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Antibiosis Components and Antioxidant Defense of Rice as Mechanism of Resistance to Brown Planthopper, *Nilaparvata lugens* (Stål)

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The role of antibiosis components and antioxidant defense of rice genotypes, namely CR3006-8-2, RP4918-221, KAUM182-1, T12, IHRT-ME-25, W1263, Ptb33 (resistant check) and TN1 (susceptible check) was studied by phenotyping them against brown planthopper (BPH). Three genotypes, namely KAUM182-1, RP4918-221 and CR3006-8-2 were resistant to BPH and significantly low damage score (1.97–3.00); honeydew excretion area (46.76–49.64 mm^2); nymphal survival (60.60–66.40%) and growth index (2.98–3.86) was recorded on them. Higher constitutive and induced level of soluble phenolics, peroxidase and polyphenol oxidase was observed in resistant genotypes without and with BPH infestation. A negative relationship between honeydew excretion, nymphal emergence, growth index and nymphal survival was observed with these biochemical constituents. Likewise, a reverse trend was observed between nymphal development period and biochemical constituents. These genotypes have emerged as a new source of resistance to BPH which can be used in hybridization programme to breed durable BPH resistant rice varieties.

Keywords: rice, brown planthopper, antibiosis, biochemical, antioxidant, resistance

Introduction

Rice, *Oryza sativa* L. (Poaceae), is cultivated extensively in the most diverse ecosystems of tropical and sub-tropical regions of the world. Among various biotic constraint of rice production, the insect pests are of prime importance and warm humid environment of the crop is also conducive for their survival and proliferation (Heong and Hardy 2009). The brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) (BPH) is one of the most damaging insect pests of rice and a typical phloem sap feeder. Being an r-strategist pest BPH increases exponentially and causes huge yield losses due to excessive removal of plant sap, the condition known as, 'hopper burn'. Susceptible cultivars suffer 40 to 70 per cent yield loss in case of serious pest infestation (Heong and Hardy 2009). BPH also transmits virus diseases like grassy stunt, ragged stunt and wilted stunt.

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By regular monitoring of rice field, farmers can manage this pest effectively by need based application of recommended insecticides (Sarao 2015). However, application of un-recommended insecticides for the control of this insect disrupts the ecological balance and cause pest resurgence and development of multiple insecticide resistance (Matsumura and Morimura 2010). Hence, cultivation of resistant varieties is an environmentally sound strategy for population management of this insect (Horgan et al. 2015; Sarao et al. 2016). The natural enemy population will also increase on the resistant varieties due to less pesticide applications (Gurr 2011). Host plant show very diverse kind of reactions upon wounding and feeding by insect pests and nutritional biochemistry of a plant will also alter in this response (Vanitha et al. 2011). Inducible resistance to insect pests is an important tool for the scientists to develop a variety resistant to insect pests (Jena and Kim 2010). But the role of antibiosis mechanisms, enzymatic and non-enzymatic antioxidants involved in plant defense against BPH feeding has been understood to a limited extent. There are suites of defense related enzymes in plants, however, peroxidases (PO), polyphenol oxidases (PPO) are key enzymes involved in oxidation of phenols and inhibit protein digestion. Catalase (CAT) and peroxidases rapidly destroy H_2O_2, but they allow low steady-state levels to persist to maintain signalling pathways (Noctor and Foyer 1998; Qiu 2011). Hence, the present studies were undertaken to quantify the antibiosis level and activity of soluble phenolics, CAT, PO and PPO molecules in selected genotypes so as to use resistant genotypes to breed BPH resistant variety which will form an integral part of integrated pest management.

Materials and Methods

Plant and insect material

Eight rice genotypes, viz. CR3006-8-2, RP4918-221, KAUM182-1, T12, IHRT-ME-25, W1263, Ptb33 and TN1 were obtained from Indian Institute of Rice Research, India. BPH was reared on 30-day-old TN1 plants under glasshouse conditions of Department of Plant Breeding and Genetics, Punjab Agricultural University, India positioned at 30°54' N and 75°48' E as per the protocol developed by Heinrichs et al. (1985) at temperature of 28 ± 2 °C, 75 ± 5% relative humidity and 14:10 h light : dark photoperiod.

