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Feeding-induced changes in defence enzymes and PR proteins and their implications in host resistance to *Nilaparvata lugens*

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Abstract

Six rice genotypes showing susceptible and resistant reactions to brown planthopper (BPH), *Nilaparvata lugens* were studied for feeding-induced changes in defence enzymes and pathogenesis-related (PR) proteins. The high resistant genotypes PTB 33, ADT 45 and ASD 7 and moderately resistant genotypes CO 43 and KAU 1661 recorded the greater expression of defence enzymes peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, total phenol and β-1,3 glucanase in response to *N. lugens* feeding at 1 day after infestation (DAI) compared with susceptible genotype TN1. The greater activity of chitinase was observed in resistant cultivars at 3 DAI and the activity was sustained for more than 1 week compared with susceptible TN1. In conclusion, the current study revealed that these defence enzymes and PR proteins might attribute to the resistance mechanisms in rice plants against BPH infestation.

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 and transmitting virus diseases like grassy stunt, ragged stunt and wilted stunt. It causes economic damage by sucking phloem sap which in turn leads to hopper-burn and severe yield loss (Pathak and Saxena 1980; AICRIP, 2008). Brown planthopper causes wide spread destruction and heavy yield loss on rice plants (Shi et al. 2003; Bark et al. 2007). Large scale cultivation of high-yielding cultivars is very conducive to *N. lugens* infestation. In addition, application of high level of nitrogenous fertilizers, continuous cropping, staggered planting and non-judicious use of insecticides have been reported for the increased outbreak of *N. lugens* (Chelliah and Heinriches 1984). Thus, it is necessary to develop the alternate methods of management for BPH. Recently, host plant resistance, which considered as cheap, ecofriendly and compatible with other methods of pest management, has become major management strategy against BPH (IRRI, 1980; Sharma 2007).

The resistant plants have constitutive and induced defence mechanisms to protect themselves from pathogen and herbivore attack. Accumulation of defence enzymes and PR proteins by insect feeding has been reported in many plant and pest interactions (Radjacommare et al. 2004; Saravanakumar et al. 2007a). Induced responses of the host plants are referred to changes in phytochemicals and developmental stages against stress originating from biotic factors. It has been established that some of the components of herbivore induced responses are defensive and negatively affect insect herbivore physiology (Baldwin and Preston 1999; Saravanakumar et al. 2007b). Stout et al. (1994) reported that the greater induction of polyphenol oxidase (PPO), peroxidase (PO), lipoxygenases and protease inhibitors in response to corn earworm [*Helicoverpa* (=*Heliothis*) *zea*], serpentine leafminer (*Liriomyza trifolii*) and tomato rust mite (*Aculops lycopersici*) feeding on tomato plants. An infestation by silver-leaf whitefly (*Bemisia argentifolii*) on tomato plant led to the higher induction of chitinase, β-1,3-glucanase and PO (McKenzie et al. 2002). Similarly, it is documented that changes in secondary metabolites of host plant induced by an insect feeding alter the nutritional

quality, suitability of the host plant and behaviour of the herbivores (Saravanakumar et al. 2007b).

However, the feeding-induced changes in defence enzymes and PR proteins of resistant and susceptible rice genotypes to suck pests were less understood. In this context, the study on accumulation of defence molecules of resistant and susceptible genotypes in response to insect feeding will have the great impact on the integrated pest management of BPH on rice. Thus, this study was carried out with an objective of assaying defence enzymes and PR proteins in rice plants and their role in host resistance to BPH infestation. More specifically, we opined that if the plant resistance is modified by herbivore feeding, it results in induced resistance. Thus, we measured the variations in induced defence responses in high resistant, moderately resistant and susceptible rice genotypes against herbivore feeding.

Materials and Methods

Plant materials

The experiment was carried out at Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India at glasshouse condition. The plastic pots were used for growing the rice seedling. The seeds of PTB 33, ASD 7, ADT 45, CO 43, KAU 1661 and TN1 rice genotypes were sown in plastic pots (15 × 45 cm) and the seedlings were watered regularly and 15-day old seedlings were transplanted in 15 cm diameter plastic pots. The NPK fertilizer was applied as per the recommendation of Crop Production Guide, 2008 of Tamil Nadu Agricultural University, Coimbatore, India. *Viz*., 1.15 : 1.06 : 0.27 g of urea, superphosphate and murate of potash was applied in each pot before transplanting. The potted plants were covered with cylindrical Mylar^{R} cages (13 × 90 cm) at 20 days after transplanting. Culturing of BPH was done as per Heinrichs et al. (1985). The plants were maintained without any BPH inoculation. To assay the activity of defence enzymes and pathogenesis-related (PR) protein in resistant and susceptible genotypes, 45 days old plants were used for all the experiments. Five replications were maintained for each treatment and 10 plants were populated for each replication.

