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Biochemical mechanism of resistance to shoot fly, *Atherigona approximata* Malloch in foxtail millet (*Setaria italica* L.)

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Abstract

Shoot fly (*A. approximata*) is one of the major insect pests of foxtail millet and host plant resistance is an important factor in reducing the damage caused by this pest. A better understanding of the mechanism of resistance in foxtail millet against shoot fly would help in developing the resistant varieties. In the present study, biochemical (total and reducing sugars, total phenols, total free amino acids, tannins and crude proteins content) parameters and major nutrients *viz*., N, P, K were analysed in the 10 selected foxtail millet genotypes representing each resistance category. The study revealed that higher amount of crude proteins, total soluble sugars, total reducing sugars, nitrogen and phosphorous were present in susceptible genotypes compared to resistant genotypes. The amount of phenols, tannins, total free amino acids and potassium in all resistant genotypes were found higher compared to susceptible genotypes. Significant positive correlation was observed between *A. approximata* damage and crude proteins (r= 0.87^{**}), total soluble sugars (r= 0.84^{**}), total reducing sugars (r= 0.93^{**}), nitrogen (r= 0.87^{**}) and phosphorous (r= 0.95^{**}). While, total phenols (r= -0.92^{**}), tannins (r= -0.91^{**}), total free amino acids (r= -0.92^{**}) and potassium (r= -0.83^{**}) showed a significant negative association with shoot fly infestation. These biochemical components in foxtail millet can be used effectively in the breeding program to develop resistant varieties against *A. approximata*.

Keywords: Biochemicals, host plant resistance, shoot fly, phenols, tannins

1. Introduction

Foxtail millet, generally referred to as German or Italian millet, among the most important small millets grown in the country and is the third largest millet crop in the world. This is drought tolerant, grows at high elevation (up to 600 ft) and is often planted as an alternative crop for sorghum on black cotton soils, where rainfall is deficient. Besides, its low yielding nature, it has unique nutritional properties, namely a rich source of carbohydrates (60.9 mg), protein (12.3 mg), fat (4.3 mg), minerals (3.3 mg) for 100 g dry weight and it has essential minerals such as calcium (31 mg), phosphorous (290 mg) per 100 g (Anon., 1991) [1]. The realistic yield gap seen between demonstration yield as well as the typical farmer yield was due to various biotic and abiotic factors, which are the key development constraints. Although pests and disease problems are minimal in this crop at times, in particular the species of shoot flies assume a serious pest status and cause a significant loss of yield. The incidence of shoot fly was recorded for the first time in south India during 1913 (Fletcher, 1914) [7]. Shoot flies (*Atherigona* sp.) infest different crops of the Poaceae mainly cereals and millets infesting only seedlings. It causes damage to seedlings of the age between 1-week up to 30 days. The common symptom of damage is central shoot drying, or 'dead heart.' Shoot fly is a major pest of economic significance. Among the major insect pests in millets, shoot flies are reported to be significant causing 25-90 percent dead heart (DH) damage (Selvaraj *et al*., 1974) [23]. The damaged plants that produce side tillers are also attacked by the maggots repeatedly (Kahate *et al.,* 2014) [11]. Host plant resistance was one of the most efficient means of keeping the shoot fly population below the economic threshold level, as no input costs from farmers are needed. Work on biochemical basis of shoot fly resistance shows that the little millet genotypes with lower amounts of moisture, nitrogen, crude protein, phosphorus and chlorophyll do not support oviposition of shoot fly, *A. pulla* (Kadire *et al.*, 1996) [10]. The higher concentrations of total and reducing sugars appeared to decrease shoot fly resistance in sorghum (Singh *et al*., 2004) [24]. There is also a decrease in the infestation of grasshoppers and shoot bugs, *Peregrinus* *maidis* in sorghum due to increased phenol levels (Woodhead *et al.*, 1980) [32]. Thus, it is important that genotypes with different mechanisms are identified in order to enhance their levels and broaden the basis of resistance against this pest. Therefore, the present study was conducted on different foxtail millet genotypes to determine the plant characteristics that influence resistance / susceptibility to shoot fly, *A. approximata.*

2. Materials and Methods

The genotypes of foxtail millet were received from All India Coordinated Research Projects on small millets. Each genotype was sown in a plot containing 3 m row of 2 lines in the field with a spacing of 30X10 cm between rows and plants, respectively in three replications at G-bock of Zonal Agricultural Research Station, V. C. Farm, Mandya. The plants of different genotypes were raised as per package of practice, except the plant protection measures (Anon., 2018) [2]. Based on the percentage infestation at 35 days after sowing, the genotypes were categorized into different resistance category *viz.*, highly tolerant, tolerant, moderately tolerant, susceptible and highly susceptible with 1-5, 5-25, 25-50, 50-85 and > 85 percent dead heart, respectively.

