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Biochemical basis of resistance in rice landraces to brown planthopper, *Nilaparvata lugens* (Stal.)

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ABSTRACT

Laboratory estimation of chemical constituents such as total phenols, total soluble protein, total soluble sugar, total reducing sugars, nitrogen, phosphorus, potassium was made in healthy and BPH infested leaf tissues of 22 landraces, one resistant check (PTB-33) and two susceptible checks (TN1 and Jaya). Total phenol content was highest in healthy tissues of resistant landrace, PTB-33 and as a result of BPH feeding, the phenol content was increased in all the entries at different degrees. Significant differences were observed in total soluble protein and total soluble sugars in healthy tissues of landraces. Both total soluble protein and total soluble sugar content was reduced in leaf tissues of BPH infested plants. Total nitrogen content was highest in susceptible landraces and susceptible checks TN 1 and Jaya. Nitrogen content was depleted in the BPH infested plants when compared to healthy tissues. Significantly higher amount of potassium was found in resistant and moderately resistant landraces when compared to TN1 and Jaya. These resistant landraces could be added as new resistant donors and utilized in resistance breeding programme against brown planthopper in rice.

KEY WORDS: Hopper burn, landraces, *Nilaparvata lugens,* resistance

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae) is one of the most serious pests of rice. The hopper causes direct damage by sucking plant sap, which often results in hopper burn and indirect damage by transmitting viral diseases such as grassy stunt (Ling *et al*., 1970) and ragged stunt (Ling *et al*., 1978). Though chemical control of BPH is commonly practiced, it is both costly and harmful to the environment. One of the most economical, efficient and environmentally friendly means to manage this pest is the use of resistant cultivars (Heinrichs, 1980). However, the development of *N. lugens* biotypes capable of surviving and damaging resistant cultivars is a constant threat (Pathak and Heinrichs, 1982). Therefore, identification and deployment of new genes for BPH resistance in modern high yielding varieties is the important strategy to reduce the yield loss.

Traditional landraces are important reservoirs of valuable traits like medicinal properties, nutrition, taste, aroma, tolerance to drought and varying level of resistance to insect pests and diseases (Hanumaratti *et al.,* 2008). Traditional rice

varieties had been documented to have contributed to the origin of 1,709 modern rice varieties (Lang et al., 2009). Though some brown planthopper (BPH) resistant rice genotypes have been identified, there has been break-down of resistance. Keeping this in view, a study was undertaken to identify new source of resistance and determine few biochemical factors associated with BPH resistance in select landraces of rice.

MATERIALS AND METHODS

The present investigations were undertaken at Zonal Agricultural Research Station (ZARS), Vishveshvaraiah Canal (V. C) Farm, Mandya, Karnataka during 2012. Two hundred and eighty landraces collected from different parts of Karnataka were screened for their reaction to BPH populations. Out of 280 landraces, 22 landraces with high degree of resistance were selected for studying biochemical basis of resistance.

Mass culturing of BPH

In order to obtain different instars of BPH required for the present investigation, the insect was mass reared in wooden cages with fine mesh wire net. Four to six weeks old plants of susceptible rice variety TN1 or Jaya were used for culturing the BPH. The potted TN1 or Jaya plants were placed in oviposition cage and gravid females obtained from maintainance cage were released on to the potted TN1 plants for oviposition. Then the oviposited plants were placed in maintenance cage for hatching of eggs. The host plants in maintenance cage were changed twice a week and replaced them with fresh plants.

