RESEARCH ARTICLE https://doi.org/10.58297/MIXG4169

Biochemical factors associated with vegetative phase resistance against yellow stem borer, *Scirpophaga incertulas* (Walker) in land races of rice

Megha CM[1], Vijaykumar L[2], Shivanna B[3], Anusha SB[4]

[1,2,4]Department of Agricultural Entomology, College of Agriculture, V. C. Farm, Mandya-571 405, Karnataka, India
[4]Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India
[2]Corresponding author email: vkumaruasb@gmail.com

Received: 29th March 2022; Accepted: 12th May 2022

Abstract

A total of 50 local land races were screened against infestation of yellow stem borer, *Scirpophaga incertulas* (Crambidae: Lepidoptera) at vegetative phase under natural infestation (0 to 65%) during kharif 2019. Among them 18 land races were selected from each category of resistance based on standard evaluation system for rice. The estimation of biochemical constituents in rice stem (60 days old plants) of different categories was done to establish a relationship between various biochemical components with resistance/ susceptibility. Studies revealed that higher amounts of total sugars, reducing sugars and crude proteins were found in susceptible lines compared to resistant land races and were positively correlated with stem borer infestation. Total free amino acids, total phenols and tannins were found to be higher in resistant than susceptible varieties and were negatively correlated. The mineral content in the stem *viz.*, nitrogen was positively correlated whereas phosphorous and potassium were found in higher quantities in resistant categories (2.19 – 2.37%) than susceptible ones and were found to be negatively correlated with infestation of yellow stem borer (-0.95).

Keywords: Biochemicals, Resistance, Rice, Yellow stem borer

Introduction

Rice (*Oryza sativa* Linn.), an ancient crop domesticated more than 8000 years ago, evolved along with man and adapted to diverse ecological conditions. Production and productivity of rice yield depends on a number of biotic and abiotic stress factors. Insects, mites and nematodes are few of the key biotic stresses which limit rice production in India (Prakash *et al.*, 2006). The annual yield loss due to insect pests in India varies from 21 to 51 per cent (Singh and Dhaliwal, 1994) andit varies between 26 to 34 per cent globally (Widowsky and O` Toole, 1996). More than 1,000 species of invertebrate pests have been reported to infest rice and about a dozen species of insects are important as key pests. Major insect pests that cause significant economic losses in Asian countries are the yellow stem borer, *Scirpophaga incertulas* (Walker), *Cnaphalocrocis medinalis* (Guenee), brown planthopper, *Nilaparvata lugens* (Stal.), Green leafhopper, *Nephotettix virescens* (Distant) and Asian rice gall midge, *Orseolia oryzae* (Wood-Mason). In South-Asia, yellow stem borer, Asian rice gall midge and brown planthoppers are the major production constraints (Ramaswamy *et al*., 1996; Vijaykumar *et al*., 2009).

The plant strategy to deter feeding insect pests has become an important aspect of insect- plant interaction studies. Feeding activities of insect pests results in physiological, morphological and chemical changes in the form of accumulation of toxic compounds with defensive properties (Amsagowri *et al*., 2018). The biochemical factors are chemicals that affect behaviour, physiology and growth of insect. Some biochemical factors are associated with repellence, deterrence or adverse effects on insect pests. Several control strategies such as field sanitation, introduction of parasitoids and use of synthetic pesticides have been employed for control of *S. incertulas* but their

deployment have not given satisfactory control particularly when the larvae were feeding inside the stalks (Kfir *et al*., 2002). In the present study the biochemical constituents in rice stem of land races were correlated with dead heart damage by yellow stem borer in so as to understand the role of biochemical basis of defense.

Materials and Methods

A total of 50 local land races were collected from Zonal Agricultural Research Station, V. C. Farm, Mandya 12^0 32'N, 760 53'E, and 690 m AMSL under AICRP (Rice) were evaluated against rice yellow stem borer under field conditions during *Kharif* 2019 at 'A' block V. C. Farm, Mandya. The estimation of biochemical constituents *viz*., total sugars, reducing sugars, total phenols, crude proteins total free amino acids, tannins and minerals *viz.,* nitrogen, phosphorous and potassium in rice stem collected from 60 days old plants were carried to establish the relationship between various biochemicals with selected resistant and susceptible genotypes *viz.,* Malpali samba- 1, Rajboga, Kari kagga, Neermullare, Malgudisanna -2, Jenugudu, Kalajeera, Mara batta- 2, China ponni, Kavekantak, Gangadale, Neermulka, Naweli, Kana kunja, Punkattkodi -1, Kundipullan, Krishna leela, Puttabatta-2.

