Reaction of Resistant and Susceptible Rice Genotypes Against Brown Planthopper (*Nilaparvata lugens*)

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The study was carried out to assess the reaction of different rice genotypes in response to brown planthopper (BPH) *Nilaparvata lugens* (Stål.) attack and the possible use of these genotypes in BPH management. The resistant genotypes PTB 33, ADT 45 and ASD 7 and the moderately resistant genotypes CO 43 and KAU 1661 recorded the lowest nymphal preference, fecundity, feeding rate, survival, growth index, population buildup and plant dry weight loss per mg of insect dry weight produced, and more unhatched eggs, longer nymphal development period, days to wilt and higher Functional Plant Loss Index compared with the susceptible genotype TN1. In resistant and moderately resistant genotypes, a greater accumulation of defense enzymes such as peroxidase and polyphenol oxidase in response to *N. lugens* infestation was recorded one day after infestation, and more pathogenesis-related protein and chitinase activity was noted 3 days after infestation. The activity was sustained for more than a week after infestation compared with the susceptible genotype TN1.
KEY WORDS: Defense enzymes; genotypes; *Nilaparvata lugens;* pathogenesis-related proteins; resistant; susceptible.

INTRODUCTION

The brown planthopper (BPH), *Nilaparvata lugens* (Stål.), is one of the most serious pests of rice. It damages the plants by sucking the sap, which leads to hopper burn and transmits grassy stunt, ragged stunt and wilted stunt virus diseases in rice, thereby causing severe yield loss (25). Large-scale cultivation of high-yielding cultivars is very conducive to *N. lugens* infestation. In addition, application of a high level of nitrogenous fertilizers, continuous cropping, staggered planting and non-judicious use of insecticides have been reported as causes of increased *N. lugens* outbreaks (6). In this context, host plant resistance, which is relatively cheap, ecofriendly and compatible with other methods of pest management, has recently become a major management strategy against brown planthopper (13).

To protect themselves from pathogen and herbivore attack, resistant plants employ defense responses and these can influence herbivore settling, feeding, oviposition, growth, development, fecundity and fertility (2). Defense responses also form the physical barriers that impede pathogen ingression or arthropod access to tissues, *i.e.*, cell walls, suberin, callose, cuticles and stored allelochemicals that will affect the insect feeding (10). In addition, accumulation of defense enzymes and pathogenesis-related proteins by insect feeding has been reported in many plant and pest interactions (28).

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In particular, Stout *et al.* (33) reported the resultant greater induction of polyphenol oxidase (PPO), peroxidase (PO), lipoxygenases and protease inhibitors in tomato plants against corn earworm (*(Helicoverpa (=Heliothis) zea)*, serpentine leafminer (*Liriomyza trifolii*) and tomato rust mite (*Aculops lycopersici*) feeding. Infestation by the silverleaf whitefly (*Bemisia argentifolii*) in tomato led to higher activity of chitinase, β-1,3-glucanase and peroxidase in tomato plants (21). However, the reaction of defense enzymes and proteins in resistant and susceptible genotypes to sucking pests was understood to only a limited extent. In this context, the accumulation of defense molecules in resistant and susceptible genotypes has been exploited to utilize the defense mechanisms in integrated pest management in rice. Rice is an important food crop in most Asian countries and its potential is hampered by key pests like *N. lugens.* Hence, the present study was undertaken and focused on the activity of defense molecules in resistant and susceptible genotypes and their effect on BPH infestation.

MATERIALS AND METHODS

Plant material The seeds of PTB 33, ASD 7, ADT 45, CO 43, KAU 1661 and TN 1 rice genotypes were sown in plastic pots (15 × 45 cm) in an experiment carried out at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India. The seedlings were watered regularly; 15-day-old seedlings were transplanted into 15-cm-diam plastic pots. The potted plants were covered with cylindrical Mylar^R cages (13 × 90 cm) at 21 days after transplanting. Culturing of BPH was done according to Heinrichs *et al.* (11). The plants were maintained without any BPH infestation. For assaying the activity of defense enzymes and protein in resistant and susceptible genotypes, 30-day-old plants were used for all the experiments. Five replications were maintained for each treatment and ten plants were used for each replication.

