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Shoot fly tolerance studies in sorghum (*Sorghum bicolor* (L.) Moench) germplasm lines

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Abstract
116 sorghum germplasm lines and four checks (One resistant check IS-18851, one susceptible check DJ-6514 and two varietal checks SPV-1411 and PVK-801) were evaluated to find out the genetic components of variation, heritability and correlation for grain yield and shoot fly resistance traits in sorghum germplasm lines. The germplasm lines were planted in randomized block design with two replications during rabi 2015-16 at Sorghum Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Observations were recorded on five randomly selected plants in each plot and replication for grain yield, yield contributing traits and following shoot fly tolerant associated traits from each replication. Wide range of variation was observed for shoot fly resistance and yield contributing characters. GCV ranged from 3.49% (days to maturity) to 111.53% (abaxial trichome density) and PCV ranged from 3.87% to 111.66%. higher GCV along with high heritability and genetic advance exhibited by traits dead hearts at 14 and 28 DAE, trichome density (abaxial and adaxial) and plumule & leaf sheath pigmentation. Glossiness, seedling vigour, leaf wetness and chlorophyll content were significant and positively correlated with dead heart percentage at 14 and 28 DAE at both the genotypic and phenotypic levels. While it had shown negative significant association with trichome density (adaxial and abaxial) surface, plant height, leaf length, leaf breadth and grain yield per plant at both genotypic and phenotypic level. From present study it is concluded that traits *viz*., number of trichomes at adaxial and abaxial surfaces of lamina, leaf glossiness, seedling vigour, plumule leaf pigmentation played important role in shootfly resistance and also exhibited high GCV, heritability and high to moderate genetic advance and significantly positive association for shoot fly dead heart percent will be effective for improving shoot fly resistance through breeding programme.

Keywords: Variability, heritability, genetic advance, correlation, *Sorghum bicolor*

Introduction
Sorghum is the fifth most important cereal crop globally after rice, maize, wheat and barley [1]. It is predominantly cultivated in semi-arid tropics (SAT) and It is extensively cultivated in both rainy (*kharif*) and winter (*rabi*) season in Maharashtra. It is the dietary staple of more than 500 million people in 30 countries. Its grain is main food source in many developing countries. Besides food, it is also used for animal feed, fuel, syrup, alcoholic beverages and ethanol. Shoot fly is a major insect pest of cultivated sorghum adoption of chemical control method is not economically feasible for most of the sorghum growing farmers. Therefore host plant resistance is itself excellent pest controlling method, and when integrated with other methods of insect control offers a sound approach to deal with insect pest. This approach holds great potential for sorghum which is known to be poor man's crop. Shoot fly resistance involves number of component traits, which are quantitative in nature and influenced by G x E interaction.
The knowledge of genetic variability and relationship among various quantitative characters is helpful in deciding the selection criteria to bringing out the possible improvement. Genetic variation though important can be used for crop improvement only when it is considered in relation to non-genetic variation. Number of studies in sorghum by several workers revealed wide variation for various traits. Yield is a polygenically controlled complex character and is determined by a number of component characters which are also quantitatively inherited. Knowledge of heritability and genetic advance of the characters indicates the scope of improvement through selection.
Though association analysis is helpful in determining the components of yield, it does not provide an exact picture of relative importance of indirect and direct influence of each of the components towards grain yield. The inclusion of large number of variables in the correlation

analysis shows more complex indirect associations. In such a situation, knowledge of major yield components is of paramount significance in formulating an effective selection programme. Information is merging exclusively on genetic variation heritability, correlation and path analysis in sorghum. Hence, this investigation was undertaken to find out the genetic variation and major yield components in sorghum imparting tolerance through correlation.

Material and Methods

The material used for this study was comprised 116 genotypes and four checks (One resistant check IS-18851, one susceptible check DJ-6514 and two varietal checks SPV-1411 and PVK-801). The germplasm lines were planted in randomized block design with two replications during rabi 2015-16 at Sorghum Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Parbhani is located at 19.27 °N to 76.78 °E. It has an elevation of 347 meters. Each genotype was sown in two rows of 4 m length with spacing of 0.45 m and 0.15 m between rows and within plants was adopted. All the agronomical practices were followed to raise good crop.

Observations were recorded on five randomly selected plants in each plot and replication for grain yield, yield contributing traits and following shoot fly tolerant associated traits from each replication.

Shoot fly resistance parameters

Leaf glossiness

Recorded visually on 1-5 scale at 10-12 DAE (fifth leaf stage), when expression of this trait was most apparent, in the morning hours, with maximum reflection of light from the leaf surface [2].

