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Genetics of brown plant hopper (*Nilaparvata lugens* Stal.) resistance in elite donors of rice (*Oryza sativa* L.)

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Abstract
The inheritance and allelic relationships of genes governing resistance to the brown plant hopper (BPH) was studied in six resistant donors of rice viz, R-RF-55, R1600-1124-2-618-1, R1546-1328-1-90-1, R1677-3473-1-4301-1, R1470-347-136-1-1 and R1670-3267-1-3920-1, in greenhouse condition. The present investigation was conducted at Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur during 2009-2011. Based on the results obtained in F_1 through F_3 generations, BPH resistance was governed by single dominant gene in R-RF-55 and single recessive gene in R1546-1328-1-90-1. Two independent dominant genes for resistance were observed in R1600-1124-2-618-1. Allelic test revealed that dominant gene of R1470-347-136-1-1 and R1670-3267-1-3920-1 while recessive gene of R1677-3473-1-430-1 were non-allelic to the genes *bph5* (ARC10550) and *bph7* (T12). Also, dominant gene of R1470-347-136-1-1 and R1677-3473-1-4301-1 was found non allelic to *Bph1* (MTU15) and *Bph6* (Swarnalata), respectively. The identification of independent genes would be helpful in pyramiding resistant genes to stabilize resistance.

Keywords: rice, inheritance of resistance, dominant gene, recessive gene, BPH, nilaparvata

Introduction
Rice (*Oryza sativa* L.), one of the agronomically and nutritionally important cereal crop in the grass family (Poaceae), is the principal staple food in developing countries. It is no longer a luxury food, but has become the cereal that constitutes a major source of calories for the urban and the rural (Sasaki and Burr, 2000) [20]. Insect pests are the major biotic constraints in crop production. The brown plant hopper (BPH) "*Nilaparvata lugens* Stal." is one of the most destructive and wide spread insect pests that can be found throughout the rice growing areas in Asia, causing significant yield losses in cultivars every year (Khush, 1979; Sogawa *et al.*, 2003) [22, 21]. The brown plant hopper is a small (2.0-3.5 mm in body length) brownish sucking insect, belonging to order Hemiptera, suborder Homoptera, super family Fulgoroidea and family Delphacidae. The estimated losses in grain yield due to this insect range from 10 per cent in moderately affected to 70 per cent in severely affected fields. The damage to the standing crop sometimes reached up to 100 per cent reveals about significance of this pest. Using its stylet, this insect ingests assimilates specifically from the phloem of rice plants and causes whole plants to yellow and rapidly dry, which is referred to as hopper burn (Otake 1978) [23]. It also transmits several viral diseases while sucking assimilates from the phloem, which causes additional crop damage (Sogawa 1973; Ling *et al*. 1978) [25, 24].

A general trend of increase in the insect infestation has been observed in the past few years. The increased infestation has been attributed to the reduced genetic variability of short-statured and high tillering varieties of rice, heavy use of nitrogenous fertilizers, the practice of continuous cropping and staggered planting. Attempts to control insect pest with chemical methods have given rise to many problems *viz.*, resurgence, insecticidal resistance, destruction of natural enemies, development of new biotype, etc. Application of insecticides towards the maturity stage of crop growth causes presence of their residues in rice bran and straw above the tolerance level (Rajukkannu *et al.*, 1988) [29]. Therefore, considering the figures of economic losses, pesticides hazards and environmental pollution; development and use of resistant variety is not only cheapest but it is safest method to control this pest. The genetics of BPH resistance is well studied and as many as 21 major genes have been identified in cultivated and wild species (Zhang, 2007 and Fujita *et al*., 2008) [26, 27].

Four BPH biotypes are known. Biotypes 1 and 2 are widely distributed in Southeast Asia, biotype 3 is a laboratory biotype produced in the Philippines, and biotype 4 occurs in the Indian subcontinent (Khush and Brar 1991) [28]. With a view to widening the genetic base so as to enable the reliable use of BPH resistance, the identification of a larger number of cultivars with BPH resistance along with which of their genotypes is desirable. New donors need to be identified and studied for inheritance of resistance and allelic relationships of genes they carry for resistance in order to provide alternate source of resistance, whenever there is a change in biotypes. Therefore, the present investigation was undertaken with the objective of understanding inheritance of brown plant hopper resistance and allelic relationships of gene (s) governing resistance to brown plant hopper in some newly identified donors.

