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SCREENING FOR THE PRESENCE OF BROWN PLANT HOPPER (*Nilaparvata lugens* Stål) RESISTANT GENES IN SELECTED RICE BREEDING MATERIALS

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ABSTRACT

Brown Plant Hopper (BPH; *Nilaparvata lugens* Stål) is a serious insect pest of rice (*Oryza sativa* L.) in Sri Lanka, and varietal resistance is the most economic, least complicated and environmentally-friendly approach for protecting crops against the pest damage. Pyramiding of resistant genes allow rice varieties to have a broad-based resistance, which minimizes the risk of epidemics. This cannot be achieved only by conventional breeding, which is often slow and laborious. Marker assisted selection (MAS) offers an expedited selection and transfer of genes to crop varieties. There is a scarcity of information on the identification of molecular markers in rice for BPH resistance in Sri Lanka. Therefore, a study was carried out to test the available molecular markers such as KAM4 (*bph2*) and RG 457 (*bph10*), and AJ09 (*BPh13*), *Bph12*(t) and a putative RAPD marker $OPA16_{938}$ were tested for potential use in MAS. The KAM, RM261 and $OPA16_{938}$ showed potential use while AJ09 and RG 457 were not useful with the materials tested. However, presence and absence of *bph2, bph10, Bph12*(t) and *Bph13* in the parental materials were detected. The study also revealed the need for carrying out investigations on resistant genes in rice breeding materials in Sri Lanka using available markers and also development of markers in Sri Lanka for resistant genes in rice to BPH biotypes.

KEYWORDS: Molecular markers, *Nilaparvata lugens*, Resistance

INTRODUCTION

The Brown Plant Hopper (BPH; *Nilaparvata lugens* Stål) has been identified as a serious pest of rice (*Oryza sativa* L.) in Sri Lanka. An average of 5-10 % of rice lands of the country is affected annually due to BPH attack. Major outbreak of this pest was recorded in *maha* season 1997/1998 (Nugaliyadda *et al.*, 2001). Infestation of the insect reduces plant height, crop vigor, number of productive tillers per plant and number of filled grains per panicle of rice. The BPH is also the vector for rice rugged stunt oryza virus, rice grassy stunt 'tenuivirus' and tungro virus (Henrichs *et al.*, 1979). Host plant resistance has been identified as the most effective way of managing BPH incidence. Varietal resistance has been reported as the most economic, least complicated and environmentally-friendly approach for protecting crops against insect damages (Pathak and Kush, 1979). Twenty one BPH resistant genes have been identified in *indica* rice cultivars and in two wild relatives namely,

O. australiensis and *O. officinalis* (Hewakapuge, 2002; Renganayaki *et al.*, 2002). Quantitative Trait Loci, which possess BPH resistance has been mapped using a mapping population derived from double haploid cross between IR 64 and Azucena (Alam and Cohen, 1998). The BPH resistant genes in traditional and wild species of *Oryza* have been incorporated to modern semi-dwarf cultivars.

Pyramiding of genes into elite cultivars cannot be achieved only by conventional breeding, which is often slow and laborious. Use of molecular markers closely linked to genes of interest has made a significant contribution to the development of improved crop varieties by gene pyramiding. Selection of target genes based on linked molecular markers is refers to as Marker Assisted Selection (MAS). The success of MAS is determined by the tightness of the linkage of the molecular marker with the target gene. The MAS offers an opportunity to expedite selection and transfer of desirable traits in segregating generations at early stages of plant development. The PCR-based markers are the most practical markers for MAS, as the PCR approach is much faster, easier, less laborious and less time consuming than the RFLP analysis. Development of own markers is costly and time consuming and hence testing of available markers would be the best option. Therefore, objective of this study was to test the available BPH resistant markers for MAS and subsequent use in pyramiding genes.

MATERIALS AND METHODS

A total of seven parental materials, which included two IR materials and five elite rice varieties, were used in the crossing program to study the BPH resistance (Table 1). The crosses made at the Rice research and Development Institute (RRDI) at Batalagoda, Sri Lanka are shown in Table 2.

