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Identification of forage sorghum lines having multiple-resistance to sorghum shoot fly and spotted stem borer

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Abstract
Sorghum shoot fly and spotted stem borer are the major pests that reduce fodder yields in forage sorghum. The present study aims at identifying resistant sources and characters that contribute resistance to this crop. The experiment consisted of 19 forage sorghum lines along with checks, studied during *Kharif* 2009-2012. The results indicated that seedling vigour, glossiness, shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts were the most reliable parameters for characterization of multiple resistance/ susceptibility to shoot fly and spotted stem borer among the damage parameters in sorghum. Based on principal component analysis, Katakhatav, Ramkel, Rampur Local depicted multiple resistances to sorghum shoot fly and spotted stem borer, while HC 308, NSSV 13, RSSV 9 and SL 44 exhibited moderate levels of resistance paving way for including them in forage sorghum improvement program.

Keywords: *Atherigona soccata*, *Chilo partellus*, Multiple pest resistance, Sorghum

Introduction

In India, forage sorghum [*Sorghum bicolor* (L.) Moench] is grown over 2.6 m ha, mainly in the states of western UP, Haryana, Punjab, Rajasthan and Delhi during *kharif* season and fulfills over two third of the fodder demand (Pandey and Roy, 2011). Insect pests are one of the main production constraints in forage sorghum production. Sorghum shoot fly (*Atherigona soccata* Rondani) and spotted stem borer (*Chilo partellus* Swinhoe) are the major pests that reduces production in many parts of the world. These two pests *viz*., shoot fly and spotted stem borer are reported to cause 40% (Pawar *et al*., 1984) and 68% (Singh, 1997) losses, respectively, in India. In North India, sorghum shoot fly and spotted stem borer is reported to inflict severe damage to fodder sorghum.

Agronomic practices and host plant resistance have been employed for minimizing the losses due to insect pests in sorghum. However, farmers cannot sow when pest damage can be avoided as sowing depends upon the monsoon. Insecticide application is not advocated in forage sorghums as it is directly fed as green fodder and stover posing health problems to animals. Host plant resistance plays a major role in reducing the extent of losses in this crop and is compatible with other pest management practices (Sharma, 1993). Shoot fly and stem borer attain damaging proportions on the same crop in a crop-growing season. A number of genotypes individually resistant to *A. soccata* and *C. partellus* have been identified, but levels of resistance are low to moderate. Under such circumstances breeding efforts largely rely on developing superior genotypes possessing multiple resistances to key pests. The present study was undertaken to identify multiple pest resistance in 19 forage sorghum lines.

Materials and Methods

Plant material: The experiments were conducted at the Indian Institute of Millets Research (IIMR) farm, Hyderabad, Andhra Pradesh, during June, 2009-2012 rainy seasons. The experimental material consisted of 19 forage sorghum lines along with two resistant checks for sorghum shoot fly (IS 2312 and IS 18551), one resistant check for spotted stem borer (IS 2205) and two susceptible checks for both shoot fly and stem borer (Swarna and DJ 6514). The entries were sown in two row plots of 4 m row length, spaced 60 cm apart. Two sets of the experiment were sown one each for shoot fly (early planting) and stem borer (late planting).The trial was laid in randomized block design with three replications. The seeds were sown at a depth of 5 cm and immediately irrigated and later maintained under rain fed conditions. Standard crop management practices were followed barring use of insecticides.

Artificial infestation

Sorghum shoot fly: The fish meal technique (Nwanze, 1997) was adopted to ensure uniform and optimum shoot fly pressure in the experimental material.

Spotted stem borer: The test material was artificially infested with the laboratory- reared neonate using a Bazooka applicator (Sharma *et al*., 1992). The freshly hatched neonate larvae were gently mixed with seeds of *Papaver* sp as carrier, and transferred into plastic bottles fixed to the Bazooka applicator. In the field, the plants at 20 days after emergence (DAE) were individually infested by placing the nozzle of the Bazooka on the leaf whorl. In each stroke, 5 to 7 larvae were released in the morning between 0800 h and 1100 h into the whorls of one row in two row plots to inflict an optimum level of leaf damage and dead-heart formation.

