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IDENTIFYING GENETIC LOCUS ASSOCIATED WITH BROWN PLANTHOPPER (*NILAPARVATA LUGENS* (STAL.)) RESISTANCE INVOLVING BULKED SEGREGANT ANALYSIS IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT:

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is one of the major insect pests causing severe damage to the rice crop. Several major genes and Quantitative Trait Loci (QTL) associated with BPH resistance in rice were identified and put into high yielding varieties of rice. In the present study, an attempt has been made to identify the genetic locus responsible for resistance to BPH involving 264 F_2 individuals from the cross between IR50 (susceptible to BPH) and Bharathi (resistant to BPH). The parental survey revealed 50.12% polymorphism between parents. Bulked Segregant Analysis method was adopted to identify the polymorphic markers associated with susceptibility/ resistance. Theses markers were surveyed on F_2 individuals to establish linkage/ association with resistance to BPH following linkage analysis. The association of putative markers identified based on DNA pooling from selected segregants was confirmed by linkage analysis using MAPMAKER/EXP 3.0. The results of linkage analysis revealed that SSR marker, RM6487 on chromosome 4 showed significant association with resistance to BPH.

KEY WORD: *Rice, Brown planthopper, Modified Seed-box Screening Test, Bulked segregant analysis.*

INTRODUCTION:

Rice (*Oryza sativa* L.) remains as the staple food for over half the people in the world for a longer period of time than any other crop and is being

damaged by many insect pests and diseases. Among the insect pests of rice, brown planthopper (BPH) is continuously threatening the rice farmers from the date of introduction of modern rice varieties till date. The exclusive deliberations made on this pest in the past (IRRI, 1979) and in the present (Heong and Hardy, 2009) narrate the pressure of this pest on rice. Historically, BPH was known as a minor pest of rice and serious BPH outbreaks were reported occasionally before 1960. However, BPH rose from the status of a secondary pest to a major yield constraint beginning in the 1960s in the tropical Asia. This made the rice breeders and entomologists to explore the genetics of BPH resistance in rice. From 1960 onwards, extensive information on BPH resistant cultivars and possible genetic control of resistance in them was generated assuming BPH resistance in rice as one of the classic examples of major gene resistance in the history of host plant resistance to insects in crop plants.

With the advent of polymerase chain reaction (PCR) and molecular mapping technologies, several major genes and quantitative trait loci (QTL) governing BPH resistance have been mapped across the rice genome and DNA markers linked to BPH resistance have been reported. Most of these genes and QTL have been mapped using simple seed-box screening test. Complex phenotypic assays, anatomical differences and biochemical pathways which would help to have a clear understanding towards the differential response to BPH among resistant and susceptible cultivars have not been attempted in gene/QTL mapping experiments. Till now 29 BPH resistance genes have been detected in rice and four (*Bph14*, *Bph26*, *Bph17* and *bph29*) have been cloned (Du *et al*., 2009; Liu *et al*., 2015; Tamura *et al*., 2014; Wang *et al*., 2015). Rice with its great diversity has more hidden source and genes for resistance to BPH. Searching for new source and understanding the genetics behind the resistance is still a continuous process. Based on this the present study was conducted for genetic analysis and location of rice BPH resistance genes, using the $F_{2:3}$ families from a cross of IR50 and Bharathi. The tested materials were identified for BPH resistance by the method of the Modified Seedling Screening Test (MSST). Based on the evaluation of BPH resistance of $F_{2:3}$ families, the genotype of each F_2 plant could be inferred from the phenotype of corresponding $F_{2:3}$ lines. Combining with the molecular marker linked gene mapping built by F_2 population, the genetic analysis and gene mapping for BPH resistance were processed. The aim of the study is to search SSR molecular markers linked gene(s) resistant to BPH.

MATERIALS AND METHODS:

Cross Combination

A $F_{2:3}$ population from the cross between IR50 (IR2153-14-1-6-2/ IR28// IR36) and Bharathi (Hybrid Rice Evaluation Centre, Gudalur) was developed at Paddy Breeding Station (PBS), Coimbatore. A set of 264 F_3 families of this cross was screened for resistance to BPH.

Mass rearing of insect source

Insects were mass reared on the susceptible rice line (host) Taichung Native 1 (TN1) following the method of Heinrichs *et al*. (1985). Initial BPH population was collected from the rice field at Paddy Breeding Station (PBS), Coimbatore. The collected insects were reared and maintained in 45 days old host plants in separate culture room which was protected with wire mesh. Adult male and female insects in ratio of 1:1 were let for ovipostion in 45 days old plants covered with milar cage, twelve to fifteen days after release. First instar nymphs were emerged out which were uniform in nature and will be of more effective for screening.

