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Studies on antibiosis basis of resistance exhibited by rice landraces to brown planthopper, *Nilaparvata lugens* (Stal.)

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Abstract
Rice landraces *viz.*, Mysore Malli, Chetty Samba, Panamara Samba, Vellai Gundu Samba, Vellai Kombi Samba and standard susceptible and resistant checks, TN 1 and PTB 33, resp., were evaluated for unveiling their biochemical constituents such as total sugars, total protein and total phenols upon infestation by the major sucking pest of rice, Brown Planthopper (BPH). Total sugars and total protein content in healthy samples of rice landraces showed significant differences in their content when compared with the susceptible check. BPH infestation reduced the total sugars and total protein content. BPH infested susceptible check, TN 1 showed highest reduction in total sugars and total protein (34.46 & 44.26%, resp.) over healthy plants and lowest reduction was observed in PTB 33 (20.67 and 25.10%, resp.). Total phenol content was highest in healthy PTB 33 (5.25 mg/g) followed by the resistant landrace, Mysore Malli (4.91 mg/g), when compared to the susceptible check TN 1 (2.41 mg/g) and as a result of BPH infestation, all the tested entries showed increase in phenol content. After BPH infestation, highest percent increase of phenol content over healthy plants was observed in PTB 33 (47.44%) followed by Vellai Kombi Samba (46.08%) and lowest in TN 1 (31.11%). Thus the lower sugar and protein content and increased phenol content in resistant landraces were responsible for the antibiosis basis of resistance against BPH.

Keywords: Rice landrace, brown planthopper, total sugars, total protein, total phenols

Introduction

Rice, *Oryza sativa* L. (Poaceae) is a staple food crop cultivated in most of the tropical and sub tropical countries of the world, fulfilling the dietary needs of nearly half of the world's population. It serves as a crucial food source, contributing to more than 21 percent of the world's total caloric consumption and accounting for as much as 76 percent of the calorie intake for people residing in Southeast Asia (Zhao *et al*., 2020) [25]. According to United States Department of Agriculture (USDA, 2022) [19] the primary rice producers during 2020-21 were China, leading with a production of 148.30 million tonnes (MT), followed by India at 120.00 MT, being the second largest producer and consumer of rice. Asia stands as the world's foremost rice producer, generating a remarkable 90.6 percent of the total global rice production (Bandumula, 2018) [1]. Due to biotic and abiotic stress experienced by the crop, the productivity of rice has been declined. In biotic stres, insect pests are of prime importance. Brown planthopper (BPH) is a monophagous pest of rice which can cause yield loss upto 60-100% in susceptible rice variety and the infested plants become brown and dry which is commonly referred as “Hopper burn” and also it damage the crop indirectly by acting as a vector for virus diseases like grassy stunt and ragged stunt. Utilizing host plant resistance is a prominent and economically valuable strategy for effectively controlling the Brown Plant Hopper (Chelliah, 1985) [3]. Understanding the mechanism of resistance is an essential prerequisite prior to the development of resistant varieties. Rice varieties with resistance appears to exhibit higher phenolic compound levels, reduced free amino acid levels, and lower concentrations of reducing sugars. The study aimed to investigate biochemical basis of resistance mechanisms and to quantify the biochemical constituents *viz.* total sugars, total protein and total phenols of resistant rice landraces against BPH.

Materials and Methods

Biochemical changes induced by BPH feeding

The present study on biochemical analysis were carried out in the Department of biochemistry, Tamil Nadu Agricultural University, Coimbatore. Total of 5 landraces, *viz. Mysore* Malli, Chetty Samba, Panamara Samba, Vellai Gundu Samba, Vellai Kombi Samba and standard check TN 1 and PTB 33 were taken to identify the biochemical aspects of resistance mechanism. Twenty 3rd to 4th instars of BPH nymphs were released on each of the 30 days old transplanted plants (1-3 tillers/pot) grown in mud pots and covered with mylar film cages to prevent the escape of nymphs. Samples were collected at 0 day (Healthy sample) and 3 days after infestation (DAI). Collected samples were used to determine the biochemical components *viz*. total sugars, total protein and total phenols.

Total sugars

Total sugars in the leaf sheath were estimated by following the anthrone method (Hodge and Hofreiter, 1962) [6]. 100 mg of sample was taken in a boiling tube and hydrolysed with 5 ml of 2.5 N HCL in the boiling water bath for three hours. To neturalize the sample, solid sodium carbonate was added until the effervescence ceases. Then the volume was made upto 100 ml with distilled water and centrifuged at 10,000 rpm for 10 min. From supernatant 0.5 ml was taken in a test tube and the volume was made upto 1 ml with distilled water. Then 4 ml of anthrone reagent was added into each test tube. The sample was observed for green to dark green colour development and absorbance was measured using UV visual spectrophotometer at 630 nm against blank. For standard curve, glucose was prepared at different concentrations. From the standard curve, the concentration of total sugars was determined and expressed in mg /g.