Nymphal settling preference on seedlings of the test genotypes was assessed using a conventional seed box test (11). Pre-germinated seeds of selected rice genotypes were sown 3 cm apart in a wooden box (60 × 40 × 10 cm) in a row of 20 seeds across the width of the box. At 7 days after sowing, *ca* 1000 2^{nd} instar BPH nymphs were released by gently tapping over the seedlings in such a way that approximately five to seven nymphs settled on each seedling in the conventional seed box covered with a Netlon cage. The number of nymphs settled on each seedling was counted at 1, 6, 12, 24, 48 and 72 h after infestation. The seedlings were disturbed after each count for reorientation of nymphs on seedlings.

Oviposition preference Number of eggs laid on the plant tissue was determined using the method described by Khan and Saxena (16). Three pairs of newly emerged adult male and female BPH were released on 30-day-old caged uninfested plants for oviposition. Five days after oviposition, the adults were removed. At 10–12 days after oviposition, nymphs started to emerge. The total number of nymphs emerged was counted and the nymphs were removed up to 15 days from the date of release of adults for oviposition; the data represented the number of viable eggs laid by the female during their lives. At the end of nymphal emergence, unhatched eggs were counted by dissecting out the plant tissues under a binocular microscope and from this total fecundity and percent unhatched eggs were recorded.

Feeding marks were used as a parameter to assess the feeding behavior of BPH, as described by Natio (23). Three newly emerged adults were confined on 30-day-old caged

uninfested plants of each test genotype. The feeding marks were stained 24 h after feeding with 0.1% Rhodamine dye for 15 min. The number of stained stylet probes made by BPH was counted for each genotype using a binocular microscope.

Feeding rate of BPH on each genotype was assessed based on honeydew excreted by the adult hoppers The feeding chamber developed earlier (31) was used for measuring the honeydew excretion. Five freshly emerged female hoppers pre-starved for 4 h were released into the chamber; after 48 h the filter papers were removed and sprayed with 0.01% ninhydrin – acetone solution. The honeydew stains appeared as violet or purple spots. The spots were delineated with tracing paper and honeydew excreted area was measured by placing the filter paper over a sheet of graph paper. The area of honeydew excretion was calculated and expressed as mm^2.

Nymphal survival and development period Ten 1^{st} instar BPH nymphs were released on 30-day-old caged seedlings of different genotypes; the number of nymphs that reached adulthood was counted and the percent nymphal survival was determined (11). Nymphal development period was studied on each genotype by releasing ten 1^{st} instar nymphs of BPH and observing the nymphs daily for ecdysis. The number of days to reach the adult stage was recorded (26).

Growth index of BPH on each genotype was computed by using the data obtained from the experiments on nymphal survival and development period following the formula:

$$Growth\ index = percent\ survival\ of\ nymphs / nymphal\ development\ period$$

Population buildup of BPH on different genotypes was studied by releasing 1^{st} instar nymphs of BPH on 30-day-old caged seedlings. The number of first generation nymphs emerged and days to wilt was recorded (11).

Assessing tolerance parameters To study the level of tolerance on 30-day-old seedlings, 50 1^{st} instar nymphs were introduced onto each plant; a control plant was maintained for each genotype. When the plants started to wilt, the hoppers were collected, oven-dried for 48 h and weighed. The infested and uninfested plants were removed from the pots along with their roots, washed thoroughly, air-dried for 3 h, then oven-dried at 70°C for 60 h and weighed. The functional plant loss index (FPLI) and plant dry weight loss per mg of insect dry weight produced were calculated for all the genotypes using the formulas of Panda and Heinrichs (24):

$$(i) FPLI = 1 - \left[\frac{Dry\ weight\ of\ infested\ plant}{Dry\ weight\ of\ uninfested\ plant} \right] \times 100$$

$$(ii) Plant\ dry\ weight\ loss\ per\ mg\ of\ N.\ lugens\ dry\ weight\ produced = \frac{Dry\ weight\ of\ uninfested\ plant - Dry\ weight\ of\ infested\ plant}{Dry\ weight\ of\ N.\ lugens\ progeny\ on\ infested\ plant}$$