Materials and Methods

Six resistant donors viz., R1600-1124-2-618-1, R1546-1328-1-90-1, R-RF-55, R1677-3473-1-4301-1, R1670-3267-1-3920-1 and R1470-347-136-1-1 were studied to know the nature of brown plant hopper (BPH) resistant gene (s) they possess, and to identify the genes responsible for confirming resistance. The experiment was carried out in the Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. Generation and maintenance of rice brown plant hopper breeding materials was done at the research farm while screening of parents, F_1's, F_2's and F_3's against brown plant hopper was made at glass house of Department of Entomology, College of Agriculture, IGKV, Raipur. The known resistant parents used in the study were ARC10550, T12, MTU15, H105 and Swarnalata, whereas the susceptible parents were Samleshwari and Mahamaya. Both susceptible parents Samleshwari and Mahamaya recorded cent per cent damage by brown plant hopper, whereas no damage was observed in the resistant donors (Table 1). Crosses were attempted between resistant donors with known resistant parents and resistant donors with susceptible parents during Kharif 2009. The F_2 and F_3 progenies were obtained by advancing the generation from half of the F_1 seeds of the crosses and tested for their reaction against BPH under greenhouse condition as per methodology suggested by Kalode *et al*. (1979) [4] in consequent summer and Kharif seasons.

The test and check varieties were pre-germinated in petridishes and these germinated seeds were transferred to wooden boxes of size 60 x 40 x 10 cm, containing well mixed homogeneous soil. Each seed box contained twenty-four test lines with 20 seedlings of each including two middle rows of resistant check (Ptb33) and susceptible check (TN1) and four border rows of susceptible check (TN1). The F_1 was screened by planting single row of each cross combination. The F_2 populations were screened by planting all twenty-four rows of each cross combination and F_3 progenies of each line were screened by planting two rows of each line. Approximately 15 plants in F_1, a population of 500 progeny in F_2 and 50 plants of each F_3 line were screened. In F_2 each individual plant was planted separately in different single tray. When the seedlings were 8-10 days old, 1st or 2nd instar hopper nymphs were released in the screening trays so that each seedling has 6-8 nymphs. Observations were recorded 7-10 days after releasing insects, when 95% of the plants in the susceptible check line TN1 were killed. The entries were scored for damage following the criteria for scoring the damage of individual plants. The observations were recorded on the basis of 0-9 scale as per the International Standard Procedure (IRRI, 1996) [2]. In F_1 and F_2, plants were individually scored. The F_3 progenies were classified as breeding true for resistance (all plants in the line being resistant), segregating (both resistant and susceptible occurring) or breeding true for susceptibility (all plants in the line being susceptible).

The reaction of F_1 indicated the dominance or recessive nature of the resistance gene(s) involved the former when resistant and the latter when susceptible.

The Chi-Square (χ^2) test was employed to test the significance of deviation of an observed segregation ratio from a theoretical one for the purpose of working out the genetic ratios in F_2 and F_3.

Thus, Ho (null hypothesis): Observed frequency distribution fits satisfactorily to the theoretical given ratio. According to Panse and Sukhatme (2000) the value of χ^2 is given by:

$$\chi^2 = \sum_{i=1}^{k} \frac{(Oi - Ei)^2}{Ei}$$

With degree of freedom (df) = k-1

Where,

O = Observed frequency of the class,

E = Expected frequency of the respective class,

$\sum$ = Summation of all classes.

When the χ^2 value significant the null hypothesis is rejected. Non-significant χ^2 values justified the agreement between the observed and expected hence; the null hypothesis is accepted, as true.

The crosses of resistant/susceptible parents were studied for segregation in F_2 and F_3. The crosses of resistant/resistant parents were studied for segregation in F_2. Non-segregating population revealed the allelic relationship between the genes, whereas, those segregating indicate that the genes are non-allelic nature.

