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Oxidative enzyme and biochemical changes in sweet sorghum infested by shootfy

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Abstract
This research investigation carried out with an objective to detecting the oxidative enzymes and protein activities in sweet sorghum plants and their role in resistance against shootfly in sweet sorghum. Total soluble protein and chlorophyll content were estimated higher in susceptible genotypes as compared to resistant. Susceptible genotypes showing maximum decreased rate of chlorophyll content as compared to resistant genotypes. The resistant genotypes viz., IS-18360, RSSV-260 and RSSV-167 recorded significantly lower soluble protein as compare to susceptible genotypes, SSV-84, RSSV-269 and RSSV-493. The average polyphenol oxidase activity was more in infected plant as compared to non-infected plant, means it was observed that activity of polyphenol oxidase increased after the infection of shootfly. It was observed that activity of peroxidase increased after the infection by shootfly which was higher in the resistant parents as compared to susceptible parents. Highest value for peroxidase and polyphenol oxidase activity recorded by the resistant parent IS-18360 and RSSV-260, it means parents showing the resistance mechanisms with higher activity of peroxidase and polyphenol oxidase enzyme.
Enzymatic activities suggested that the shootfly feeding leads to a loss in POX (Plant Oxidative Enzymes) activity in susceptible sweet sorghum genotypes. However, resistance genotypes may be able to tolerate shootfly feeding by increasing their POX activity. These biochemical characters can be used as marker traits in shootfly resistance breeding programme to broaden the genetic base and increase the level of resistance to sorghum shootfly.

Keywords: Sweet sorghum, shootfly, oxidative enzymes, protein, host plant resistance

1. Introduction
Sweet sorghum [*Sorghum bicolor* (L.) Moench] is an emerging special purpose sorghum with a sugar-rich stalk, simply like sugarcane. Besides having rapid growth, high sugar content and maximum biomass production potential, sweet sorghum has wider adaptability (Reddy and Sanjana, 2003) [17]. Given that water availability is poised to become a major constraint to agricultural production in coming years (Rayan and Spencer, 2001) [16], cultivation of sugarcane becomes difficult. Sweet sorghum would be a rational crop option on the behalf of sugarcane in such situations. Sweet sorghum can be grown with minimum irrigation, rainfall and acquire inputs in relation to sugarcane. The sugar content in the juice extracted from sweet sorghum varies from 16-23 per cent Brix. It has a great potential for jaggery, syrup and most importantly bio fuel alcohol production (Ratnavathi *et al*., 2004) [15].
Insect cause huge loss in grain and forage yields of sorghum worldwide. There are over 150 insect pest species damaging sorghum crop from sowing to harvest. As many as 25 pest s have been reported to damage sorghum crop in Maharashtra State (Dhumal, 1967) [3] but the important among these are shootfly (*Atherigona soccata* Rondani), stem borer (*Chilo partellus* Swinhoe) and earhead midge (*Contarinia sorghicola* Coquillett) which cause considerable damage to the crop. Among several species of shoot fly recorded in India, the sorghum shoot fly, *Atherigona soccata* Rondani has gained importance with the introduction of high yielding varieties and hybrids.
Shootfly (*Atherigona soccata* Rondani) is one of the major biotic factors which affect the productivity from 20-50% in sorghum. Insect causes enormous loss in grain, juice yield and forage yield of sweet sorghum world-wide. Sorghum shootfly causes an average loss of 50% in India (Jotwani and Shrivastava, 1982) [6], but the infestation may be over 90%. In Maharashtra more than 25% have been reported to damage sorghum crop by shootfly, which causes major considerable damage.