Seedlings of 22 landraces, resistant check PTB-33 and susceptible checks, Jaya and TN 1 were grown separately in plastic pots in two sets. Fifteen days after sowing one set of all the 22 landraces were caged with 10 BPH nymphs and another set of plants were maintained to serve as control without infestation. Number of insects was kept constant by replacing the dead nymphs daily. Thirty days after release the leaf sheaths infested with BPH were sampled along with control plants. These samples were dried at 32 ^{0}C in a hot-air oven for 24-48 hours and leaf sheaths of the test entries powdered using pestle and mortar. These powdered samples were sieved through a 100 mesh screen and stored in the sealed containers at 4 ^{0}C, for estimation of biochemical constituents. The total phenol content in each of the samples was estimated by following the procedure suggested by Malick and Singh (1980). The total soluble protein content of rice plant samples were determined as per Lowry *et al.* (1951). Similarly total soluble sugar content in each of the samples were estimated by adopting the procedure suggested by Dubois *et al.* (1956).

Nitrogen was estimated by using kjeldahl method (Bremner, 1965) and data were expressed in percentage. For estimation of phosphorus and potassium 1g ground plant material was digested using di-acid mixture on low heat hot plate in a digestion chamber until liquid become colourless. After cooling, the volume was made up with glass distilled water and solution was filtered through Whatman No.1 filter paper. Aliquots of this solution were used for the determination of phosphorus and

potassium. The phosphorus content of samples were determined by the wet oxidation procedure as outlined by Jackson (1973) and di-acid digested sample was used to estimate potassium by using digital flame photometer method.

RESULTS AND DISCUSSION

The results of analysis of various biochemical constituents *viz*., total phenols, total soluble protein, total soluble sugar, total reducing sugars, nitrogen, phosphorus and potassium in leaf sheaths of different test entries in both healthy and BPH infested plants has been presented in the table 1 and 2

The results revealed that the total phenolic content in the healthy leaves of landraces was significantly more when compared to susceptible check Jaya and TN 1. Increased level of phenols was observed in landraces Ratnachoodi-1(0.63 mg/g), JBT 36/14(0.62 mg/g) and Rajamudi (0.63 mg/g). However BPH infestation resulted in increased phenolic production in all the landraces with varying levels. Significant increase in phenol content after BPH feeding was observed in Akkalu-1 (0.49 to 1.65 mg/g), JBT 36/14(0.62 to 2.12 mg/g), Kottayam(0.54 to 1.87 mg/g), Nazarbatta (0.49 to 1.73 mg/g), PS-339(0.50 to 1.70 mg/g) and Raibhog (0.48 to 1.67 mg/g). In resistant check (Ptb-33) the phenol content increased from 0.62 to 2.31 mg/g but in susceptible check Jaya and TN-1 also the phenol content was found to increase from 0.27 to 0.91 mg/g and 0.21to 0.76 mg/g respectively. Earlier workers reported that increase in phenolic compound in plants as a result of infestation is considered to be a common reaction to herbivory (Karban and Baldwin, 1977). The present findings lies in conformity with findings of Mishra and Misra (1991) and they reported that infestation of BPH on rice resulted in the increased phenolic content in resistant varieties. In the present study the increase in phenolic content after infestation in susceptible checks and also resistant landraces indicates that the increase in phenolic content is injury specific.

As regards to total soluble protein content, it was observed that the susceptible TN 1 and Jaya had higher amount of total soluble protein recording 5.02 and 5.62 mg/g respectively than the rest of the entries. In contrast the most of the landraces (except Akkalu-2, Chinnaponni, Karpoorakeli and Ugibatta) and resistant check Ptb-33 was recorded to have lesser amount of total soluble protein ranging from 1.63 to 4.91 mg/g tissue. Higher amount of protein content was negatively correlated with resistance There was a decrease in total soluble protein content as a result of BPH feeding in all the entries with different magnitudes. In susceptible check, TN 1 and Jaya the total soluble protein decreased from 5.02 to 4.20 mg/g and 5.62 to 4.78 mg/g, respectively. These results are in agreement with the observations made by Sogawa (1971), wherein, BPH infestation was reported to cause decrease in soluble protein. As chlorosis increased, the protein content of the leaves decreased steadily.