Phenotyping

Seed box screening

The seeds (15–20) of test genotypes were sown in seed box (0.45×0.35×0.10 m) containing well-puddled soil in rows of 3.5 cm apart as per standard protocol (Heinrichs et al. 1985). Each genotype was replicated thrice in complete randomized block design in the tray. Ten-day-old seedlings were infested with 2nd–3rd instar stage nymphs at a rate of 6–8 insects per seedling (Heinrichs et al. 1985). Ptb33 and TN1 were used as standard resist-

ant and susceptible checks, respectively. Damage score on each seedling was recorded on a 0–9 scale when 90–100% plants of TN1 were dead following Standard Evaluation System of IRRI (2014). A score of 0 represents no visible damage, whereas a score of 9 represents complete drying of plant. Each genotype with a mean rating of 0–3.49, 3.50–5.49 and 5.50–9.00 was designated as resistant, moderately resistant and susceptible, respectively (Heinrichs et al. 1985).

Honeydew excretion

Feeding rate of BPH female adult on each genotype was assessed according to Heinrichs et al. (1985). Five one-day-old BPH females starved for 2 h before release were allowed to feed for 24 h at the leaf sheath portion of 30-day-old seedlings in glass chimneys. There were five replications for each genotype. Circular pieces of Whatman filter paper no. 1 were dipped in the dye solution of 0.1% bromocresol green in ethyl alcohol. These coloured papers were placed on a plastic plate and the plant was covered with glass chimney. The insect honeydew stains were appeared as blue colour spots on contact with filter paper. The filter paper was removed after 24 h and spot area was measured on graph paper.

Nymphal emergence

Two pairs of newly emerged insects were released on 30-day-old plants of each genotype kept in the glass chimneys. There were five replications for each genotype. The daily emerged nymphs were counted till the day they were stopped emerging (Khan and Saxena 1985).

Nymphal survival and development period

Twenty newly emerged nymphs from nymphal emergence experiment were released on 30-day-old plants of each test genotypes covered with glass chimneys. There were five replications for each genotype. For nymphal development period, ecdysis was observed on daily basis. The observations were recorded for different instars till the BPH reached the adult stage.

Growth index

Growth index of each tested genotype was computed by dividing the data obtained from the experiment on nymphal survival with that from nymphal development period (Alagar and Suresh 2007).

Biochemical constituents

Soluble phenolics

It was estimated as per the method given by Swain and Hills (1959). The leaves of 30-day-old rice test genotypes were collected and dried in incubator at 55–65 °C. The dried leaf samples (40 mg) were refluxed with 5 ml of 80% aqueous methanol for 1 h at 70–75 °C. The refluxed content was filtered and final volume was made to 10 ml by adding 80% of methanol. It was estimated by using Folin phenol and saturated Na_2CO_3. Absorbance was recorded at 760 nm against blank. Concentration of soluble phenolics was determined from standard curve made by using gallic acid with a range of 10–50 µg.

Catalase (CAT)

It was estimated as per the method given by Chance and Maehly (1955). Catalase was extracted from leaves of 30-day-old rice genotypes with 0.05 M sodium phosphate buffer (pH 7.5) having 1% polyvinyl pyrolidone. The catalase activity was determined by using 0.05 M sodium phosphate buffer, 0.05 ml of enzyme extract and 1 ml of H_2O_2. The decrease in absorbance was measured at 240 nm. The mixture without H_2O_2 was used as blank.

Peroxidase (PO)

It was determined as per method given by Shannon et al. (1966). Peroxidase was extracted from leaves of 30-day-old rice genotypes with 2 ml of 0.1 M potassium phosphate buffer (pH 7.5), 1% PVP along with 1 mM EDTA and 10 mM β-mercaptoethanol. The enzyme activity was determined by appearance of brown colour from guaiacol to tetra-guaiacol in presence of H_2O_2. Reagent used was 0.05 M guaiacol in 0.1 M potassium phosphate buffer (pH 6.5). The reaction was started by adding 0.1 ml of 0.8 M H_2O_2 and the change in absorbance was recorded at wavelength of 470 nm of spectrophotometer continuously for 3 min at interval of 30 s. The reaction mixture without H_2O_2 was used as blank.

