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Biochemical bases of resistance to brown planthopper (*Nilaparvata lugens*) (Stål) in different rice accessions

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ABSTRACT

Host plant resistance has been a valuable tool in the management of planthoppers. The experiment was carried out to assess the level of total phenols, Ortho-dihydroxy phenols, Phenols, crude silica and total sugars in BPH (Brown Plant Hopper) affected rice leaves. Resistant varieties showed high amount of phenols, O.D. Phenols and crude silica as against low quantity of total sugars. These, biochemical basis of resistant method is also identifying the resistant variety against brown planthopper (*Nilaparvata lugens)* in rice.

Key words: Brown planthopper, Crude silica, Rice accessions, Total phenols, Total sugars.

INTRODUCTION

The brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae) was formerly a minor pest of rice in south and south east Asia but now has become a major problem throughout these regions (Dyck and Thomas, 1979). Following the introduction of high yielding varieties and chemical insecticides the BPH has become serious constrain in rice production. Host plant resistance is a major economic and desirable practice for the management of Brown Plant Hopper (BPH) (Chelliah, 1985). Understanding the mechanism of resistance is important before evolving resistant varieties. Analyses of bio-chemical constituents revealed low content of total sugars as against higher quantities of total phenols, Ortho-dihydroxy phenols and silica in all the moderately resistant varieties. Resistant rice varieties appear to have higher levels of phenolic compounds, lower levels of free amino acids, and lower concentrations of reducing sugars (Thayumanavan *et al* 1990). Yesuraja and Mariappan (1993) reported that the resistant varieties had higher amount of total phenols and O.D. Phenols. Sogawa (1971) reported that no change in level of free sugars in the rice leaf blades prior to the drying up of the infested plants. Certain amino acids, sucrose, and organic acids were found to act as feeding stimulants (Sakai and Sogawa 1976). The content of reducing sugars in the resistant varieties was less when compared to the susceptible ones and the amount of total and non-reducing sugars was high in resistant varieties (Saxena, 1986; Bharathi, 1991). Yoshihara *et al*. (1979 and 1980) reported that the soluble silicic acid act as a sucking inhibitor for brown planthopper. There is still no evidence to indicate that oxalic acid content is higher in the phloem of Mudgo compared with susceptible variety, TN1. Silicic acid occurs in both resistant and susceptible rice varieties (Saxena, 1986).

MATERIALS AND METHODS

Plant samples of selected rice accessions/varieties were collected at 30, 45 and 60 DAT. The following methods were used to determine the biochemical components from the collected samples and each biochemical analysis was replicated thrice. The following varieties were used for biochemical analysis. BG 367-2, IR 72, ACK 09030, GEB 24, Kattanur, PB 1460, Thogai samba, HKR 47, TN-1 (Susceptible check).

Total phenols: Total phenol content was estimated by the method suggested by Malick and Singh (1980). A quantity of 100 mg of plant sample was extracted with 80 percent ethanol and was centrifuged at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness and the residue was dissolved in 5 ml of distilled water. The aliquots of 0.2 to 2 ml were pipetted out and the volume was made up to 3 ml with distilled water. A quantity of 0.5 ml of folin-ciocalteau reagent and 2 ml of 20 percent sodium carbonate solution was added. Then it was kept in a boiling water bath for one minute, cooled and the colour developed was measured at 650nm wave length using Spectronic 20. Phenol content was calculated by drawing a standard graph with catechol as standard and expressed as catechol equivalents.
Ortho-dihydroxy phenols

The method suggested by Arnow (1937) was followed for the estimation of OD phenols. Ethanol extract was prepared as mentioned in the estimation of total phenols. To 1 ml of the extract, 1 ml of 0.5 N hydrochloric acid, 1 ml of Arnow's reagent and 2 ml of 1 N sodium hydroxide were

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added. The volume was made up to 25 ml with distilled water and the colour developed was read in Spectronic 20 and OD phenol was calculated from the standard graph with phyrogallol as standard.