Host plant resistance is an effective measure of keeping shoot fly population below threshold level as it does not required any cost input by the farmer. Host plant resistance to sorghum shoot fly appears to be polygenic character and depends on the interactions of number of quantitative characters which finally sum up in the expression of resistance to shoot fly. Host plant resistance provide a promising approach for managing shootfly because it is sustainable, economical and environmentally reliable. When we go for developing insect-resistant varieties, by understanding of the mechanism of the resistance is critical for formulating optimal strategies for identifying and exploiting resistant sources from germplasm. Where considerable improvement has been made in identifying germplasm resistance to shootfly, improvement towards characterization of physiological and biochemical mechanisms to resistance endure limited. Thus the present study was carried out with an objectives of detecting the role of protein and enzymes in sweet sorghum plant and their activity in host plant resistance to shootfly infestation. For analysis, different parameters were selected *viz*., Chlorophyll content (mg/g), Soluble protein (mg/g fresh weight), Polyphenol oxidase (ΔO.D./min/g) and Peroxidase (ΔO.D./min/g), these parameters were analysed separately from infected and healthy plants of sorghum among six genotypes from which three are resistant and remaining are susceptible for shootfly.

2. Materials and Methods

The present investigation was conducted at Botany Farm, Post Graduate Institute, M.P.K.V., Rahuri, during the period *Kharif* - 2018. The experimental material for present study was obtained from Senior Sorghum Breeder, Sorghum Improvement Project, MPKV, Rahuri. Six sweet sorghum genotypes were selected for the experiment, from which three shootfly resistant *viz*., IS-18360, RSSV-260 and RSSV-167 and three shootfly susceptible sweet sorghum genotypes *viz*., SSV-84, RSSV-269 and RSSV-493. For this study four different parameters were selected *viz*., Chlorophyll content (mg/g), Soluble protein (mg/g fresh weight), Polyphenol oxidase (ΔO.D./min/g) Peroxidase (ΔO.D./min/g), These parameters were analysed separately from infected and healthy plants of sorghum among six genotypes from which three are resistant and remaining are susceptible for shootfly.

The experimental genotypes was planted in two rows with four meter length and the rows were 60 cm apart. The experiment was carried out in a randomized block design with three replications. Normal agronomic practices were followed for raising the sweet sorghum crop and no insecticide were applied in experimental plot. Overall resistance was recorded as the percentage of dead heart (DH%) and density of trichome caused by shootfly infestation. Plant with dead heart were recorded in all plots at 28 DAE.

Sorghum seedling (leaves and stem) (2 g) of each six of genotypes infested with shootfly were collected from field for biochemical analysis at 21 DAE. Plant without dead heart symptoms served as uninfested control. Soluble protein From the seedling were estimated by methods suggested by Kamala Jayanthi *et al*., Kumar and Khan and Kamakkar *et al*., respectively. The enzyme extractions was done by following the procedure given by Nwanze *et al.* and enzyme activity was expressed as change in absorbance per minute per gram fresh weight (ΔO.D./min/g).

3. Results and Discussion

The genotypes differed significantly for dead heart percentage and trichome density, which indicated substantial amount of variability present in the material selected for this investigation. It was recorded that the parent RSSV-260 (12.13) exhibited maximum mean value trichome density and least percentage of dead heart (17) followed by genotype IS-18360 (21.86) and RSSV-167 (27.20). RSSV-260 and IS-18360 was as the good resistant as the genotypes SSV-84 and RSSV-269 for shootfly infestation, whereas the dead heart formation was greater on the genotypes SSV-84, RSSV-269 and RSSV-493 (Table 1).

Table 1: Performance of genotypes resistance to shootfly (*Atherigona soccata*) in Sweet Sorghum

	Genotypes	Trichome density (mm^2)	Dead heart percentage (%) at 21 DAE
41	SSV-84 (S)	0.74	68.00
22	RSSV-269 (S)	0.775	68.23
33	RSSV-493 (S)	1.23	67.00
44	RSSV-260 (R)	12.14	17.00
55	IS-18360 (R)	10.6	21.86
66	RSSV-167 (R)	8.53	27.20
	mean	5.66	44.69
	SE	0.23	0.72
	CD@1%	1.31	4.15

3.1 Chlorophyll Content (mg/g fresh wt)

Result shown in Table 2, revealed that chlorophyll content observed more in healthy samples as compared to infected ones, whereas it was observed higher in susceptible genotype (SSV-84), while lowest in the resistant genotype (RSSV-167). Chlorophyll content was decreased after infestation of shootfy, it was observed that chlorophyll content in infested sample was minimum or decreased.