Polyphenol oxidase (PPO)

It was estimated as per the method given by Bastin and Unluer (1972). Polyphenol oxidase was extracted from leaves of 30-day-old rice genotypes with 5 ml of ice cold 0.1 M tris HCL buffer (pH 7.5) having 5 mM β-mercaptoethanol. The reagent used was 0.01 M catechol in 0.1 M phosphate buffer (pH 6.0). For analysis, 2.5 ml of 0.01 M catechol was taken and 0.2 ml of enzyme extract was added in it. The increase in absorbance was recorded at 495 nm for 3 min. at interval of every 30 s.

Data analysis

The data obtained from various experiments related to antibiosis parameters and biochemical factors were analysed in a CRD design using ANOVA with the help of SAS 9.2 software. The different treatment means were separated by least significant difference test (LSD) at $p = 0.05$ (Gomez and Gomez 1984). Pearson's correlation coefficient was used as a measure of the relationship between different study parameters.

Results

Antibiosis studies

The damage score differed significantly among different selected genotypes (1.58 to 9.00) (Table 1). Significantly least damage score was recorded on KAUM182-1, CR3006-8-2 and RP4918-221. Similarly, IHRT-ME-25, W1263 and T12 fall under moderate resistant

Table 1. Seed box screening, honeydew excretion and nymphal emergence of *N. lugens* on selected rice genotypes

Genotype	Designation/Gene	Seed box score (Mean±SE)	Honeydew excretion (mm^2) (Mean±SE)	Nymphal emergence (Mean±SE)
RP4918-221	Swarna × *O. nivara* (U)	3.00 ± 0.33^{c}	49.64 ± 0.47^{c}	78.20 ± 0.35^{ef} (8.83)
KAUM182-1	Gouri × Thavalakannan (U)	1.97 ± 0.34^{cd}	46.76 ± 0.17^{cd}	69.20 ± 0.06^{fg} (8.31)
T12	Acc.56989 (*bph7*)	5.39 ± 0.25^{b}	84.52 ± 0.37^{b}	117.60 ± 0.50^{b} (10.83)
IHRT-ME-25	JKRH2064 (U)	5.46 ± 0.43^{b}	79.48 ± 0.22^{b}	101.40 ± 0.56^{cd} (10.04)
W1263	Eswarakorra × MTU15 (U)	5.44 ± 0.040^{b}	51.76 ± 0.34^{c}	110.60 ± 0.32^{bc} (10.51)
CR3006-8-2	Pusa44 × Salkathi (U)	2.86 ± 0.40^{cd}	48.56 ± 0.47^{c}	88.00 ± 0.46^{de} (9.36)
Ptb33	Arikkirai *(bph2 + Bph3)*	1.58 ± 0.09^{d}	35.08 ± 0.36^{d}	62.40 ± 0.22^{g} (7.88)
TN1	None	9.00 ± 0.00^{a}	190.40 ± 0.26^{a}	230.80 ± 0.44^{a} (15.17)
Df		7	7	7
Error df		16	32	32
F-value		62.85	99.82	22.84
p-value		<0.0001	<0.0001	<0.0001
CD ($p = 0.05$)		1.32	8.06	(0.75)

U = unknown; Figures in parentheses are the means of √n+1 transformations; Means with the same letter within a column are not significantly different (LSD: $P > 0.05$).