Observations

Sorghum shoot fly: The intensity of glossiness was recorded in the morning hours, when there was maximum light reflection at 10 DAE on a scale of 1–5, where 1 is high intensity of glossiness and 5 is non-glossy. Seedling vigour was scored at 10 DAE on a scale of 1–5 where 1 is highly vigorous (plants showing maximum height, leaf expansion and robustness) and 5 is low vigour (plants showing minimum growth, less leaf expansion and poor adaptation). The proportion of plants with shoot fly eggs and dead hearts was recorded twice, at 21 and 28 DAE. The total number of plants in each entry and the number of plants with shoot fly eggs and dead hearts were recorded and the proportion calculated. The data set showing dead hearts ($\geq$ 70 % infestation in susceptible check) was considered since there was contrast in the infestation.

Spotted stem borer: The observations on number of plants with *C. partellus* induced dead hearts were recorded at 45 DAE and expressed as percentage of the total number of plants. Leaf feeding by *C. partellus* larvae was assessed, two weeks after artificial infestation on a 1 to 9 rating scale (Sharma *et al*., 1992).

***Selection index*:** A selection index based on shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts, stem borer leaf damage score and stem tunneling/ stalk was computed by summing up the ratios between values and overall mean and dividing by the number of parameters considered as followed by Tefera *et al*. (2011) in maize. The entries with selection index values less than 0.8 were regarded as resistant and those with a selection index greater than 0.8 as susceptible.

Statistical analysis: The three years data on morphological traits (seedling vigour, glossiness), dead-hearts caused by shoot fly, stem borer, stem borer induced leaf damage score, stem tunneling were subjected to pooled analysis and ANOVA calculated, The means were compared by LSD at 5% level. The data on dead-hearts caused by shoot fly and borer, seedling vigour, glossiness, stem borer leaf damage score, stem tunneling, were subjected to principle component analysis and genotypes classified using SPSS, 1999.

Results and Discussion

Nineteen forage sorghums along with two resistant checks for shoot fly, one resistant check for stem borer and two susceptible checks were evaluated for resistance to shoot fly and stem borer during rainy seasons (2009-12). The parameters considered for evaluating resistance *viz*., seedling vigour, glossiness, shoot fly, stem borer induced dead hearts, stem borer leaf damage score, borer induced stalk tunneling are tabulated (Table 1).

Morphological traits

Seedling vigour: The data generated on 19 lines and checks showed a seedling vigour from 2.9 - 4.9 on scale of 1-5, with a mean of 3.9. IS 2312 (RC) recorded low value (2.9) indicating that it was vigorous in terms of growth (Table 1). The susceptible check swarna recorded score of 4.9 indicating poor seedling vigour thus becoming prone to shoot fly infestation as slow growing entries are preferred by shoot fly for oviposition. The entries SL 44, GFS 5 and SSG 59-3 were at par with IS 2312 (RC). Faster seedling growth and toughness of the leaf sheath are associated with shoot fly resistance (Singh and Jotwani, 1980), obstructing the first instar larvae from reaching the growing tip (Mote *et al*., 1986). Faster growing plants remain in the favourable height (susceptible stage) for a relatively shorter period than the slower growing susceptible plants (Khurana and Verma, 1985).

Glossiness: The data on glossiness of the test entries ranged from 2.7 - 4.4 on a scale of 1-5, with an average of 3.9. The genotypes, IS 2312 (RC), RSSV 9, MP Char and IS 2205 (RC) recorded low values indicating that they were more towards glossiness (Table 1). The susceptible check recorded score of 4.4 indicating susceptibility for shoot fly infestation. Traits such as leaf glossiness, trichome density were found to be associated with resistance and chlorophyll content, leaf surface wetness, seedling vigour, and waxy bloom with susceptibility to shoot fly (Dhillon *et al.*, 2005). Colour of leaves, glossiness of leaves and presence of trichomes are prominent attributes which confer resistance to shoot fly in sorghum (Jadhav *et al.*, 1986). However, it was reported earlier by several workers that entries having greater plant height, narrower leaves, greater trichome density and greater trichome length, yellowish green colour of leaves, glossiness of leaves, and faster initial plant growth rate were found highly resistant to shoot fly while, entries having less plant height, broad leaves, dark green leaf colour, non-glossy leaves, absence of trichomes and slow initial plant growth rate were found highly susceptible to shoot fly.