Table 1. Parental materials and their sources of BPH resistance

Parental material	*Source of BPH resistance*
IR 54751-2-34-10-6-2	Introgression line *O. officinalis* (*bph11, bph12, Bph13*)
IR 65482-4-136-2-2	Introgression line *O. australiensis* (*Bph10*)
Bg 352	Ptb 33 (*bph2* and *Bph3*)
Bg 358	Ptb 33
Bg 360	Ptb 33 (*bph2*)
Bg 300	Ptb 33 (*bph2* and *Bph3*)
Bg 357	Ptb 33 (*bph2* and *Bph3*)

Table 2. Crosses made at RRDI at Batalagoda

Parent Materials		
IR 54751-2-34-10-6-2	X	Bg 352
		Bg 358
		Bg 360
		Bg 300
IR 65482-4-136-2-2	X	Bg 300
		Bg 357
		Bg 360

A total of 150 progenies (F_3) from seven single crosses were screened by the standard seed box screening test (Heinrichs *et al.*, 1985) for the parental germplasm using BPH reared on Bg 379/2. The leaf samples from the parental material and three resistant and three susceptible progenies from each cross were randomly taken for the DNA marker analysis. The DNA from above samples namely, Ptb 33 and Bg 94-1 was extracted using the miniprep method (Anonymous, 2003). The presence of DNA was confirmed by agarose gel electrophoresis and UV spectrophotometer (Biomate 3). Depending on the intensity of DNA bands, the original DNA solutions and 2-3 times diluted samples, which contained about 20-100 ng μl^{-1} of DNA were used in the polymerase chain reaction (PCR). All parents and 36 DNA bulks (three susceptible and three resistant of six crosses) were screened for the STS markers namely, KAM 4 F/KAM 4 R [for *bph2* gene (Murai *et al.*, 2001)], RG 457 FL/RL and RG 457 RB/RL [for *Bph10* (Nguyen *et al.*, 1999)], AJ 09 [for *Bph13* (Renganayaki *et al.*, 2002)], and $OPA16_{938}$ putative RAPD marker (Jena *et al.*, 2003). In addition, 80 individuals of the F_2 population of IR 54751-2-34-10-6-2 x Bg 300 was tested for identification of polymorphism for RM 261 for *Bph12*(t) (Yang *et al.*, 2002) and potential use for MAS. The PCR mixture contained 20-100 ng template DNA, 1 pmol of each primers, 0.25 mM dNTP's each, 1x PCR buffer (20 mM Tris pH 8.0 including 20 mM $MgCl_2$ and 0.5 units of *Taq* polymerase in a total volume of 10-15 µl). The PCR reactions were initially denatured at 94 °C at 5 min followed by 35- 40 cycles of PCR amplification (Eppendorf Master Cycler Gradient) using the parameters namely, denaturation at 94 °C (30 sec), primer annealing at 66 °C (1 min) and primer extension at 72 °C (1 min). After completion of the PCR, amplification products of RG 457 primers were subjected to restriction digestion using *Hinf* at 37 °C for 1.5 hr. For RM 261, the initial denaturation of 5 min followed by 10 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min and extension at 72 °C for 1 min. Following these, another 10 cycles of denaturation was carried out at 94 °C for 1 min, annealing at 60 °C for 1min and extension at 72 °C for 2 min followed by 15 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min with a final

extension at 72 °C for 10 min. The PCR products were separated and detected on 1-1.4 % agarose gels stained with EtBr using BIORAD gel documentation apparatus. For RM 261, the polyacrylamide gel electrophoresis followed by silver staining procedure (Samarasinghe *et al.*; 2002) was carried out to separately to detect bands.

RESULTS AND DISCUSSION

Screening of progenies for reaction to BPH insect attack at the RRDI using the standard seed box screening test

The reactions observed in the test rice varieties to the BPH that was reared on the variety Bg 379/2 using the standard seed box screening is shown in Table 3.