Table 2: Biochemical parameters associated with shootfly resistance in sweet sorghum

	Genotypes	Soluble protein (mg/g fresh weight)			Polyphenol Oxidase (ΔO.D./min/g)			Peroxidase (ΔO.D./min/g)			Chlorophyll Content (mg/g)		
		Fresh	Infected	Per cent increase	Fresh	Infected	Per cent increase	Fresh	Infected	Per cent increase	Fresh	Infected	Per cent decrease
1	SSV-84 (S)	2.16	2.49	13.25	0.3	0.35	14.29	0.71	0.81	12.35	2.17	1.33	63.2
2	RSSV-269 (S)	2.09	2.35	11.06	0.28	0.33	15.15	0.63	0.79	20.25	2.36	1.47	60.5
3	RSSV-493 (S)	2.51	2.92	14.04	0.28	0.32	12.50	0.54	0.66	18.18	1.99	0.91	54.3
4	RSSV-260 (R)	1.73	1.98	12.63	0.46	0.55	16.36	1.1	1.48	25.68	1.94	1.14	41.2

5	IS-18360 (R)	1.29	1.59	18.87	0.53	0.62	14.52	1.09	1.32	17.42	1.72	0.77	55.2
6	RSSV-167 (R)	1.9	2.75	30.91	0.52	0.6	13.33	0.95	1.28	25.78	1.51	0.9	40.4
	mean	1.95	2.35	17.02	0.4	0.46	13.04	0.84	1.06	20.75	1.95	1.09	44.1
	SE	0.032	0.023		0.013	0.01		0.018	0.024		0.016	0.013	
	CD@1%	0.096	0.066		0.038	0.03		0.051	0.069		0.044	0.037	

(S) Susceptible & (R) Resistant

3.2 Soluble protein (mg/g fresh wt.)

Soluble protein was observed more in the susceptible genotype (SSV-84) (2.49mg/g) while lower in the resistant genotype (IS-18360) (1.29). From the analysis it was observed that after the infection of shootfly, level of soluble protein increased and level increased activity were higher in the susceptible parents as compared to resistant parents. The percent increased rate of soluble protein was higher in the susceptible genotypes as compared to the resistant genotypes (Table 2).

3.3 Polyphenol oxidase (ΔO.D./min/g)

Result of enzymes activities shown in Table 2 and depicted in fig. 1 and 2, revealed that **t**he average polyphenol oxidase activity in healthy samples ranged from 0.28 to 0.53 and in an infected samples it was ranged from 0.32 to 0.62, it was more as compared to non-infected plant, means it was observed that activity of polyphenol oxidase increased after the infection of shootfly. Activity of this enzyme observed higher in the resistant genotype (IS-18360) (0.53 F and 0.62 In) as compared to susceptible genotype (SSV-84) (0.30F and 0.35 In).

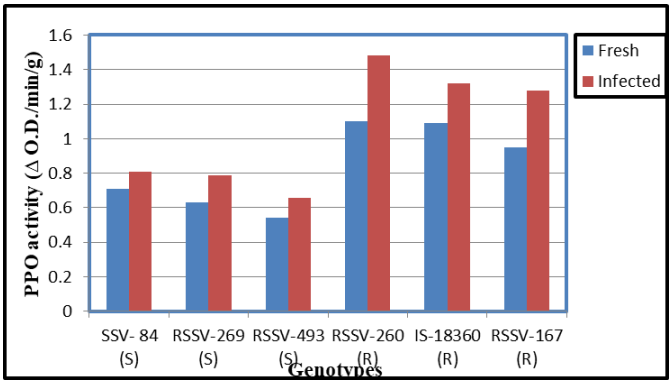

Fig 1: Polyphenol oxidase activity in sweet sorghum genotypes after infestation by shootfly