Preparation of plant samples for analysis

The samples of stem were dried at 35 °C in hot air oven for 24 to 48 hrs. The dried samples were ground using mixer grinder. The powdered samples were stored in plastic covers until analysis.

Extraction of plant tissues in alcohol

The stem samples of 18 selected rice land races were collected and analyzed from the pooled sample of 3 replications. The samples were thoroughly washed with distilled water and dried under shade. 10 gram of plant sample was taken in a separate conical flask and 150 ml of 80 per cent ethanol was added and refluxed for 30 minutes on hot water bath. After boiling, the extract was cooled and tissues were ground thoroughly in a mortar with pestle in slight amount of ethanol. The supernatant was decanted into another flask and the residue was again re-extracted with small quantity of hot ethanol and decanted. This extract was filtered through Whatman's No.1 filter paper and made up to a known volume with 80 per cent ethanol. The ethanol part of (alcoholic) extract was stored in refrigerator at 4 °C, and used for the estimation of total sugars, reducing sugars and phenols.

The total and reducing sugars in each test genotype were estimated by following the method suggested by Somogyi (1952). Estimation of total phenols in stem samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954). The amount of total free amino acids present in the samples was estimated by following Ninhydrin method developed by Moore and Stein (1948). Estimation of tannins in the stem samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954). Further, the data collected were subjected to Analysis of Variance (ANOVA) and means were separated by Tukey's HSD test (Tukey, 1953).

Estimation of nitrogen and crude proteins

Finely powdered oven dried Na_2CO_3 samples (0.5 g) weretaken in the digestion tubes. To this 1-2 g of digestion mixture and 10-15 ml of concentrated sulphuric acid was added and digested. Then the samples were placed in Kjeldahl digestion assembly till a light bluish green residue is obtained. Then the content was cooled by adding 5 to 10 ml of distilled water. The receiving flask was placed at the receiving end of distillation unit. The digested mixture was loaded on tube for distillation apparatus one at a time. By keeping all reserve tanks loaded with appropriate reagents such as 4% boric acid with mixed indicator and 40% NaOH, the content was distilled for 6 minutes and the released ammonia was collected in boric acid solution by programming the Kjeldahl distillation unit (make: Borosil KDI 1300W, BLFAKDI010). Once the distillation was completed, the receiving flask was removed and titrated against standard H_2SO_4 till the color changed from green to pink. Titer value (TV) was noted and nitrogen content was calculated using the equation

$$\% \text{ N in plant sample} = \frac{\text{TV x N.of H2S04x0.0014}}{\text{wt.of sample}} \text{ x } 100$$

Crude protein was calculated by the formula:

Crude protein (g %) = % N x 5.95 (conversion factor for rice)

Then, the percentage crude protein was expressed in terms of mg/gm of the sample.

Phosphorous content

Five ml of digested sample was pipetted out into a 25 ml volumetric flask and 5 ml of vanadomolybdate reagent was added. The volume was made up with distilled water and the content was mixed thoroughly. The absorbance was read after 30 minutes at 420 nm on UV double beam spectrophotometer (make: Shimadzu UV-1900i). The phosphoric acid (Himedia) was used for the preparation standard curve. Further, the phosphorous concentration in the stem samples was estimated with the help of standard curve (Graph ppm) by using the following formula, and expressed in percentage.

$$\% \text{ Phosphorous} = \frac{\text{Graph ppm} \times \text{vol. of digested sample} \times \text{vol. made} \times 100}{\text{Weight of sample} \times \text{Aliquot taken} \times 10^6}$$

Potassium content

The potassium content in di-acid digested plant sample was determined by flame photometric method after appropriate dilution (10^6). Flame photometer (Systronics 130) was switched on and K filter was selected. The blank was fed to the instrument and the reading was adjusted to zero with the blank in the instrument. Then 40 ppm K solution (standard Himedia) was fed to the flame photometer and adjusted to 100 to run the standards. After this, readings were taken for other intermediate concentrations for standardization. The acid digested plant sample was fed into the atomizer and the flame photometer reading was noted down. If the concentration of the sample exceeded the range, the sample was diluted to the suitable concentration range so that final concentration lies between 0 to 40 ppm. The curve was plotted using graph sheet by taking K concentration on X-axis and flame photometer readings on Y-axis. Acid digested plant sample reading was located on the standard curve, which will give the K concentration in the extract (Graph ppm). From this graph concentration, the amount of K in the sample was calculated by using the following formula.

