INHERITANCE OF RESISTANCE IN RICE TO BROWN PLANTHOPPER: ITS IMPLICATIONS ON RICE VARIETAL IMPROVEMENT IN SRI LANKA

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ABSTRACT

Six dominant and four recessive genes responsible for resistance in rice to brown planthopper (BPH) (*Nilaparvata lugens*) have so far been identified on the basis of the damage reactions at seedling stage of parents, F1, F2 and F3 generations of crosses between resistant and susceptible varieties. As such the methodologies used for breeding and selection of BPH resistant rice varieties have been based on the assumption that BPH resistance in rice is monogeneic. Since BPH resistance in rice is a result of a series of interaction between the host plant and the insect the simple monogenic nature of resistance as suggested by many authors has been criticised. BPH resistance in Ptb 33 was found to be monogenic dominant on the basis of the damage reactions at seedling stage of F1, F2 generations of cross between Ptb 33 and a susceptible variety, TN1. However, on the basis of the honeydew production the resistance was found to be determined by a polygenic system. Therefore, efforts are being made to utilise Ptb33 as a parent in hybridisation and to rationally explore its resistance genes into a wide range of commercial cultivars and prevent development of biotypes of the pests.

KEYWORDS: Brown planthopper, Inheritance, Ptb 33, Rice, Varietal resistance

INTRODUCTION

The rice brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) is a phloem feeder with a high degree of host specificity to feed and survive

exclusively on rice (*Oryza* spp.) and its cultivars (Wilson and Claridge, 1991). Resistance to BPH has been found in a wide range of traditional rice varieties and wild rice species (Pathak and Khush, 1979; Heinrichs *et al*., 1985; Woodhead and Padgham, 1988). Some of this resistances have successfully been incorporated into varietal gene-bases and has helped reduce BPH out breaks and increase rice production in many Asian countries (Panda and Khush, 1995).

Virulence pattern of BPH populations vary from region to region and could change over time due to rapid adaptation of the pests to previously resistant varieties (Roderick, 1994). This has been a major constraint to the continued national rice improvement programs in Asia. Of the several BPH resistant donors used in the rice varietal improvement programme in Sri Lanka only the BPH resistance of Ptb 33 has successfully been incorporated into high yielding varieties (Kudagamage and Nugaliyadde, 1995; Nugaliyadde *et al.,* 2000). At present more than 10 improved varieties with BPH resistance derived from Ptb33 are grown in more than 60% of the total rice extent in the country.

Ptb 33 - a traditional cultivar from Pathambi, India is found to be highly resistant to the BPH populations in many Asian countries (Seshu and Kauffmann, 1980). Khush (1979) identified one dominant and one recessive gene responsible for BPH resistance in Ptb33 based on the reaction to the Philippine-strain of BPH biotype 1. A similar study conducted with a BPH population from Sri Lanka indicated the presence of a single dominant gene in Ptb 33 (Nugaliyadde, 1994).

The genetic analysis, conducted so far on BPH resistance in donor varieties, are based on the reactions at seedlings stage of F_1, F_2 and F_3 generations of crosses between resistant and susceptible parents (Khush, 1977; Khush, 1979; Khush *et al.*, 1985; Kabir and Khush, 1988; Nemato *et al.,* 1989). Since BPH resistance in rice is a result of a series of interactions between the host plant and the insect, the simple monogenic nature of the plant resistance as suggested by the above authors has been widely criticized (Den Hollander and Pathak 1981; Woodhead and Padgham, 1988). Den Hollander and Pathak (1981) who studied the inheritance of virulence of different biotypes of BPH stated that 'the virulence in BPH is polygenic and that the widely assumed gene-for-gene correspondence between resistance on the part of the plant and virulence on the part of the insect could not be established'.

Therefore, efforts were taken to analyze the inheritance of BPH resistance in Ptb 33 using honeydew production as the evaluation criteria to help design breeding and selection strategies to efficiently utilize BPH resistance and slow down the development of biotypes.

MATERIALS AND METHODS

Experiments were conducted in the greenhouse at Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka (25^0-35^0 C, 60-85% r.h.). A BPH population maintained on in the greenhouse on TN1 was used for the experiments. Ptb 33 (resistant) and TN 1 (susceptible) were crossed and a part of the F_1 seeds were grown in the field to generate F_2 to form seeds.