Reactions of rice genotypes to BPH infestation by standard seed box screening test

The pre-germinated seeds of test genotypes were sown 3 cm apart in a wooden seed box (60 × 40 × 10 cm), filled with 5–7 cm depth of soil. Each genotype was sown in a row (20 seeds per row) across the width of the seed box. The seed box was transferred to a galvanized iron tray filled with water. At seventh day after sowing, the seedlings were infested with first to second instar nymphs of BPH by gently tapping over the seedlings in such a way that approximately 5–7 nymphs settled on each seedling. Each seed box was covered with a Netlon cage to prevent any escape of BPH and to prevent the entry of natural enemies. Damage rating of the test genotypes was done based on 0–9 scale of Standard Evaluation System for rice (IRRI, 2002). Five replications were maintained for each genotypes and the experiment was conducted three times.

Assay of defence enzymes and pathogenesis-related proteins

The freshly emerged adults were randomly selected and five per plant were released on 45 day old rice plants. Before releasing the adult, all the old and dried leaf sheaths were removed. Only the fresh leaf sheaths were kept. Single tiller were used for experiment. Samples were collected from stem portions along with leaf sheaths at 0, 1, 3, 5 and 7th day after inoculation of BPH. One gram of rice plant sample was macerated with 1 ml of 0.1 M sodium phosphate buffer (pH 7) at 4°C. The homogenate was centrifuged for 20 min at 6 000 ***g*** at 4°C and the supernatant were used for the assay of PO and PPO.

Assay of peroxidase

Peroxidase activity was assayed as given in Hammerschmidt et al. (1982). 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 the absorbance/min/g fresh tissue.

Assay of polyphenol oxidase

Polyphenol oxidase activity was determined as per Mayer et al. (1965). 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 and the changes in absorbance was read at 495 nm at 30 s

interval for 3 min. The activity was expressed as changes in absorbance/min/g fresh tissue.

Assay for phenylalanine ammonia lyase

The phenylalanine ammonia lyase (PAL) assay was conducted as per the method described by Ross and Sederoff (1992). The assay mixture containing 100 μl of enzyme, 500 μl of 50 mM Tris–HCl (pH 8.8) and 600 μl of 1 mM L-phenylalanine was incubated for 1 h. The reaction was arrested by adding 2N HCl. Later, 1.5 ml of toluene was added, vortexed for 30 s, centrifuged (6000 ***g***, 5 min) and toluene fraction containing *trans*-cinnamic acid was separated. The toluene phase was measured at 290 nm with toluene as blank. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as n-moles of *trans*-cinnamic acid/min/g fresh sample.

Assay of total phenol

The total phenol content was estimated following the method of Zieslin and BenZaken (1993). One gram of stem tissue was homogenized in 10 ml of 80% methanol and diluted for 15 min at 70°C. One millilitre of the methonalic extract to 5 ml of distilled water and 250 μl of Folin-Ciocalteu reagent (1N) was added and the solution was kept on a boiling water bath. After 3 min, 1 ml of saturated solution of Na_2CO_3 and 1 ml of distilled water were added and the reaction mixture was incubated for 1 h at 25°C. The absorbance was measured at 725 nm. Total soluble phenol was calculated according to a standard curve obtained from a Folin-Ciocalteu reagent with catechol and expressed as μg of catechol equivalent/g sample.

Assay for β-1,3 glucanase

The enzyme activity was assayed as per Pan and Kuc (1991). The crude enzyme extract of 62.50 μl was added to 62.50 μl of laminarin (4%) and then, it was incubated at 40°C for 10 min. The reaction was stopped by addition of 375 μl of dinitrosalicylic acid (DNS) and heated for 5 min on a boiling water bath (DNS was prepared by addition of 300 ml of 4.50% NaOH with 8.80 g of DNS and 22.5 g of potassium sodium tartarate). The resulting coloured solutions were diluted with distilled water, vortexed and the absorbance was read at 500 nm. The crude extract preparation mixed with laminarin with zero time incubation served as blank. The enzyme activity was expressed as μg equivalents of glucose/min/g of fresh sample.