Seedling vigour

Recorded at 10 DAE on 1-5 grade scale. The scale (1) indicates high vigour with maximum height, leaf expansion and robustness. The scale (5) indicates low vigour with poor growth, less leaf expansion and poor adaptation.

Shoot fly dead heart incidence (%)

In every genotypes total shoot fly affected plants with dead hearts symptoms were recorded after 14 and 28 days after emergence (DAE) and expressed as percentage of the total number of plants per plot. Dead heart percentage was calculated.

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

Leaf angle (Degrees)

Leaf angle from randomly selected five plants of each genotype, from each replication was recorded at 50% flowering. Angle of fifth leaf (from the base) was recorded degrees with the help of scale (Semi-circle).

Trichome density (10x magnification)

Central portion of the fifth leaf (from the base) was taken from the seedling after 22 days of emergence. The small leaf pieces of (approximately 2 cm^2) were placed in acetic acid and ethyl alcohol solution (2:1) in a stoppered glass vials (10 ml capacity) for dechlorophication. The leaf pieces were kept in this solution for 24 hours and there after transferred into Lactic acid (90%). Leaf segments cleared of the chlorophyll content were observed for the Trichome density. The leaf sections were mounted on a slide in a drop of lactic acid and observed under Stereomicroscope at a magnification of 10 x.

The trichomes on both Adaxial (Upper) and Abaxial (Lower) surfaces of the leaf were counted from two different microscopic fields selected at random and expressed as number of trichomes/10x microscopic fields.

Chlorophyll content

The chlorophyll content of the third leaf (from the top) of 80 days old plants were measured with the help of Chlorophyll meter (SPAD-502 Plus, KONICA MINOLTA).

Plumule and leaf sheath pigmentation (1-3 scale)

The pink and green colored pigment on Plumule and leaf sheath were visually scored on a 1-3 rating scale at 7 days after emergence [3]. The Plumule and leaf sheath pigmentation are the most reliable parameters, and these can be used as marker traits to screen and select for resistance to sorghum shoot fly.

Leaf wetness

Cultivars with a high transpiration rate are preferred for oviposition. Shoot fly resistance lines have low leaf surface wetness, and are characterized by a smooth amorphous wax layer and sparse wax crystals [2]. The seedlings at fifth leaf stage (12 DAE) were brought to laboratory in early morning and central whorls was opened and mounted on slide with sticky tape and observed under the microscope (10 x magnifications) for leaf surface wetness. Leaf surface wetness was rated on 1-5 scale.

1 = Leaf blade without water droplets and 5 = Entire blade with densely water droplets.

The analysis of variance was done as suggested by Panse and Sukhatme [4]. Phenotypic and genotypic coefficients of variation were calculated according to Burton [5]. Correlation studies were carried out as suggested by Johnson [6].

3. Results and Discussion

In the present investigation, wide range of variation was observed for shoot fly resistance and yield contributing characters *viz*., dead heart per cent at 14 DAE and at 28 DAE, trichome density at both abaxial adaxial surface, glossiness, vigour, leaf wetness, leaf length, leaf angle, chlorophyll content, Days to 50% flowering and grain yield per plant. These are in close proximity with the earlier findings [7].

3.1. Genotypic, phenotypic variance and heritability

Although the phenotypic variance was greater than genotypic variance, the differences between them were of lower magnitude suggesting the least influence of environment in the expression of these characters similar results have also been reported by [8]. GCV ranged from 3.49% (days to maturity) to 111.53% (abaxial trichome density) and PCV ranged from 3.87% to 111.66%. High estimates of genotypic and phenotypic variance depicting high variability, were observed for the traits dead heart percent at 14 and 28 DAE, trichome density, Plumule and leaf sheath pigmentation, number of tillers, plant height, grain yield per plant. High GCV & PCV values for shoot fly tolerant traits reported earlier [9]. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) not only useful for comparing the relative amount of variations among different traits but also for estimating the scope for improvement by selection as the differences between genotypic and phenotypic coefficient of variability indicate the environmental influence.