Table 2. Nymphal development period of *N. lugens* on selected rice genotypes

Genotypes	1st instar	2nd instar	3rd instar	4th instar	5th instar	Total nymphal duration
RP4918-221	1.80±0.12^{b} (1.33)	2.40±0.25^{cd} (1.54)	3.00±0.22^{bcd} (1.72)	4.70±0.20^{b} (2.16)	5.10±0.10^{b} (2.25)	17.00±0.24^{cd} (4.12)
KAUM182-1	2.00±0.11^{ab} (1.41)	3.00±0.22^{ab} (1.72)	4.30±0.26^{a} (2.06)	5.20±0.12^{a} (2.27)	5.90±0.15^{a} (2.42)	20.40±0.39^{b} (4.51)
T12	1.20±0.20^{c} (1.08)	2.20±0.12^{cd} (1.48)	2.90±0.10^{cd} (1.70)	3.70±0.13^{c} (1.92)	4.40±0.11^{c} (2.09)	14.40±0.29^{f} (3.79)
IHRT-ME-25	1.30±0.21^{c} (1.12)	2.20±0.13^{cd} (1.48)	3.30±0.20^{bc} (1.81)	4.10±0.10^{b} (2.02)	4.40±0.10^{c} (2.09)	15.30±0.32^{ef} (3.90)
W1263	2.00±0.05^{ab} (1.41)	2.50±0.16^{bc} (1.57)	3.60±0.10^{b} (1.89)	3.80±0.12^{c} (1.94)	4.40±0.19^{c} (2.09)	16.30±0.30^{de} (4.03)
CR3006-8-2	2.00±0.04^{ab} (1.41)	3.00±0.22^{ab} (1.72)	3.60±0.29^{b} (1.89)	4.60±0.19^{b} (2.14)	4.70±0.30^{bc} (2.16)	17.90±0.23^{c} (4.22)
Ptb33	2.20±0.12^{a} (1.48)	3.50±0.22^{a} (1.86)	4.70±0.12^{a} (2.16)	5.50±0.04^{a} (2.34)	6.00±0.08^{a} (2.44)	21.90±0.37^{a} (4.67)
TN1	1.10±0.10^{c} (1.04)	1.90±0.10^{d} (1.37)	2.60±0.19^{d} (1.60)	3.20±0.12^{d} (1.78)	3.70±0.12^{d} (1.92)	12.50±0.35^{g} (3.53)
Df	7	7	7	7	7	7
Error df	32	32	32	32	32	32
F-value	12.38	8.26	13.12	34.21	28.16	45.69
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Figures in parentheses are the means of $\sqrt{n+1}$ transformations; Means with the same letter within a column are not significantly different (LSD: $P>0.05$).

category. The area of honeydew excretion varied significantly among genotypes (35.08–190.40 mm^2) and it was in the ascending order among genotypes as Ptb33, KAUM182-1, CR3006-8-2, RP4918-221, W1263, IHRT-ME-25, T12 and TN1 (Table 1).

The total nymphal emergence was significantly low in resistant genotypes than in other genotypes (Table 1). The nymphal development period of all the five instars of BPH varied significantly (Table 2). The nymphal duration was prolonged on resistant genotypes than on moderately resistant genotypes (Table 2). Nymphal survival differed significantly among different selected genotypes and average survival ranged from 49.40–98.79%. Significantly least nymphal survival was observed on Ptb33 followed by KAUM182-1, RP4918-221 and CR3006-8-2 (Table 3). Likewise, significantly least growth index was observed on Ptb33 and KAUM182-1 followed by CR3006-8-2 and RP4918-221 whereas, the index was highest on TN1 (Table 3).

Table 3. Nymphal survival and growth index of *N. lugens* on selected rice genotypes

Genotype	Nymphal survival (%) (Mean±SE)	Growth index (Mean±SE)
RP4918-221	65.20±0.31^{c} (53.83)	3.86±0.84^{de}
KAUM182-1	60.60±0.34^{d} (51.10)	2.98±0.11^{ef}
T12	74.20±0.30^{b} (59.57)	5.16±0.25^{b}
IHRT-ME-25	73.80±0.28^{b} (59.27)	4.85±0.27^{bc}
W1263	74.80±0.25^{b} (59.94)	4.59±0.19^{bc}
CR3006-8-2	66.40±0.38^{c} (54.32)	3.04±0.52^{de}
Ptb33	49.40±0.13^{e} (44.63)	2.26±0.10^{df}
TN1	98.79±0.38^{a} (84.21)	7.93±0.23^{a}
Df	7	7
Error df	32	32
F-value	69.16	45.70
p-Value	<0.0001	<0.0001
LSD (p = 0.05)	(0.87)	1.09

Figures in parentheses are the means of arc sine √percentage transformations; Means with the same letter within a column are not significantly different (LSD: P>0.05).