Table 3. BPH reaction observed against parental materials

Parental material	*Reaction to BPH*
IR 54751-2-34-10-6-2	Resistant (R)
IR 65482-4-136-2-2	Resistant (R) /Moderately resistant (MR)
Bg 352	Moderately resistant (MR)
Bg 358	Moderately resistant (MR)
Bg 360	Moderately resistant (MR)
Bg 300	Moderately resistant (MR)
Bg 357	Resistant (R)

Reaction shown to the BPH infestation by the variety Bg 379/2, new BPH resistant donors *viz.* IR 54751-2-34-10-6-2 (R) and IR 65482 – 4 –136 –2 –2 (MR) indicated the presence of useful resistant genes for breeding for BPH resistance in rice in Sri Lanka. Reaction by elite cultivars *viz.* Bg 300 (MR), Bg 352 (MR), Bg 358 (MR), Bg 357 (R) and Bg 360 (MR) depicted the presence of some resistant genes in the BPH biotype reared in the variety Bg 379/2.

Screening of parental materials and progenies by molecular markers

KAM 4F/KAM 4R (bph2) - The STS marker (300 bp) specific for resistant gene *bph2* was observed in Bg 352, Bg 360, Ptb 33 and Bg 300 (Photo 1). The *Bph2* is a recessive gene (Athwal *et al.*, 1971) and the marker KAM 4 cannot distinguish between the homozygots and heterozygots. Therefore, this marker may be positive with heterozygous susceptible lines. However, if the marker present in the resistant lines, such plants can be selected for presence of the *bph2* gene. In this analysis. KAM 4 marker was observed segregating among the progeny lines derived from the crosses that involve Bg 360, IR 547 and IR 654. Therefore, those resistance lines can be selected for further advancement in a breeding program. This is a potential

marker for MAS involving the crosses between Bg 360, Bg 300, Bg 352 *vs* IR 54751 and IR65482.

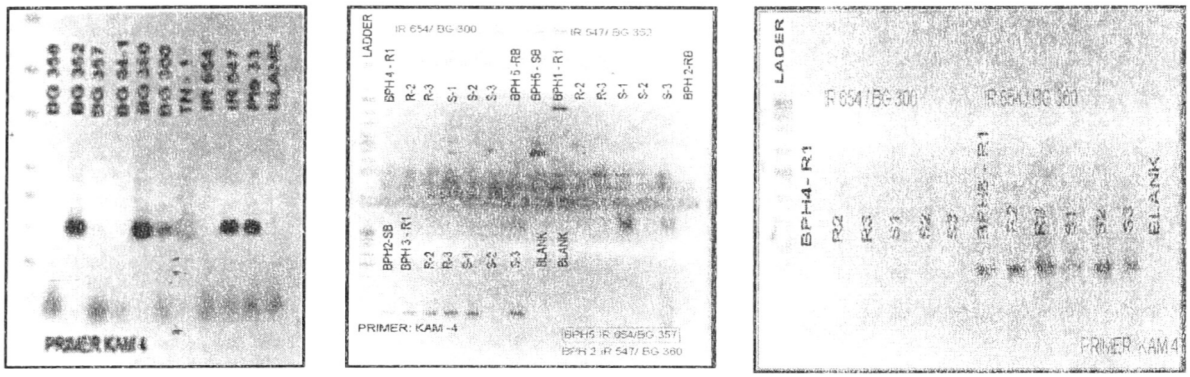

Photo 1. The PCR products of parental materials and F_3 progenies using KAM 4 F/R

RG457 -Markers (Bph10) – The PCR products of RG makers (RG 457 FL/RL and RG 457 RB / RL) generated amplification products (Photo 2) for all parental accessions. The digestion of PCR products with *Hin*f1 yielded restriction fragments polymorphism. Both markers showed homozygosity for *Bph10* in IR65482-4-136-2-2 (new sample), Bg 352, Bg 357 and Bg 360 (Photo 3) indicating the presence of *Bph*10 gene in the aforesaid parents. In progenies, homozygosity and heterozygosity of marker for resistance were observed in both resistant and susceptible lines (Plate 3). This indicates that the *Bph10* is not resistant to BPH used in these experiments and hence is not a useful marker to be used in the breeding program. However, IR lines used in this study showed R\MR to insects depicting the presence of other resistant genes in the tested germplasm.