Heritability and genetic advance

Heritability estimates in conjunction with genetic advance were reliable in predicting the resultant effect from selecting the best individuals [6]. High heritability coupled with high genetic advance as per cent mean was observed for dead heart percent at 14 DAE (88.59 & 61.28) and 28 DAE (96.09 & 72.39), trichomes density at Adaxial (99.73 & 152.99) and at Abaxial (99.77 & 229.50) surface, plumule and leaf sheath pigmentation (89.75 & 81.83) and grain yield per plant (84.89 & 80.78) respectively, suggesting lesser influence of environments in expression of characters and prevalence of additive gene action in their inheritance. High heritability with high genetic advance for dead heart per cent and grain yield, hence these traits can be improved through simple selection procedure [10]. High heritability with moderate genetic advance as per cent of mean was recorded for seedling vigour, leaf wetness, leaf length, number of tillers, leaf angle and 100 seed weight. Similar results were obtained [9, 11]. The results indicate that these characters were less influenced by environment but governed by additive and non-additive gene action. While the characters leaf length, leaf breadth, chlorophyll content, days to 50 per cent flowering and 100 seed weight showed high heritability but low genetic advance as per cent of mean, thereby indicating that expression of these characters is governed by non-additive gene action and selection on phenotypic basis will be non-effective.

In present study selection for traits dead hearts at 14 and 28 DAE, trichome density (abaxial and adaxial) and plumule & leaf sheath pigmentation exhibiting higher GCV along with high heritability and genetic advance will be efficient for shoot fly tolerance.

Correlation coefficient

Correlation indicates inherent association between various traits and hence, leads to a directional model for direct and/or indirect improvement in grain yield [12]. Genotypic correlation was generally of higher magnitude than phenotypic correlation (Table-1) indicating that inherent association between various characters studied. In present study results indicated significant and positive association ($P < 0.01$) of grain yield per plant with panicle dry weight (r=0.784), days to 50*%* flowering (r=0.770) and total biomass (r=0.635) at genotypic level.

In present study grain yield per plant exhibited significantly positive association ($P < 0.01$) with plant height (0.128*) and leaf length (0.239) at genotypic level while leaf breadth (0.239** and 0.183*) and 100 (0.306* and 0.267*) seed weight at both genotypic and phenotypic level. Positive and significant association of grain yield with plant height and 100 seed weight was also reported [13, 14]. Leaf length and breadth accumulate more dry matter in terms of more photosynthetic area.

Dead heart per cent at 14 and 28 days after sowing, days to maturity, no. of tillers and chlorophyll content showed significant negative association with grain yield per plant both at genotypic and phenotypic level. Negative correlation for days to maturity was reported earlier [15]. This will help to isolate early genotypes with higher grain yield. However, in contrast to this, significant positive correlation between days to flowering and grain yield are reported by earlier findings [Patel 1980]. Dead heart percent; symptom of shoot fly damage adversely affects the grain yield. Increase in 1% dead heart would result in loss of 143 kg grain yield per hectare [16]. Shoot fly tolerant contributing traits; glossiness and seedling vigour exhibited negative and significant association with grain yield in positive direction. Mote and Bapat 1983 reported that in promising derived genotype shoot fly resistance have desirable yield contributing characters like height, maturity and seed weight.

Regarding to traits imparting shoot fly tolerance; glossiness, seedling vigour, leaf wetness and chlorophyll content were significant and positively correlated with dead heart percentage at 14 and 28 DAE at both the genotypic and phenotypic levels. Leaf glossiness acts as non-preference mechanism. Intensity of Leaf glossiness at seedling stage is positively associated with level of resistance to shoot fly [2]. There is positive association between seedling vigour and shoot fly damage parameters (oviposition and dead hearts) [17]. Vigourous growth rate at seedling stage act as escape mechanism for shoot fly attack in tolerant genotypes [2]. More leaf wetness and high chlorophyll content are preferred for shoot fly oviposition. Shoot fly resistance lines have low leaf surface wetness, and are characterized by a smooth amorphous wax layer and sparse wax crystals [2]. Susceptible varieties contain significantly higher chlorophyll over resistance [18].

Whereas, dead heart percentage both at 14 and 28 DAE showed negative significant association with trichome density (adaxial and abaxial) surface, plant height, leaf length, leaf breadth and grain yield per plant at both genotypic and phenotypic level. Trichomes may contribute to the expression of antibiosis to shoot fly in sorghum as trichomed cultivar hinders the movement of newly hatched larvae to the base of whorl [19]. Physiological traits such as leaf glossiness, trichome density, plumule and leaf sheath pigmentation were found to be associated with resistance and chlorophyll content, leaf surface wetness, seedling vigour and waxy bloom with susceptibility to shoot fly [20]. First instar larvae of shoot fly move along the shoot to the growing point of the seedling and cuts the growing points resulting in wilting and drying of the central leaf causing the typical “Deadheart” symptoms [21] this might result in reduction in plant height and leaf area during further growth stage. Negative but non-significant association was observed for Dead heart percent at 14 and 28 DAE and days to 50% flowering both at genotypic and phenotypic level. Negative correlation between shoot fly dead hearts and time to 50% flowering (r = −0.49**) reported earlier [22].