Reaction to BPH of Ptb33, TN1 and F_1 and F_2 populations of the cross Ptb33/TN1 at seedling stage

Seeds of Ptb 33, TN 1 and F_1 and F_2 populations of the cross Ptb33/TN1 were sown in a galvanised tray (60 X 40 X 10 cm) filled with sterilised soil 5 cm deep. The seeds were sown in 40 cm long-rows demarcated width-wise and spaced 5 cm among rows. Within a row, the seeds were placed equidistant (5 cm) apart. In each seed box, there were 9 rows of F_2 population, 1 row each of parents (Ptb33 and TN1) and F_1 generation. When the seedlings were seven-days old (at 3-leaf stage), they were infested with 2nd instar nymphs at the rate of 3 nymphs/ seedling. All test seedlings were rated individually for damage in about 7 days after infestation, when TN1 seedlings show >90% damage symptoms (damage score 9). The Standard Evaluation System for rice (IRRI, 1980) was used to score the seedling for BPH damage. The experiment was replicated 5 times.

Honeydew production of BPH on Ptb33, TN1 and F_1 and F_2 populations of the cross Ptb33/TN1

The standard experimental set up designed to measure the amount of honeydew excreted in 24 h by a 1-day old brachypteous female was used (Heinrichs *et al.,* 1985). Ptb 33, TN 1 and F_1 and F_2 populations were planted individually on 15cm diameter clay pots. When the test plants were 6-weeks old, the plant bases were enclosed individually with a feeding chamber (an inverted plastic cup). One-day old BPH females (at the rate of one female/ feeding chamber) were introduced into feeding cups and allowed to feed for 24h. Honeydew excreted during this period was collected on to a bromocresol-green treated filter paper (Whatman No. 1) placed at the bottom of the feeding chamber. The area stained (blue) on the filter paper due to honeydew was measured with the help of a square mm grid. Honeydew measurements were taken on 20 plants each for Ptb33, TN1 and F1 population and on 148 plants for the F2 population. Ten honeydew measurements were taken on each plant spaced over 10 consecutive days.

RESULTS AND DISCUSSION

Inheritance of BPH resistance in Ptb33 based on the seedling reaction

The reaction of F_1 and F_2 populations of the cross Ptb33/ TN1 indicated that the resistance in Ptb33 to BPH is monogenic dominant (table 1). In a similar genetic analysis, BPH resistance of Ptb 33 and two of its derived line, Bg 300 and Bg 379-2 (with moderate level of resistance to BPH) was found to be monogenic dominant possibly due to a common gene (Nugaliyadde, 1994). On the contrary, the factors responsible for BPH resistance of Bg 300 and Bg 379-2 were found to be entirely different to each other. BPH resistance of Bg 300 originates from the composition of the surface lipids, which interferes with the ability of the insect to locate suitable feeding sites on the plant. On the other hand, the phloem sap of Bg 379-2 is found to deter sustained feeding of the insect and thereby it derived its resistance (Nugaliyadde *et al.*, 2000).

Table 1. Reaction to brown planthopper of Ptb33, TN1 and F_1 and F_2 populations from the cross Ptb33/ TN1 at the seedling stage.

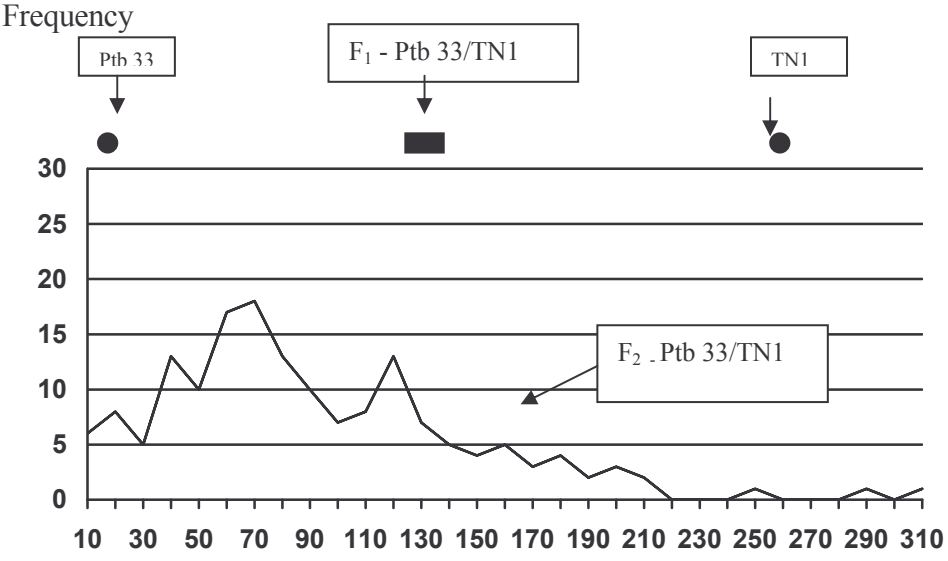
Variety/ Cross	*No. seedlings*		*P value Chi square analysis 3:1*
	Resistant	*Susceptible*	
Ptb 3311	27	3	-
TN1	2	28	-
F_1 - Ptb33/TN1	27	2	-
F_2 - Ptb33/TN1	436	125	0.25-0.10