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 12 000 ***g*** for 15 min at 4°C and the supernatant was used in the enzyme assay. The colorimetric assay of chitinase was carried out as per the method described by Boller and Mauch (1988). Colloidal chitin was prepared according to Berger and Reynolds (1958) from crab shell chitin (Sigma, Mumbai, India). 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 5000 ***g*** for 3 min. An aliquot of the supernatant (0.3 ml) was pipetted onto 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. 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/g of fresh tissue.

Native polyacrylamide gel electrophoresis analysis

The isoform profiles of PO, PPO and chitinase were studied by discontinuous native polyacrylamide gel electrophoresis (Native PAGE) (Laemmli 1970). The protein extract was prepared by homogenizing 1 g of stem sample in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 16 000 ***g*** for 20 min at 4°C. The protein content of the sample was determined (Bradford 1976) and samples (50 μg protein) were loaded into 8% polyacrylamide gels (Sigma). After electrophoresis, PO isoforms were visualized by soaking the gels in staining solution containing 0.05% benzidine (Sigma Aldrich, Mumbai, India) and 0.03% H_2O_2 in acetate buffer (20 mM, pH 4.2) (Nadolny and Sequira 1980). For assessing PPO isoform profiles, the gels were equilibrated for 30 min in 0.1% *p*-phenylene diamine followed by addition of 10 mM catechol in the same buffer (Jayaraman et al. 1987). Chitinase activity was detected in PAGE gel according to Trudel and Asselin (1989) with slight modifications. Gels were incubated in 150 mM sodium acetate buffer at pH 5.0 for 5 min and then in 100 mM sodium acetate

buffer at pH 5.0, containing 0.01% glycol chitin for 30 min at 37°C. The gels were finally transferred onto a solution containing 0.01% (w/v) calcofluor white M2R (Fluorescent brightener; Sigma Aldrich) in 500 mM Tris–HCl (pH 8.9). After 5 min, the brightener solution was removed and gels were rinsed with distilled water for more than 1 h. Lytic zones were visualized and photographed under UV light using gel documentation system (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis

The data obtained from various experiments were statistically analysed in a completely randomized block design. The percentage data were transformed using arc sine transformation before statistical analysis. Further, different parameters observed in the experiments were subjected to Duncan's Multiple Range Test (Gomez and Gomez 1984) analysis using IRRISTAT version 92-a developed by International Rice Research Institute, Biometrics Unit, The Philippines.

Results

Reactions of rice genotypes against BPH

Among six genotypes evaluated for their resistance against *N. lugens* by standard seed box screening test, ADT 45, ASD 7 and PTB 33 showed resistant reactions. The genotypes, CO 43 and KAU 1661 were found to be moderately resistant and TN1 showed susceptible reaction to BPH infestation (table 1).

PO, PPO and PAL activity

Among different genotypes assayed for the accumulation of POs in response to *N. lugens* feeding on 45 day old plants, PTB 33 (424.45 Ab/min/g tissue) and ADT 45 (399.32 Ab/min/g tissue) recorded the higher activities at 1 DAI when compared with susceptible TN1 (207.23 Ab/min/g). At 3, 5 and 7 DAI, the activity of PO was declined in sustained manner in resistant genotypes compared with the susceptible one. The increase in PO activity was higher (37.49%) in CO 43 at 1 DAI followed by ADT 45 (33.21%) (table 2). Polyphenol oxidase activity was higher in ADT 45 (49.45 Ab/min/g) at 1 DAI, when compared with susceptible TN1 (38.45 Ab/min/g) and the activity declined gradually in all the tested genotypes at 3, 5 and 7 DAI. The per cent increase in PPO activity was also higher in ADT 45 (67.18%) at 1 DAI (table 3). Further, ADT 45 showed the greater accumulation of PAL (192.47 n-mole of *trans*-cinnamic acid/g tissue) at 1 DAI. It was followed by ASD 7 (157.24), KAU 1661 (150.47), CO 43 (130.13) and the susceptible TN1 (100.12 n-mole of *trans*-cinnamic acid/g tissue). The induction level and per cent increase of PAL had declined in sustained manner in resistant genotypes than the susceptible one at 3, 5 and 7 DAI. Further, PAL induction was greater in ADT 45 followed by ASD 7 and CO 43 compared with TN1 (table 4).