$$\% \text{ Potassium} = \frac{\text{Graph ppm} \times \text{vol. of digested sample} \times \text{vol. made} \times 100}{\text{Weight of sample} \times \text{Aliquot taken} \times 10^6}$$

Results and Discussion

To study the biochemical basis of resistance among land races of rice against rice yellow stem borer, 18 landraces representing each resistance category were selected from the screening trial. The resistance categories include high resistance (0 percent damage; SES score 0), resistance (1 to 10 % damage; SES score 1), moderate resistance (11 to 20 % damage; SES score 3), moderately susceptible (21 to 30 % damage; SES score 5), susceptible (31 to 60 % damage; SES score 7) and highly susceptible (>61 % damage; SES score 9) **(Table 1)**.

Among the landraces, amount of total soluble sugars varied from 3.81 to 3.94 mg/g and was lower in highly resistant genotypes. However, in highly susceptible genotypes the amount of total soluble sugars was found significantly higher and varied from 8.11 to 8.59 mg/g. Similarly, among the genotypes, lower amount of total reducing sugars (6.79 to 7.44 mg/g) was observed in highly resistant genotypes, however in highly susceptible genotypes the amount of total reducing sugars (14.12 to 14.78 mg/g) was found significantly higher. Likewise, among the genotypes, lower amount of crude proteins was observed in highly resistant genotypes which varied from 2.89 to 3.10 mg/g. However in highly susceptible genotypes the amount of crude proteins was found significantly higher and varied from 6.70 to 6.80 mg/g. Also, among the genotypes, lower amount of total free amino acids was observed in highly resistant genotypes varied from 23.59 to 25.00 mg/g and in highly susceptible genotypes the amount of total free amino acids was found to be significantly higher which varied from 15.31 to 15.47 mg/g.

Among the genotypes, the lower amount of total soluble sugars was observed in highly resistant genotypes that varied from 3.81 to 3.94 mg/g, however in highly susceptible genotypes the amount of total soluble sugars was found significantly higher which varied from 8.11 to 8.59 mg/g. Among the genotypes, lower amount of nitrogen was observed in highly resistant genotypes which varied from 0.48 to 0.52 per cent. However, in highly susceptible genotypes the amount of nitrogen was found to be significantly higher that varied from 1.12 to 1.14. Similarly, among the genotypes, higher amount of phosphorus was observed in highly resistant genotypes which varied from 0.38 to 0.43 per cent. However, in highly susceptible genotypes the amount of phosphorous was found to be significantly lower and varied from 0.14 to 0.16. Among the genotypes, higher amount of potassium was observed in highly resistant genotypes which varied from 2.37 to 2.41 per cent. However, in highly susceptible genotypes the amount of potassium was found to be significantly lower which varied from 1.54 to 1.60. Also, among the genotypes, higher amount of tannins was observed in highly resistant genotypes that varied from 4.85 to 5.85 mg/g, however in highly susceptible genotypes the amount of tannins was found to be significantly higher and varied from 0.66 to 0.98 mg/g. Likewise, among the genotypes, higher amount of phenols was observed in highly resistant genotypes which varied from 0.54 to 0.69 mg/g.However in highly susceptible genotypes the amount of phenols was found to be significantly higher and which varied from 0.18 to 0.19 mg/g (**Table 1**) (**Figure 1**).