Results

Inheritance of Resistance

The resistant donors namely R-RF-55, R1546-1328-1-90-1, and R1600-1124-2-618-1 were studied for inheritance of gene(s) for brown plant hopper resistance present in these donors. The donors namely R-RF-55 and R1546-1328-1-90-1 were crossed with susceptible parents Samleshwari and Mahamaya. The resistant donor R1600-1124-2-618-1 was crossed with susceptible parent Samleshwari. Reaction of F_1, F_2 and F_3 population of above generated crosses are presented in Table 2. The F_1 populations of the crosses of R-RF-55 with Samleshwari and Mahamaya and 1600-1124-2-618-1 with Samleshwari showed resistant reaction against the brown plant hopper population and showing inheritance of a single dominant gene for resistance in these donors. Whereas, the F_1 plants of crosses R1546-1328-1-90-1 with Samleshwari and Mahamaya showed susceptible reaction and showing the inheritance of recessive gene pattern in resistant donor R1546-1328-1-90-1. The reaction of BPH evaluated for segregation in F_2 population of the crosses R-RF-55 with their susceptible parents was observed in a frequency of three resistant: one susceptible (3R: 1S) and showing the presence of single dominant gene in the resistant parent. Further, the F_3 progenies of these crosses for each resistant parent were also analyzed for segregation pattern. The F_3 families from these crosses segregated in the ratio of 1 resistant: 2 segregating: 1

susceptible expected for monogenic control of resistance. Thus, resistance in R-RF-55 is conferred by a single dominant gene. The segregation behavior of F_2 population of the cross of R1600-1124-2-618-1 with Samleshwari fit well in ratio of fifteen resistant: one susceptible (15R: 1S) signifying the possibility of two independent dominant genes controlling resistance, further, the F_3 progenies of above cross was evaluated and classified into the ratio of 7 resistant: 8 segregating: 1 susceptible (7R: 8Sg: 1S) conferring the existence of two independent dominant genes in resistant parent R1600-1124-2-618-1. The F_2 populations of the crosses R1546-1328-1-90-1 with susceptible parents segregated as 1 resistant: 3 susceptible (1R: 3S). This indicated the presence of single recessive gene for resistance in donor R1546-1328-1-90-1. A segregation pattern of 1 resistant: 2 segregating: 1 susceptible was observed for these crosses in F_3 families. This confirmed the inheritance of a single recessive gene present in this donor.

Allele Tests

Allelic tests help us to determine whether the gene for resistance presents in two or more different donors are same or different. Identification of non-allelic source of resistance against any insect pest is necessary for increasing the durability of resistance. Allelic relationship studies of gene for brown plant hopper resistance present in unknown resistant parents with known resistant donors are presented in Table 3. To work out the allelic relationship regarding BPH resistance genes, the unknown resistant parents R1677-3473-1-4301-1, and R1470-347-136-1-1 were crossed with resistant donors viz., ARC10550 (*bph5*) and T12 (*bph7*) having known genes for resistance for BPH. Also, the resistant parent R1677-3473-1-4301-1 was crossed with H105 (*bph2*). Data recorded on BPH reaction revealed that, the F_1 of these crosses showed resistant reaction against brown plant hopper. The F_2 populations of these crosses showed a ratio of 13 resistant: 3 susceptible segregation behavior. The resistant lines R1677-3473-1-4301-1 crossed with Swarnalata (Bph6). The unknown resistant lines R1470-347-136-1-1 crossed with MTU15 (*Bph1*). Data of the F_2 reactions of these crosses were classified in 15 resistant: 1 susceptible segregation ratio. Such ratios are obtained when two independently dominant genes are involved for resistance. The unknown resistant parent R1670-3267-1-3920-1 was also tested for their allelic relationship with known resistant donors ARC10550 (*bph5*) and T12 (*bph7*). The F_1 population of these crosses showed susceptible reaction and in F_2 generation data also revealed the segregation ratio of 7 resistant: 9 susceptible progenies for BPH resistance and indicating that two independent recessive genes control the resistant reaction to brown plant hopper.

Table 1: Reaction of parental genotypes to brown plant hopper under greenhouse conditions

S. No.	Parents	Parentage	Plant damage score during Kharif 2009	Reaction to BPH
1	ARC10550	N/A	2.73	R
2	T12	N/A	2.37	R
3	MTU15	N/A	2	R
4	Swarnalata	N/A	0	HR
5	H105	N/A	2.57	R
6	R1600-1124-2-618-1	MTU1010 / Triguna	0.81	R
7	R1546-1328-1-90-1	R574-11 / BR240-47	1.17	R
8	R-RF-55	Dagada Deshi / R1102-27-93-3-1	3.00	R
9	R1677-3473-1-4301-1	R1037-469-1-1 / Danteshwari	1.71	R
10	R1670-3267-1-3920-1	R1027-2282-2-1 / Poornima	1.70	R
11	R1470-347-136-1-1	K64 / R302-111	1.60	R
12	Samleshwari	R310-37 / R308-6	9	S
13	Mahamaya	Asha / Kranti	9	S

a: HR – Highly Resistant; R - Resistant; S - Susceptible; N/A - Not available

Table 2: Inheritance pattern of F_1, F_2 and F_3 populations of crosses resistant parents with susceptible parents in rice for BPH resistance