Assay of defense enzymes and proteins The freshly emerged adults of BPH were released on 30-day-old rice plants. Samples were collected at 24-h intervals starting from the day of infestation (0 day) to 7 days after infestation (DAI). One gram of rice plant

sample was extracted with 1 ml of 0.1 M sodium phosphate buffer (pH 7) at 4°C. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C and the supernatants were used for the assays of PO and PPO.

Peroxidase (PO) activity was assayed spectrophotometrically. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28±2°C). The changes in absorbance were recorded at 420 nm at 30-s intervals for 3 min. The boiled enzyme preparation served as blank. The enzyme activity was expressed as changes in absorbance min^{-1} g^{-1} fresh tissue (9).

Polyphenol oxidase (PPO) activity was determined according to the procedure described by Mayer *et al.* (19). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 μl of the enzyme extract. To start the reaction, 200 μl of 0.01 M catechol was added, the changes in absorbance were read at 495 nm at 30-s intervals for 3 min, and the activity was expressed as changes in absorbance min^{-1} g^{-1} fresh tissue.

Assay of chitinase Rice leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged at 16,000 rpm for 15 min at 4°C and the supernatant was used in the enzyme assay. The colorimetric assay of chitinase was carried out according to the method described by Boller and Mauch (4). Colloidal chitin was prepared according to Berger and Reynolds (3) from crab shell chitin (Sigma, St. Louis, MO, USA). The reaction mixture consisted of 10 μl of 1 M sodium acetate buffer (pH 4.0), 0.4 ml of enzyme extract and 0.1 ml of colloidal chitin (10 mg). After 2 h incubation at 37°C, the reaction was stopped by centrifugation at 8000 rpm for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube containing 30 μl of 1 M potassium phosphate buffer (pH 7.1) and incubated with 20 μl desalted snail gut enzyme (Helicase, obtained from Sigma Aldrich, France). Finally, the mixture was incubated with 2 ml of dimethyl amino benzaldehyde for 20 min at 37°C and the absorbance was measured at 585 nM. The enzyme activity was expressed as μmol GlcNAc min^{-1} g^{-1} of fresh tissue.

Statistical analysis The data obtained from various experiments were statistically analyzed in a completely randomized block design and different parameters observed in the experiments were subjected to Duncan's Multiple Range Test (DMRT) (8) analysis using IRRISTAT version 92-a, developed by International Rice Research Institute Biometrics Unit, The Philippines.

RESULTS

Nymphal preference Among the genotypes tested for nymphal preference, resistant and moderately resistant genotypes such as ADT 45 (4.30), PTB 33 (5.58), CO 43 (12.20) (5.79) and ASD 7 (5.86) recorded the lowest nymphal preference compared with the susceptible genotype TN 1 (7.72) (Table 1).

Nymphal emergence The number of nymphs emerged was lower in resistant genotypes such as PTB 33 (93.60), ASD 7 (154.40) and KAU 1661 (158.20), than in susceptible genotype TN 1 (314.60) (Table 1).

TABLE 1. Reaction of resistant and susceptible rice genotypes in response to *Nilaparvata lugens* feeding (values are means of five replications)