A significant, negatively correlation with the infestation of yellow stem borer and the contents of the total phenols (r= -0.85**), total free amino acids (r= -0.87**), tannins (r= -0.94**), total phosphorous (r= -0.96**) and total potassium (r= -0.95**) contents of the rice stem was recorded. Likewise, a significant positive correlation with the infestation of yellow stem borer, and the contents of total soluble sugars

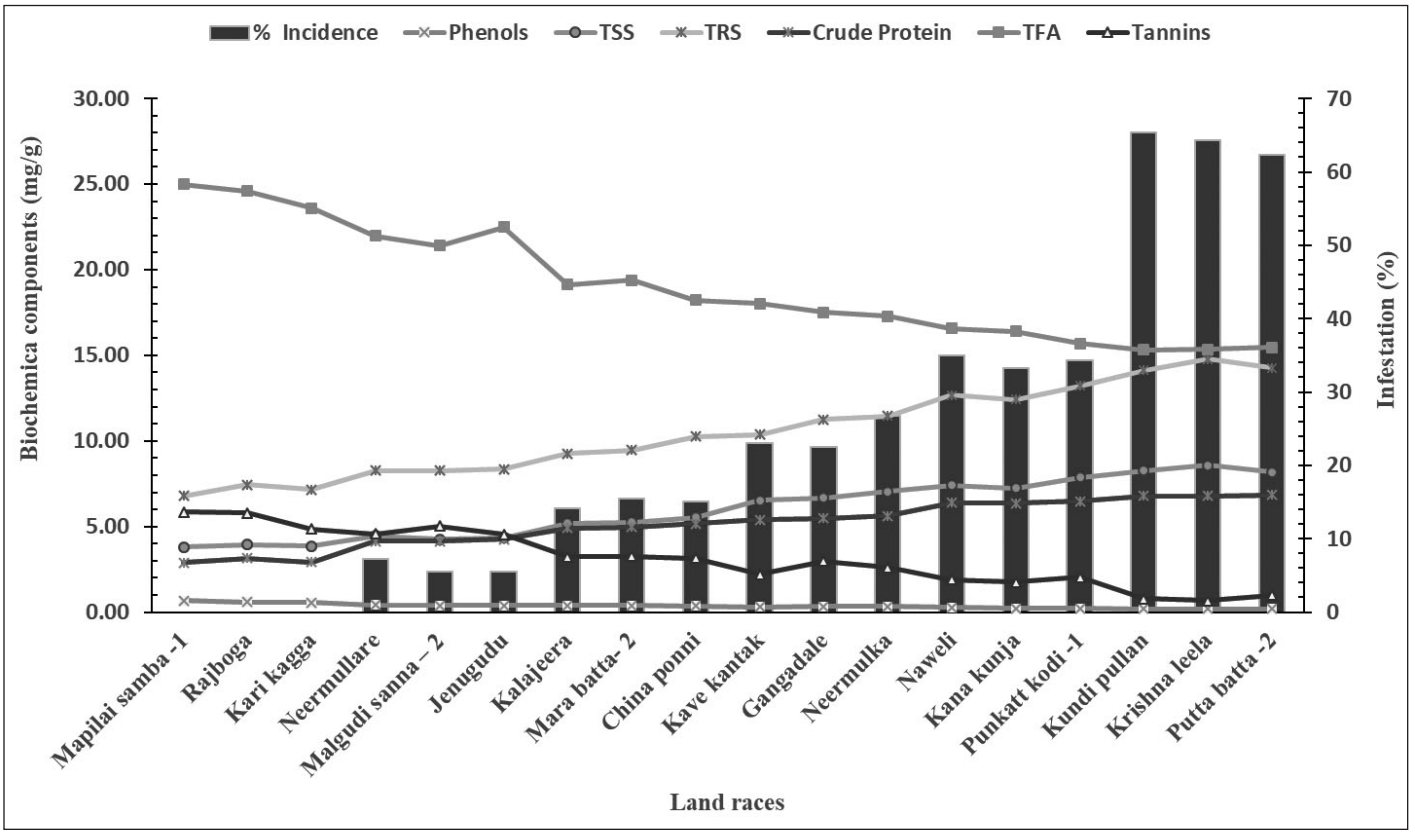

Figure 1. Relationship between biochemical components of rice stem and yellow stem borer infestation, *Kharif* 2019

Table 1. Dead heart damage by *S. incertulas* and biochemical constituents in rice stem samples of local land races