Total protein

Protein present in leaf sheath was estimated by following the procedure given by Lowry *et al.* (1951) [8]. 100 mg of leaf sheath was weighed and homogenized with 0.1 M sodium phosphate buffer (pH:7). Homogenized samples were centrifuged at 10,000 rpm for 10 min. The supernatant was collected and used for protein estimation. In a test tube 0.5 ml of supernatant was taken and the volume was made upto 1 ml using distilled water. Then 5 ml of alkaline copper tartarate solution and 0.5 ml of folin-ciocalteau reagent were added and kept in dark for 10 - 20 min for development of blue colour and absorbance was measured at 660 nm using UV visual spectrophotometer. Standard curve was prepared using bovine serum albumin fraction V at different concentrations. From the standard curve, the concentration of protein was determined and expressed in mg /g.

Total phenols

Total phenol content in rice leaf was estimated by the method given by Malik and Singh (1980) [10]. 100 mg of leaf sheath was weighed and extracted with 80 percent ethanol and then extracted sample was centrifuged at 10,000 rpm for 20 minutes and supernatant was collected. Collected supernatant was evaporated to dryness on a boiling water bath at 80 °C. The dried residue was dissolved in 5 ml of distilled water. The aliquot sample of 0.5 ml was taken in separate test tube and the volume was made upto 3 ml using distilled water. Then 0.5 ml of folin-ciocalteau reagent were added and after 3 minutes, 2 ml of 20 percent sodium carbonate was added. Test tubes were kept in a boiling water bath for one minute, cooled and the colour developed was measured at 650 nm using UV visual spectrophotometer. Standard curve was prepared using catechol at different concentrations. From the standard curve, the concentration of phenols was determined and expressed in mg /g.

Results and Discussion

The results of the biochemical analysis *viz*. total sugars, total protein and total phenols in selected landraces of healthy and BPH infested plants were given in the table 1.

Total sugars

Total sugar content on 30 days old healthy plant of selected entries significantly differed from each other. Among the tested entries, sugar content ranged from 3.94 to 8.17 mg/g. PTB 33 recorded significantly lower amount of total sugars (3.94 mg/g) followed by *Mysore Malli* (4.95 mg/g). TN 1 recorded the highest amount of total sugars (8.17 mg/g) followed by Vellai Kombi Samba (6.81 mg/g), Vellai Gundu Samba (6.26 mg/g), Chetty Samba (6.13 mg/g) and Panamara Samba (5.63 mg/g). BPH infestation reduced the sugar content present in the landraces. PTB 33 showed significantly lesser percent reduction in sugar content (20.67%) compared to the other entries tested, followed by Chetty Samba (22.24%) and Panamara Samba (24.24%). The highest percent reduction in total sugar content was recorded in TN 1 (34.46%) followed by Vellai Gundu Samba (29.04%), Vellai Kombi Samba (28.28%), Mysore Malli (27.58%). According to Watanabe and Kitagawa (2000) [22], BPH infestation induced physiological changes that can reduce photosynthesis or disrupt the translocation of photosynthates, ultimately leading to a decrease in the sugar content of BPH-infested plants. Sujatha *et al*. (1987) [16] reported that BPH-susceptible varieties like Tellahamsa and Jaya had higher levels of total sugars and concluded that sugar content in varieties served as a potent stimulant for BPH feeding. Reddy *et al.* (2004) [14] observed notable quantitative differences in sugar content between healthy and BPH-infested rice varieties. WBPH populations have shown a positive correlation with the levels of total sugars and amino acids, as noted by Rath *et al.* (1999) [13]. Similar observations reported by Thayumanavan *et al.* (1990) [17], Maheshwari *et al.* (2006) [9], Basanth (2012) [2], Udayasree *et al.* (2020) [18] and Jayasimha *et al.* (2015) [7] are corroborating with the present findings.

Total proteins

Protein content on 30 days old healthy plant of selected entries ranged from 2.71 to 5.99 mg/g. TN 1 contained the highest amount of protein (5.99 mg/g), followed by Panamara Samba (4.49 mg/g) and Mysore Malli (3.79 mg/g). The lowest amount of protein was recorded on PTB 33 (2.71 mg/g) followed by Vellai Kombi Samba (3.28 mg/g), Chetty samba (3.41 mg/g) and Vellai Gundu Samba (3.56 mg/g). BPH infestation influenced the amount of protein present in the respective landraces. The higher percent reduction in protein content was observed on TN1 (44.26%) followed by Vellai Kombi Samba (32.99%), Vellai Gundu Samba (29.51%), Panamara Samba (29.33%), Mysore Malli (26.77%) and Chetty Samba (27.50%) whereas PTB 33 recorded 25.10 percent decrease which was significantly lower than the rest of the entries. The current study draws support from the previous study reported by various authors. Higher protein content was negatively correlated with resistance. BPH infestation led to a decrease in soluble protein. As chlorosis became more pronounced, the protein content in the leaves steadily decreased. Sogawa (1971) [15] and Vanitha *et al.* (2011) [21] found that susceptible rice plants had higher protein content in their basal stems compared to resistant plants.