S. No.		Reaction of F_1 plants	Reaction of F_2 plants						Reaction of F_3 progenies						
			No. of plants			Expected ratio	χ^2 value	Table value	No. of progenies				Expected ratio	χ^2 value	Table value
			R	S	Total	R : S			R	Sg	S	Total	R:Sg:S		
1.	R-RF-55x Samleshwari	R	398	147	545	3:1	1.068	3.841*-6.635**	29	58	22	109	1:2:1	1.347	5.991*-9.210**
2.	R-RF-55 x Mahamaya	R	447	153	600	3:1	0.080	3.841*-6.635**	33	67	26	126	1:2:1	1.284	5.991*-9.210**
3.	R1600-1124-2-618-1 x Samleshwari	R	537	28	565	15:1	1.614	3.841*-6.635**	61	78	8	147	7:8:1	0.592	5.991*-9.210**
4.	R1546-1328-1-90-1 x Samleshwari	S	159	432	591	1: 3	1.142	3.841*-6.635**	35	80	37	152	1:2:1	0.473	5.991*-9.210**
5.	R1546-1328-1-90-1 x Mahamaya	S	143	463	606	1: 3	0.636	3.841*-6.635**	32	80	31	143	1:2:1	2.031	5.991*-9.210**

Note: R - Resistance, S - Susceptible, Sg - Segregating

** 1% level of significance *5% level of significance

Table 3: Segregation pattern of F_1 and F_2 populations using resistant donors for allelic studies in rice for BPH resistance

S. No.	Cross combination	reaction of F_1 plants	Reaction of F_2 plants					
			No. of plants			Expected ratio	χ^2 value	Table value
			R	S	Total	R : S		
1.	R1677-3473-1-4301-1 x ARC10550	R	492	99	591	13:3	1.551	3.841*-6.635**
2.	R1677-3473-1-4301-1 x T12	R	498	103	601	13:3	1.011	3.841*-6.635**
3.	R1677-3473-1-4301-1 x Swarnalata	R	456	36	492	15:1	0.954	3.841*-6.635**
4.	R1677-3473-1-4301-1 x H105	R	492	98	586	13:3	1.668	3.841*-6.635**
5.	R1470-347-136-1-1 x ARC10550	R	461	87	552	13:3	1.857	3.841*-6.635**
6.	R1470-347-136-1-1 x T12	R	395	79	474	13:3	1.336	3.841*-6.635**
7.	R1470-347-136-1-1 x MTU15	R	469	32	501	15:1	0.016	3.841*-6.635**

8.	R1670-3267-1-3920-1 x ARC10550	S	214	308	522	7:9	1.604	3.841*-6.635**
9.	R1670-3267-1-3920-1 x T12	S	199	285	484	7:9	1.367	3.841*-6.635**

** 1% level of significance *5% level of significance

Discussion

Two genes for resistance to brown plant hopper were identified earlier by Athwal *et al.* 1971 [1]. One is dominant and was designated *Bph 1*. This gene is present in Mudgo, MTU 15, MGL 2, and CO 22. The other gene is recessive and was designated bph 2. It is found in ASD 7, H 105, and Ptb 18. Kabir and Khush, (1988) identified the dominant gene *Bph6* in Swarnalata and recessive gene *bph5* in ARC10550 and *bph7* in T12 governing resistance to brown plant hopper. The resistant donor R-RF-55 possess only single dominant gene for resistance which is indicated by 3 resistant: 1 susceptible (3: 1) segregation ratio observed in F_2 generation. This is also supported by F_1 showing resistance and classification of F_3 progenies in the ratio of 1 resistant: 2 segregating: 1 susceptible. In many of the earlier studies Krishna *et al*., 1977 [7]; Velusamy and Chelliah, 1985 [18]; Tomar and Prasad 1996 [17]; Rana *et al.,* 2009 [13] has also been reported brown plant hopper resistance to be governed by one dominant gene.