Sl. No.	Genotypes		Score	Incidence (% dead hearts)	Biochemical components (mg/g)						Minerals (%)		
					Phenols	TSS	TRS	CP	TFA	Tannins	N	P	K
1	HR	Malpali samba- 1	0	0.00 (0.00) [a]	0.68[a]	3.81[a]	6.79[a]	2.90[a]	25.00[a]	5.85[b]	0.48[a]	0.43[a]	2.41[a]
2		Rajboga	0	0.00 (0.00) [a]	0.59[b]	3.94[a]	7.44[b]	3.10[b]	24.60[a]	5.79[a]	0.52[a]	0.39[ab]	2.40[a]
3		Kari kagga	0	0.00 (0.00) [a]	0.54[b]	3.87[a]	7.17[ab]	2.89[a]	23.59[b]	4.85[ab]	0.48[a]	0.38[ab]	2.37[a]
4	R	Neermullare	1	7.27 (15.65) [b]	0.43[c]	4.46[c]	8.28[c]	4.10[c]	21.97[cd]	4.57[de]	0.68[b]	0.35[bc]	2.23[b]
5		Malgudisanna -2	1	5.47 (13.53) [b]	0.42[c]	4.25[b]	8.27[c]	4.10[c]	21.41[d]	5.02[c]	0.67[b]	0.34[bc]	2.24[b]
6		Jenugudu	1	5.50 (13.57) [b]	0.41[c]	4.33[c]	8.36[c]	4.15[c]	22.48[b]	4.54[cd]	0.70[b]	0.34[bc]	2.19[bc]
7	MR	Kalajeera	3	14.11 (22.07) [c]	0.38[cd]	5.19[d]	9.27[d]	4.87[d]	19.14[e]	3.23[f]	0.81[c]	0.31[cde]	2.16[bc]
8		Mara batta- 2	3	15.45 (23.16) [c]	0.37[cd]	5.25[d]	9.46[d]	4.90[de]	19.38[e]	3.25[g]	0.82[c]	0.32[cd]	2.15[bc]
9		China ponni	3	15.06 (22.85) [c]	0.36[de]	5.53[e]	10.27[e]	5.15[ef]	18.21[f]	3.16[e]	0.86[cd]	0.32[cd]	2.11[c]
10	MS	Kavekantak	5	23.08 (28.73) [d]	0.32[f]	6.54[f]	10.38[e]	5.39[fg]	18.01[fg]	2.21[gh]	0.90[de]	0.28[def]	1.92[d]
11		Gangadale	5	22.49 (28.32) [e]	0.33[fe]	6.67[f]	11.27[f]	5.46[fg]	17.52[fg]	2.97[h]	0.91[de]	0.26[efg]	1.87[d]
12		Neermulka	5	26.8 (31.19) [d]	0.32[fe]	7.05[g]	11.45[f]	5.52[g]	17.27[gh]	2.60[h]	0.92[e]	0.25[fg]	1.83[de]
13	S	Naweli	7	35.01 (36.30) [f]	0.27[g]	7.40[h]	12.67[g]	6.39[h]	16.56[hi]	1.89[i]	1.07[f]	0.21[gh]	1.74[e]
14		Kana kunja	7	33.2 (35.20) [f]	0.23[gh]	7.25[g]	12.43[g]	6.20[h]	16.41[hi]	1.74[j]	1.04[f]	0.20[h]	1.74[e]
15		Punkattkodi -1	7	34.28 (35.86) [f]	0.22[gh]	7.88[i]	13.20[h]	6.45[h]	15.70[ij]	2.03[i]	1.08[f]	0.21[gh]	1.71[ef]
16	HS	Kundipullan	9	65.35 (53.97) [g]	0.18[h]	8.27[j]	14.12[i]	6.77[i]	15.32[j]	0.78[j]	1.13[g]	0.16[hi]	1.60[fg]
17		Krishna leela	9	64.31 (53.34) [g]	0.19[h]	8.59[k]	14.78[j]	6.80[i]	15.36[j]	0.66[k]	1.14[g]	0.14[i]	1.54[g]
18		Puttabatta -2	9	62.34 (52.17) [g]	0.18[h]	8.18[j]	14.27[i]	6.70[i]	15.47[j]	0.98[k]	1.12[g]	0.14[i]	1.56[g]
SE m ±			-	**0.38**	**0.01**	**0.04**	**0.07**	**0.05**	**0.17**	**0.05**	**0.02**	**0.03**	**0.81**
CD @p=0.05				**1.12**	**0.04**	**0.11**	**0.23**	**0.13**	**0.50**	**0.16**	**0.08**	**0.10**	**2.47**

Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1953); TSS- Total soluble sugars; TRS- Total reducing sugars; CP- crude proteins; TFA- Total free amino acids; N- Nitrogen; P- Phosphorous; K- Potassium; Figures in the parentheses indicate arcsine transformed values.