Modified Seed-box Screening Test (MSST)

Wooden box of standard size (50x40x10 cm) was filled with fine wet soil and leveled properly. Pre-germinated seeds of each F_3 family (at least 15 to 20 seeds per entry) were sown (seeds were placed 3cm apart in the wooden box) in such a way that it could accommodate 12 lines per seed-box including the resistant and susceptible checks PTB33 and TN1, respectively. On seventh day after sowing, the wooden seed-boxes were transferred to galvanized iron trays (62 x 47 x 15cm) containing 5 cm depth of water. The seedlings were infested with second instar nymphs at the rate of three to five nymphs per seedling on 12th day after sowing. After infestation, the wooden seed-boxes were covered with wire mesh wooden cages in order to protect the released nymphs from other predators and also prevent the escape of nymphs from seed-box. Each F_3 family replicated thrice and the test plants were observed daily for damage by BPH. Damage rating of the test lines was done on individual plant basis when 90 per cent of the plants either in the susceptible check row (TN1) in the seed-box were died. The test lines were scored on a 1-9 scale using the Standard Evaluation System for Rice (SES) scale (IRRI, 1996) (Table 1).

Bulked Segregant Analysis and Identification of Polymorphic Markers

Genomic DNA was isolated from frozen fresh leaf tissue of both the parents and 264 F_2 progenies following the procedure described by McCouch *et al*. (1988). The final DNA concentration was adjusted to 25 ng/μl. Following the BSA method put forward by Michelmore *et al.*(1991), twenty plants were selected from the F_2 population representing ten resistant and ten susceptible to BPH according to the evaluation on $F_{2:3}$ families to BPH response. DNA was extracted separately and mixed by equal amount for setting up BPH resistant and susceptible DNA pools respectively, which were used for identifying molecular markers linked to the target gene(s).

First, the polymorphism of parents was tested by 395 pairs of SSR primers covering all 12 rice chromosomes. Then, the genotypes of F_2 individual plants were identified by the polymorphic primers between the bulks. Amplification was carried out in 10µl reaction volume

containing 20-30ng of genomic DNA, 1.0µM of primer, 1.0mM dNTPs consisting of dATP, dGTP, dCTP and dTTP, 1.0mM assay buffer and 0.03 units of Taq DNA polymerase. Amplification was done using a PTC Thermal Cycler (MJ Research Inc.,) programmed for initial denaturation at 94ºC for 2 minutes, 35 cycles of 30 seconds denaturation at 94ºC, 45 seconds annealing at 55ºC and 45 seconds extension at 72ºC and final extension at 72ºC for 10 minutes and then at 4ºC till storage. The PCR products (2µl) were run in a four per cent denaturing polyacrylamide gel electrophoresis (PAGE) at 200 volts for 2.5 hours and resolved by silver staining procedure (Panaud *et al.*, 1996). Through Mapmaker/Exp 3.0 genetic loci associated with BPH resistance was conducted for F_2 population. Co-segregation of SSR markers with BPH resistance was analyzed by using MAPMAKER/EXP3.0 (Lander *et al.*, 1987) software to determine the linkage relationships between them. The Kosambi function was transformed into Centimorgan (cM).

RESULTS AND DISCUSSION:

Seedling screening showed that IR50 was susceptible (damage rating of 1.0) and ASD 16 was resistant (damage rating of 8.8) to BPH. F_3 families showed considerable variation for seedling resistance to BPH with the damage ratings ranging from 1.00 to 9.00. All the 264 F_3 were classified into three categories on the resistance scores as resistance, segregating or susceptibility. Out of the 264 F_3 families, 211 were resistant and 53 were susceptible. The phenotypic performance of F_3 families in MSST is presented in (Figure 1). Out of the 395 microsatellite markers, 198 primers were polymorphic between the parents. Out of these 198, four primers (Table 2) *viz*., RM6933 (chromosome 2), RM6487, RM3317 (chromosome 4) and RM32 (chromosome 5) were polymorphic between the bulks and were tested for co-segregation among the individual F_2 population constituting the bulks. Genotypic data for all the 264 F_2 individuals were scored and used for analysis. Based on resistance scores, 264 F_2 were classified into three categories as resistance, segregating and susceptibility. The numbers of resistance, segregating and susceptibility F_2 are 73, 128 and 53 respectively. The F_2 segregation of RR, RS, and SS fit in the expected 1:2:1 ratio.

Based on the F_2 genotypic data and F_3 phenotypic data, the two point analysis was carried out using MAPMAKER/EXP3.0 by coding the observed phenotype in the same manner as a segregating SSR marker allele. Linkage analysis with Mapmaker/EXP 3.0 revealed that SSR marker RM6487 was linked to the BPH resistance gene. The map distance between the BPH resistance gene and the closest SSR marker RM6487 was 8.9 cM. In two point analysis, RM6487 revealed linkage with phenotype at distance of 8.9 cM. Though RM6933, RM3317 and RM32 detected polymorphism between the bulks of phenotypic extremes for BPH resistance significant association was found only between RM6487 marker data and phenotypic data for BPH resistance. The segregation pattern of SSR locus in a set of

F_2 population is shown in Figure. 2 Though other markers showed polymorphism between the bulks, no association was established between markers and phenotype involving the F_2 segregants.