Shoot fly oviposition: There were significant differences among the genotypes tested. The shoot fly oviposited seedlings ranged from 18.7 - 56.0% and the mean of the test entries was 39.3%. Almost all the entries were preferred for oviposition. The genotype IS 2312 (RC) recorded lowest oviposition (18.7% seedlings) and was significantly different from all other test entries. Resistance to shoot fly is a cumulative effect of non-preference and antibiosis (Raina *et al.* 1981).

Shoot fly dead hearts: The dead-hearts (DH) ranged from 35.4 - 73.9% and the mean damage was 54.4% at 28 DAE. The genotypes RSSV 9 was at par with the resistant check (IS 2312) which recorded 35.4% dead-hearts. Rests of the genotypes were susceptible with damage exceeding 45.3%. The susceptible check Swarna recorded 73.9% DH. Retardation of growth and development, prolonged larval and pupal periods and poor emergence of adults on resistant varieties provides direct evidence of antibiosis. Larval and total growth indices were significantly lowered in resistant compared

Table 1. Evaluation of forage sorghum lines for multiple resistance under field conditions (2009-12)

Entry	SV	GL	SFOVI	SFDH (%)	SBDH (%)	SB LDS	ST (%)	S I
CO(FS)-29	4.0	4.1	42.0	49.7	43.1	7.6	23.3	1.2
CSV 21F	3.8	4.0	35.3	48.7	25.5	6.8	25.4	1.0
GFS 5	3.6	3.9	35.9	60.9	26.2	7.1	27.9	1.1
HC 136	3.9	4.2	49.0	69.9	50.5	7.9	26.1	1.4
HC 171	3.7	3.8	35.9	54.7	30.4	7.1	21.9	1.0
HC 308	3.8	3.6	42.1	51.5	20.5	5.1	14.6	0.8
Katakhatav	4.4	4.0	44.1	54.9	15.5	4.8	11.6	0.7
MP Chari	3.7	3.4	29.7	48.8	31.3	6.8	24.6	1.0
NSSV 13	4.1	4.4	42.4	57.6	16.2	5.8	15.8	0.8
PSC 1	4.1	4.3	40.7	48.8	30.7	7.1	19.9	1.0
Ramkel	3.9	4.0	35.0	48.0	19.2	4.9	11.2	0.7
Rampur Local	4.1	3.9	35.2	55.7	18.5	4.8	11.1	0.7
RSSV 9	3.8	3.3	32.2	43.0	17.3	4.9	12.0	0.7
S 541	4.1	3.9	40.4	52.2	41.6	7.1	26.8	1.2
Sangoli Hundi	4.9	4.4	49.0	67.1	39.8	7.8	25.3	1.3
SL 44	3.4	3.6	31.0	45.3	18.5	6.0	16.8	0.8
SSG 59-3	3.6	4.0	37.4	53.0	30.5	7.3	17.7	1.0
SSV 74	3.7	4.0	43.2	56.3	40.8	8.2	29.5	1.3
SSV 84	3.7	3.7	39.2	60.9	52.2	8.1	30.8	1.4
Swarna	4.3	4.4	56.0	73.9	57.8	8.3	33.3	1.5
DJ 6514	4.0	3.8	54.7	71.4	55.8	8.1	33.6	1.5
IS 2205	3.4	3.4	36.7	49.6	11.1	4.8	8.5	0.6
IS 18551	4.1	4.0	39.4	49.0	12.9	4.7	9.4	0.6
IS 2312	2.9	2.7	18.7	35.4	14.4	4.9	10.8	0.6
Mean	3.9	3.9	39.3	54.4	30.0	6.5	20.3	
C.D. (5%)	0.7	0.7	10.4	8.8	5.4	0.6	5.2	
C.V. (%)	20.4	19.1	28.5	17.4	19.1	9.4	27.4	

SV- Seedling vigour (1-5 scale); GL- Leaf glossiness (1-5 scale); SF OVI- Shoot fly oviposited seedlings (%); SF DH (%)- Shoot fly dead hearts (21 DAE); SBDH (%) - Stem borer dead hearts (45 DAE); SB LDS- Stem borer leaf damage score (1-9); ST (%) - Stem tunneling; SI – Selection index with susceptible varieties. The percentage pupation on resistant lines was significantly lower compared with that on susceptible lines (Dhillon *et al*., 2005).