Table 1: Biochemical constituents in the selected landraces of healthy and BPH infested plants

S.No	Landrace	Plant age (30 days)								
		Total sugars (mg/g)*			Total protein (mg/g)*			Total phenols (mg/g)*		
		Healthy	Infested	Percent decrease over healthy plant	Healthy	Infested	Percent decrease over healthy plant	Healthy	Infested	Percent increase over healthy plant
1	Chetty Samba	6.13^{c}	4.76^{b}	22.24	3.41^{de}	2.47^{e}	27.50	3.64^{d}	5.06^{d}	39.49
2	Mysore Malli	4.95^{e}	3.58^{d}	27.58	3.79^{c}	2.78^{c}	26.77	4.91^{b}	6.90^{b}	40.51
3	Panamara Samba	5.63^{d}	4.26^{c}	24.24	4.49^{b}	3.17^{b}	29.33	2.93^{f}	4.02^{e}	37.77
4	Vellai Gundu Samba	6.26^{c}	4.45^{c}	29.04	3.56^{d}	2.51^{d}	29.51	3.37^{e}	4.85^{d}	43.70
5	Vellai Kombi Samba	6.81^{b}	4.88^{b}	28.28	3.28^{e}	2.20^{f}	32.99	4.08^{c}	5.95^{e}	46.08
6	PTB 33	3.94^{f}	3.13^{e}	20.67	2.71^{f}	2.03^{g}	25.10	5.25^{a}	7.73^{a}	47.44
7	TN 1	8.17^{a}	5.36^{a}	34.46	5.99^{a}	3.34^{a}	44.26	2.41^{g}	3.25^{f}	31.11
	SEd	0.280	0.161	-	0.222	0.100	-	0.210	0.327	-
	CD (0.05)	0.210	0.205	-	0.175	0.125	-	0.277	0.164	-

In a column, means with the same letter are not significantly different at 5% level by DMRT

*Average of three replication

Furthermore, the susceptible variety exhibited a greater percentage reduction in protein content compared to the resistant variety. Similar results were observed by Udayasree *et al.* (2020) [18] and Jayasimha *et al.* (2015) [7]. The lower protein content in the resistant genotypes in this study can be regarded as a contributing factor to the lower feeding rate and reduced growth and development of BPH.

Total phenols

Phenol content on 30 days old healthy plants of selected entries ranged from 2.41 to 5.25 mg/g. PTB 33 recorded the highest phenol content (5.25 mg/g) and it was more than double and significantly different from the susceptible check TN 1 (2.41 mg/g), followed by Mysore Malli (4.91 mg/g), Vellai Kombi Samba (4.08 mg/g), Chetty Samba (3.64 mg/g), Vellai Gundu Samba (3.37 mg/g), Panamara Samba (2.93 mg/g). BPH infestation increased the phenol content in all the tested landraces with varying levels. PTB 33 recorded the highest percent increase in phenol content (47.44 percent) followed by Vellai Kombi Samba (46.08%), Vellai Gundu Samba (43.70%), Mysore Malli (40.51%). The lowest percent increase in phenol content after BPH infestation was recorded on TN 1 (31.11%), followed by Panamara Samba (37.77%) and Chetty Samba (39.49%). Previous studies suggested that the rice varieties having higher levels of phenolic compounds exhibited resistance to sucking pests (Pathak and Khush, 1979 [12]; Mishra and Misra, 1991 [11]; Grayer *et al.*, 1994 [5]; Yesuraja and Mariappan, 1993 [24]). Reddy *et al.* (2004) [14] observed a notable rise in phenolic content in resistant rice varieties INRC 8815 and INRC 7069 after BPH infestation. The phenolic content increased from 0.157 to 0.470 mg/g tissue in INRC 8815 and from 0.160 to 0.430 mg/g tissue in INRC 7069. As per findings by Usha Rani and Jyothsna (2010) [20], Yasur *et al.* (2009) [23] and Felton *et al.* (1992) [4], the heightened concentration of phenolic compounds can be attributed to the extent of damage of tissues caused by insects or pathogen infections. The present study corroborates with the previous study suggesting that infestation of rice by BPH led to an elevated phenolic content in resistant varieties. In the current study, the rise in phenolic content after BPH infestation was observed in both susceptible and resistant varieties, suggesting that the increase in phenolic content was due to herbivore infestation**.**

Conclusion

Present findings revealed that the rice landraces with lowest levels of total sugar and total protein content and highest levels of total phenols were resistant to BPH infestation. This might be due to the fact that the nutrition *viz.* sugars and proteins, required by BPH for their growth and development could not be provided by the resistant check and landraces due to their significantly lower sugar and protein content. Future studies need to focus on the secondary metabolites possessed by the landraces and those induced after BPH infestation to precisely understand the mechanism of defense.

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