Genotypes	NP	NNE	UNEGG	FECUN	FDMK	FRAT	SUR	NDP	GI	IGN	DWT	FPLI	PD
ASD 7	5.86 b	154.40 b	18.39 a	191.60 b	16.20 b	313.00 c	54.00 a	16.40 b	5.40 c	221.00 c	21.40 c	55.82 c	30.83 c
ADT 45	4.30 a	194.40 d	15.64 b	108.60 a	16.40 b	360.00 d	52.00 a	18.53 a	5.00 c	196.00 b	28.40 b	61.54 cd	34.22 d
KAU 1661	5.79 b	158.20 b	16.61 ab	208.00 c	20.40 a	216.50 b	64.00 b	16.00 b	3.40 ab	319.40 e	25.40 c	22.47 b	17.83 b
CO 43	5.79 b	169.20 c	5.83 c	193.80b	12.20 c	308.50 c	76.00 c	13.41 c	5.67 c	236.60 d	21.40 d	64.25 d	41.30 e
PTB 33	5.58 b	93.60 a	17.71 a	105.40 a	18.00 b	86.25 a	54.00 a	19.74 a	2.74 a	119.40 a	39.40 a	13.33 a	8.33 a
TN 1	7.72 c	314.60 e	2.51 d	325.00 d	7.20 d	811.25 e	82.00 d	11.67 d	7.03 c	370.00 f	17.00 e	90.37 e	120.43 f
CD (0.05)	0.45	6.59	1.51	6.82	1.83	11.26	3.49	1.38	0.68	12.59	1.38	6.12	3.21

Within columns, means followed by a common letter do not differ significantly at P=0.05 (DMRT).

NP – Nymphal preference (number per plant)
NNE – Number of nymphs emerged per plant
UNEGG – Unhatched eggs (%)
FECUN – Number of eggs laid per plant (fecundity)
FDMK – Number of feeding marks per plant
FRAT – Feeding rate – honeydew excreted (mm^2)
SUR – Survival of nymphs (%)
NDP – Nymphal development period (days)
GI – Growth Index
IGN – Number of first generation nymphs emerged per plant
DWT – Days to wilt
FPLI – Functional Plant Loss Index (%)
PD – Plant dry weight loss per mg of insect dry weight produced.

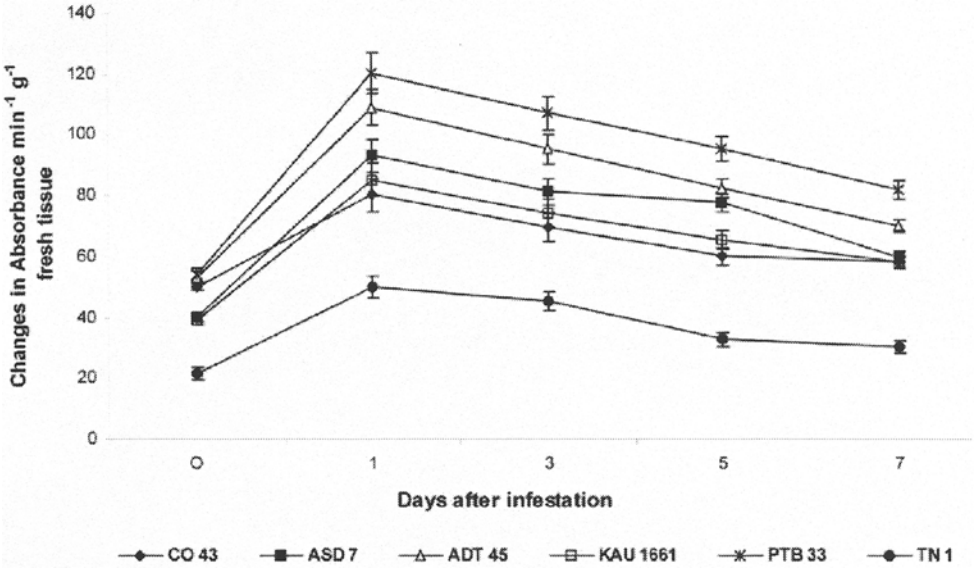

Fig. 1. Activity of peroxidase in the resistant and susceptible rice genotypes against brown planthopper infestation. Vertical bars indicate the standard deviation of three replications.

Egg laying behavior The number of unhatched eggs was significantly higher in ASD 7 (18.39), PTB 33 (17.71), KAU 1661 (16.61) and ADT 45 (15.64) than in the susceptible TN 1 (2.51). Among the genotypes tested to assess the egg laying behavior of BPH, it was found that PTB 33 (105.40), ADT 475 (108.60), ASD 7 (191.60) and KAU 1661 (208.00) had the lower fecundity, and that in susceptible TN 1 a higher number of eggs was laid (325.00) (Table 1).