$$\text{Dead heart }(\%) = \frac{\text{Number of plants with dead heart/plot}}{\text{Total number of plants/plot}} \times 100$$

The genotypes of foxtail millet representing each resistance category were selected for sampling. The uninfested stem portion of selected foxtail millet genotypes were sampled at 20-25 days after sowing. The sampled genotypes were collected separately in a butter paper for the estimation of the important biochemicals *viz.*, the total and reducing sugars, total phenols, total free amino acids, tannins and crude proteins. Further, the major nutrients *viz.*, N, P, K in the selected genotypes representing each resistance category were estimated. The stem samples of selected genotypes were dried at 35 ^{0}C in hot air oven for 24-48 hours. The dried samples were ground using grinder. The powdered samples were stored in plastic covers until analysis.

2.1 Extraction of plant tissues in alcohol

The stem samples of selected foxtail millet genotypes were collected and thoroughly washed with distilled water and dried under shade. 10 g of plant sample was taken in separate conical flask and 150 mL of 80 percent 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 ethanol. The supernatant was decanted in to another flask and residue were 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 percent ethanol. The ethanol part of extract was stored in refrigerator at 4 ^{0}C and used for the estimation of biochemical components present in plant sample.

The total and reducing sugars in each test genotype were estimated by the method suggested by Somogyi (1952) [26]. Estimation of total phenols and tannins in stem samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954) [4]. The amount of total free amino acid present in the samples were estimated by following Ninhydrin method developed by Moore and Stein (1948) [16]. Nitrogen and crude proteins were estimated by micro-Kjeldahl method, phosphorous by spectrophotometric method and potassium by flame photometric method. The mean data was processed after suitable transformation, and was subjected for ANOVA (Gomez and Gomez, 1984 [8]; Hosmand, 1988 [9]) and means were separated by Tukey's HSD (Tukey, 1953) [28] for interpretation.

3. Results and Discussion

3.1 Total soluble sugars (TSS)

Among the screened genotypes of foxtail millet, total soluble sugars varied significantly and a lower amount of TSS was observed in highly tolerant genotypes *viz.*, IIMR FXM 4 and GPUF 2, which recorded 0.72 and 0.74 mg g^{-1} (Figure 1). However, in highly susceptible genotypes the amount of TSS was found significantly highest in IIMR FT 1 (1.28 mg g^{-1}) and SIA 382 (1.42 mg g^{-1}). TSS in different genotypes showed a significant positive impact on percent infestation (r=0.84**) at 35 DAS (Table 2). The present study is in close agreement with Patel *et al.* (2015) [19] and Vijaykumar *et al.* (2009) [30] where they reported that the amount of total soluble sugars in all susceptible genotypes of rice against *Orseolia oryzae* was found higher compared to tolerant genotypes. Umeshkumar *et al.* (2011) [29] reported that less percentage of total sugars was identified as the factor that impart resistance to shoot fly in foxtail millet. Sekar *et al.* (2018) [22] also found that the susceptibility to shoot fly was associated with high soluble sugars composition in susceptible genotypes of sorghum.

Table 1: Biochemical constituents in stem samples of foxtail millet genotypes against shoot fly, *Atherigona approximate*

