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Influence of types of sterile cytoplasm on the resistance to sorghum shoot fly (*Atherigona soccata*)

Umakanth V. Akula[1], Padmaja G. Poluru[1], Ashok Kumar Jangam[2] and Patil V. Jagannath[1]

[1]Directorate of Sorghum Research, Rajendranagar, Hyderabad, Andhra Pradesh 500 030, India, E-mail: umakanth@sorghum.res.in; [2]ICAR Research Complex for Goa, Ela, Old Goa 403 402, Goa, India

With 2 figures and 3 tables

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Abstract

Shoot fly *Atherigona soccata* (Rondani) is one of the most important insect pests affecting sorghum during early stages of crop growth. Commercial production of hybrid seed in sorghums relies on a single source of male sterility (A_1) resulting in restricted nuclear genetic diversity of male-sterile (A) as well as restorer (R) lines. Alternative cytoplasms should be exploited to avoid insect pest outbreaks that might be related to the use of single source of cytoplasm. Therefore, this study was carried out to identify the non-milo cytoplasms that are less susceptible to shoot fly. Four isogenic lines in four male-sterile backgrounds, viz. A_1, A_2, A_3 and A_4, and their corresponding maintainer (B lines) lines were studied using a fishmeal technique across rainy and postrainy seasons of 2006–07. The A_4 cytoplasm was found to be least susceptible to shoot fly as it was comparatively less preferred for oviposition and had lower deadheart formation across seasons than the other cytoplasms tested and thus can be exploited for developing shoot fly-resistant hybrids.

Key words: *Atherigona soccata* — cytoplasmic male sterility — sorghum — resistance

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world after wheat, rice, maize and barley. Globally, sorghum is cultivated over an area of 42 million hectares with an annual production of 58.5 million tons. India is a major sorghum producer with an area of 7.5 million hectares and production of 7.2 million tons (FAOSTAT 2009). Productivity of sorghum in India is very low (783 kg/ha) as compared to global yield (1373 kg/ha). Losses in yield are very high because of various kinds of stresses beginning from seedling stage to crop maturity. Although many high-yielding hybrids and varieties were released, damage by insect pest is a key constraint, which limits the production and productivity in sorghum (Anandan et al. 2009). More than 150 insect species damage the crop from sowing till harvest causing a loss of over US$1 billion in grain and forage yield worldwide (Sharma and Nwanze 1997). Nearly 32% of the actual produce of sorghum is lost because of insect pests in India (Board and Mittal 1983). Sorghum shoot fly *Atherigona soccata* (Rondani) (Diptera: Muscidae) is one of the most important insect pests affecting the crop during early stage in Eastern Africa, Asia and the Mediterranean Europe. It infests the crop during both rainy and postrainy seasons in India and is a major constraint to profitable sorghum cultivation (Padmaja et al. 2010). Loss because of shoot fly damage was estimated as 5–50% in India (Jotwani 1982, 1983). Lack of acceptable levels of genetic tolerance/resistance to this insect in parental lines compounded further with unacceptability of chemical control measures by the farmers. Host plant resistance is the most relevant pest management strategy under subsistence farming conditions, as it involves no extra cost to the farmers.

Shoot fly resistance in terms of deadheart percentage is a quantitative character, which is predominantly governed by additive genes, while tillering consequent to deadheart formation was controlled by non-additive genes (Borikar and Chopde 1982). The primary mechanism of resistance is ovipositional non-preference. The direct and indirect effects, correlation coefficients and multiple and stepwise regression analyses suggested that deadhearts, plants with eggs, leaf glossiness, trichomes on the abaxial surface of the leaf and leaf sheath pigmentation could be used as marker traits to select for resistance to shoot fly *A. soccata* in sorghum (Dhillon et al. 2005). Resistance to shoot fly is attributable to the gradual accumulation of desirable alleles rather than attributable to the presence of one or two major genes. Breeding for shoot fly resistance is a slow process needing several cycles of crossing to combine high levels of resistance with yield (Rana et al. 1985). Shoot fly resistance showed a systematic gradation in a series of crosses among the susceptible, intermediate and resistant varieties. The resistant sources available in the germplasm were found to be poor combiners for resistance to shoot fly and the associated traits (Aruna and Padmaja 2009). This may be another reason for low progress in shoot fly resistance breeding. It is important to identify the sources and understand the mechanisms conferring resistance to shoot fly. For the development of shoot fly-resistant hybrids, resistance is required in both the parental lines (Sharma et al. 2006). Most of the hybrids grown in India are based on milo cytoplasm (A_1 cytoplasm) that is highly susceptible to shoot fly (Dhillon 2004). Commercial production of hybrid seed in sorghums relies on a single source of male sterility (A_1) and the analogy of this situation to that existing in corn (*Zea mays* L.) before the 1970 epidemic of southern corn leaf blight, caused by *Helminthosporium maydis* Nisik and Miy., is inescapable. In addition to cytoplasmic uniformity in the hybrids, the use of single cytoplasm restricts nuclear genetic diversity of male-sterile (A) as well as restorer (R) lines. Therefore, to prevent such eventualities and to broaden the genetic base, the need for the diversification of cytoplasmic male sterility (CMS) base of sorghum hybrids was felt long back and as a result, several non-milo CMS systems were identified and developed (Schertz 1994) for use in hybrid breeding programmes. Clearly,