Number of feeding marks More feeding marks were observed on the resistant genotypes because the BPH does not find a suitable feeding site on resistant genotypes and moves around, making numerous probes in an attempt to locate a suitable feeding site (17). The number of feeding marks was highest in KAU 1661 (20.40), followed by PTB 33 (18.00), ADT 45 (16.40), ASD 7 (16.20) and CO 43 (12.20), compared with 7.20 in susceptible TN 1, indicating the struggle by BPH to find suitable sites for feeding in resistant genotypes (Table 1).

Feeding rate The rate of honeydew excretion was measured in order to assess the feeding rate of sap-sucking insects. The amount of food intake is directly proportional to the amount of honeydew excreted by BPH. The lowest feeding rate was recorded in PTB 33 (86.25 mm^2) (Table 1), which was ten times lower than the susceptible TN 1 (811.25 mm^2). Other genotypes, such as KAU 1661 (216.50 mm^2), CO 43 (308.50 mm^2), ASD 7 (313.00 mm^2) and ADT 45 (360.00 mm^2), also had low feeding rates compared with the susceptible genotype TN 1.

Nymphal survival and development period Survival rate of BPH nymphs on resistant genotypes was lower than on the susceptible one. Resistant genotypes such as ADT 45 (52%), PTB 33 (54%) and ASD 7 (54%) had the lowest survival rates, which were

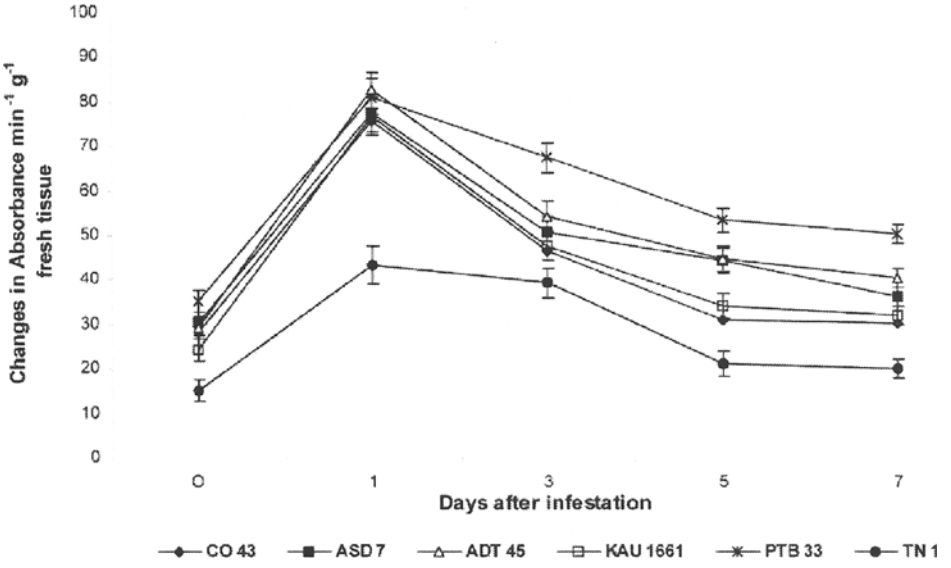

Fig. 2. Activity of polyphenoloxidase in the resistant and susceptible rice genotypes against brown planthopper infestation. Vertical bars indicate the standard deviation of three replications.