Selection of traits showing Positive correlation improves both the traits simultaneously. On other hand negative association impedes improvement of dependable trait by indirect selection.

From present study it is concluded that traits *viz*., number of trichomes at adaxial and abaxial surfaces of lamina, leaf glossiness, seedling vigour, plumule leaf pigmentation played important role in shootfly resistance and also exhibited high GCV, heritability and high to moderate genetic advance and significantly positive association for shoot fly dead heart percent will be effective for improving shoot fly resistance through breeding programme.

Table 1: Analysis of variance for eighteen characters studied for resistance to sorghum shoot fly

Characters	Source of variation		
	Treatments	Replication	Error
Dead hearts% at 14 DAE	107.35**	65.312	6.49
Dead hearts% at 28 DAE	416.28**	112.27	8.30
Trichome Density (Adaxial)	1630.25**	4.86	22.46
Trichome Density (Abaxial)	7579.35**	34.50	8.61
Leaf Glossiness	1.34	0.05	0.10
Seedling Vigour	0.822	0.85	0.11
Leaf Wetness	0.683	0.75	0.10
Plumule and Leaf sheath pigmentation	1.477*	0.37	0.07
Plant Height	1092.30**	36.81	10.91
Leaf Length	71.80**	0.17	3.10
Leaf Breadth	1.39*	0.08	0.06
Number of Tillers	0.54	0.006	0.26
Leaf Angle	190.95**	18.70	4.65
Chlorophyll content	96.50**	0.36	3.50
Days to 50% Flowering	95.91**	16.53	4.10
Days to Maturity	41.42**	81.66	4.33
100-seed weight (g)	0.41	0.002	0.03
Grain yield (g/plant)	174.04**	39.04	14.22

Table 2: Genetic Variability Parameters for Eighteen characters studied for resistance to *sorghum* shoot fly.

Characters	Range		MEAN	σ^2 (g) (Genotypic variance)	σ^2 (p) (Phenotypic variance)	GCV (%)	PCV (%)	h^2 b. s. (%)	GA	GA as% of mean
	Minimum	Maximum								
Deadheart% 14 DAE	8.30	38.40	22.4700	50.4310	56.9256	31.6043	33.5777	88.59	13.7693	61.2785
Deadheart% 28 DAE	17.90	68.55	39.8357	203.9720	212.2761	35.8519	36.5745	96.09	28.8395	72.3960
Tichome Density (adaxial)	0.00	277.50	121.3833	8148.9057	8171.3694	74.3688	74.4712	99.73	185.7031	152.9889
Tichome Density (abaxial)	0.00	194.00	55.1625	3785.3704	3793.9839	111.5348	111.6616	99.77	126.5983	229.5007
Leaf Glossiness	1.50	5.00	2.9938	0.6220	0.7256	26.3444	28.4531	85.73	1.5043	50.2475
Seedling Vigour	1.75	5.00	3.1388	0.3518	0.4711	18.8973	21.8671	74.68	1.0559	33.6415
Leaf Wetness	2.00	4.75	3.1771	0.2909	0.3927	16.9762	19.7245	74.07	0.9562	30.0983
Plumule and Leafs heath Pimentation	1.00	3.00	1.9938	0.6990	0.7788	41.9347	44.2644	89.75	1.6317	81.8391
Plant Height (cm)	67.50	198.00	130.3833	540.6956	551.6131	17.8342	18.0134	98.02	47.4245	36.3731
Leaf Length (cm)	38.95	70.45	54.3425	34.3499	37.4549	10.7851	11.2620	91.71	11.5622	21.2764
Leaf Breadth (cm)	3.20	7.90	5.4942	0.6628	0.7308	14.8177	15.5593	90.69	1.5971	29.0696
Number of Tillers	0.30	2.40	1.3800	0.1389	0.4088	27.0094	46.3336	33.98	0.4476	32.4340
Leaf angle (degree)	37.00	84.50	56.3125	93.1482	97.8019	17.1389	17.5618	95.24	19.4030	34.4559
Chlorophyll content	34.00	63.40	45.1850	46.4991	50.0087	15.0914	15.6505	92.98	13.5453	29.9775
Days to 50% flowering	64.00	96.00	77.2458	45.9077	50.0082	8.7714	9.1547	91.80	13.3731	17.3124
Days to Maturity	108.50	138.00	123.4750	18.5442	22.8831	3.4876	3.8742	81.04	7.9858	6.4675
100-seed weight (g)	1.45	3.90	2.4433	0.1922	0.2262	17.9417	19.4644	84.97	0.8324	34.0684
Grain yield (g/plant)	6.40	52.85	21.0017	79.9107	94.1354	42.5646	46.1979	84.89	16.9666	80.7871