(r= 0.94**), total reducing sugars (r= 0.94**), crude proteins (r= 0.91**) and total nitrogen (r= 0.93**) and these parameters which were significant was found influencing the infestation level to the extent of 92 per cent (R^2= 0.92) (**Table 2**).

The results of the present study corroborate with Vijaykumar *et al.,* (2012) where they reported higher amount of total sugars in all susceptible rice genotypes compared to resistant ones. Likewise, Rajdurai and Kumar (2017) also reported lower amount of sugars and soluble proteins in all resistant genotypes imparting resistance against the yellow stem borer. Similar observations on increased level of sugars were reported in all susceptible rice genotypes against Asian rice gall midge (Vijaykumar *et. al.,* 2009; 2012), rice leaf folder (Vanitha *et al.,*2015) and rice brown planthopper (Ashrith *et al.,* 2017). The present findings are in line with the results of Punithavalli *et al.,* (2013) who reported that lower protein content was evident invariably in all the infested rice genotypes. Further, Lokesh and Mehla (2017) reported that crude proteins were positive and significantly associated with growth, development and life cycle of maize stem borer, *Chilo partellus*. Similarly, from the correlation studies of Vijaykumar *et al.,* (2012) it was observed that the total free amino acids had significant negative influence on per cent incidence of rice gall midge.

The present findings are in close conformity with Punithavalli *et al.,* (2013) who reported that the contents of biochemicals such as phenol, orthodihydroxy phenol and tannins were negatively correlated with leaf folder damage indicating that these are defensive compounds contributing towards the rice yellow stem borer resistance. Further, Elanchezhyan *et al.,* (2017) reported that higher concentration of total phenols observed in the resistance group could be one of the factors contributing towards tolerance with antibiotic effect against yellow stem borer. Likewise, the study (Facknath and Lalljee, 2005) indicated that the phosphorus decreases the host suitability against various insect-pests by changing secondary metabolites such as phenolics, terpenes and accumulation of phenolics (tannins and lignin) acts as a barrier which has feeding deterrent and insecticidal effects on herbivores. Similarly, Bala *et al.,* (2018) reported higher levels of potassium in enhancing the secondary metabolites and reducing accumulation of carbohydrate during plant damage from various insect pests. The presently identified resistant genotypes are highly useful in breeding resistant varieties against rice yellow stem borer.

Table 2. Correlation matrix between the infestation of *S. incertulas* and biochemical constituents of rice stem at 60 DAT, *Kharif* 2019

Parameters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	R^2 value
Y – Dead hearts (%) by YSB	-0.85**	0.94**	0.96**	0.91**	-0.87**	-0.94**	0.93**	-0.96**	-0.95**	
X_1 – Phenols	1.00	-0.91	-0.93	-0.97	0.96	-0.93	-0.96	0.95	0.94	
X_2 – TSS		1.00	0.99	0.97	-0.96	0.96	0.970	-0.97	-0.99	
X_3 – TRS			1.00	0.98	-0.95	0.97	0.98	-0.99	-0.99	
X_4 –Crude Proteins				1.00	-0.98	0.97	0.99	-0.97	-0.97	
X_5 – TFA					1.00	-0.93	-0.97	0.94	0.95	0.92
X_6 – Tannins						1.00	-0.97	00.96	0.96	
X_7– Nitrogen							1.00	-0.98	-0.98	
X_8– Phosphorous								1.00	0.99	
X_9– Potassium									1.00	

N = 18; ** Significant at P ≤ 0.01; YSB- yellow stem borer; TSS- Total soluble sugars; TRS- Total reducing sugars; TFA- Total free amino acids; YSB- Yellow stem borer

Acknowledgements

The authors are thankful to Science and Engineering Research Board (SERB), Department of Science and Technology, Ministry of Science and Technology, Government of India under the Grant No. EEQ/2017/000484/26/3/2018 for providing funding and laboratory facilities. The authors are also thankful to University of Agricultural Sciences, GKVK, Bangalore and B.Sc (Agri.) students of Agricultural College, V. C. Farm, Mandya for their help.