Till date, ten BPH resistance genes and QTLs (*Bph 12, Bph 15, Bph 17, Bph 20(t), Bph 6, Bph 27, Bph 27(t), bph 12(t), QBph 4.1* and *QBph 4.2*) have been identified in chromosome 4. Among this, *Bph 12, Bph 15, Bph 17, Bph 20(t), QBph 4.1* and *QBph 4.2* are closely linked in a region of 5–9 Mb on short arm of chromosome 4 (Hu *et al*., 2016). Comparison of genomic region identified in the present study and the already reported genes and QTLs for BPH resistance using bi-parental progenies indicated correspondence between the genomic regions. The linkage established between RM6487 and BPH resistance in the present study established a genomic region for BPH resistance which has been already reported based on the mapping of genes *viz*., *Bph 12, Bph 15* and *Bph 17*(Rahman *et al*., 2009).

CONCLUSION:

Bulked segregant analysis (BSA) has been established as one of the efficient strategies for mapping major genes conferring resistance to many pest and disease resistance in crop plants. This strategy was use to map one of the major genes conferring resistance to BPH by Renganayaki *et al.* (2002). Identification of linkage between RM 6487 with BPH resistance from the present study and other reports already made for this association establishes strong evidence for hot spot in the rice genome conferring resistance to BPH in rice. Fine mapping of the gene(s) and cloning of the gene in this genomic region will facilitate Marker Assisted Selection (MAS) for BPH resistance and evolve breeding strategies to develop varieties with durable resistance to BPH in rice.

REFERENCES:

Du, B., W. Zhang, B. Liua, J. Hua, Z. Weia, Z. Shia, R. Hea, L. Zhua, R. Chena, B. Hanb and G. Hea. 2009. Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. Proc. **Natl. Acad. Sci.,** 1-6.

Heinrichs, E.A, F.G. Medrana and H.R. Rapusas. 1985. Genetic evaluation for insect resistance in rice. Los Banos, Philippines, International Rice Research Institute. 356p.s

Heong, K.L and B. Hardy. 2009. **Planthoppers: new threats to the sustainability of intensive rice production systems in Asia**. Los Baños (Philippines): International Rice Research Institute. 460p.

Hu, J., C. Xiao and Y.He. 2016. Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. **Rice, 9 (1)**: 1-12.

IRRI. 1979. **Brown planthopper: Threat to rice production in Asia**. International Rice Research Institute, Los Baños, Philippines. 376p.

IRRI. 1996. Standard Evalution System for Rice.fourth ed., Manila, The Philippines.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincon and L. Newburg. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. **Genomics, 1**: 174-181.

Liu, Y.Q., H. Wu and H. Chen. 2015. A gene cluster encoding lectin receptor kinases confers broadspectrum and durable insect resistance in rice. **Nat Biotechnol., 33**:301–305.

McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Coffman and S.D. Tanksley. 1988. Molecular mapping of rice chromosomes. **Theor. Appl. Genet., 76:** 815-829.

Michelmore, R.W., I. Paran and R.V., Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. **Proc. Nat. Acad. Sci. 88**:9828-9832.

Panaud O., X. Chen and S.R. McCouch. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.). **Mol. Gen. Genet., 252**: 597-607.

Rahman, M.L., W. Jiang, S.H. Chu, Y. Qiao, T.H. Ham, M.O. Woo, J. Lee, M.S. Khanam, J.H. Chin, J.U. Jeung, D.S. Brar, K.K. Jena and K.J. Koh. 2009. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*. **Theor. Appl. Genet., 119:** 1237–1246.

Renganayaki, K., A.K. Fritz, S. Sadasivam, S. Pammi, S.E. Harrington, S.R. McCouch S.M. Kumar and A.S. Reddy. 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:** 2112-2117.

Tamura, Y., M. Hattori and H. Yoshioka. 2014. Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52. **Sci. Reports, 4:**5872.

Wang, Y., L.M. Cao and Y.X. Zhang. 2015. Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. **J. Exp. Bot., 66**:6035–6045.