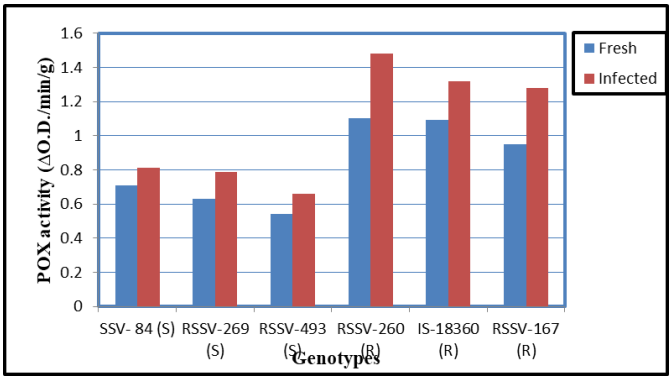

Fig. 2: Peroxidase activity in sweet sorghum genotypes after infestation by shootfly

3.4 Peroxidase (ΔO.D./min/g)

The average peroxidase activity ranged from 0.54 to 1.10 in healthy sample and in an infected samples it was ranged from 0.66 to 1.48, it was observed that activity of peroxidase increased after the infection by shootfly higher in the resistant genotypes as compared to susceptible genotypess. Highest value for peroxidase activity recorded by the resistant genotype IS-18360, it means genotypes showing the resistance mechanism with higher activity of peroxidase enzyme.

It was observed that the genotypes having lower chlorophyll content were less susceptible to shootfly. The higher chlorophyll content led to susceptibility because of increased preference of shootfly larvae towards the higher chlorophyll containing plant.

Chlorophyll content was found to be decreased after infestation of shootfy, it was observed that chlorophyll content in infested sample was minimum or decreased. From the present investigation susceptible genotypes showing maximum rate of chlorophyll content decrease as compared to resistant genotypes. Similar results were reported by Patil *et al*., (2017) [14], also reported significant differences for chlorophyll content among susceptible and resistant genotypes in sorghum.

The resistance genotypes *viz*., IS-18360, RSSV-260 and RSSV-167 recorded significantly lower soluble protein as compare to susceptible genotypes, SSV-84, RSSV-269 and RSSV-493. This finding is in accordance with the earlier workers, Padmja *et al*., (2014) who reported an overall increase in total protein content compared with uninfested plant in sorghum, also recorded highest protein content in SSV-84. Bhoge *et al*., (2017) [1] and Patil *et al*., (2017) [14], also reported similar finding in sorghum. Activity of this enzyme observed higher in the resistant parent (IS-18360) (0.53 F and 0.62 In) as compared to susceptible parent (SSV-84) (0.30F and 0.35 In). It has been earlier reported by Padmja *et al*., (2014 [13], Bhoge *et al*., (2017) [1] and Patil *et al*., (2017 [14]. It was observed that activity of peroxidase increased after the infection by shootfly was higher in the resistant parents as compared to susceptible parents. Highest peroxidase activity was recorded by the resistant parent IS-18360, it means parents showing the resistance meachanism with higher activity of peroxidase enzyme. This finding confined similar to the earlier research of Padmja *et al*., (2014) [13], Bhoge *et al*., (2017) [1] and Patil *et al*., (2017) [14].

4. Conclusion

From the studies of enzyme activities, protein and chlorophyll content, it was concluded that the shootfly feeding leads to a loss in POX activity in susceptible sweet sorghum genotypes. However, resistant genotypes may be able to tolerate shootfly feeding by increasing their POX activity. Hydrogen peroxide is through to be increased in response to plant stress such as insect feeding (Dowd and Lagrimini, 1997) [2]. The quantity of hydrogen peroxide is conciliated by the presence of oxidative enzymes such as POX (Levine *et al*., 1994) [9]. Polyphenol oxidase lessen the nutritional quality of shoot fly infested plants by converting soluble phenolic compound into quinines that finally stop the digestion of proteins in insect. Similarly considerable evidence from the earlier work implicates that increased accumulations of PPO in plant against tomato fruit borer has affected growth and

development of these insects (Isman and Duffey 1982) [5].
Present study revealed that higher enzyme activity and protein activity imparted resistance in sweet sorghum. These biochemical characters can be used as marker traits in shootfly resistance breeding programme to broaden the genetic base and increase the level of resistance to sorghum shootfly.

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