References

Amsagowri V, Muthukrishnan N, Muthiah C, Mini ML and Mohankumar S. 2018. Biochemical changes in rice yellow stem borer infested rice accessions. *Indian Journal of Entomology,* 80(3): 926-934.

Ashrith KN, Sreenivas AG, Guruprasad GS, Hanchinal SG and Krishnamurthy D. 2017. Biochemical basis of resistance in rice against brown and white backed plant hopper. *International Journal of Current Microbiology and Applied Sciences,* 6(10): 1699-1706.

Bala K, Sood AK, Pathania VS and Thakur S. 2018. Effect of plant nutrition in insect pest management: *A Review of Journal of Pharmacognosy and Phytochemicals*, 7(4): 2737-2742.

Bray HG, and Thorpe WV. 1954. Analysis of phenolic compounds of interest in metabolism, *Methods of Biochemical Analysis (Eds. David Glick),* Volume 1, pp 27-52.

Elanchezhyan K. 2017. Evaluation of promising rice cultures against yellow stem borer, *Scirpophaga incertulas* Walker (Crambidae: Lepidoptera). *Journal of Entomology and Zoology Studies*, 5(5): 1903-1905.

Facknath S and Lalljee B. 2005. Effect of soil-applied complex fertilizer on an insect–host plant relationship: *Liriomyza trifolii* on *Solanum tuberosum*. *Entomologia Experimentalis et Applicata*, 15(1): 67-77.

Kfir R, Overhalt WA, Khan ZR and Palaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology,* 47:701-731.

Khurana AD and Verma AN. 1983. Some biochemical plant characters in relation to susceptibility of sorghum to stem borer and shoofly. *Indian Journal of Entomology*, 45(1): 29-37.

Lokesh and Mehla JC. 2017. Study on morphological and biochemical features associated with maize (*Zea mays* L.) resistance against *Chilo partellus. International journal of pure and applied bioscience*, 5(4): 1011-1021.

Moore S and Stein WH. 1948. Photometric ninhydrin method for use in the chromatography of amino acids method. *Journal of Biological Chemistry*, 176(1): 367-388.

Prakash A, Rao J, Rath PC and Behera KS. 2006. Pests of rice. *Indian Farming*, 56(7): 4-9.

Punithavalli M, Muthukrishnan NM and BalajiRajkuma M. 2013. Defensive responses of rice genotypes for resistance against rice leaf folder *Cnaphalocrocis medinalis. Rice Science,* 20(5): 363-370.

Rajadurai G and Kumar K. 2017. Evaluation of 193 rice entries against yellow stem borer, *Scirpophaga incertulas. Journal of Entomological Research*, 41(2): 133-144.

Ramaswamy C, Shanmugam TR and Suresh D. 1996. Constraints to higher rice yields in different rice production environments and prioritization of rice research in south India. *Evanson, RE, Herdt, RW and Hussain, M. (Edt.). Rice Research in Asia: Progress and Priorities. International Rice Research Institute, Manila, Philippines*., 146-160.

Singh J and Dhaliwal GS. 1994. Insect pest management in rice: a perspective agriculture. In: *Trends in agricultural insect pest management* (Eds: Dhaliwal, G.S. and Ramesh Arora). Common Wealth Publishers, New Delhi, India, pp. 56-112.

Somogyi M. 1952. Estimation of sugars by colorimetric method. *Journal of Biological Chemistry*, 195(1):19-23.

Tukey JW. 1953. The problem of multiple comparison, Princeton, New Jersey, Princeton University, Department of Statistics. 396.

Vanitha BK, Kumar CTA, Kumar LV and Prashantha C. 2015. Association of biochemical factors in rice against infestation of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Journal of Experimental Zoology* (India),18(1): 373-376.

Vijaykumar L, Chakravarthy AK, Patil SU and Rajanna D. 2009. Resistance mechanism in rice to the midge *Orseolia oryzae* (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, 102(4), 1628-1639.

Vijaykumar L, Patil SU and Chakravarthy AK. 2012. Biochemical basis of resistance in rice against Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera :Cecidomyiidae). *Current Biotica*, 6(2): 163-170.

Widowsky DA and O'toole JC. 1996. Prioritization of rice research agenda for eastern India, In: *Rice research in Asia: Progress and Priorities* (*Eds*: Evanson, R. E., Herdt, R.W. and Hussain, M.)., *International Rice Research Institute, Manila Philippines*., 109-129.