

Biochemical basis of resistance in rice to yellow stem borer, *Scirpophaga incertulas* Wlk.

Abstract: A field study was conducted by screening 196 rice accessions at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, for identifying the resistant entries against rice yellow stem borer, *Scirpophaga incertulas*. In the identified resistant entries and susceptible check, biochemical factors such as chlorophyll content, total sugars, reducing sugars, total phenols, total soluble protein, and proline were analyzed. Higher amounts of total phenols, moderate chlorophyll content, and few amounts of sugars were identified as the factors imparting resistance against this pest in the resistant entries

Keywords: screening, stem borer, biochemical factors, total phenols, sugars

Introduction:

The yellow stem borer (YSB), *Scirpophaga incertulas* is a serious pest of rice causing considerable damage to the plant from seedling to maturity, thus accounting for a large share of crop losses. The use of insecticides is also not easy for its control due to their cryptic habits and is very expensive, as it requires repeated applications. Hence, other avenues of control measures should be explored, of which varietal resistance is one such approach. Resistance to the pest may be due to physical, chemical, or combined factors. Several approaches exploring the biochemical mechanisms of resistance for crop pests in several crops have been reported. In rice, Kumar 1997 reported a higher incidence of borer in rice during the maximum tillering stage during which the sugar content was also high. To ascertain some of the biochemical factors responsible for resistance in rice to YSB, 196 entries were taken for conducting the field trial at Karaikal. Resistant entries were identified using IRRI standard evaluation procedure. In the identified resistant entries and susceptible check, chlorophyll content, total sugars, reducing sugars, total phenols, total soluble protein, and proline content were estimated and correlated with the resistance.

Materials and Methods

Rice accessions numbering 196 have been received from the Directorate of Rice Research (DRR), Hyderabad with two controls (TN 1 and Suraksha). Accessions were screened using a standard evaluation system for rice developed by IRRI for rice yellow stem borer, *Scirpophaga incertulas*. Fertilizers viz., N: P₂O₅: K₂O were applied @ 120:60:60 kg/ha in the form of Urea, Di-ammonium phosphate (DAP), and Muriate of potash (MOP) respectively. Full doses of P₂O₅ and K₂O along with a half dose of N were applied before transplanting as a basal application, while the remaining half dose of N was applied in two equal splits at tillering and panicle initiation stages of the crop. Standing water was maintained continuously to a height of 2 to 5 cm throughout the crop season by irrigating the field on a need basis. Weeds were manually removed from the experimental field to avoid crop-weed competition during the crop period.

Comment [d1]: It should be short and lucid. Add one or two sentences about challenges of the study and later discuss about the contributions made. Brief the methods and result obtained in the experiment performed.

Comment [d2]: Enrich review of literature part in introduction with updated data. Try to identify the research gap

Comment [d3]: Write detail methodology of evaluation against stem borer.

Comment [d4]: No need to give detail of Agronomic practices. It should write in one sentence.

After screening the accessions, the resistant entries with the least white ear damage (70 DAT) were identified. The top five entries based on the ranking of damage and a susceptible check TN 1 was taken for analysis. To determine the biochemical factors responsible for imparting resistance in the promising genotypes, estimation of total chlorophyll, total sugars, reducing sugars, total phenols, protein, and proline were carried out along with the check. The biochemical factors were estimated from the leaf samples. Total chlorophyll was estimated following Hiscox and Israelstam, 1979. For total and reducing sugars Nelson-Somogyi method was followed (Eric Fournier, 2001), while total phenol was estimated following Sadasivam and Manikkam, 1996. For the estimation of protein, Lowry's method was followed (Lowry *et al.*, 1951) and proline was estimated employing Bates *et al.*, 1973.

Results and Discussion

Greenness has an attraction towards insects, to find its relevance in infestation, total chlorophyll was analyzed in the selected entries (Table 1). Significant variation was observed among the raised entries for total chlorophyll content. The susceptible check TN 1 exhibited higher chlorophyll content than the resistant varieties, implying the fact that entries with higher chlorophyll content attracted the insects for feeding. A similar type of result was reported by Xu *et al.*, 2010 for leaf folder incidence in rice.

Comment [d5]: Support your results with suitable and updated references in discussion.

Comment [d6]: Support your results with suitable and updated references in discussion.

Table 1. Total chlorophyll of selected rice genotypes showing differential reaction to rice stem borer

S. No.	Accession	(%) white ear	Total chlorophyll (mg/g)
1.	OR 2324-8	1.23	2.21
2.	RTN 62-6-7-1	1.67	4.16
3.	R 1138-688-3-533-1	1.82	3.22
4.	CR 2698	1.94	2.72
5.	HUR-913	2.14	2.29
6.	TN-1	8.32	4.83
Mean	--	--	3.24
C.D (P=0.05)	--	--	0.42
C.V%	--	--	7.06

Since sugars help the yellow stem borer to survive and cause infestation, the total and reducing sugars were analyzed to find their effect in the selected entries. When the entries were analyzed for total and reducing sugars, interestingly it was found that the susceptible variety TN 1 was observed to contain significantly more amount of total and reducing sugars than resistant entries (Tables 2 & 3). Total sugar content ranged from 11.53 to 51.90 mg/g in the resistant entries which was significantly lower than the susceptible check (129.86 mg/g). It was evident from the data that susceptible TN 1 had higher total sugars and the resistant entries had comparatively lower concentrations of total sugars. These findings are in line with the study of Nanda *et al.*, 2000; Padhi, 2004; Chandramani *et al.*, 2009. Nutrients especially sucrose and certain amino acids may function as potent sucking stimulants for stem borer.