Sl. No.	Category	Genotype	Incidence (%)	Biochemical components (mg g^{-1})						Minerals (%)		
				Phenols	TSS	TRS	Crude protein	TFA	Tannins	N	P	K
1	HT	IIMR FXM 4	1.82 (7.75)^{a}	3.64^{a}	0.72^{a}	0.22^{a}	8.50^{a}	10.79^{a}	0.67^{a}	1.36^{a}	0.12^{a}	3.82^{a}
2		GPUF 2	3.25 (10.38)^{a}	3.46^{ab}	0.74^{a}	0.27^{ab}	9.06^{b}	10.23^{ab}	0.57^{b}	1.45^{ab}	0.14^{ab}	3.26^{b}
3	T	SIA 3159	14.18 (22.12)^{b}	3.32^{bc}	0.78^{ab}	0.26^{ab}	9.31^{bc}	9.49^{c}	0.53^{bc}	1.49^{abc}	0.16^{bc}	2.99^{bc}
4		DHFT 109-3-1	22.06 (28.01)^{b}	3.41^{bc}	0.82^{abc}	0.24^{ab}	9.50^{c}	8.98^{cd}	0.48^{cd}	1.52^{bc}	0.15^{bc}	3.08^{bc}
5	MT	FIAVT 153	42.10 (40.45)^{c}	3.25^{c}	0.81^{abc}	0.28^{b}	9.63^{c}	9.06^{cd}	0.49^{cd}	1.54^{bcd}	0.17^{cd}	2.96^{c}
6		TNSI 364	43.12 (41.04)^{c}	2.76^{d}	0.88^{bc}	0.52^{c}	10.13^{d}	8.75^{cd}	0.46^{d}	1.62^{cd}	0.19^{de}	2.93^{cd}
7	S	SIA 3156	64.36 (53.36)^{d}	2.92^{d}	0.86^{bc}	0.56^{c}	10.44^{d}	8.44^{d}	0.43^{d}	1.67^{de}	0.19^{de}	2.88^{cd}
8		IIMR FXM 5	71.26 (57.59)^{d}	2.21^{e}	0.93^{c}	0.54^{c}	11.06^{e}	8.59^{d}	0.45^{d}	1.77^{ef}	0.21^{ef}	2.91^{cd}
9	HS	IIMR FT 1	86.50 (68.62)^{e}	1.38^{f}	1.28^{d}	0.78^{d}	11.38^{e}	6.53^{e}	0.34^{e}	1.82^{f}	0.22^{f}	2.68^{d}
10		SIA 382	88.15 (70.40)^{e}	1.56^{f}	1.42^{e}	0.85^{e}	14.06^{f}	6.16^{e}	0.29^{e}	2.25^{g}	0.25^{g}	2.40^{e}
SE m ±			1.41	0.04	0.02	0.01	0.08	0.15	0.01	0.03	0.01	0.06
CD @p=0.05			4.18	0.12	0.07	0.04	0.25	0.45	0.04	0.09	0.02	0.16

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 sugar; TRS-Total reducing sugar; TFA-Total free amino acid; N-Nitrogen; P-Phosphorous; K-Potassium.

3.2 Total reducing sugars (TRS)

Among different categories, a significant and lower amount of TRS was recorded in highly tolerant categories *viz*., IIMR FXM 4 and GPUF 2 which recorded 0.22 and 0.27 mg g^{-1}. However, in highly susceptible genotypes total reducing sugar varied between 0.78 and 0.85 mg g^{-1} (Table 1). Correlation studies shows that TRS had positive significant influence (r= 0.93^{**}) on percent incidence at 35 DAS (Table 2). The present findings were in agreement with the reports given by Sonone *et al*. (2015) [27]. Bhavani *et al*. (2012) [3] reported that total reducing sugar content in sugarcane showed significant positive relationship with top borers susceptibility. Singh *et al.* (2004) [24] also found that the low levels of reducing sugars seemed to enhance the degree of resistance to shoot fly in sorghum. Likewise, similar results were observed against *Orseolia oryzae* in rice by Vijaykumar *et al.* (2009) [30].

3.3 Crude proteins

Crude proteins were found lower in highly tolerant genotypes of about 8.50 and 9.06 mg g^{-1} in IIMR FXM 4 and GPUF 2, respectively. However, the highest percent crude proteins were observed in highly susceptible genotypes which recorded 11.38 mg g^{-1} (IIMR FT 1) and 14.06 mg g-1 (SIA 382) (Table 1). An increasing trend of crude proteins with increase susceptibility and it was positively correlated with percent incidence (r= 0.87^{**}) (Table 2). The present findings are in line with the results of Umeshkumar *et al.* (2011) [29] reported that the crude proteins contents were positively and significantly correlated with percent dead heart at different growth period of foxtail millet. Kadire *et al.* (1996) [10] also recorded a significant and positive correlation of percent dead heart and ovipositional rate of shoot fly with crude proteins content at different growth periods in little millet. Likewise, Vijaykumar *et al.* (2009) [30] and Vijaykumar *et al.* (2012) [31] found that in majority of resistant genotypes, lower amount of crude proteins content was recorded compared to susceptible genotypes of rice against *Orseolia oryzae*.