Table1. Scale based on Standard Evaluation System for Rice (SES)

Grade	Criteria
0	No damage
1	Very slight damage
3	First and Second leaves of most of the plants partially turns yellowing
5	Pronounced yellowing and stunting or about half the plants wilted or dead
7	More than half of the plants dead
9	All plants dead

Table 2. Details of SSR markers polymorphic between bulks of extreme phenotype

Marker	Linkage group	Forward 5' to 3'	Reverse 5' to 3'	Annealing Temp (°C)
RM6933	2	TGTAGCAGAAACCAATGCTC	GTCACTCCACTTCGCTTATC	55
RM6487	4	AGAAGCTGTAGACGATGGCC	CTAGACCTCATCCCCTTCCC	61
RM3317	4	AGCAACCTGACAGAAGAATG	TCTCGTTGAGTTGGAAGAAG	55
RM32	5	AGTCTACGTGGTGTACACGTGG	TGCGGCCTGCCGTTTGTGAG	55

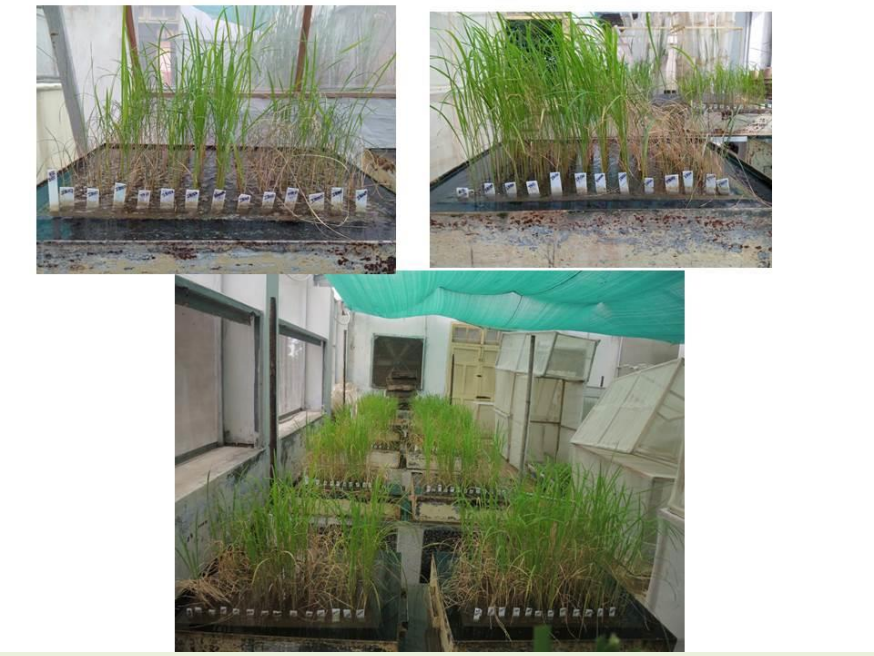

Figure 1. Phenotypic performance of F_3 families in MSST

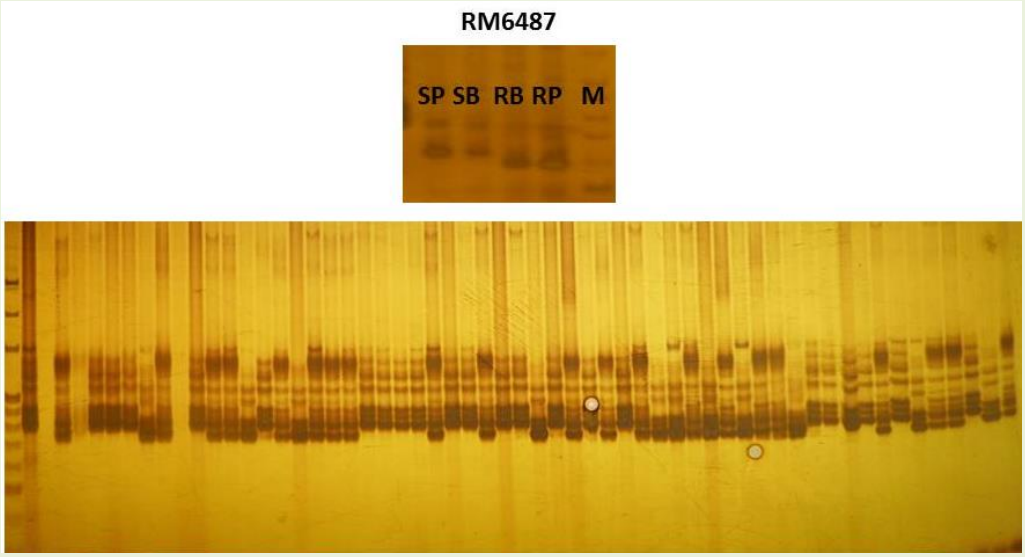

Figure 2. a. Resistant and Susceptible Bulks showing polymorphism for RM6487
(SP- Susceptible Parent, SB- Susceptible Bulk, RB-Resistant Bulk and RP- Resistant Parent)
b. Segregation pattern of SSR marker RM6487 in F_2 population of IR50 x Bharathi