Table 2. Total sugars of selected rice genotypes showing differential reaction to rice stem borer

S. No.	Accession	(%) white ear	Total sugars (mg/g)
1.	OR 2324-8	1.23	51.90
2.	RTN 62-6-7-1	1.67	12.35
3.	R 1138-688-3-533-1	1.82	17.62
4.	CR 2698	1.94	53.02
5.	HUR-913	2.14	11.53
6.	TN-1	8.32	129.86
Mean	--	--	46.051
C.D (P=0.05)	--	--	4.80
C.V%	--	--	5.73

Table 3. Reducing sugars of selected rice genotypes showing differential reaction to rice stem borer

S. No.	Accession	(%) white ear	Reducing sugars (mg/g)
1.	OR 2324-8	1.23	47.31
2.	RTN 62-6-7-1	1.67	32.81
3.	R 1138-688-3-533-1	1.82	19.22
4.	CR 2698	1.94	34.93
5.	HUR-913	2.14	27.72
6.	TN-1	8.32	53.55
Mean	--	--	35.93
C.D (P=0.05)	--	--	1.08
C.V%	--	--	1.66

Varieties with higher amounts of phenolic compounds make the plant resistant as this compound cause a barrier for the borer larvae to utilize the plant nutrients (Kind, 1954), so the total phenol was estimated. Total phenol content was less in the susceptible entry (TN 1) and the amount of phenol is significantly higher in the resistant entries (Table 4) which corroborated with the findings of Panda *et al.*, 1975; Padhi 2004; Suchita *et al.*, 2011. Several workers have reported the presence of more phenolic compounds in the rice varieties resistant to sucking pests. (Pathak and Khush, 1977; Grayer *et al.*, 1994). Brown plant hopper (BPH) infestation resulted in increased phenolic production in most of the resistant and moderately resistant cultures whereas in the BPH susceptible TN 1 the total phenol was reduced (Loka Reddy *et al.*, 2004). A similar phenomenon had also been reported in other crops like tomato (Sivaprakasam, 1996), brinjal (Kumar, 1997), and sorghum (Kalappanavar and Hiremath, 2000).

Table 4. Total phenols of selected rice genotypes showing differential reaction to rice stem borer

S.No.	Accession	(%) white ear	Phenols (mg/100g)
1.	OR 2324-8	1.23	12.76
2.	RTN 62-6-7-1	1.67	15.71
3.	R 1138-688-3-533-1	1.82	14.03
4.	CR 2698	1.94	12.49
5.	HUR-913	2.14	17.67
6.	TN-1	8.32	5.67
Mean	--	--	13.05
C.D (P=0.05)	--	--	2.85
C.V%	--	--	12.01

Total soluble protein in leaves of rice entries is tested as the protein act as an important defense mechanism against insect pests Garcia Olmedo *et al.*, 1987; Ryan, 1990; Lawrence and Koundal, (2002). Even though significant variation was noticed, the susceptible check had on-par protein content with some resistant varieties. This result deviated from the studies of Garcia Olmedo *et al.*, 1987; Ryan, 1990; Lawrence and Koundal, (2002). But the entries OR 2324-8 and HUR-913 which showed on-par protein content with susceptible check TN 1, showed a higher phenol content of 12.76 and 15.71 mg/100 g when compared with the phenolic content of 5.67 mg/100 g of TN 1 (Table 5). So the higher phenolic content helped in the defense mechanism of the resistant entries.

The data from previous studies suggested that proline has a regulatory function, controls plant development, and acts as a signal molecule (Laszlo Szabados and Arnould Savoure, 2004). Proline metabolism can also influence programmed cell death in plants. In *Arabidopsis*, incompatible plant-pathogen interactions trigger a hypersensitive response (HR) via reactive oxygen species (ROS) signals, which is accompanied by local activation of *P5CS2* and proline accumulation (Fabro, G. 2004). Proline was recently proposed to modulate the plant defense response to *Agrobacterium tumefaciens*. Proline accumulates in plant tumors, and functions as a competitive antagonist of gamma-aminobutyric (GABA)-dependent plant defense, interfering with the GABA-induced degradation of quorum-sensing signal (Haudecoeur, *et al.*, 2009).

Very few or nil reports are found for the role of proline against a pathogen or pest incidence. In order to investigate the role of proline against pest damage, the proline content was analyzed in the rice entries. Interestingly the susceptible check TN 1 was found to have a significantly higher level of proline when compared to resistant entries (Table 6) implying the fact that more damage induces the synthesis of proline which may act as a signal molecule for plant defense mechanism. Further studies may be proved.

So it is suggested that rice genotypes having high phenolic compounds, moderate chlorophyll content, and lower sugar content could be utilized in the breeding program for developing resistant varieties for stem borer. |

Comment [d7]: Add the conclusion of your result and future scope in the research right direction.

Table 5. The total soluble protein of selected rice genotypes showing differential reaction to rice stem borer

S.No.	Accession	(%) white ear	Protein (mg/g)
1.	OR 2324-8	1.23	12.85
2.	RTN 62-6-7-1	1.67	19.36
3.	R 1138-688-3-533-1	1.82	15.76
4.	CR 2698	1.94	20.21
5.	HUR-913	2.14	10.66
6.	TN-1	8.32	11.84
Mean	--	--	15.11
C.D (P=0.05)	--	--	1.96
C.V%	--	--	7.15

Table 6. Proline of selected rice genotypes showing differential reaction to rice stem borer

S.No.	Accession	(%) white ear	Proline (ppm)
1.	OR 2324-8	1.23	24.95
2.	RTN 62-6-7-1	1.67	31.26
3.	R 1138-688-3-533-1	1.82	32.68
4.	CR 2698	1.94	52.72
5.	HUR-913	2.14	34.30
6.	TN-1	8.32	113.03
Mean	--	--	48.15
C.D (P=0.05)	--	--	9.39
C.V%	--	--	10.72

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