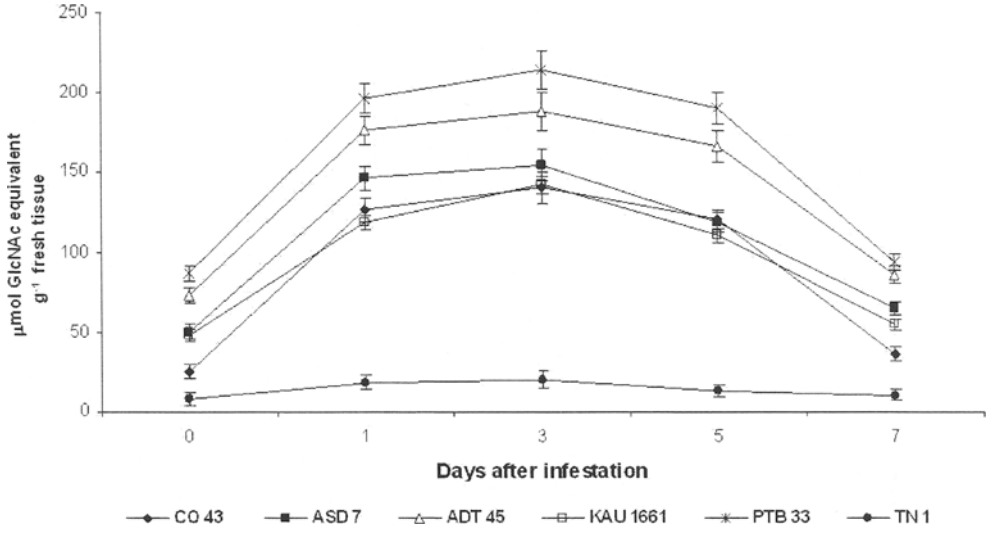

Fig. 3. Activity of chitinase in the resistant and susceptible rice genotypes against brown planthopper infestation. Vertical bars indicate the standard deviation of three replications.

significantly different from susceptible TN 1 (82%) (Table 1). Similarly, the nymphal development period was more prolonged on resistant genotypes than on the susceptible one. Among the genotypes tested, the nymphal development period was significantly higher in resistant PTB 33 (19.74 days) and ADT 45 (18.53 days) than in susceptible TN 1 (11.67 days) (Table 1).

Growth index and population buildup The lowest growth index of BPH was recorded in PTB 33 (2.74) and it was significantly different from that of susceptible TN 1 (7.03). In the present study, significant differences were observed in the emergence of first generation nymphs of BPH. The lower numbers of first generation nymphs were observed on PTB 33 (119.40), ADT 45 (196.00) genotypes compared with higher numbers on susceptible TN 1 (370.00). The days to wilt was significantly appreciable in PTB 33 (39.40 days), ADT 45 (28.40 days) and KAU 1661 (25.40 days), whereas in the susceptible genotype the figure was only 17 days (Table 1).

Tolerance The Functional Plant Loss Index (FPLI) was lowest in PTB 33 (13.33%) and KAU 1661 (22.47%), and significantly different from that in the susceptible TN 1 (90.37%). The plant dry weight loss per mg of insect dry weight produced was lower in PTB 33 (8.33 mg) and KAU 1661 (17.83 mg) than in susceptible TN 1 (120.43 mg) (Table 1).

Peroxidase (PO) and polyphenol oxidase (PPO) Among the genotypes assayed for the activity of peroxidase (Fig. 1) against BPH feeding in 30-day-old plants, PTB 33 and ADT 45 recorded significantly higher peroxidase activity at 1 DAI, followed by ASD 7 and KAU 1661, than in the susceptible genotype TN 1. The same trend was observed at 3, 5 and 7 DAI (Fig. 1). Figure 2 shows that there was higher activity of PPO at 1 DAI in PTB 33 and ADT 45 than in the susceptible TN 1. In addition, sustained and higher activity was recorded up to 7 DAI in resistant genotypes than in the susceptible TN 1.

Chitinase The induction of plant chitinase reached a peak at 3 DAI. Among the genotypes tested, PTB 33 and ADT 45 had higher levels of plant chitinase activity compared with susceptible genotype TN 1 at 3 DAI. Similarly, higher activity was observed in resistant genotypes than in susceptible genotypes up to 7 DAI (Fig. 3).

DISCUSSION

Resistant cultivars are sought as the major tactic in an integrated approach to rice insect pest management. The identification of insect-resistant rice germplasms and its resistance mechanisms to insect pests has been an integral part of the success of the 'Green Revolution' and has increased the profitability of rice production, minimized safety risks to farmers, and contributed to a more healthful environment.