Stem borer dead-hearts: There were significant differences among the genotypes studied. The dead-hearts ranged from 11.1 - 57.8% with a mean damage of 30.0%. The genotypes Katakhatav, NSSV 13 were at par with the resistant check IS 2205 which recorded 11.1% dead-hearts. The genotype, SSV 84 was highly susceptible and per cent dead-hearts (52.4) were at par with susceptible check Swarna (57.8%).

Dead-heart parameter was reported to be one of the most stable parameters for differentiating degrees of resistance with respect to borer. Several workers have used dead hearts as a criterion to select for stem borer resistance (Taneja and Leuschner, 1985; Singh and Rana, 1989). The major mechanism of resistance to stem borer in sorghum is antibiosis (Singh and Rana, 1984). Prem Kishore (1987a) indicated that some plant characters affect the establishment of stem borer larvae on the plant. The erect ligular hairs on whole ligular length as well as hairy carpet base of ligule where it joins the lamina restricted the entry of larvae in stem. Undulating leaf base and hairy midrib allowed the larvae to fall down in later stages of plant growth (Prem Kishore, 1987b).

Leaf damage score: The data on leaf damage score indicated that there were significant differences among the genotypes studied. The mean leaf damage score was 6.5 which ranged from 4.7 - 8.3. The lines Katakhatav, Rampur local, Ramkel, RSSV 9, HC 308 were at par with resistant check IS 2205 which recorded low leaf damage score of 4.8 suggesting low larval establishment in the whorl leaves. Low leaf feeding and low larval establishment are important components of resistance to *C. partellus* in sorghum (Van den Berg and Westhuizen, 1998). The genotype SSV 74, SSV 84, HC 136 and Sangoli Hundi showed heavy leaf damage. Most of the genotypes were susceptible recording damage score above 6.0. However, Starks and Doggett (1970) suggested that leaf feeding by *C. partellus* in sorghum is a poor indicator of expected grain yield as production of newer leaves may compensate the leaf damage.

Stem borer stalk tunneling: The stem tunneling due to borer was expressed as proportion of stem tunneled. There were significant differences in tunneling of the stem in various genotypes. The tunneling ranged between 8.5 - 33.6 % and the mean tunneling being 20.3 %. The genotypes Rampur Local, Ramkel, Katakhatav and RSSV 9 were on par with resistant check IS 2205 which recorded 8.5% stem tunneling. Low tunneling suggests that the larvae either took more time to enter inside the stems of these genotypes or fewer larvae survived on these genotypes. Ajala *et al*. (1995) suggested that stem tunneling rather than leaf feeding damage is the most important factor for estimating tolerance. Extent of stem tunneling is influenced by antibiosis and has been used to measure genotypic susceptibility to *C. partellus* (Alghali, 1987).

***Selection index*:** A selection index based on shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts, stem borer leaf damage score and stem tunneling/ stalk was computed by summing up the ratios between values and overall mean. The entries Ramkel, Rampur Local and RSSV 9 recorded low selection index values (0.7), hence regarded as resistant while the entries SL 44, NSSV 13 and Katakhatav were moderately resistant. The entries recording selection index values above 0.8 are regarded as susceptible.

In view of complexity of interactions between the shoot pests and sorghum, principal component analysis (PCA) was applied for assessing the reliable traits by taking into consideration seven parameters *viz*., seedling vigour, seedling glossiness, shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts, stem borer leaf damage score and stem borer stalk tunneling. Two principal components (PCs) were extracted with eigen value $\geq$1.0, after varimax rotation with Kaiser normalization procedure which converged in three iterations. The extraction communalities for all the variables tested were $\geq$0.5 indicating that the variables were well represented by the two extracted PCs which together explained a cumulative variation of 88.6%. PC_1 explained 66.7% of the variation while PC_2 explained 21.9% of variation. PC_1 had the loadings for seedling vigour (0.79), glossiness (0.74), shoot fly dead hearts (0.89), stem borer dead hearts (0.86), whereas stem borer stalk tunneling (0.73) and Stem borer leaf damage score (0.72) were loaded in PC_2 (Table 2). When compared to the first component, PC_2 explained lesser variation and the characters loaded in this component could be of less importance in explaining the overall variability. Thus, PCA brought out five characters/variables *viz*., seedling vigour, glossiness, shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts as most important in explaining variability close to 50% of total variation in the genotypes tested. Therefore, these characters are of utmost importance in assigning the genotypes into different classes. Several similar studies have used multivariate analysis to study insect-plant relationships (Caillaud *et al.*, 1995).