3.4 Total phenols

In highly tolerant genotypes, phenols content varied from 3.46 to 3.64 mg g^{-1} and in highly susceptible genotypes, significantly lower amount of phenol was observed which recorded 1.38 to 1.56 mg g^{-1}. The correlation studies between percent infestation and phenol showed that there was significant negative influence (r= -0.92^{**}) on percent infestation (Table 2). The present results are in close agreement with Vijaykumar *et al.* (2009) [30] and Vijaykumar *et al.* (2012) [31], where they observed negative correlation of incidence of gall midge with total phenol content in rice. Kamatar *et al.* (2003) [12] also reported that the higher levels of phenols are desirable in a sorghum plant to resist the shoot fly infestation. Biochemical analysis of different sugarcane genotypes by Bhavani *et al.* (2012) [3] observed a higher phenol content in the shoot tissues of highly resistant genotypes compared to susceptible genotypes against early shoot borer, *Chilo infuscatellus*.

Table 2: Correlation matrix between infestation of *Atherigona approximata* and biochemical constituents of stem in foxtail millet, *Kharif* 2019

Parameters	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**	X_6	X_7	X_8	X_9
Y – Shoot fly infestation	-0.92**	0.84**	0.93**	0.87**	-0.92**	-0.91**	0.87**	0.95**	-0.83**
X_1 – Phenols	1.00	-0.94	-0.95	-0.88	-0.85	0.88	-0.88	-0.93	0.78
X_2 – TSS		1.00	0.92	0.93	0.81	-0.88	0.93	0.88	-0.78
X_3 – TRS			1.00	0.91	0.82	-0.89	0.91	0.94	-0.79
X_4 – Protein				1.00	0.85	-0.90	0.99	0.94	-0.84
X_5 – TFA					1.00	-0.95	0.85	0.94	-0.94
X_6 – Tannin						1.00	-0.90	-0.94	0.95
X_7– Nitrogen							1.00	0.94	-0.84
X_8– Phosphorous								1.00	-0.91
X_9– Potassium									1.00

N = 10; ** Significant at $P \leq 0.01$; TSS-Total soluble sugar; TRS-Total reducing sugar; TFA-Total free amino acid; DAS-Days after sowing.

3.5 Total free amino acid (TFA)

In highly tolerant category TFA was varied from 10.23 to 10.79 mg g^{-1}. While, low levels of TFA was recorded in IIMR FT 1 (6.53 mg g^{-1}) and SIA 382 (6.16 mg g^{-1}) and were categorized as highly susceptible genotypes. A decreasing trend of TFA in tested foxtail millet genotypes showed significant negative (r =-0.92^{**}) impact on percent infestation at 35 DAS. (Table 2). These results are in similar with findings of Vijaykumar *et al.* (2009) [30] and Vijaykumar *et al.* (2012) [31] reported that in rice shoot apices of resistant genotypes recorded higher levels of total free amino acids compared to susceptible genotypes. Further Praveen *et al.* (2013) [20] also reported that maize genotypes resistant to stem borer recorded higher amount of total free amino acids against susceptible genotypes. Mohammad *et al.* (2017) [15] also found that the correlation between total free amino acids content in chickpea and growth index of grub of *Callosobruchus chinensis* showed a negative relationship.

3.6 Tannins: In highly tolerant categories significantly higher amount of tannins was observed *i.e.* 0.67 and 0.57 mg g^{-1} in IIMR FXM 4 and GPUF 2, respectively. However, in highly susceptible genotypes significantly lower amount of tannins was recorded in IIMR FT 1 (0.34 mg g^{-1}) and SIA 382 (0.29 mg g^{-1}) (Table 1). The tannin content among the genotypes had a negative correlation (r= -0.91^{**}) with percent infestation at 35 DAS (Table 2). Chamarthi *et al.* (2011) [5] also reported that the tannins content was negatively associated with shoot fly damage imparting that, it was a defensive compound contributing towards the shoot fly resistance. Likewise, Sanjay and Singh (1998) [21] observed that the higher concentration of tannins had been reported to impart resistance in sorghum against damage from shoot fly and it has been positively correlated with shoot fly resistance. Further, Mohammad *et al.* (2017) [15] found that the susceptible varieties of chickpea with less tannins content recorded more growth index of grub of *Callosobruchus chinensis* as compared to resistant varieties.