Total sugars: Total sugar content of the selected rice accessions was estimated by the procedure given by Hedge and Hofreiter (1962). One gram of the sample was hydrolyzed by keeping it in a water bath for three hours with 5 ml of 2.5 N HCl. The sample was neutralized using solid sodium carbonate and the volume was made upto 100 ml. The sample was centrifuged and the supernatant was used for analysis. Aliquots of 0.5 and 1.0 ml were taken from the sample and the volume was made upto one ml using distilled water in all the tubes. Four ml of anthrone reagent was added and the tubes were kept in boiling water bath for eight minutes. Tubes were then cooled immediately and the green colour was read at 630 nm by using spectrophotometer. A standard curve was prepared using different concentrations of glucose. The amount of soluble sugars present in the sample was calculated using the standard graph.

Crude silica: Crude silica was estimated by the method suggested by Yoshida *et al*.,(1959). One gram of oven dried sample was taken in a 75 ml pyrex test tube and 10 ml of acid mixture (Conc. Nitric acid, hydrochloric acid and sulphuric acid in the ratio 5: 2: 1) was added and digested in a fume hood for 2 hrs. Then heated over a low gas flame gradually increase the heat until the mixture became clear. The contents were then cooled and the test tube was filled with distilled water up to 50 ml mark and filtered the sample extract through an acid washed filter paper of whatmann No.44. The filter paper and the residue of the sample extracts were dried in an oven at 80^{o}c. Then the filter paper with residue was ashed by placing in a muffle furnace and the amount of crude silica was calculated by

$$\text{Crude silica} = \frac{\text{Wt. of crude silica (g)}}{\text{Wt of sample}} \times 100$$

RESULTS AND DISCUSSION

Total phenols: The data on the phenol content in the selected rice accessions at different ages of the crop brought out the significant variability among the accessions as well as plant age. Significant interaction was also observed. The amount of total phenol was observed to be maximum in the leaf sheath of HKR 47 (10.460 mg/g) compared to the susceptible check TN-1 (3.887 mg /g) (Table.1). In general the total phenol content was three times higher than TN-1 in moderately resistant accessions. Similar was the trend at each plant age also. The results revealed that total sugars had a negative relationship and phenols, O.D phenols and silica had a positive relationship with resistance. Sujatha *et al*.(1987) suggested that the phenol and silica contents were positively correlated with resistance. Regarding phenolics there was high accumulation of total phenols in the leaf sheath of varieties namely, HKR 47, PB 1460, BG 367-2 and GEB 24. It was further observed that the sheaths of resistant plants contained more of phenols than in susceptible check TN-1.

Ortho- dihydroxy phenol: In contrast to the total phenols the content of O.D. Phenol was uniformly lower in the leaf sheath of all the accessions. Here again among the accessions BG367-2 had higher O.D.phenols (4.717 mg/ g) and it was very low in the susceptible check TN-1 (1.669 mg/g) (Table.2). The O.D. Phenol content was also increased as the plant age increased.

Total sugars: Data on the level of total sugar content of leaf sheath of different ages of the crop are presented in Table 3. There was a significant difference in the total sugar content between the different accessions in all the three ages of the plant. Decrease in content of the total sugars with increase in plant age was observed. The mean amount of total sugars was lowest in Thogai Samba accession (7.409 mg/ g) and highest in susceptible check TN-1 (16.578 mg/g). Sugars have been implicated in host- plant resistance to insect pests in a number of instances (Susidko and Felko, 1977). Sogawa (1977) reported the preference of BPH under laboratory conditions for various sugars like sucrose, glucose and dextrose. This forms the basis for resistance in the crop plants