Inheritance of BPH resistance in Ptb33 based on honeydew production

BPH excreted significantly less amount of honeydew (mean and s.e. 11.2 $\pm$ 1.6 mm^2) on Ptb33 than on F_1 plants (mean and s.e. 132 $\pm$ 42 mm^2) and TN1 (mean and s.e. 267 $\pm$ 51 mm^2) (figure 1). The honeydew excreted on F_1 was intermediate to the parents.

Honeydew excreted on the 148 F_2 plants (replicated 10 times) ranged from 0 to 309 $\pm$ 40 mm^2. The frequency distribution for honeydew production on F_2 population followed a skewed distribution towards Ptb33 (resistant parent) indicating a polygenic nature of BPH resistance in Ptb33. These observations suggest that a major gene and a number of minor genes or a polygenic system are responsible for BPH resistance of Ptb 33.

In rice, the genetic analysis studies on the resistance to planthopper and leafhopper pests is based on the 'Standard Seedling Test' where the damage symptoms of the parents, F_1, F_2 and F_3 populations are evaluated on a 1-9 scale when the susceptible check get the damage score of 9 (death). Insect damage of parents, F_1 hybrids and F_3 families are scored on row basis and classified as resistant, susceptible or segregating. In the case of F_2 populations each seedling is classified as resistant or susceptible. Those seedlings and rows with a damage score from 1 to 5 are considered as resistant and from 7 to 9 as susceptible. This grouping is done with the assumption that hopper resistance in rice is a qualitative trait. As a result, several genes for resistance to brown planthopper, white-backed planthopper, green leafhopper have been identified (Panda and Khush, 1995). Ten genes for BPH resistance have already been defined i.e. Mudgo (Bph 1), ASD 7 (bph 2), Rathuheenati (Bph 3), Babawee (bph 4), ARC 10550 (bph 5), Ptb 33 (1d,1r), Swarnalatha (Bph 6), ARC15831 (bph 7), Chin saba (bph 8), Balamawee (Bph 9).



Honeydew area (mm^2/ female BPH/ 24h)

Figure 1. Amount of honeydew excreted by a femele BPH in 24 h on Ptb 33, TN1 and F_1 plants of the cross, Ptb33/TN1, and the frequency distribution of the honeydew area on 148 F_2 plants of the cross Ptb 33/TN1 (frequency distribution plotted at intervals of 10)

In view of the increasing demand and greater potential that exist for BPH management through plant resistance, the conventional breeding approaches need to be redesigned with inputs from biotech tools that are available or easily developed to make the breeding more effective and efficient and meaningful. At present the breeding and selection strategies for the development of BPH resistant varieties are designed with the assumption that the resistance is monogenic dominant or recessive. As such segregating populations are subjected to standard seedling tests to identify resistant progenies and to advance the generation and re-testing for resistance. The procedure does not facilitate selection of progenies with minor-genic resistance. Hence, many varieties that would have been selected with such resistance against this pest may have been discarded making breeding efforts inefficient.

It is therefore important that the evaluation procedure for BPH resistance is redesigned to enable identify those segregants possessing different genes or gene-combinations and different resistant mechanisms to the pest. Marker aided selection would be the best choice of techniques that can be used to select varieties with the desired trait characters. This would facilitate development of varieties with a wider genetic variation for the resistant trait and to cultivate them concurrently within rice systems thereby reduce the selection pressure on the insect to form virulent 'biotypes'. (Kudagamage and Nugaliyadde, 1984; Woodhead and Padgham, 1988; Roderick, 1994).