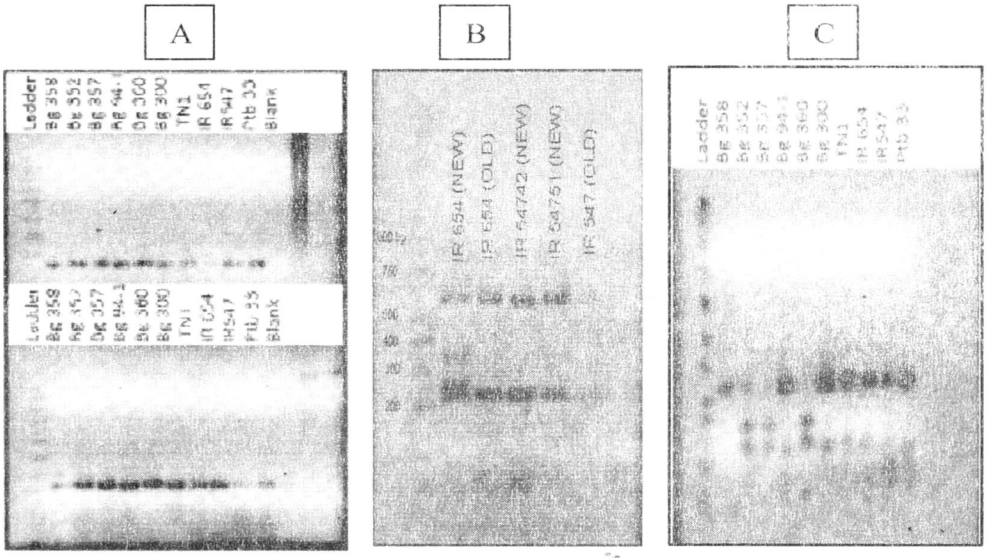

Photo 2. A- PCR products of 10 varieties (including parental material) using 457 FL/RL (A - Top, 750 bp) and RG 457 FL / RB (A – bottom, 800 bp), markers and their *Hinf*-digested products: B- IR65482-4-136-2-2 (New), and C- Bg 352, Bg 357 and Bg 360 (300, 250, 500bp) and the rest of the varieties (550, 200bp)

