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Morphological and biochemical assessment of sweet sorghum genotypes for shootfy resistance

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Abstract
This research conducted with an objective to study the morphological and biochemical parameters in sweet sorghum genotypes for shoot fly resistance. It was recorded that the genotype RSSV-260 (12.13) exhibited maximum mean value of 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. 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

Introduction
Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a special purpose sorghum with a sugar-rich stalk, almost like sugarcane. Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability (Reddy and Sanjana, 2003). Given that water availability is poised to become a major constraint to agricultural production in coming years (Rayan and Spencer, 2001), cultivation of sugarcane becomes difficult. Sweet sorghum would be a logical crop option in lieu of sugarcane in such situations. Sweet sorghum can be grown with less irrigation and rainfall and purchased inputs compared 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 fuel alcohol production (Ratnavathi *et al*., 2004). The silage after extraction of juice from sweet sorghum can be used for co-generation of power.
Insect cause enormous 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) 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), 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 one of most effective means of keeping shoot fly population below threshold level as it does not involve any cost input by the farmer. Plant resistance to sorghum shoot fly appears to be complex character and depends on the interplay of number of componential characters which finally sum up in the expression of resistance to shoot fly (Dhillon *et al.,* 2006). Plant resistance offers a promising approach for managing shootfly because it is sustainable, economical and environmentally responsible. When developing insect-resistant crops, a thorough understanding of the underlying mechanism of the resistance is critical for formulating optimal strategies for identifying and exploiting resistant sources. Although considerable progress has been made in identifying germplasm resistance to shootfly, progress towards characterization of physiological and biochemical mechanisms conferring the resistance remain 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 parents from which three are resistant and remaining are susceptible for shootfly.

Material 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 this studies 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 test material was planted in 4-m row plot 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. (Ratio of the number of dead heart to the total number of plant x 100) recorded at 28 DAE was used for evaluating resistance.

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 symptom served as uninfested control. The total sugar, 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). Data obtained from field experiment were subjected to analysis of variance using the statistical software Windostat.

Result 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 genotype RSSV-260 (12.13) exhibited maximum mean value for 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). Leaf glossiness is one of the most important measurement of shootfly resistance during seedling stage at 14 DAE was found minimum in resistance genotype IS-18360 (1.73) and RSSV-260 (1.46) as compared to susceptible genotype SSV-84 (3.13) and RSSV-269 (2.93). Leaf surface wetness is also important measurement of shootfly resistance during seedling stage at 14 DAE. Resistance genotype IS-18360 (1.53) exhibited lowest mean value of leaf leaf surface wetness as compared to susceptible genotype SSV-84 (3.33). Based on the substantial information obtained from mean performance of genotypes, *viz.*, RSSV-260 and IS-18360 can be considered in developing shootfly tolerant hybrid (Table 1).

Chlorophyll Content (mg/g fresh wt)

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.

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.

Polyphenol oxidase (ΔO.D./min/g)

The 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).

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.

Plant resistance offers a promising approach for managing shootfly because it is sustainable, economical and environmentally responsible. When developing insect-resistant crops, a thorough understanding of the underlying mechanism of the resistance is critical for formulating optimal strategies for identifying and exploiting resistant sources. Although considerable progress has been made in identifying germplasm resistance to shootfly, progress towards characterization of physiological and biochemical mechanisms conferring the resistance remain 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. It indicated 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 Mate *et al*., (1988), Bapat *et al*., (1987) and Patil *et al*., (2017) [13], 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) [12] 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) [13], 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) [12], Bhoge *et al*., (2017) [1] and Patil *et al*., (2017) [13]. 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) [12], Bhoge *et al*., (2017) [1] and Patil *et al*., (2017) [13].

From the enzyme activities, protein and chlorophyll content, study suggested 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 produced in response to plant stress such as insect feeding (Dowd and Lagrimini, 1997) [2]. The level of hydrogen peroxide is mediated by the presence of oxidative enzymes such as POX (Levine *et al*., 1994) [8]. Polyphenol oxidase reduces the nutritional quality of infested plants by converting soluble phenolic compound into quinines that eventually prevent 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. Based on the substantial information obtained from mean performance of genotypes, *viz.*, RSSV-260 and IS-18360 can be considered in developing shootfly tolerant hybrid in future.

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

Sr. No.	Genotypes	Trichome density (mm^2)	Dead heart percentage (%) at 21 DAE	Leaf glossiness (score)	Leaf surface wetness (score)	Seedling vigor (score)
1	SSV-84 (S)	0.74	68.00	3.13	3.33	3.40
2	RSSV-269 (S)	0.775	68.23	2.93	3.13	2.93
3	RSSV-493 (S)	1.23	67.00	3.20	3.26	3.40
4	RSSV-260 (R)	12.14	17.00	1.46	1.60	1.53
5	IS-18360 (R)	10.6	21.86	1.73	1.53	1.73
6	RSSV-167 (R)	8.53	27.20	1.80	1.73	1.86
	mean	5.66	44.69	2.38	2.49	2.44
	SE	0.23	0.72	0.040	0.034	0.0084
	CD@1%	1.31	4.15	0.128	0.107	0.0265

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

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