Table 1 Reaction of rice genotypes against *Nilaparvata lugens* using SSST*

Genotypes	Scoring**	Reaction
PTB 33	2.13	HR
ASD 7	2.50	HR
ADT 45	2.50	HR
CO 43	3.25	MR
KAU 1661	3.50	MR
TN 1	8.67	S

*Mean of five replications (individual plant scoring) and pool of three trials. **Damage rating as per the Standard Evaluation System for rice; HR, high resistant; MR, moderately resistant; S, susceptible, SSST, standard seed box screening test.

Total phenols

The accumulation of total phenol was higher in ADT 45 (97.33 μg/g) at 1 DAI as compared with 22.12 μg/g in healthy sample before feeding of *N. lugens* and declined to 67.62 μg/g at 7 DAI. It was followed by PTB 33 (97.45 μg/g) and ASD 7 (93.23 μg/g), and susceptible TN 1 (15.16 μg/g) at 1 DAI. At 3, 5 and 7 DAI, the gradual decrease in the expression of phenols was noticed in all the genotypes. The per cent increase in accumulation of total phenol was higher in ADT 45 (77.27%) at 1 DAI followed by PTB 33 (74.22%), ASD 7 (70.79%) and CO 43 (69.21%) (table 5).

β-1,3 glucanase

Induction of β-1,3 glucanase was higher on 45 day old plants of KAU 1661 (35.72 μg of glucose released/min/g) compared with other genotypes at 1 DAI (table 6). At 3 DAI, β-1,3 glucanase activity started to decline but the activity was sustained to 1 week after infestation. The induction was lower on susceptible TN 1 (15.41 μg of glucose released/min/g) compared with resistant PTB 33 (38.13 μg of glucose released/min/g) at 1 DAI. The per cent increase in accumulation of β-1,3 glucanase was

Table 2 Peroxidase activity in different rice genotypes against *Nilaparvata lugens* feeding on 45 day old plants

	Peroxidase activity*								
Genotypes	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	298.28^{a}	424.45^{a}	29.73 (33.04)^{e}	419.80^{a}	28.95 (32.55)^{b}	371.69^{a}	19.75 (26.36)^{d}	342.13^{a}	12.82 (20.98)^{c}
ASD 7	253.45^{d}	373.00^{c}	32.05 (34.48)^{c}	339.35^{c}	25.31 (30.21)^{d}	316.15^{c}	19.83 (26.42)^{c}	285.13^{c}	11.11 (19.47)^{e}
ADT 45	266.69^{c}	399.32^{b}	33.21 (35.08)^{b}	363.30^{b}	26.59 (31.04)^{c}	344.43^{b}	22.57 (28.34)^{b}	336.18^{b}	20.67 (27.04)^{a}
CO 43	217.00^{e}	347.17^{e}	37.49 (37.23)^{a}	315.15^{e}	31.14 (33.92)^{a}	295.20^{d}	26.49 (30.97)^{a}	270.25^{d}	19.70 (26.35)^{b}
KAU1661	278.60^{b}	366.27^{d}	23.94 (30.99)^{d}	332.85^{d}	16.30 (23.81)^{f}	289.00^{e}	3.60 (10.72)^{f}	285.25^{c}	2.33 (8.78)^{f}
TN 1	166.14^{f}	207.23^{f}	19.83 (26.44)^{f}	200.00^{f}	16.93 (24.29)^{e}	190.25^{f}	12.67 (20.83)^{e}	187.48^{e}	11.38 (19.72)^{d}

*Change in absorbance/min/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

Table 3 Polyphenol oxidase activity in rice genotypes against *Nilaparvata lugens* feeding on 45 day old plants