In the present study the resistant genotypes PTB 33, ADT 45 and ASD 7 and the moderately resistant genotypes CO 43 and KAU 1661 had the lowest nymphal preference, fecundity, feeding rate, survival, growth index, population buildup and plant dry weight loss per mg of insect dry weight produced, and higher unhatched egg number, nymphal development period, days to wilt and FPLI compared with the susceptible genotype TN 1. Similar to our study, previous research work carried out with a series of parameters to evaluate the degree of resistance (11,24), gave an idea about the susceptibility and resistance nature of a genotype. In addition, the present study demonstrated the higher expression of proteins and defense enzymes against BPH feeding in resistant genotypes compared with the susceptible genotype. Our results are in agreement with the previous findings that activity of defense proteins and enzymes such as chitinase, peroxidase, β-1,3 glucanase and lysozymes was higher in tomato against *L. trifolii* and *Bemisia argentifolii* (Genn.) insect attack (12,21). The activity of PO has been correlated with pest and disease resistance in several plants (7). Peroxidase has been implicated in a number of physiological functions that may contribute to resistance in plants against herbivore

and pathogen attack, including oxidation of hydroxy-cinnamyl alcohol into free radical intermediates, phenol oxidation, polysaccharide crosslinking, crosslinking of extension monomers and lignification, production and polymerization of phenolics, hypersensitive response, negative effects on food digestibility and protein availability to sucking pests (7). The early and increased expression of PO enzyme involved in the biochemical reaction necessary for lignification has protected plants from *Cnaphalocrosis medinalis* in rice (27). From the above evidence, it is assumed that PO activity might contribute to the reduced BPH attack and preference for rice seedlings.

The PPO activity contributed to a decrease in the nutritional quality of infested plants by converting soluble phenolic compounds into quinones that eventually prevent the digestion of proteins in insects. Similarly, considerable evidence from earlier work points to the probability that increased accumulation of PPO in plants due to feeding by *Helicoverpa (=Heliothis) armigera* and *H. zea* (14,15) has affected the growth and development of these insects. From the above, it is suggested that PPO might interfere with the insect fecundity and digestion which might ultimately lead to a delay in the development period.

In the present study, greater activity of plant chitinase was recorded in resistant genotypes than in the susceptible genotype. Similar to our results, higher activity of defense enzymes and proteins was observed in resistant plants rather than susceptible plants after infestation with whitefly, aphid and mite pests (5,18,32). The literature reporting insect control through the activity of plant chitinases indicates that chitinases could have played an important role by hydrolyzing the chitin, as chitin constitutes a major structural component of the gut lining of insects. Also, chitin is an integral part of insect peritrophic matrices, which function as a permeability barrier between the bolus and the midgut epithelium, enhance digestive processes and protect the brush border membrane from mechanical disruption as well as from attack by toxins and pathogens (18,34). During the periods of starvation and molt, some insects completely cease peritrophic matrix production (22). Our results are in agreement with the findings of Srinivasan and Uthamasamy (32), who reported a 3.5-fold increase in the chitinase activity in tomato by whitefly feeding. Chitinase may severely affect insects by affecting the chitin-based structures. In addition, chitinases can act as an α-amylase inhibitor and interfere with digestion in insects (1). From the above, it is assumed that greater chitinase activity in resistant plants interfered with insect development, feeding, growth and nymphal development period (29,30).

Greater activity of defense proteins and enzymes in response to herbivores attack was demonstrated in resistant genotypes compared with the susceptible genotypes (35). Similarly, the present study is also in agreement with previous findings that greater and timely activity of PO, PPO and chitinase in resistant genotypes compared with susceptible genotypes might interfere with different growth and development of BPH. Hence, it is proposed that activity of defense enzymes and proteins in resistant genotypes might reduce the nymphal preference, fecundity, feeding rate, survival, growth index, and population buildup of BPH which may in turn be useful in formulating a strategy for integrated pest management of BPH in rice. Our results point to a strong possibility of their promotion under field conditions following testing in various hot-spot locations.

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