alternative cytoplasms should be exploited to avoid insect pest outbreaks that might be related to the use of single source of cytoplasm by adding nuclear diversity in new parental combinations. In addition to the milo cytoplasm (A_1 cytoplasm), cytoplasmic male-sterile lines are also available in A_2, A_3, A_4, A_4M, A_4VzM and A_4G1, A_5, A_6, 9E and KS cytoplasmic backgrounds (Schertz et al. 1997, Xu et al. 1998). However, the utilization of these non-milo CMS systems at commercial level depends on factors such as stability of male sterility, restorer gene frequency in the germplasm, effect of male sterility-inducing cytoplasm on agronomic traits and the availability of commercially viable heterosis (Reddy et al. 2005). This study was carried out to identify the non-milo cytoplasms that were less susceptible to shoot fly.

Materials and Methods

Plant material: The experimental material consisted of four isogenic lines in four male-sterile backgrounds, viz. A_1, A_2, A_3 and A_4, and their corresponding maintainer lines (B lines) were obtained from University of Nebraska-Lincoln. Similar isogenic lines in different cytoplasms were not available in the national programme for this study, and hence, these lines were used to decipher the effect of cytoplasm on shoot fly resistance as the information on these aspects is limited. The experiments were carried out in the shoot fly nursery at the Directorate of Sorghum Research, Hyderabad, India, between 2006–07 and 2007–08, and the material was evaluated during two successive rainy and postrainy seasons. The test material was planted in two row plots (4 m), and the rows were 60 cm apart. The test material was evaluated in a randomized complete block design along with shoot fly-resistant (IS 18551) and susceptible (DJ 6514) checks in three replications. The plants were thinned at 7 days after seedling emergence (DAE) to maintain a spacing of 10 cm between plants. The optimum levels of shoot fly infestations were maintained by manipulating the sowing dates and through the use of interlard fish-meal technique (Sharma et al. 1992). Normal agronomic practices were followed for raising the crop. No insecticide was applied to the crop during the vegetative phase.

Oviposition and deadheart formation: Data on numbers of plants with eggs were recorded at 14 and 21 DAE and expressed as mean number of eggs per plant. The numbers of plants with deadhearts were recorded at 14, 21 and 28 DAE and expressed as percentage of total number of plants (Sharma et al. 2006).

Physico-morphological traits associated with resistance to shoot fly: Leaf glossiness was observed in the morning hours when there was maximum light reflection. The intensity of glossiness was recorded at 10 DAE on a scale of 1–5, where 1 = high intensity of glossiness (light green, shining, narrow and erect leaves) and 5 = non-glossy (dark green, dull, broad and drooping leaves). Seedling vigour was scored at 10 DAE on a scale of 1–5, where 1 = high vigour (plants showing maximum height, leaf expansion and robustness) and 5 = low vigour (plants showing minimum growth, less leaf expansion and poor adaptation). To record leaf trichome density, the central portion of the 5th leaf from the base was taken from three randomly selected seedlings in each entry at 12 DAE. Leaf samples (5 mm^2) were placed in 20 ml of acetic acid/alcohol (2 : 1) in small vials overnight. The cleared samples were transferred into 90% lactic acid and stored for observation. The leaf samples were mounted on a slide in a drop of lactic acid and observed under the microscope at a magnification of 20× (Maiti and Bidinger 1979). The number of trichomes was counted in three microscopic fields selected at random and expressed as trichome density/mm^2.

Statistical analysis: The data were subjected to analysis of variance to test the significant difference between the genotypes, cytoplasms and their interactions. The *F*-test at P = 0.05 was considered for checking the significance. Least significant difference test was used to compare the different mean effects.

Results

Oviposition

The ovipositional preference in the seedlings of different CMS and their maintainers was higher during rainy season compared with postrainy season, and oviposition increased from 14 DAE to 21 DAE (Fig. 1). Cytoplasms differed significantly at 14 DAE during rainy season and at 14 and 21 DAE during postrainy season. A_4 cytoplasm recorded significantly lower mean number of eggs during rainy season (14 DAE) and postrainy season (14 DAE and 21 DAE) and was less preferred for oviposition.

There were significant differences among genotypes during rainy season at 21 DAE and at 14 and 21 DAE during postrainy season. C × G interaction was significant at both 14 DAE and 21 DAE during postrainy season. During rainy season, plants of N122 recorded significantly lower number of eggs per plant in A_2, A_3 and A_4 cytoplasmic backgrounds compared with other isogenic lines in similar cytoplasms at 21 DAE. The maintainer lines of N122 and KS 57 were significantly superior to the other two maintainers, viz. Tx 3042 and Wheatland for low oviposition. During postrainy season, plants of N122 in A_3 and A_4 cytoplasms (21 DAE) and KS57 in A_1 and A_2 cytoplasms (14 DAE) recorded significantly low oviposition as compared to other isogenic lines and respective maintainer lines.