Total soluble sugar content was found highest in TN1(82.59 mg/g) and Jaya (81.82 mg/g), where as the resistant check Ptb-33 found to have least amount of total soluble sugar content recording 23.10 mg/g. The rest of the entries possessed 24.23 to 46.50 mg/g of total soluble sugar. Due to BPH feeding,

depletion in total sugars in TN1 and Jaya was significantly high. In landraces the depletion was observed from 26.51 to 25.27 mg/g in JBT 36/14 and 37.89 to 33.85 mg/g in Mysore mallige. The results are in agreement with the results of Sujatha *et al.,* (1987). They mentioned that higher amounts of total sugars have been reported in BPH susceptible Tellahamsa and Jaya varieties. Sugars functions as potent sucking stimulants for BPH (Koyama, 1981)

The data on nitrogen content revealed that significantly higher per cent of N in susceptible check TN1 (1.21%) and Jaya (1.25%) and the values are on par with the values of Akkalu-2 (1.19%) and Chinnaponni (1.20%). In other landraces nitrogen content varies from 0.31 to 1.12 per cent. After BPH infestation the nitrogen content depletes by higher percentage in susceptible check Jaya and TN1 where as in other landraces and resistant check depletion was less. This indicates that less depletion of proteins in resistant landraces compared to susceptible check. The present findings lies in conformity with findings of Reddy *et al.,* (2004).

There was no significant difference in phosphorus content between resistant and susceptible check. The phosphorus content ranges from 0.31 to 0.59% in landraces even though the differences were statistically significant. After BPH feeding also no significant difference in per cent phosphorus was observed indicating that phosphorus had no role in offering resistance by rice landraces against BPH.

The potassium content in landraces varies from 0.83 to 1.88 per cent but the variation among them was marginal. Also found that the decrease in potassium content after BPH infestation was significantly high in susceptible check Jaya (1.39 to 1.28%) and TN1 (1.42 to 1.30%). The decrease in potassium content in landraces after BPH feeding was found significantly less. Samiayyan and Janarthanan (1988) suggested that the reduction in populations of BPH, WBPH and GLH at high dosa of potassium iwas partly due to the fertilizer enhancing protein synthesis and making plant less favourable for reproduction of sucking pests.

CONCLUSION

The overall results revealed that the rice landraces with lower levels of total soluble protein, total soluble sugars, nitrogen content and higher levels of total phenol and potassium content were resistant to BPH feeding. These landraces with high level of resistance to BPH infestation possibly serves as resistance source for further development of resistant varieties.

Table 1: Certain chemical constituents present in healthy and BPH infested plants of selected landraces of rice[@]

