGATTCCACCC	170	185
		Reverse	GAGAAGGTGGTTGCAGAAC		
7.	RM281	Forward	ACCAAGCATCCAGTGACCAG	130	140
		Reverse	GTTCTTCATACAGTCCACG		
8.	RM515	Forward	TAGGACGACCAAAGGGTGAG	210	230
		Reverse	TGGCCTGCTCTCTCTCTCTC		
9.	RM152	Forward	GAAACCACCACACCTCACCG	115	135
		Reverse	CCGTAGACCTTCTTGAAGTG		
10.	RM410	Forward	GCTCAACGTTTCGTTCCTG	130	150
		Reverse	GAAGATGCGTAAAGTGAAG		

Table 2 : Banding pattern of the RILs with different primers

Sl. 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	Number of lines with 'O' banding pattern
1.	RM 242	10	9	1	–
2.	RM 17	7	5	6	1
3.	RM 553	11	4	5	–
4.	RM 251	4	11	3	2
5.	RM 566	5	3	12	–
6.	RM 517	9	7	4	–
7.	RM 515	8	12	–	–
8.	RM 281	7	11	2	–
9.	RM 152	9	8	3	–
10.	RM 410	10	8	–	2

cycles of 1 min. at 94°C (denaturation), 1 min. at 55°C (annealing), 1 min. at 72°C (extension) with final extension of 7 min. at 72°C. Amplified products were resolved by 5% Polyacrylamide gel electrophoresis (PAGE) with 50bp DNA ladder as molecular weight marker and finally stained with silver staining method.

RESULTS AND DISCUSSION

The average plant damage score of Danteshwari was found to be 9.0 and that of Dagad Deshi was 2.5. These parental reactions were expected based on pilot experiments in which Danteshwari was found to be susceptible and Dagad Deshi as tolerant.

PCR analysis was done using a random set of forty-nine SSR markers to identify the polymorphic loci between the two parental lines *i.e.* Danteshwari and Dagad Deshi. Out of these forty-nine markers, only 10 SSR markers showed parental polymorphism.

These 10 SSR markers showing polymorphism were further used for PCR amplification on selected 20 lines along with parents using standardized PCR protocol (Table 1). The PCR products were separated on 5% Polyacrylamide gel electrophoresis and further stained by using silver nitrate. Electrophoresis was carried out for sufficient time (usually 50-70 minutes) at 250 volts. Scoring of the observed bands of DNA was done separately based on the banding pattern. *i.e.* 'A' banding pattern (Dagad Deshi like allele), 'B' banding pattern (Danteshwari like allele), 'H' banding pattern (both alleles) and 'O' banding pattern (other allele).

The result showed that the segregation pattern of marker deviated from the normal Mendelian 1:1 ratio and exhibited distorted segregation pattern. Distorted segregation of molecular markers was also reported to observe in many mapping populations (Causse *et al.*, 1994 and Xiao *et al.*, 1996). The genetic basis of segregation distortion may be the abortion of male or female gametes, or the selective fertilization of particular gametic genotypes (Xu *et al*., 1997) or because of less number of population used for generating genotypic data. The marker RM 251 produced more female type alleles (61.11%) than the male (22.22%) with 16.66% heterozygosity. RM 553 on the other hand produced 20%, 55% and 25% female, male and heterozygous allele, respectively. RM 515 and RM 410 marker exhibited 0% *i.e.* no any heterozygosity in the population (with 60%, 44.44% female and 40%, 55.55% male allele respectively) whereas high percentage of heterozygosity was 60% by RM566 (with 15% female and 25% male allele, respectively).

Data in Table 2 indicate that the presence of resistant bands for all markers was also found in susceptible and *viceversa,* hence there was no clear cut linkage/association shown between these markers and the phenotype. QTLs are reported earlier by many workers on different chromosomes. On the same population Kar (2010) has reported 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. Liu *et al.* (2009) mapped four quantitative trait loci (QTLs) on chromosome 2, 4, 7 and 9, respectively. The QTL need to be identified for further validated on other large mapping population having same donors. The marker exhibiting maximum association is further used for MAS to develop BPH resistant varieties.

REFERENCES

Causse, M.A., Fulton, T.M., Cho, Y.G., Ahn, S.N., Chunwongse, J., Wu, K., Xiao, J.,Yu Z., Ronald, P.C., Harrington, S.E., Second, G.A., McCouch, S.R. and Tanksley, S.D. (1994). Saturated molecular map of the rice genome based on an interspecific back cross population. *Genet.*, **138** (4) : 1251-1274.

Chen, J. W., Wang, L., Pang, X.F., and Pan, Q.H., (2006). Genetic analysis and fine mapping of a rice brown planthopper *Nilaparvata lugens* (Stal.) resistance gene bph19 (t). *Molecular Genetics & Genomics*, **275**(4): 321-329.

Dellaporta, S.L., Wood, J., and Hicks, J.B. (1983). A plant DNA minipreparation: Version II, *Plant Mol.Bio., Rep.*, **1**(4) : 19-21.

Kalode, M. B., Krishna, T. S. and Gour, T. B. (1978). Studies on pattern of resistance to brown plant hopper (*Nilaparvata lugens* Stal.) in some rice varieties. *Pro. Indian. Nat. Sc. Acad.*, **44** (1) :43-48.

Kar, S. (2010). Identification of QTLS for brown planthopper *Nilaparvata lugens* (Stal.) resistance in RIL mapping population of rice (*Oryza sativa* L.) M.Sc. (Ag.) Thesis, Indira Gandhi Krishi Vishwavidyalaya, C.G., pp. 5-21.

Liu, Y., Su, C., Jiang, L., He, J., Wu, H., Peng, C., and Wan, J. (2009). The distribution and identification of brown planthopper resistance genes in rice. *Hereditas*., **146**(2): 67-73.

Renganayaki, K., Fritz, A. K., Sadasivam, S., Pammi, S., Harrington, S.E., McCouch, S.R., Kumar, S.M., and Reddy, A.S. (2002). Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. *Crop Sci.*, **42**(6): 2112-2117.

Xiao, J., Li, J., Yuan, L., and Tanksley, S.D. (1996). Identification of QTLs affecting traits agronomic importance in recombinant inbred population derived from a subspecific cross. *Theor. Appl. Genet.*, **92** (2) : 230-244.

Xu, Y., Zhu, L. Xiao, J., Huang, N. and McCouch, S.R. (1997). Chromosomal regions associated with segregation distortion of molecular markers in F_2 backcross double haploid and recombinant inbred population in rice (*Oryza sativa* L.). *Mol. Gen. Genet.*, **253** (5) : 535-545.