Table 1: Estimation of total phenols in selected rice accessions

Rice accessions	Total phenols (mg/g) at indicated days after transplanting*			Mean
	20 DAT	40 DAT	60 DAT	
BG 367-2	9.254	10.266	11.536	10.356^{ba}
IR 72	4.987	7.051	8.161	6.733^{d}
ACK 09030	5.097	7.839	9.156	7.364^{d}
GEB 24	8.059	10.147	10.825	9.677^{c}
Kattanur	5.055	7.769	9.107	7.310^{d}
PB1460	8.606	10.769	11.728	10.367^{a}
Thogai Samba	6.843	8.326	9.859	8.342^{cd}
HKR 47	9.956	10.072	11.353	10.460^{bc}
Kuruvai Kalanjiyan	5.036	7.156	8.441	6.878^{e}
TN-1 (Susceptible check)	3.441	3.941	4.280	3.887^{f}

* Mean of three replications in a column, means followed by the common letter are not significantly different (P = 0.05) by LSD

Table 2: Estimation of Ortho- dihydroxy phenols in selected rice accessions

Rice accessions	Ortho- dihydroxy phenols (mg/g) at indicated day after transplanting*			Mean
	20 DAT	40 DAT	60 DAT	
BG 367-2	3.842	4.737	5.573	4.717[a]
IR 72	2.037	3.249	3.978	3.088[a]
ACK 09030	2.005	2.970	3.761	2.912[a]
GEB 24	3.489	3.842	4.479	3.936[a]
Kattanur	3.642	4.160	5.745	4.515[a]
PB1460	2.065	3.478	4.779	3.440[a]
Thogai Samba	4.275	5.053	5.856	5.061[a]
HKR 47	3.486	3.861	4.479	3.942[a]
Kuruvai Kalanjiyan	3.097	3.021	4.939	3.685[a]
TN-1 (Susceptible check)	1.089	1.654	2.265	1.669[b]

* Mean of three replications in a column, means followed by the common letter are not significantly different (P = 0.05) by LSD

Table 3: Estimation of total sugars in selected rice accessions

Rice accessions	Total sugars (mg/g) at indicated days after transplanting*			Mean
	20 DAT	40 DAT	60 DAT	
BG 367-2	11.859	9.872	7.134	9.622[a]
IR 72	14.772	11.047	8.965	11.594[d]
ACK 09030	14.771	11.068	9.359	11.732[d]
GEB 24	12.637	10.063	8.468	10.389[c]
Kattanur	10.343	9.048	6.992	8.794[b]
PB1460	14.574	11.953	8.951	11.826[d]
Thogai Samba	9.649	7.250	5.327	7.409[a]
HKR 47	13.590	10.884	8.345	10.508[c]
KuruvaiKalanjiyan	12.431	10.752	8.342	10.508[c]
TN-1 (Susceptible check)	17.181	17.115	15.979	16.758[e]

* Mean of three replications in a column, means followed by the common letter are not significantly different (P = 0.05) by LSD

and hence low sugar has been always considered as one of the desirable qualities of a resistant plant (Samal *et al.*, 1982).

Crude silica

The data on the crude silica content in the selected rice accessions brought out significant variability between the accessions and the susceptible check TN-1. The amount of silica was found to be higher in BG 367-2 (14.99) (Table.4), ACK 09030 (14.95) and Thogai Samba (14.52) in compared to the susceptible check TN-1 (9.00). The moderately resistant varieties had more amount of crude silica content when compared to the susceptible check in TN-1. Similarly Yoshihara *et al.*, (1979) reported positive correlation for silica content.

Table 4: Estimation of crude silica in selected rice accessions

Rice accessions	Silica*(%)
BG 367-2	14.99[a]
IR 72	11.47[d]
ACK 09030	14.95[ab]
GEB 24	12.67[cd]
Kattanur	13.46[cd]
PB1460	12.61[d]
Thogai Samba	14.52[bc]
HKR 47	11.50[e]
Kuruvai Kalanjiyan	14.26[ab]
TN-1 (Susceptible check)	9.00[f]

* Mean of three replications in a column, means followed by the common letter are not significantly different (P = 0.05) by LSD

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