Entry	Total phenols (mg/g)		Total soluble protein (mg/g)		Total soluble sugars (mg/g)		Total reducing sugars (mg/g)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
Akkalu 1	0.49^{de}	1.65^{fg}	3.38^{fg}	3.07^{ef}	38.61^{h}	35.80^{h}	16.98^{i}	13.57^{h}
Akkalu 2	0.40^{fgh}	1.28^{hi}	5.18^{ij}	4.53^{j}	41.87^{i}	37.72^{i}	21.08^{m}	15.87^{j}
Anilamanil	0.57^{bc}	1.62^{fg}	3.87^{h}	3.57^{gh}	31.97^{f}	29.58^{ef}	14.78^{g}	12.86^{g}
Baiganmanji	0.60^{ab}	1.9^{d}	2.04^{bc}	1.96^{bc}	30.24^{d}	28.81^{def}	13.87^{f}	12.77^{g}
Chinnaponni	0.36^{i}	1.08^{kl}	5.23^{ij}	4.52^{ij}	43.50^{j}	39.73^{j}	18.87^{k}	14.90^{i}
Honasu 1	0.57^{bc}	1.86^{de}	1.98^{bc}	1.93^{bc}	25.29^{b}	23.83^{bc}	11.28^{cd}	10.27^{cd}
Honasu 2	0.38^{ghi}	1.22^{ijk}	4.91^{i}	4.48^{ij}	35.97^{g}	33.23^{h}	19.78^{l}	15.96^{j}
JBT 36/14	0.62^{a}	2.12^{bc}	1.72^{ab}	1.64^{ab}	26.51^{c}	25.27^{c}	12.02^{de}	10.63^{d}
Kalakolli	0.39^{fghi}	1.10^{kl}	3.99^{h}	3.69^{h}	30.68^{de}	28.23^{def}	13.97^{f}	12.01^{ef}
Karpoorakeli	0.37^{hi}	1.12^{jk}	5.57^{k}	4.96^{k}	42.17^{i}	37.90^{i}	20.82^{m}	16.18^{j}
Kottayam	0.54^{c}	1.87^{de}	2.79^{d}	2.63^{d}	31.78^{fe}	29.45^{ef}	12.47^{e}	11.68^{e}
Manila	0.41f^{g}	1.25^{hij}	3.72^{h}	3.28^{fg}	36.60^{g}	33.27^{h}	14.75^{g}	13.56^{h}
Mysore mallige	0.39^{fghi}	1.20^{ijk}	3.51^{fg}	3.16^{f}	37.89^{h}	33.85^{h}	17.81^{j}	14.67^{i}
Nazarbatta	0.49^{de}	1.73^{ef}	2.91^{d}	2.78^{de}	26.81^{c}	25.17^{c}	13.89^{f}	12.79^{g}
PS 339	0.50^{d}	1.70^{fg}	1.87^{abc}	1.78^{abc}	24.23^{ab}	22.78^{ab}	11.20^{c}	9.20^{b}
Ratnachoodi 1	0.63^{a}	1.98^{cd}	2.18^{c}	2.10^{c}	24.83^{b}	23.37^{ab}	13.71^{f}	12.47^{fg}
Ratnachoodi 2	0.47^{de}	1.56^{g}	3.68^{gh}	3.49^{gh}	32.21^{f}	30.27^{g}	13.82^{f}	11.98^{ef}
Raibhog	0.48^{de}	1.67^{fg}	1.98^{bc}	1.90^{abc}	30.09^{d}	28.13^{de}	10.17^{b}	9.63^{bc}
Rajamudi	0.63^{a}	1.88^{d}	2.95^{de}	2.82^{de}	31.86^{f}	29.65^{f}	10.97^{c}	10.39^{d}
Selamsanna	0.42^{f}	1.33^{hi}	3.27^{ef}	3.07^{ef}	30.56^{d}	28.30^{def}	15.79^{h}	12.46^{fg}
Ugibatta	0.40^{fgh}	1.31^{hi}	5.47^{jk}	4.72^{jk}	46.50^{k}	42.15^{k}	19.73^{l}	14.73^{i}
Moradde	0.46^{e}	1.39^{h}	3.30^{f}	3.10^{ef}	29.67^{d}	27.71^{d}	15.53^{gh}	13.02^{gh}
PTB 33	0.62^{a}	2.31^{a}	1.63^{a}	1.59^{a}	23.10^{a}	22.12^{a}	7.87^{a}	7.46^{a}
TN 1	0.27^{j}	0.91^{m}	5.02^{i}	4.20^{i}	82.59^{l}	73.35^{m}	31.79^{n}	22.67^{k}
Jaya	0.21^{k}	0.76^{n}	5.62^{k}	4.78^{jk}	81.82^{l}	71.81^{l}	34.81^{o}	23.77^{l}
CD@p=0.05	0.033	0.14	0.329	0.327	1.161	1.519	0.718	0.699

In a column, means with same letter are not significantly different at 5% level by DMRT, @= 30 days old plants : Average of 3 replications

Table 2: Mineral contents in healthy and BPH infested plants of selected landraces