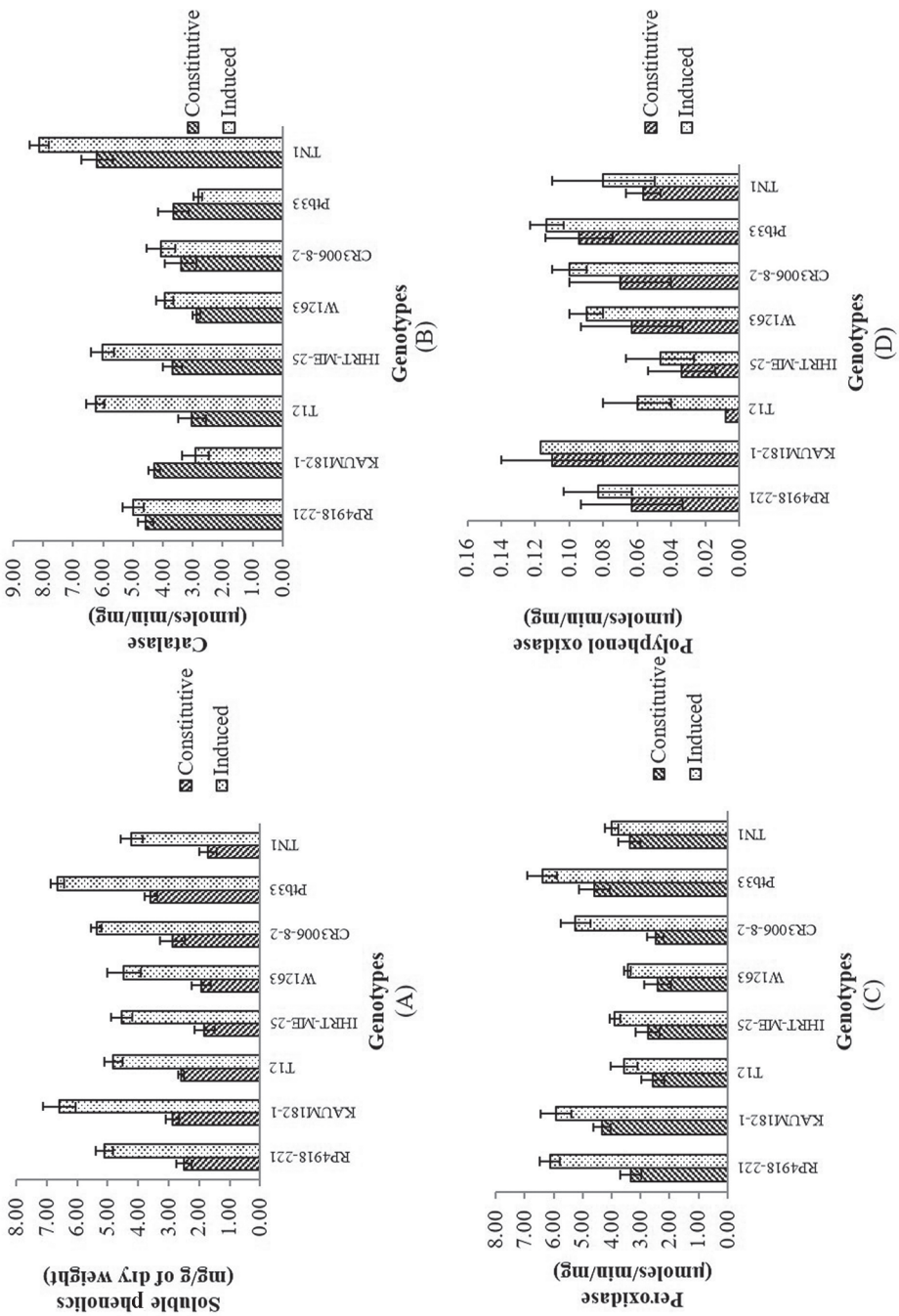

Figure 1. Quantitative changes in soluble phenolics (A), catalase (B), peroxidase (C) and polyphenol oxidase (D) in selected rice genotypes at constitutive and induced level after BPH infestation. Bars indicate biochemical content (mean ± SE). Error bars indicate standard error

Biochemical constituent studies

The soluble phenolic content at constitutive and induced level in plants was observed to be maximum in Ptb33 followed by KAUM182-1 and CR3006-8-2 (Table S1*; Fig. 1(A)). The activity of CAT in Ptb33 and KAUM182-1 decreased 22.53-32.91% from initial level after BPH infestation and partially increased in RP4918-221 (9.19%) and CR3006-8-2 (19.41%) (Table S1; Fig. 1(B)). The constitutive and induced activity of PO was maximum in resistant genotypes (Table S1; Fig. 1(C)). No significant difference was observed in the activity of PPO among genotypes at constitutive and induced levels of BPH infestation (Table S1; Fig. 1(D)).