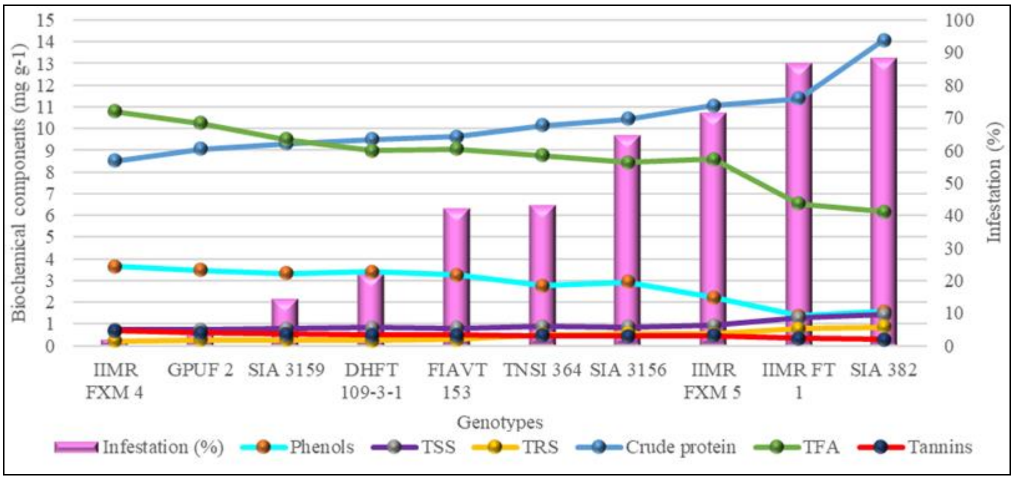

Fig 1: Biochemical constituents in stem samples of foxtail millet genotypes against shoot fly, *Atherigona approximata* at 35 DAS

3.7 Nitrogen (N)

Nitrogen content was found lower in tolerant genotypes than in susceptible genotypes. In highly tolerant categories, it was recorded 1.36 to 1.45 percent and in highly susceptible genotypes percent nitrogen was recorded 1.82 and 2.25 percent in IIMR FT 1 and SIA 382, respectively. By correlation studies it was found that, nitrogen percent had positive significant influence (r= 0.87$^{**)}$ on percent infestation at 35 DAS. (Table 2). Greater uptake of nitrogen in susceptible genotypes results in higher production of nitrogen metabolites that were used by the shoot fly larvae for rapid growth and development and in turn increase in dead hearts during early stage of growth (Khurana and Verma, 1983) [13]. Similarly, low levels of nitrogen in plants were reported to be associated with the shoot fly resistance in sorghum (Singh and Jotwani, 1980; Chavan *et al*., 1990) [25, 6].

3.8 Phosphorous (P)

Highly tolerant genotypes had low percent phosphorous about 0.12 and 0.14 percent in IIMR FXM 4 and GPUF 2, respectively. However, in highly susceptible genotypes phosphorous was found lower in IIMR FT 1 (0.22%) and SIA 382 (0.25%) and percent phosphorous in foxtail millet stem sample increased the susceptibility and showed significant positive correlation (r=0.95**) with percent infestation at 35 DAS (Table 2). Positive association of phosphorous with oviposition by the shoot fly females during the seedling stage may be due to their association with production and release of chemical cues influencing the oviposition behavior of sorghum shoot fly (Khurana and Verma, 1983) [13]. Kadire *et al.* (1996) [10] also reported that nitrogen content in susceptible genotypes were found to be higher compared to that in tolerant genotypes. Low concentrations of nitrogen in sorghum seedlings greatly enhanced the degree of antixenosis for oviposition, feeding and dead heart formation and can be used as selection criteria for resistance to shoot fly (Singh *et al*., 2004) [24].

3.9 Potassium (K)

In Highly tolerant genotypes percent potassium was varied among the genotypes *viz*., IIMR FXM 4 (3.82%) and GPUF 2 (3.26%). However, in highly susceptible genotypes *viz*., IIMR FT 1 and SIA 382 recorded lower percent potassium *i.e*. 2.68 and 2.40, respectively. The results of correlation studies revealed that percent potassium had negative and significant correlation (r= -0.83**) on percent incidence at 35 DAS (Table 2). Kiran *et al.* (2018) [14] reported that potassium offers high resistance to insect pests as well as high potassium rates improve secondary metabolite compounds, minimise carbohydrate deposition and damage from insect pests to the plants. However, varieties with high content of potassium were less preferred by delphacids and aphids in sorghum (Mote and Shahane, 1994) [17]. Further, Paras *et al.* (2017) [18] also found that the potassium content was higher in resistant genotypes compared to susceptible genotypes of bitter gourd against fruit fly, *Bactrocera cucurbitae*.

4. Conclusion

Biochemical analysis of different foxtail millet genotypes indicated that higher phenols, tannins, total free amino acids and potassium contents and lower total and reducing sugars, nitrogen, phosphorous and crude protein contents in the shoot tissues of highly resistant genotypes (IIMR FXM 4 and GPUF 2) had increased its resistance to shoot fly by influencing the biology and played a significant role in the antibiosis mechanism.

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