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Antibiosis and tolerance mechanisms of resistance in rice varieties carrying brown planthopper resistance genes

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Abstract

Brown planthopper (BPH), *Nilaparvata lugens* (Stal.) is a major pest in rice production. Development of BPH-resistant varieties is an economical and effective way to control this pest. In the present investigation, 15 rice varieties were screened in greenhouse along with resistant and susceptible checks *viz.,* Ptb 33 and TN1 respectively. They were also studied for BPH honeydew excretion, nymphal development period as indicators of antibiosis and days to wilt to identify level of tolerance to BPH in rice varieties. Among the 15 rice varieties, one is highly resistant five were resistant and five were moderately resistant. The rice varieties, T 12 and Ptb 33 showed high level of antibiosis and tolerance to BPH. Swarnalatha, Babawee, Pokkali, IR 64, IR 65482-7-216-1-2-3 and MUTNS-1 showed moderate level antibiosis and tolerance. IR 36 exhibited moderate level of antibiosis and high level of tolerance. ASD 7 showed low level of antibiosis and moderate level of tolerance.

Key words : Rice, BPH (*Nilaparvata lugens*), antibiosis, tolerance

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) is a typical piercingsucking insect pest of rice (*Oryza sativa* L.; Poaceae), which feeds on phloem sap and thus affects the growth of rice and results in 'hopperburn' in rice fields [1]. In addition to direct damage, BPH also transmits viruses, such as the ragged stunt virus and grassy stunt virus, and associated diseases to rice plants [2]. In recent years major out breaks of BPH were recorded in several rice growing countries like China, Korea, Japan, India, Indonesia, Malaysia, Philippines, Thailand and Vietnam and have caused heavy rice yield losses [3]. To control this pest, the applications of chemical insecticides have not been a satisfactory tactic in practical rice production, because insecticides can cause BPH resurgence and may play a major role in inducing outbreaks [4]. Alternatively, growing of resistant variety is an economical and efficient way for control of BPH pest. Further, since the release of IR 26 in 1973, several resistant varieties to BPH have been developed and released in India; they became susceptible to BPH within a few years after their introduction, because of breakdown of resistance or development of biotypes [5]. So, understanding the mechanisms and genetics of resistance is important before evolving resistant varieties. Host plant resistance to insects has been classified into three mechanisms *viz*., antixenosis, antibiosis and tolerance [6]. Antixenosis, antibiosis and tolerance have all been observed as mechanisms of resistance against *N. lugens* in various rice cultivars. The resistance mechanisms are believed to be associated with minor genes. However, the resistance types of most BPH-resistance genes identified remains largely unknown. Therefore, it is necessary to identify the levels of antibiosis and tolerance in rice varieties carrying BPH-resistance genes, which would favor resistance breeding in rice. Hence, the present investigation was conducted to identify the levels of antibiosis and tolerance in rice varieties carrying various BPH-resistance genes.

MATERIALS AND METHODS

The present investigations were carried out in the greenhouse at Andhra Pradesh Rice Research Institute and Regional Agricultural Research Institute, Maruteru, West Godavari district, Andhra Pradesh, India during the year 2013.

Plant material

The fifteen rice varieties carrying varying BPH resistant genes *viz*., ASD 7 (Bph2), Rathu Heenati (Bph3+Bph17), Babawee (Bph4), Milyang 63, Swarnalatha (Bph6), T12 (Bph7), Chins Saba (Bph8), Pokkali (Bph9), IR64 (Bph1), IR 65482-7-216-1-2B (Bph18), IR36 (Bph2), MUTNS-1, OM 4498, RP 2068-18-3-5, IR 7033-121-15 (Bph 20/21) were used in these studies. They were screened against BPH in the greenhouse through standard seed box screening test and studied for their levels of antibiosis and tolerance. The rice varieties Ptb 33 (Bph2+Bph3) and TN1 (no resistant gene) were taken as resistant and susceptible checks respectively.