Table 3: Genotypic and phenotypic correlation coefficient studied insorghum

Characters		Deadheart 14	Deadheart 28	Trichome den US	Trichomeden LS	Leaf glossin	Seedling vig	Leaf wetness	Plumule & Pigm	Leaf length	Leaf breadth	No. of Tillers	Leaf angle	Chlorophyll	Daysto 50 flo	Grain yield
Deadheart 14 DAE	G	1.000	0.918**	-0.441**	-0.715**	0.748**	0.672**	0.627**	0.088	-0.183**	-0.136*	-0.059	-0.125	0.422**	-0.081	-0.225**
	P	1.000	0.861**	-0.416**	-0.672**	0.642**	0.543**	0.521**	0.072	-0.157*	-0.112	-0.029	-0.103	0.386**	-0.077	-0.195**
Deadheart 28 DAE	G		1.000	-0.486**	-0.733**	0.776**	0.763**	0.704**	0.128*	-0.237**	-0.164*	0.011	-0.073	0.433**	-0.082	-0.235**
	P		1.000	-0.476**	-0.717**	0.699**	0.638**	0.593**	0.126	-0.219**	-0.150*	0.011	-0.067	0.409**	-0.077	-0.205**
Trichome density U	G			1.000	0.548**	-0.341**	-0.336**	-0.332**	0.046	0.133*	0.089	-0.162*	-0.067	-0.163*	0.125	0.038
	P			1.000	0.547**	-0.314**	-0.292**	-0.286**	0.039	0.127*	0.086	-0.096	-0.064	-0.159*	0.120	0.034
Trichome density L	G				1.000	-0.543**	-0.520**	-0.476**	-0.034	0.178**	0.184**	-0.107	-0.062	-0.288**	0.154*	0.100
	P				1.000	-0.498**	-0.449**	-0.411**	-0.033	0.170**	0.173**	-0.058	-0.062	-0.276**	0.148*	0.091
Leaf Glossiness	G					1.000	0.888**	0.780**	0.117	-0.199**	-0.224**	-0.056	-0.108	0.554**	0.027	-0.190**
	P					1.000	0.690**	0.585**	0.082	-0.196**	-0.213**	-0.053	-0.113	0.490**	0.036	-0.156*
Seedling Vigor	G						1.000	0.858**	0.166*	-0.215**	-0.311**	-0.122	-0.088	0.494**	0.086	-0.220**
	P						1.000	0.692**	0.161*	-0.157*	-0.272**	0.005	-0.079	0.418**	0.052	-0.143*
Leaf Wetness	G							1.000	0.143*	-0.140*	-0.329**	0.159*	-0.164*	0.537**	0.200**	-0.254**
	P							1.000	0.148*	-0.101	-0.256**	0.095	-0.133*	0.442**	0.151*	-0.198**
Plumule Leafpigmn	G								1.000	-0.088	-0.032	-0.156*	-0.071	-0.013	-0.053	0.017
	P								1.000	-0.083	-0.023	-0.060	-0.064	-0.006	-0.037	0.016
Leaf length	G									1.000	0.436**	-0.186**	-0.033	-0.186**	0.054	0.239**
	P									1.000	0.397**	-0.100	-0.032	-0.162*	0.046	-0.001
Leaf breadth	G										1.000	-0.240**	-0.120	-0.073	0.037	0.239**
	P										1.000	-0.138*	-0.107	-0.063	0.046	0.183**
No of tillers	G											1.000	0.065	0.030	0.048	-0.411**
	P											1.000	0.028	0.065	0.034	-0.146*
Leaf angle	G												1.000	-0.196**	0.007	0.066
	P												1.000	-0.196**	0.010	0.051
Chlorophyll content	G													1.000	0.221**	-0.190**
	P													1.000	0.199**	-0.144*
Daysto 50 flowering	G														1.000	-0.056
	P														1.000	-0.062

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