The F_1 progenies from the crosses of R1546-1328-1-90-1 with susceptible parent Mahamaya and Samleshwar showed susceptible reaction. The F_2 populations from these crosses segregated in the ratio of 1 resistant to 3 susceptible, thereby indicating that resistance in these cultivars is conferred by single recessive genes. The F_3 families from the crosses segregated in the ratio of 1 resistant: 2 segregating: 1 susceptible, thus confirming the conclusion about the monogenic recessive control of resistance in these cultivars. The monogenic recessive nature of resistance against brown plant hopper was also reported earlier by Sidhu and Khush, 1978 [16]; Rao *et al.,* 1980 [14]; Khush *et al.,* 1986 [5]; Li *et al.,* 2001 [8]; Rao *et al.,* 2005 [15] and Rana *et al.,* 2009 [13] in rice. The resistant donor R1600-1124-2-618-1 was crossed with Samleshwari and possessed resistant reaction in F_1 generation. It has given a 15 resistant: 1 susceptible (15: 1) and 7 resistant: 8 segregating: 1 susceptible (7: 8: 1) segregation ratio in F_2 and F_3 families, respectively. These ratios are attained when two dominant genes segregate simultaneously. Thus, it can be concluded that the resistant parent R1600-1124-2-618-1 has two dominant genes for resistance. Resistance to brown plant hopper has been noted by Kim, 1985 and Padmavathi *et al.,* 2005 [10] to be governed by two dominant genes.

The F_2 segregation of crosses R1470-347-136-1-1 and R1677-3473-1-4301-1 with ARC10550 (*bph5*) and T12 (*bph7*) was obtained in a ratio 13 resistant: 3 susceptible. The cross between R1677-3473-1-4301-1 and H105 was also segregated in ratio of 13 resistant: 3 susceptible plants. This indicated that resistance is governed by two genes i.e. one dominant gene present in R1470-347-136-1-1, and R1677-3473-1-4301-1 and a recessive gene present in ARC10550 (*bph5*), T12 (*bph7*) and H105 (*bph2*). Thus, the gene for brown plant hopper resistance indentified in R1677-3473-1-4301-1 and R1470-347-136-1-1 were different from those found in ARC10550 (*bph5*) and T12 (*bph7*). Also, the brown plant hopper resistant gene identified in R1677-3473-4301-1 was non allelic to H105 (*bph2*). Angeles *et al.,* (1986) [5] in Ptb21 and Ptb33; Murty *et al*., 1988 [9] in Velluthacheera and Ptb21; Tomar and Prasad (1996) [17] in Babawee and CR266-407-6-1 also reported the similar results. The F_2 populations of the cross between R1470-347-136-1-1 with MTU15 (*Bph1*) and the cross of R1677-3473-1-4301-1 with Swarnalata (*Bph6*) segregated in a ratio of 15 resistant: 1 susceptible demonstrating that two independently dominant gene were involved in these crosses. The gene present in R1470-347-136-1-1 was inherited independently and non allelic to the gene present in MTU15 (*Bph1*). Also, the gene present in R1677-3473-1-4301-was different from the gene present in Swarnalata (*Bph6*). Pophaly *et al.,* (2001) [12] in crosses of OR1334-8 and TTB148-174-3-2-1 also reported the duplicate dominant gene governing resistance to brown plant hopper.

The F_2 population from the crosses of R1670-3267-1-3920-1 with ARC10550 and T12 segregated in the ratio of 7 resistant: 9 susceptible indicating that, two independent recessive genes control the resistant reaction to brown plant hopper. The results thus indicated that the one recessive gene responsible for resistance in R1670-3267-1-3920-1 and one recessive gene in ARC 10550(*bph5*) and T12 (*bph7*) are non allelic to each other and therefore are independent source of resistance. Pophaly *et al.,* (2001) [12] in the cross combinations of Lal Basant/Budhiya Banko, Lal Basant/Basangi; Verma *et al*., (2001) [19] in crosses of Barhi /Budhiya Banko, Barhi /Lal Basant, and Barhi /Basangi; Rao *et al.,* (2005) [15] in crosses of Bakiya/ Pandri Ajan, Chopdo/ Dhori, Chopdo/Pandri Ajan also reported with similar results. In the present study, the inheritance in all cases has been found to be simple. The incorporation and selection of single recessive or dominant genes are easier in breeding population. In depth understanding of the inheritance of the resistance gene greatly enhances the breeder's ability to plan an appropriate breeding strategy to exploit/transfer the target gene(s). Since, the resistance genes in the parents studied is inherited independently; they are expected to be transferred quite easily. In the present age of biotechnology, the identification of independent (non-allelic) genes would be helpful in pyramiding the resistant genes to further stabilize resistance.

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