	Polyphenol oxidase activity*								
Genotypes	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	16.30^{c}	49.40^{a}	67.00 (54.93)^{b}	37.43^{ab}	56.45 (48.71)^{b}	33.20^{a}	50.90 (45.52)^{a}	19.45^{c}	16.20 (23.73)^{f}
ASD 7	23.15^{a}	46.75^{ab}	50.40 (45.23)^{d}	35.20^{c}	34.23 (35.80)^{f}	31.22^{ab}	25.85 (30.55)^{f}	29.20^{a}	20.72 (27.08)^{d}
ADT 45	16.23^{c}	49.45^{a}	67.18 (55.05)^{a}	38.17^{a}	57.48 (49.31)^{a}	32.45^{ab}	49.98 (44.99)b	29.23^{a}	44.47 (41.83)^{a}
CO 43	19.60^{b}	41.85^{c}	53.17 (46.82)^{c}	36.25^{b}	45.93 (42.67)^{e}	30.17^{b}	35.03 (36.29)^{e}	25.75^{b}	23.88 (29.25)^{c}
KAU1661	18.45^{b}	41.65^{c}	55.70 (48.27)^{b}	35.00^{c}	47.29 (43.45)^{d}	30.13^{b}	38.77 (38.51)^{d}	26.40^{b}	30.11 (33.28)^{b}
TN 1	12.62^{d}	38.45^{d}	67.18 (55.05)^{a}	28.45^{d}	55.64 (48.24)^{c}	23.42^{c}	46.11 (42.77)^{c}	15.45^{d}	18.32 (25.34)^{e}

*Change in absorbance/min/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

Table 4 Phenylalanine ammonia lyase activity in rice genotypes against *Nilaparvata lugens* feeding on 45 day old plants

	Phenylalanine ammonia lyase activity*								
Genotypes	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	128.15^{b}	158.13^{b}	18.96 (25.79)^{e}	147.12^{b}	12.89 (20.99)e	142.32^{b}	9.96 (18.32)e	138.15^{b}	7.24 (15.48)e
ASD 7	112.15^{c}	157.24^{b}	28.68 (32.37)^{b}	148.25^{b}	24.35 (29.55)^{c}	138.48^{c}	19.01 (25.83)^{c}	132.15^{c}	15.13 (22.86)^{c}
ADT 45	127.88^{b}	192.47^{a}	33.56 (35.39)^{a}	183.13^{a}	30.17 (33.31)^{a}	180.25^{a}	29.05 (32.60)^{a}	170.25^{a}	24.89 (29.91)^{a}
CO 43	101.26^{d}	130.13^{d}	22.19 (28.09)^{d}	142.15^{c}	28.77 (32.43)^{b}	137.25^{d}	26.22 (30.79)^{b}	130.12^{c}	22.18 (28.08)^{b}
KAU1661	130.12^{a}	150.47^{c}	13.52 (21.55)^{f}	138.45^{d}	6.02 (14.12)^{f}	135.13^{e}	3.71 (10.90)^{f}	133.85^{d}	2.79 (9.28)^{f}
TN 1	77.47^{e}	100.12^{e}	22.62 (28.39)c	98.12^{e}	21.05 (27.29)^{d}	90.44^{f}	14.34 (22.23)^{d}	85.12^{e}	8.99 (17.43)^{d}

*n-moles of *trans*-cinnamic acid/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

higher in PTB 33 (46.32%) at 1 DAI followed by KAU 1661 (34.97), ADT 45 (33.45%) and ASD 7 (29.29%) compared with 19.21% in susceptible TN1.

Chitinase

The induction of plant chitinase attained a peak at 3 DAI on 45 day old plants. Among the genotypes tested, ADT 45 recorded the high level of chitinases (135.65 μmoles of GlcNAc equivalent/g) at 3 DAI. It was followed by ASD 7 (120.32), CO 43 (85.45), KAU 1661 (73.93) and susceptible TN 1 (40.83 μmoles of GlcNAc equivalent/g). At 5 and 7 DAI, the gradual decrease in expression of chitinase was measured in all genotypes. The per cent increase in accumulation of chitinase was higher in ASD 7 (83.25%)

Table 5 Total phenol accumulation in rice genotypes against *Nilaparvata lugens* feeding on 45 day old plants