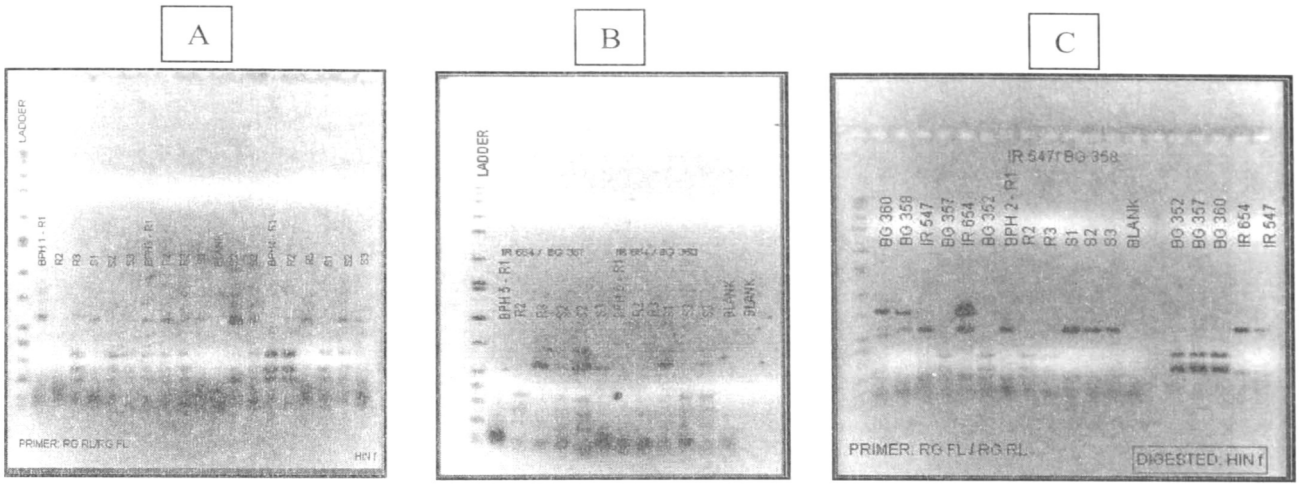

Photo 3. PCR products of F3 progenies using 457RG primers and digested by *Hinf* Lanes : BPH1- IR54751-34-10-6-2/Bg 352, BPH2- IR54751-2-34-10-6-2/Bg 358, BPH3- IR54751-2-34-10-6-2/Bg 360, BPH4- IR 65482-4-136-2-2-/Bg 300, BPH5-IR 65482-4-136-2-2-/Bg 357 and BPH6-- IR 65482-4-136-2-2-/Bg 360

AJ 09 marker (Bph13) - This marker has been detected in a *O. officinalis* line IR54745-2-21-12-17-6 (Renganayake *et al.,* 2002). As this study used *O. officinalis* line 54751–2–34–10–6–2, the particular marker was tested for potential use. The PCR product (230 bp) was observed in all parental types and no polymorphism was observed, thus indicating that the *Bph13* may present in all parental germplasm and the AJ09 cannot be used in gene pyramiding in this program.

RM 261 marker [Bph12(t)] - The *O. officinalis* derived introgression line, IR 54751– 2 – 34 – 10 – 6 – 2 showed resistance to BPH collected from the damaged field of Bg 379-2 in Kegalle. The *Bph12(t)* is a BPH-resistant dominant gene found in *O. latifolia*, which shares the same basic genome constituent (*C*) with the *O. officinalis*. The marker showed polymorphism among the parents and in 80 individuals (Photo 4) and was segregated as 1:2:1 Mendalian ratio proving tight linkage with the gene. This indicates the possibility of using RM 261 for pyramiding BPH genes in Bg 300.

OPA 16_{938} RAPD marker – The $OPA16_{938}$ has been identified from the introgression line IR54741-3-21-22 derived from *O. sativa* and *O. officinalis* using a RAPD primer OPA16 (Jena *et al.,* 2001). The $OPA16_{938}$ was a putative marker closely linked to the gene conferring resistance to BPH biotype in India. As another *O. officinalis* introgression line namely, IR54751-2-34-10-6-2 was used in this study, the particular marker $OPA16_{938}$ was tested for potential use. Results (Photo 5) showed that IR54751 and the resistant bulk of the cross IR54751/Bg 352 and a

susceptible bulk of IR54751/Bg 358 carry the $OPA16_{938}$ maker. This is a potential marker and needs further verification with progenies.