Narrow genetic variability for resistance to BPH (Saxena and Khan, 1989), and lack of knowledge of the genetics of resistance also limits the progress of the breeding programmes aiming to develop high yielding varieties with resistance to this pest (Cook *et al*., 1987). Moreover, the undesirable agromorphological characteristics linked with resistance in the donor varieties make it difficult to combine insect resistance into high yielding varieties through conventional breeding. Similarly, it was not possible to combine all genes complementing the high level of BPH resistance in Ptb 33 into improved gene bases. All improved varieties developed so far using Ptb 33 as the resistance source (Bg 300, Bg379-2, Bg 352, Bg 304, Bg 403, Bg 357, Bg 359, Bg 360) are moderately resistant to the insect. Therefore, efforts are to be made to tag the BPH resistant genes in Ptb33 and to facilitate selection of segregating progenies.

Several methods were suggested to maximize the use of host-plant resistance for BPH (and in general homopteran pests) management. Sequential release of varieties with different resistant traits; use of multilines with vertical resistance; polygenic resistance with moderate resistance -horizontal resistance; received wide acceptability (Khush, 1979; Heinrichs *et al.*, 1985; Panda and Khush, 1995). However, these methods failed in practical applications, mainly due to the difficulties in developing a spectrum of varieties that could satisfy the above criteria. The situation, in general, has been to recommend any improved variety having acceptable level of resistance to BPH. The main criteria in recommending varieties has been the higher yield potential, adaptability and grain quality rather than the resistant traits for

pests and diseases. These decisions would have greatly influences to the series out breaks of BPH biotypes in Southeast Asia since 1980s. Therefore, it is imperative that a decision making process be adopted when selecting varieties for commercial cultivation in a given area/ or season. Such decisions would inevitably help reduce out breaks of BPH.

In crosses between demes of BPH associated with different varieties of rice, den Hollender and Pathak (1981) found that all F_1 and F_2 phenotypes for traits that determines host plant use were intermediate. This phenomenon on the insect's virulence reflects the high degree of variation that exists in BPH and in general among homopteran pests and the ability for the insect to adopt rapidly to resistant plant trait that they are being exposed to (Roderick, 1994). Despite the importance of homopteran (BPH) as major pests of crops, very few studies deal with the genetic foundation for host plant adaptation and to explain whether the traits for host plant use are monogenic or polygenic (several genes each with smaller effects). Such information is important to determine how pest adaptation in homopterans (BPH in this case) proceeds and how best the plant genetic variation could be utilized for resistant management (Claridgte and den Holender, 1982).

Some allelochemicals present in the surface layer of plants as volatiles and lipids and the chemicals of internal tissues and of the phloem sap itself known to use by plant sap feeding homopterns (as BPH) as cues for host acceptance. *In vivo* and *in vitro* studies enabled quantify the influence of these factors on behavioural and biological effects on the insect (Claridge *et al*., 1991). For instance, the chemical composition of surface-lipids and phloem-sap of Ptb33 is known to influence feeding behaviour of BPH (Padgham *et al.,* 1989; Nugaliyadde, 1994). This lead to the understanding that several genes are involved in expressing BPH resistance in Ptb33, which could be used to develop varieties with different resistant traits in them as exemplified by two improved varieties like Bg 300 and Bg 379-2 (Nugaliyadde *et al*., 2000).

Contrarily, many doubts have arisen about the possibilities of developing high yielding crop cultivars with higher level of resistance to insect pests. This assumption is based on the fact that the energy or other resources that the plants divert for resistance would not be available for the growth and reproduction of the plant. For instance, van Emden (1991) found a strong negative correlation between the yield of pigeon pea cultivars and their degree of resistance to pod damaging insects. Based on this and similar other observations he concluded that 'partial host-plant resistance was more important than high level of resistance to insects'. For instance, it would be possible to differentiate different factors responsible for BPH resistance in Ptb33 and to incorporate them separately into improved variety gene bases.

ACKNOWLEDGEMENTS

Mrs. Malkanthi Samarakoon, Ms. Pushpa Kumari and the laboratory staff of Rice Research and Development Institute, Batalagoda are acknowledged for their assistance.

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