Entry	Total nitrogen(%)		Phosphorus (%)		Potassium (%)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested
Akkalu 1	0.81^{i}	0.74^{f}	0.412^{cde}	0.409^{cd}	1.39^{h}	1.31^{kl}
Akkalu 2	1.19^{m}	0.98^{ij}	0.501^{g}	0.497^{fghi}	1.45^{fgh}	1.35^{jk}
Anilamanil	0.70^{fg}	0.66^{e}	0.481^{fg}	0.475^{efgh}	1.60^{de}	1.54^{f}
Baiganmanji	0.40^{bc}	0.38^{b}	0.392^{cd}	0.389^{cd}	1.66^{cd}	1.62^{e}
Chinnaponni	1.20^{m}	1.02^{k}	0.480^{fg}	0.476^{efgh}	1.47^{fgh}	1.37^{ij}
Honasu 1	0.39^{bc}	0.36^{b}	0.563h	0.557^{ijk}	0.83^{i}	0.79^{m}
Honasu 2	0.98^{k}	0.89^{h}	0.500^{g}	0.497^{fghi}	1.50^{ef}	1.42^{gh}
JBT 36/14	0.31^{a}	0.29^{a}	0.312^{ab}	0.310^{ab}	1.71^{c}	1.67^{d}
Kalakolli	0.68^{f}	0.65^{e}	0.570^{h}	0.566ijk	1.67^{cd}	1.61^{e}
Karpoorakeli	1.12^{l}	0.98^{ij}	0.381^{cd}	0.378^{bcd}	1.47^{fgh}	1.37^{ij}
Kottayam	0.50^{d}	0.47^{c}	0.480^{fg}	0.476^{efgh}	1.69^{c}	1.65^{de}
Manila	0.87^{j}	0.80^{g}	0.591^{h}	0.586^{k}	1.52^{f}	1.44^{gh}
Mysore mallige	0.73^{gh}	0.66^{e}	0.300^{a}	0.298^{a}	1.53^{f}	1.45^{g}
Nazarbatta	0.53^{d}	0.49^{c}	0.420^{de}	0.417^{cdef}	1.69^{c}	1.65^{de}
PS 339	0.38^{bc}	0.35^{b}	0.311^{ab}	0.309^{ab}	1.82^{ab}	1.78^{bc}
Ratnachoodi 1	0.37^{b}	0.35^{b}	0.431^{def}	0.428^{cdefg}	1.79^{b}	1.75^{c}
Ratnachoodi 2	0.76^{h}	0.72^{f}	0.490^{g}	0.486^{efgh}	1.58^{e}	1.52^{f}
Raibhog	0.41^{c}	0.38^{b}	0.450^{efg}	0.447^{efg}	1.88^{a}	1.84^{a}
Rajamudi	0.52^{d}	0.49^{c}	0.381cd	0.512^{hij}	1.86^{ab}	1.82^{ab}
Selamsanna	0.81^{i}	0.75^{f}	0.400^{cde}	0.397^{cd}	1.59^{de}	1.53^{f}
Ugibatta	1.09^{l}	0.96^{i}	0.390^{cd}	0.387^{bcd}	1.50^{ef}	1.40^{hi}
Moradde	0.59^{e}	0.53^{d}	0.420^{de}	0.417^{cde}	1.60^{de}	1.54^{f}
PTB 33	0.30^{a}	0.28^{a}	0.380^{cd}	0.377^{bcd}	1.71^{c}	1.69^{d}
TN 1	1.21^{m}	0.99^{ij}	0.421^{de}	0.418^{cdef}	1.42^{gh}	1.30^{l}
Jaya	1.25^{n}	1.01^{jk}	0.360^{bc}	0.357^{abc}	1.39^{h}	1.28^{l}
CD@p=0.05	0.032	0.035	0.052	0.076	0.084	0.047

In a column, means with same letter are not significantly different at 5% level by DMRT, @= 30 days old plants : Average of 3 replications

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