Correlation studies

A negative relationship between honeydew excretion (–0.61, –0.50, –0.40), nymphal emergence (–0.70*, –0.58, –0.36), growth index (–0.81**, –0.70*, –0.59) and nymphal survival (–0.84**, –0.73*, –0.53) was observed between soluble phenolics, peroxidase and polyphenol oxidase activity, respectively, while a positive relationship was observed with catalase (Table 4). Likewise, a reverse trend was observed between nymphal development period and biochemical constituent (Table 4).

Table 4. Correlation between antibiosis components and biochemical constituents

Parameters	Soluble phenolics	Catalase	Peroxidase	Polyphenol oxidase
Honeydew excretion	–0.61	0.89**	–0.50	–0.40
Nymphal development period	0.94**	–0.93**	0.80**	0.78*
Nymphal emergence	–0.70*	0.85**	–0.58	–0.36
Growth index	–0.81**	0.93**	–0.70*	–0.59
Nymphal survival	–0.84**	0.89**	–0.73*	–0.53

*Significant at 5 per cent level of significance; **Significant at 1 per cent level of significance.

Discussion

Phenotyping for antibiosis

The seedbox screening method is widely adopted worldwide to phenotype the genotypes against BPH (Horgan et al. 2015; Sarao et al. 2016) due to its high throughput efficiency. This study mainly measures nymphal feeding response and is very subjective in terms of time of scoring the entries in relation to damage noted on the TN1. So this method has been widely adopted in reporting new sources of BPH resistance and studies on inheritance of resistance (Jena et al. 2002; Jena and Kim 2010) as well as used for map based cloning and characterization of BPH resistance genes (Wang et al. 2015; Hu et al. 2015).

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Measurement of honeydew excreted by BPH is a best method for complementing the seedbox screening in several studies (He et al. 2013; Jena et al. 2015). By computing the area of honeydew excretion, the feeding of the BPH could be determined. Resistant varieties inhibit the feeding activity of BPH due to different plant metabolites which make them less preferred for feeding and this was reflected in low honeydew excretion (He et al. 2013; Jena et al. 2015). Less nymphal emergence and prolong nymphal development period was found in resistant genotypes as compared to others due to nutritionally deficient food (Maheshwari et al. 2006). The nymphal survival is the best test for studying the antibiosis component of plant resistance. Even the resistant genotypes in the current study had shown a nymphal survival to the range of 49–66% (Seo et al. 2009; Qiu et al. 2011; He et al. 2013), which will put less selection pressure on the insect population and thus avoids the rapid adaptation of BPH. Lower growth index on resistant genotypes conveyed the adverse influence of plant nutrition on nymphal survival and development rate (Syobu et al. 2011; Kumar et al. 2012).

Enzymatic and non-enzymatic antioxidants related to defense

In our studies we observed that the changes in soluble phenolic content at induced level after BPH infestation was negatively correlated with BPH development. Similar observations were also reported by the other scientists (Vanitha et al. 2011; Dharshini and Gowda 2014). Rani and Jyotsna (2010) found that increase in the concentration of phenolic compounds is according to the extent of tissue damage caused by the feeding insects. Higher and timely activity of PO in resistant genotypes as compared to susceptible genotypes may interfere with the growth and development of BPH (Castillo 1992). PO may induce the formation of structural barriers such as thickened cell wall that inhibit feeding (Wei et al. 2009). PPPO activity decreases the nutritional quality of infested plants by converting soluble phenolic compounds into quinones that eventually prevent the digestion of proteins in insects (Alagar et al. 2007). We observed higher activity of CAT in susceptible genotypes which is an anti-oxidative enzyme and its inhibition leads to elevated levels of H_2O_2, which has a role in signalling pathways (Noctor and Foyer 1998; Grant and Loake 2000).

Rice genotypes KAUM182-1, RP4918-221 and CR3006-8-2 have displayed high level of antibiosis against BPH. These genotypes has emerged as a new sources of resistance to BPH which can be used in hybridization programme to breed durable BPH resistant rice varieties, which will ultimately form the core of IPM strategy for BPH management.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. Biochemical constituent of selected rice genotypes at constitutive and induced level after infestation by *N. lugens*