Genotypes	Total phenol*								
	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	25.12^{b}	97.45^{a}	74.22 (59.51)^{b}	86.45^{a}	70.94 (57.39)^{b}	83.26^{a}	69.83 (56.69)^{a}	77.25^{a}	67.48 (55.24)^{a}
ASD 7	27.23^{a}	93.23^{b}	70.79 (57.30)^{c}	77.55^{b}	64.89 (53.67)^{d}	51.00^{d}	46.61 (43.07)^{e}	35.28^{d}	22.82 (28.51)^{e}
ADT 45	22.12^{c}	97.33^{a}	77.27 (61.56)^{a}	87.13^{a}	74.61 (59.77)^{a}	72.45^{b}	69.47 (56.47)^{b}	67.62^{b}	67.29 (55.13)^{b}
CO 43	25.39^{b}	82.45^{c}	69.21 (56.30)^{d}	76.60^{b}	66.85 (54.86)^{c}	67.23^{c}	62.23 (52.08)^{d}	42.45^{c}	40.19 (39.34)^{c}
KAU1661	27.18^{a}	72.12^{c}	62.31 (52.13)^{e}	62.12^{c}	56.25 (48.59)^{e}	51.00^{d}	46.71 (43.11)^{d}	35.55^{d}	23.54 (29.02)^{d}
TN 1	7.45^{d}	15.16^{d}	50.86 (45.49)^{f}	11.23^{d}	33.66 (35.46)^{f}	10.64^{e}	29.98 (33.19)^{f}	7.83^{e}	4.85 (12.60)^{f}

*μg/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

Table 6 β-1,3 glucanase accumulation in rice genotypes against *Nilaparvata lugens* feeding on 45 day old plants

Genotypes	β-1,3 glucanase*								
	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	20.47^{b}	38.13^{a}	46.32 (42.89)^{a}	35.42^{a}	46.32 (42.89)^{a}	35.15^{a}	41.76 (40.26)^{a}	30.28^{a}	32.40 (34.68)^{a}
ASD 7	20.13^{b}	28.47^{d}	29.29 (32.75)^{d}	26.28^{d}	29.29 (32.75)^{d}	25.43^{c}	20.84 (27.14)^{c}	21.83^{d}	7.79 (16.09)^{d}
ADT 45	20.25^{b}	30.43^{c}	33.45 (35.32)^{c}	28.38^{c}	33.45 (35.32)^{c}	25.81^{c}	21.54 (27.63)^{b}	24.28^{c}	16.60 (24.00)^{b}
CO 43	18.45^{c}	23.48^{e}	21.42 (27.55)^{e}	22.12^{e}	21.42 (27.55)^{e}	20.45^{d}	9.78 (18.17)^{e}	19.82^{e}	6.91 (15.14)^{e}
KAU1661	23.23^{s}	35.72^{b}	34.97 (36.25)^{b}	31.87^{b}	34.97 (36.25)^{b}	28.45^{b}	18.35 (25.35)^{d}	25.87^{b}	10.20 (18.59)^{c}
TN 1	12.45^{d}	15.41^{f}	19.21 (25.98)^{f}	13.23^{f}	19.21 (25.98)^{f}	13.69^{e}	9.06 (17.47)^{f}	13.21^{f}	5.75 (13.78)^{f}

*μg of glucose released/min/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

Table 7 Chitinase accumulation in rice genotypes against *Nilaparvata lugens* on 45 day old plants

Genotypes	0 DAI	1 DAI	% increase	3 DAI	% increase	5 DAI	% increase	7 DAI	% increase
PTB 33	30.43^{b}	55.60^{c}	45.27 (42.28)^{b}	127.25^{b}	76.09 (60.74)^{c}	115.45^{b}	73.64 (59.12)^{c}	90.97^{ab}	66.55 (54.67)^{c}
ASD 7	20.15^{c}	90.60^{a}	77.76 (61.88)^{a}	120.32^{c}	83.25 (65.88)^{a}	110.23^{c}	81.72 (64.72)^{a}	98.65^{b}	79.57 (63.16)^{a}
ADT 45	40.25^{a}	63.33^{b}	36.44 (37.13)^{c}	135.65^{a}	70.33 (57.00)^{f}	121.12^{a}	66.77 (54.81)^{f}	110.62^{a}	63.61 (52.91)^{f}
CO 43	15.32^{d}	22.25^{d}	31.15 (33.92)^{d}	85.45^{d}	82.07 (64.97)^{b}	72.43^{d}	78.85 (62.64)^{b}	60.75^{d}	74.78 (59.87)^{b}
KAU1661	20.45^{c}	22.24^{e}	8.05 (16.42)^{e}	73.93^{e}	72.34 (58.28)^{e}	65.69^{e}	68.87 (56.09)^{e}	58.15^{e}	64.83 (53.63)^{e}
TN 1	10.22^{e}	10.62^{f}	3.77 (11.01)^{f}	40.83^{f}	74.97 (59.99)^{d}	35.60^{f}	71.29 (57.61)^{d}	30.32^{f}	66.29 (54.51)^{d}