Mass culturing of BPH

To obtain different instar nymphs and adults of BPH required for various studies, the insect was mass reared in the greenhouse and iron framed rearing cages covered with fine mesh wire net. Four to six weeks old potted plants of susceptible rice variety, Taichung Native1 (TN1) were used for culturing the BPH. To start the culture of BPH, the potted TN1 plants were cleaned and the dried outer leaf sheaths were removed. They were then placed in an oviposition cage. Gravid females of BPH obtained from the maintenance cage were released on to the potted TN1 plants for oviposition and exposed for 3 days. Then the oviposited plants were placed in maintenance cage for hatching of the eggs. The host plants in culture maintenance cage were changed twice a week and replaced them with fresh potted plants [7].

Standard Seed box screening test (SSST)

The pre germinated seeds of the rice varieties were sown 3 cm apart in a seed box filled with mud soil. Each test variety was sown in three replications in a row across the width of the seed box with at least 20 plants per row. At seven days after sowing, the seedlings were infested with second and third instar nymphs of BPH at the rate of eight to ten nymphs per seedling. After

Table 1. Standard evaluation system for rating damage by brown planthopper

Grade	Criteria	Reaction
0	No damage	Immune (I)
1	Slight yellowing of few plants	Highly resistant (HR)
3	First and 2nd leaves of most of plant partially yellowing	Resistant (R)
5	Pronounced yellowing and stunting or about 10 to 25 % of the plants wilting	Moderately resistant (MR)
7	More than half of the plants wilting or dead and remaining plants severely stunted or drying	Moderately susceptible (MS)
9	All plants dead	Highly Susceptible (HS)

infestation each seed box was covered with a wire mesh cage to prevent any escape and to prevent entry of natural enemies. The test varieties were observed daily for the damage by the BPH. Damage rating of the test varieties was observed when 90 per cent of the seedlings in TN1 were killed. The test varieties were graded using standard evaluation system for rice on 0-9 (SES) scale as given in Table 1 [8].

Feeding rate

The preference of BPH for each selected rice varieties was assessed by estimating the amount of honeydew excreted by the adult hoppers as an indication of the feeding preference. What man No.1 filter paper was dipped in a 0.02% bromocresol green solution in ethanol and allowed to dry for one hour and dipped again till the filter paper turned yellowish orange. The treated paper was then placed on the wooden plank kept at the base of 30-days old plants. A plastic cup was placed over the filter paper and five freshly emerged female hoppers, pre-starved for four hours and were released into the feeding chamber having bromocresol green treated filter paper [9]. The BPH adults were allowed to feed for 24 hours at the base of the stem. The honeydew droplets excreted by the adults when came in contact with the filter paper turned into blue spots. The area of blue spots appeared on filter paper as a result of honeydew excretion was measured by graph method. The antibiosis effect on feeding among the rice varieties were determined by comparing the average area of honeydew excreted in mm^2.

Nymphal development period

Nymphal development period on selected rice varieties along with resistant and susceptible checks was studied by releasing five first instar BPH nymphs on 30-days old Mylar film caged plants. From 9th day onwards, nymphs on each rice variety were observed daily for ecdysis and recorded the number of days taken for the nymphs to reach adult stage [10].

Days to wilt

Tolerance to BPH was estimated by counting the number of days required to kill the plants after release of 25 hoppers per plant on 30 days aged plants [11]. The first instar nymphs were released on the plants and allowed to feed. The day on which the plant wilted completely was recorded.

The data was subjected to ANOVA in simple RBD analysis [12] after transforming the data into square root transformations.

RESULTS

BPH resistant scores of rice varieties

The fifteen rice varieties were scored as 1.73 to 8.53 in the standard seed box screening test, varying highly resistant to highly susceptible. Among the rice varieties, MUTNS-1 (8.53), Chin Saba (7.60) and susceptible check, TN1 (8.33) showed high susceptibility to BPH recorded more than 7.0 score. The rice varieties *viz.,* Rathu Heenati (5.37), Milyang 63 (5.30) showed moderate susceptibility to BPH with more than 5.0 score. The rice variety, T 12 with a score of 1.7 considered as highly resistant to BPH. The rice varieties *viz.,* RP 2068-18-3-5 (2.33), ASD 7 (2.53), Swarnalatha (2.60) and IR 7033-121-15 (2.80) along with resistant check, Ptb 33 (2.47) displayed resistant reaction to BPH with a score between 2.0 to 3.0. The other rice varieties like IR 36 (3.33), Pokkali (3.33), OM 4498 (4.13), IR 64 (4.43) and IR 65482-7-216-1-2-B (5.0) were found to be moderately resistant to BPH. This result revealed that rice varieties carrying different resistant genes vary in resistant level to BPH in Andhra Pradesh, India.

