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Relevance of Chemical Constituents in Conferring Tolerance to Yellow Stem Borer, *Scirpophaga incertulas* Wlk in Rice

Anil Varma Nalla [a*], S. Nadaradjan [a], D. Adiroubane [a] and K. Kumar [a]

[a] *Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal 609 603, U.T. of Puducherry, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Tolerant rice accessions were identified from a field study involving 196 rice accessions at Pandit Jawaharlal of Agriculture and Research Institute, Karaikal for rice yellow stem borer, *Scirpophaga incertulas*. Tolerant accessions in field study along with the susceptible check (TN1) were considered for analyzing biochemical parameters viz., chlorophyll content, total sugars, reducing sugars, total phenols, total soluble protein, and proline. Higher amounts of total phenols, moderate chlorophyll content, and lesser amounts of sugars were identified as the factors imparting resistance against this pest in the resistant entries. The correlation among the infestation percentage and biochemical parameters, revealed strong positive correlation between total sugars and infestation percentage; strong negative correlation between total phenols and infestation percentage implying the role of phenols in plant defense against rice yellow stem borer.

**Corresponding author: E-mail: anilvarmanalla@gmail.com;*

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1. 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. India, the second-largest rice-growing country has a production of 104.32 million tonnes and a cultivation area of about 44.6 million hectares with an average productivity of 2.34 tonnes per hectare" [1]. "The potential to yield is dented due to a lack of inbuilt resistance to different biotic stresses as discernible in ~1,000 rice cultivars across the country" [2]. "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. Plant genotypes, either due to environmental stress or genetic makeup, possess physiological and biochemical differences which alter the nutritional value (primary metabolites) for plant-feeding insects" [3,4,5,6]. "The identification of resistant/tolerant rice varieties will help breeders for future use in developing multiple resistant new breeding rice lines" [7]

"Plants employ different defence tactics and this will influence herbivore settling, feeding, oviposition, growth, development, fecundity and fertility. Accumulation of chemicals by insect feeding had been revealed in many insect- plant interactions" [8]. The plant strategy to deter feeding insect pests has become an important aspect of insect- plant interaction studies. A wide range of semio-chemicals present in the plants also plays an important defensive role against insects pests. An understanding of the defensive biochemical compound present in plants might be required for development of varieties with more durable resistance. In this light, the presences of defence compounds in resistant and susceptible accessions need to be exploited. 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.

2. MATERIALS AND METHODS

Rice accessions numbering 196 have been received from the Indian Institute of Rice Research (DRR), Hyderabad with a susceptible check (TN 1). Accessions were screened using a standard evaluation system for rice developed by IRRI for rice yellow stem borer, *Scirpophaga incertulas.* 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. Recommended Fertilizer dosage has been incorporated in the field trial plot. Accessions were sown in the raised seed bed & each accession is transplanted in two replications with a minimum of 20 hills in each replication. Weeds were removed manually from experimental field to avoid crop-weed competition during crop period.

Assessment has been done based on damage symptoms for stem borer at two stages such as vegetative stage (30 DAT) and reproductive stage (70 DAT). For each entry five hills were selected randomly in each replication for identifying the damage symptom at 30 DAT (dead heart) & 70 DAT (white ear) was recorded in all entries. Per cent damage was estimated by counting the number of tillers and damaged tillers. 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.

2.1 Estimation of Biochemical Factors

The biochemical factors were estimated from the leaf samples. Total chlorophyll was estimated following Hiscox and Israelstam, [9]. For total and reducing sugars Nelson Somogyi method was followed [10], while total phenol was estimated following Sadasivam and Manikkam [11]. For estimation of protein, Lowry's method was followed [12] and Proline was estimated employing Bates et al., [13].

2.2 Statistical Analysis

Data on Biochemical factors were analysed using AGRES software for its significance. Correlation analysis is carried out between percent infestation of white ears & biochemical parameters and the results were presented.

3. RESULTS AND DISCUSSION

Screening of Rice genotypes against Yellow Stem Borer indicates the white ear damage ranges from 1.23 % to 8.32%.

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.

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. [14]; Padhi, [15]; Chandramani et al., [16]. Dharshini et al., [17] reported that the total sugar content was the maximum in TN1 and Jaya, whereas the resistant check Ptb-33 had least amount of total soluble sugar content. Nutrients especially sucrose and certain amino acids may function as potent sucking stimulants for stem borer.

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 [18], 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., [19]; Padhi [15]; Suchita et al., [20]. Several workers have reported the presence of more phenolic compounds in the rice varieties resistant to sucking pests [21,22]. 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 [23]. A similar phenomenon had also been reported in other crops like tomato [24], brinjal (Kumar, 1997), and sorghum [25]. Dharshini et al., [17] reported “increase in phenolic content after infestation in susceptible checks and also resistant landraces and indicated that the increase in phenolic content was injury specific”.

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., [26]; Ryan, [27]; Lawrence and Koundal, [28]. 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., [24]; Ryan, [27]; Lawrence and Koundal, [28]. 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.

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**

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**

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

S. No.	Accession	(%) white ear	Phenols (mg/100 g)
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**

"The data from previous studies suggested that proline has a regulatory function, controls plant development, and acts as a signal molecule" [29]. "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" [30]. "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" [31].

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.

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**

3.1 Correlation Analysis

When correlation was done between the percent infestation and the various biochemical parameters, total sugars ($r = 0.88$; $n=4$; $p>0.01$) showed a positive correlation with per cent infestation and phenol ($r = -0.85$; $n=4$; $p>0.01$) showed a negative correlation with per cent infestation, implying the fact the accessions with low total sugars and high phenol showed resistance to yellow stem borer.

4. CONCLUSION

Rice genotypes having high phenolic compounds, moderate chlorophyll content, and lower sugar content showed resistance to the yellow stem borer and hence the rice lines having similar pattern of biochemical parameters, could be utilized in the breeding program for developing resistant varieties for stem borer.

CONFERENCE DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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