*μmoles of GlcNAc equivalent/g of fresh sample. Values are mean of five replications. In a column, mean followed by common letter are not significantly different at 5% level by DMRT. Values in parenthesis are arcsine transformed values. DAI, days after infestation; DMRT, Duncan's multiple range test.

followed by CO 43 (82.07%) and PTB 33 (76.09%) (table 7).

PAGE analysis of defence enzymes

Isozyme pattern of different defence enzymes in rice genotypes were studied on 45 day old plants at 1 and 3 DAI of BPH. Native gel electrophoretic separation of enzyme extract from different genotypes of rice plants showed different PO isoform patterns. Two prominent isoforms of peroxidase (PO1 and PO2) were observed on 45 day old plants of CO 43, ASD 7, KAU 1661 and PTB 33 where in only one isoform was observed in susceptible TN1 at 1DAI (fig. 1a).

In case of PPO, two unique isoform patterns were detected on 45 day old plants of CO 43, ASD 7, KAU 1661 and PTB 33 at 1 DAI (fig. 1b). In susceptible TN1, the intensity of isoforms was faint when compared with the isoform patterns observed in resistant genotypes. Three distinct isoforms of chitinase (Chi 1, Chi 2 and Chi 3) were observed in resistant

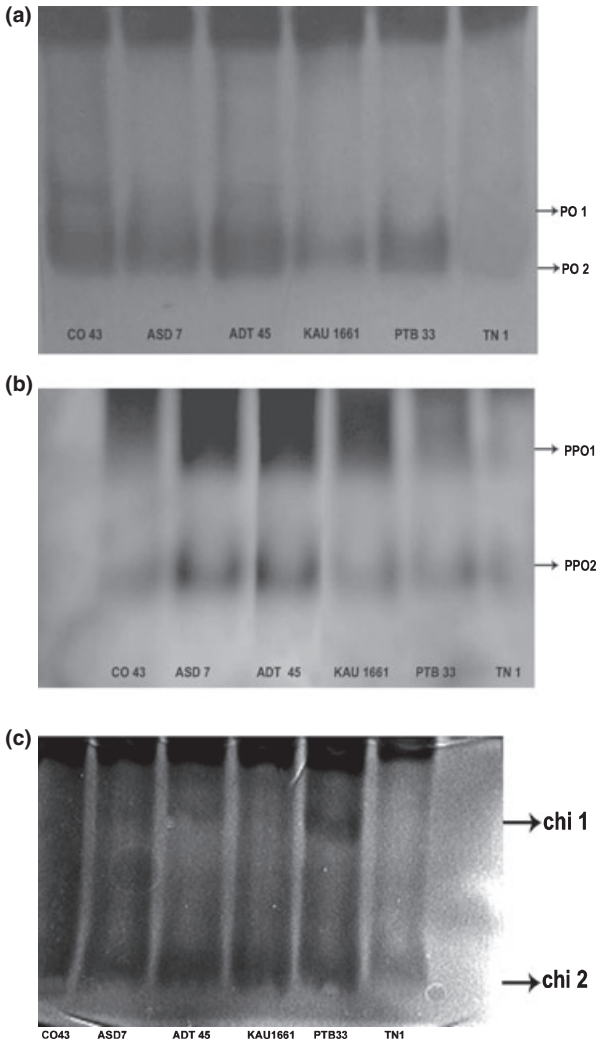

Fig. 1 Native polyacrylamide gel electrophoresis analysis of defense enzymes in resistant and susceptible rice genotypes against BPH (*Nilaparvata lugens*) infestation. (a) Peroxidiase, PO. (b) Polyphenol oxidase, PPO. (c) Chitinase, Chi.

genotypes at 45 day old plants on 3 DAI. In all the resistant genotypes, the intensity of isoforms of chitinases was discrete where in susceptible TN1, less intensity was observed (fig. 1c).