Honeydew excretion on rice varieties

Honeydew excretion measured by colour area ranged from 79.00 mm^2 on resistant check, Ptb 33 to 1461.0 mm^2 on susceptible check, TN1. Among the rice varieties significantly

Table 2. Resistant scores of rice genotypes carrying different resistant genes against brown planthopper in Seed box screening test

S.No.	Designation	Resistant gene	Damage Score*	Reaction
1	ASD7	Bph2	2.53	R
2	Rathu Heenati	Bph3+Bph17	5.37	MS
3	Babawee	Bph4	2.87	R
4	Milyang 63	-	5.30	MS
5	Swarnalatha	Bph6	2.60	R
6	T12	Bph7	1.73	HR
7	Chin Saba	Bph8	7.60	HS
8	Pokkali	Bph9	3.33	MR
9	IR 64	Bph1+	4.43	MR
10	IR65482-7-216-1-2-B	Bph18	5.00	MR
11	IR 36	Bph2	3.33	MR
12	MUTNS-1	-	8.53	HS
13	OM4498	-	4.13	MR
14	RP 2068-18-3-5	-	2.33	R
15	IR 7033-121-15	Bph20/21	2.80	R
16	Ptb 33	Bph2+Bph3+	2.47	R
17	TN1	-	8.33	HS
	F test		Sig	
	CD (0.05)		1.4	
	CV (%)		41.9	

*Mean of 10 individual plants and three replications; **= Mean of three replications

lowest honeydew excretion area was measured for BPH adults feeding on Ptb 33 (79.0 mm^2), RP 2068-18-3-5 (307.0 mm^2) and highest was on ASD 7 (1235.0 mm^2), Milyang 63 (1113.0 mm^2) and Pokkali (1069.0 mm^2). On the other rice varieties BPH adults recorded moderate honeydew excreted area. Apparently the honeydew excreted area by BPH different among rice varieties with different resistant genes.

Nymphal development period

Nymphal development period was significantly varied on resistant, moderately resistant and susceptible varieties. It was ranged between 10-14 days. Among the rice varieties significantly prolonged development period (days) of BPH nymphs was observed in Ptb 33 (14), IR 36 (13.33) and ASD 7 (13). The other rice varieties on which BPH recorded

Table 3. RHoneydew excretion, nymphal development period of BPH on rice genotypes and days to wilt of rice genotypes infested with BPH

S.No.	Designation	Honeydew excretion (mm^2)**	Nymphal development period (days)**	Days to wilt**
1	ASD7	1235 (34.93)	13.00	20.67
2	Rathu Heenati	350 (18.21)	12.67	23.67
3	Babawee	665 (25.79)	12.67	18.33
4	Milyang 63	1113 (32.98)	12.00	22.33
5	Swarnalatha	955 (30.88)	10.67	15.33
6	T12	389 (19.53)	12.00	24.00
7	Chin Saba	722 (26.82)	12.00	8.67
8	Pokkali	1069 (32.68)	12.00	16.67
9	IR 64	893 (29.71)	11.67	20.33
10	IR65482-7-216-1-2-B	1040 (31.83)	12.00	20.33
11	IR 36	937 (30.60)	13.33	23.33
12	MUTNS-1	600 (24.29)	11.67	21.00
13	OM4498	475 (21.60)	10.33	24.00
14	RP 2068-18-3-5	307 (17.29)	10.00	24.00
15	Ptb 33	79 (8.83)	14.00	24.00
16	IR 7033-121-15	561 (23.48)	11.67	23.00
17	TN1	1461 (38.21)	10.00	12.00
	F test	Sig	Sig	Sig
	CD (0.05)	5.7	1.1	3.2
	CV (%)	35.3	15.3	23.8

**= Mean of three replications; Figures in parenthesis are square root transformed values

significantly prolonged nymphal development period than susceptible check, TN1 (10) were Rathu Heenati and Babawee (12.67 days); Milyang 63, T 12, Chin Saba, Pokkali, IR 65482-7-216-1-2-B (12 days); IR 64, MUTNS-1 and IR 7033-121-15 (11.67 days). The rice varieties on which nymphal development period significantly lowest were Swarnalatha (10.67 days), OM 4498 (10.33 days) and RP 2068-18-3-5 (10 days).