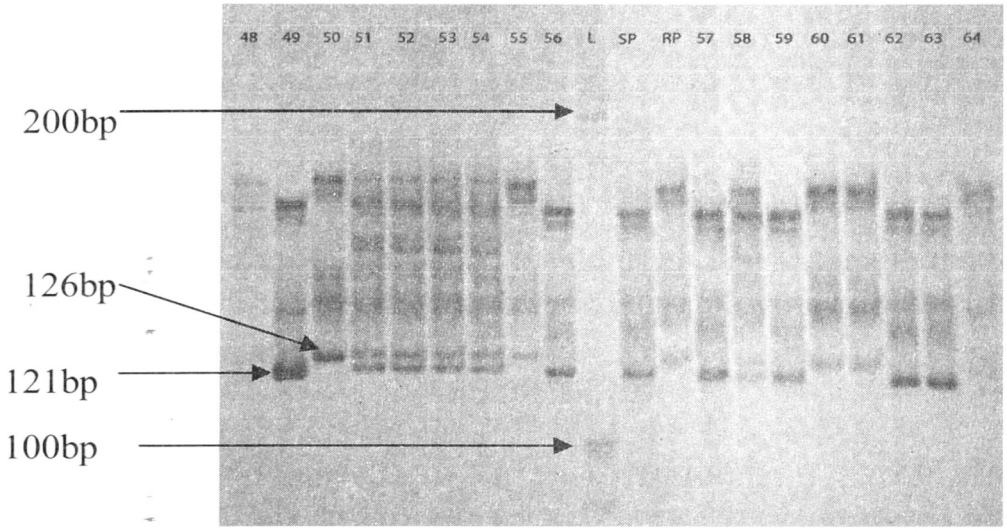

Plate 4. Silver-stained 8% denaturing PAGE electrophoresis of PCR products from the amplification of BPH resistant and susceptible parents and 48-64 F_2 individuals (heterozygote and homozygote individuals) with RM 261 microsatellite locus RP- Resistant Parent [IR54751-2-34-10-6-2 *O. officinalis* derived introgression line, (126 bp)], SP- Susceptible Parent [Bg 300 (121 bp)], L – Ladder, 48-64 - individual segregants

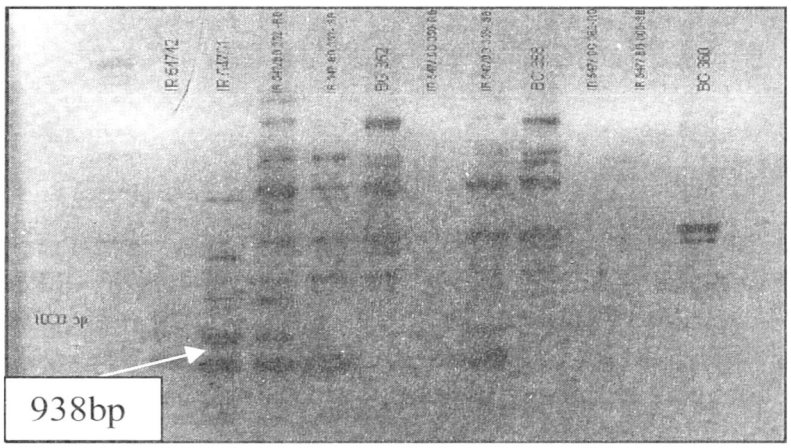

Plate 5. Agarose gel of PCR products of the parental materials and DNA bulked progenies (B) amplified using RAPD primer OPA16 at annealing temperature of 35 °C

CONCLUSIONS

According to the molecular markers tested, the *bph2* gene is present in rice varieties Bg 352, Bg 360, Ptb 33 and Bg 300. The KAM 4 is a potential marker for MAS involving the crosses between Bg 360, Bg 300, Bg 352 vs IR 54751-2-34-10-6-2 and IR 65482-4-136-2-2. The RG457 marker showed homozygosity for *Bph10* in IR65482-4-136-2-2, Bg 352, Bg 357 and Bg 360 and Sri Lankan BPH is virulent to *Bph10* gene. The AJ 09 marker indicated that the *Bph13* is present in all parental materials used thus, cannot be used as marker for MAS. The RM 261 marker is a

potential marker for the *Bph12(t)* dominant gene in IR 54751-2-34-10-6-2 to be used in marker-assisted improvement of rice variety Bg 300. The OPA 16_{938} RAPD marker was detected in IR54751-2-34-10-6-2 and it is a potential marker that needs further verification with progenies. Efforts should be made to develop specific markers in Sri Lanka to resistant genes for BPH biotypes fond in the country.

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