Table 2. Component loadings of parameters governing resistance against shoot fly and stem borer in forage sorghum (2009-12)

Parameter	Principal Components	
	1	2
Seedling vigour (1-5)	0.896	0.311
Glossiness (1-5)	0.945	0.147
Shoot fly dead hearts (%)	0.935	0.254
Stem borer dead hearts (%)	0.915	0.110
Stem borer stalk tunneling (%)	0.469	0.734
Stem borer exit holes/stalk (no.)	0.790	0.302
Stem borer larvae/stalk (no.)	0.070	0.918

Extraction Method: Principal component analysis.
Rotation Method: Varimax with Kaiser normalization, rotation converged in 3 iteration

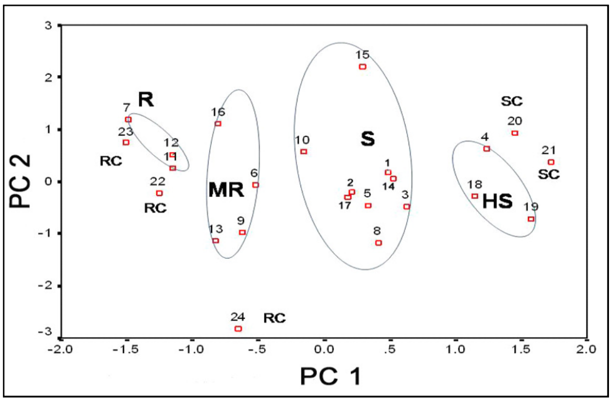

Fig 1. Plot of PC1 and PC2 showing clusters of forage sorghum entries depicting multiple resistance to Shoot fly and Spotted stem borer.

The entry corresponding to number in scatter plot is detailed below

Response	Genotype
Resistant check (RC)	22-IS 2205 (Stem borer); 23-IS 2312 (Shoot fly); 24- IS 18551 (Shoot fly)
Resistant (R)	7-Katakhatav; 11-Ramkel; Rampur local-12
Moderately resistant (MR)	6-HC 308; 9- NSSV 13; 13-RSSV 9; 16- SL 44
Susceptible (S)	1- CO(FS)-29; 14- S 541; 3-GFS-5; 5-HC 171; 8- MP Chari; 2- CSV 21F; 17- SSG 59-3; 10- PSC 1; 9- NSSV 13
Highly susceptible (HS)	4- HC 136; 18- SSV 74; 19- SSV 84
Susceptible check (SC)	20- Swarna; 21- DJ 6514

The PC_1 and PC_2 were plotted for separating the genotypes based upon the seven characters considered. The plot showed six clusters of genotypes grouped into resistant check (RC), resistant (R) moderately resistant (MR) and susceptible (S), highly susceptible (HS) and susceptible checks (SC) (Fig.1).

The present study suggests that seedling vigour, glossiness, shoot fly oviposition, shoot fly dead hearts, stem borer dead hearts are the most reliable parameters for characterization of multiple resistance/ susceptibility to *A. soccata* and *C. partellus* among the damage parameters in sorghum. The forage sorghum lines Katakhatav; Ramkel, Rampur Local were resistant to both the pests while, HC 308; NSSV 13; RSSV 9; SL 44 were having moderate levels of resistance. The entries (CO(FS)-29, S 541,GFS-5,HC 171, MP Chari, CSV 21F, SSG 59-3, PSC 1, NSSV 13, HC 136, SSV 74 and SSV 84) were susceptible to shoot fly and stem borer (Fig 1; Table 1). However, it may be difficult to combine resistance to some group of insect pests. To quote, genotypes resistant to shoot fly and spotted stem borer were susceptible to sorghum midge and vice versa (Sharma, 1993) while, the sorghum genotypes IS 2205 (Patel *et al.*, 1989), IS 18551, IS 2195, PS 28060-3 have been reported to be resistant to both sorghum shoot fly and spotted stem borer, implying that some of the sources are common to both the insects (Taneja and Leuschner, 1985).

Conclusion

The findings of the present study have indicated that the genotypes Katakhatav, Ramkel, Rampur Local, HC 308, NSSV 13, RSSV 9 and SL 44 possessed moderate to high levels of resistances to sorghum shoot fly and spotted stem borer holding promise for their inclusion in forage sorghum improvement program to elevate resistance levels in agronomically superior genotypes.

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