Days to wilt

The number of days taken to wilt at the population of 25 numbers of BPH nymphs per plant was significantly high in highly resistant, resistant and moderately resistant rice varieties than susceptible check, TN1. Among the rice varieties, the highly resistant (T 12) and resistant (OM 4498, RP 2068-18-3-5, IR 7033-121-15) and resistant check, Ptb 33 took 24 days to

complete wilt. The other rice varieties except Chin Saba (8.67 days) took 15.33 to 23.67 days; significantly higher than the TN1 (12 days).

DISCUSSION

In the present investigation the rice varieties *viz*., Pokkali was moderately resistance; Swarnalatha was resistance; T12 was highly resistance; Mudgo was moderately resistant, ASD 7 was resistant. These findings were partially in agreement with the findings of Qiu *et al*. [13] who reported Pokkali and Swarnalatha as highly resistant; T 12 as moderately resistant; Mudgo as moderately susceptible and ASD 7 as highly susceptible. This might be due to difference in BPH biotypes and their virulence levels to rice varieties carrying different resistance genes. For example, rice varieties carrying the gene bph1 displayed resistance to BPH biotype 1, but they were highly susceptible to BPH biotype 2 and lacked antibiosis or tolerance [14].

The quantity of excretion of honeydew by brown planthopper, in general, is directly related to intake of plant sap. Therefore, the amount of honeydew excreted by the insect in unit time when fed on different rice varieties is considered as an index for its feeding preference. Similarly the duration of the nymphal development period is another criterion to assess antibiosis mechanism of resistance. The expression of these mechanisms of resistance is controlled by the associated genes. In the present study, the resistant check Ptb 33 with Bph2 and Bph 3 resistant genes recorded higher area of honeydew excretion, prolonged development period of nymphs and longer days to wilt. The other two rice varieties with Bph2 gene *viz*., ASD 7 and IR 36 also recorded prolonged nymphal development period indicated that this gene may confers antibiosis that prolonged the development period. In addition to Ptb 33, the other variety, Rathu Heenathi though it showed moderate susceptibility in the present study recorded lower honeydew excreted area indicated that the resistant gene Bph 3 may confers antibiosis that reduces feeding. The little sap intake or lower honeydew excreted area might be due to occurrence of certain undesirable gustatory factors that block the sustained sucking by the insect.

It should be noted that this study examined only the levels of antibiosis and tolerance in rice varieties carrying major BPH-resistance genes. We did not identify whether resistance mechanisms (antibiosis, tolerance) in the various varieties were only conferred by these major resistance genes. The rice varieties ASD 7 and Swarnalatha have shown BPH resistant score but recorded higher honeydew excreted area than that of some moderately resistant rice varieties. Similar studies were conducted earlier by several workers. Cohen *et al*. [15] found that rice variety IR64 with resistant gene Bph1 showed antixenosis, antibiosis and tolerance to the BPH. Recent studies conducted by Qiu *et al*. [13, 16] also indicated that several BPH-resistant varieties *viz.,* Mudgo, Pokkali, Swarnalata and B 5, exhibited antibiosis and tolerance or antixenosis to the tested BPH populations.

CONCLUSION

In the present investigation, the honeydew excretion area and nymphal development period in days were applied as indicators to detect the level of antibiosis to BPH and days to wilt could be used to detect tolerance levels in rice varieties. The rice varieties with different BPH-resistant genes exhibited different levels of antibiosis and tolerance. However, to test whether the antibiosis and antixenosis were conferred by the resistance genes, it should better construct the near-isogonics lines of the resistance gene or clone the resistance genes.

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