Discussion

Resistant cultivars are considered as the major tactic of an integrated approach for the management of rice BPH. The identification of insect resistant rice germplasms and study of its resistant mechanisms were an integral part in the success of 'Green Revolution'. In this study, the high resistant genotypes PTB 33, ADT 45 and ASD 7, and moderately resistant genotypes CO 43 and KAU 1661 recorded higher expression of defence enzymes and PR proteins against BPH feeding compared with susceptible genotype TN1. This study are in agreement with the previous findings that activity of defence enzymes and PR proteins, such as chitinase, PO, β-1,3 glucanase and lysozymes were higher in tomato against *L. trifolii* and *B. argentifolii* (Genn.) feeding (Inbar et al. 1999; McKenzie et al. 2002). The activity of PO has been correlated with pest and disease resistance in several plants (Duffey and Stout 1996; Saravanakumar et al. 2007a, 2009). The PO 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 cross linking, cross linking of extension monomers and lignification, production and polymerization of phenolics, hypersensitive response, negative effects on food digestibility and protein availability to sucking pests (Duffey and Stout 1996). The early and increased expression of PO reported to involve in the lignification processes that have protected plants from *Cnaphalocrosis medinalis* G. in rice (Radjacommare et al. 2002). From the above evidences, it is assumed that activity of PO might attribute to the reduced BPH attack and their preference to rice seedlings.

Polyphenol oxidase reduces 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 the earlier work implicates that increased accumulation of PPO in plants against tomato fruit borer (*Helicoverpa armigera* and *H. zea*) has affected the growth and development of these insects (Isman and Duffey 1982a,b). Recently, Saravanakumar et al. (2007b) demonstrated that increased activity of PPO reduced the growth and development of *C. medinalis* on rice plants. From the earlier reports, it is suggested that PPO might interrupt with the insect fecundity and digestion which ultimately leads to delay in developmental period. Further, in spite of the low activity of PPO at 7 DAI in PTB 33, it showed resistance to BPH attack. This indicated that timely induction and expression of PPO at 1 DAI might be responsible for the resistant reaction in PTB 33 genotype.

The accumulation of phenolic compounds was reported to be growth inhibitory effect on *H. armigera* and *B. tabaci* and on *C. medinalis* in tomato and rice, respectively (Srinivasan and Uthamasamy 2004;

Radjacommare et al. 2002). Similarly, the greater accumulation of phenol could attribute to the resistant reactions in this study. Conspicuously, the higher accumulation of chitinases and β-1,3-glucanases was noticed in resistant cultivars when compared with susceptible genotypes. Similarly, Inbar et al. (1999) reported the induction of chitinase, PO, β-1,3 glucanase and lysozymes in response to leaf miner and whitefly infestation. In this study, though genotypes ADT 45 and PTB 33 showed the high resistant reactions, ADT 45 expressed the higher activity of PAL compared with PTB 33. On the other hand, PTB 33 showed the greater expression of other defence enzymes compared with ADT 45. This indicated that expression of defence enzymes is specific to the genotypes and their regulation may not be inter-dependent.

Pathogenesis-related proteins are reported to play a defensive role against plant pests. It was observed in this study that 3.37 folds increase in the chitinase activity in response to BPH feeding in high resistant and moderately resistant rice genotypes. Similar to our results, high activity of defence enzymes and proteins were observed in resistant plants rather than susceptible plants after infestation with whitefly, aphid and mite pests (Kramer and Muthukrishnan 1997; Srinivasan and Uthamasamy 2004). The existing literatures on insect control through the activity of plant chitinases revealed that chitinases could play an important role by hydrolyzing the chitin, as chitin constitutes a major structural component of gut linings of insects. Also, chitin is an integral part of insect pertitrophic 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 (Tellam 1996; Kramer and Muthukrishnan 1997). During the periods of starvation and moult, some insects completely cease peritrophic matrix production (Merzendorfer and Zimoch 2003). Also, chitinase severely affects insects by affecting the chitin based structures. In addition, chitinases can act as α-amylase inhibitor and interferes with digestion of insects (Ary et al. 1989). From the above evidences, it is assumed that high chitinase activity in resistant plants might interfered with insect development, feeding, growth and nymphal developmental period (Sampson and Gooday 1998). The evidences from previous and current studies proposed that up-regulated defence enzymes and PR proteins in resistant genotypes could involve in resistant mechanisms and could be useful in formulating the strategy for integrated pest management of BPH on rice.

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