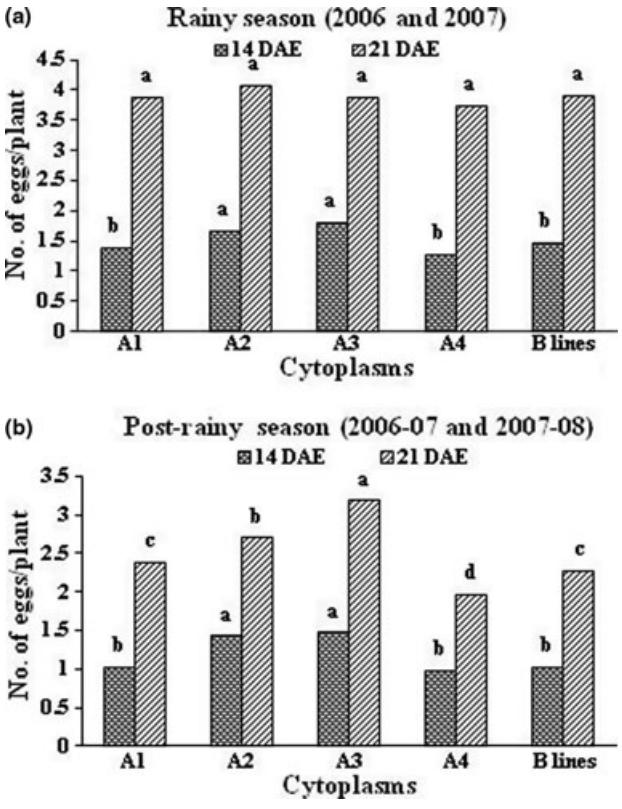

Fig. 1: Oviposition by shoot fly in different cytoplasmic male-sterile systems of sorghum. (a) Rainy season; (b) Postrainy season. Bars with the same letters indicate that the cytoplasms are not significantly different at P = 0.05

Deadhearts

Deadheart formation in different cytoplasms during rainy season ranged from 28.9% to 44%, 40.7% to 56.6% and 58.6% to 80.4% at 14, 21 and 28 DAE, respectively (Table 1). The range of deadhearts at different stages was less in postrainy season compared with rainy season (24.1–40.4%, 29.5–46.9% and 31.8–63.6% at 14, 21 and 28 DAE, respectively) (Table 2). It was also more in CMS lines compared with maintainer lines during both the seasons. Cytoplasms differed significantly at 28 DAE during both the seasons. Among the cytoplasms tested, A_4 cytoplasm had recorded its superiority for lower deadheart formation over the other cytoplasms and maintainers across seasons.

During rainy season, the genotypes differed significantly at 14, 21 and 28 DAE. All genotypes recorded lower deadhearts in their respective A_4 backgrounds at 28 DAE. N122 recorded lower mean deadheart percentage compared with other genotypes at 28 DAE. During postrainy season, the genotypes differed significantly at 14, 21 and 28 DAE and the trend of lower deadheart formation in genotypes with A_4 cytoplasm was similar to rainy season. The genotypes N122 and KS 57 were significantly superior for lower deadheart formation at 28 DAE. Among the maintainers, N 122 and Wheatland were superior for lower deadheart formation.

Trichomes

Across seasons, the genotypes and cytoplasms differed significantly for trichomes on both adaxial (upper) and abaxial (lower) leaf surfaces (Fig. 2). During rainy season, significantly more number of trichomes on adaxial surface in A_2 and abaxial surface in A_3 cytoplasms were observed. On the contrary, during postrainy season, A_1 and A_4 cytoplasms recorded more number of trichomes on both adaxial and abaxial surfaces. During rainy season, the plants of KS 57 had significantly higher number of trichomes on adaxial (A_4 cytoplasm) and abaxial surfaces (A_1, A_2 and A_4 cytoplasms), while N122 was promising for trichomes on adaxial surface (A_1 and A_2 cytoplasms) and abaxial leaf surface (A_3 cytoplasm). The maintainer of KS 57 recorded highest number of trichomes on both adaxial and abaxial surfaces. In postrainy season, the plants of N122 had significantly higher number of trichomes on both adaxial and abaxial surfaces in all cytoplasms except on abaxial surface in A_3 cytoplasm. The maintainer of N122 recorded highest number of trichomes on adaxial and abaxial surfaces.

Glossiness and seedling vigour

Plants of A_4 and A_3 cytoplasms were significantly more glossy compared with other cytoplasms and were on par to the B lines (Table 3). The genotypes N122, KS 57 and Tx 3042 were significantly more glossy compared with Wheatland. Similar to glossiness score, A_4 cytoplasms followed by A_3 cytoplasm recorded significantly higher seedling vigour (Table 3). However, the genotypes did not differ significantly for this trait.

Discussion

Several authors have discussed the importance of broadening the range of cytoplasms employed in the production of commercial sorghum hybrids to increase genetic variability

Table 1: Reaction of different cytoplasmic and maintainer lines to shoot fly in terms of deadhearts (%) (Rainy seasons of 2006 and 2007)

	14 DAE						21 DAE						28 DAE					
S. No.	A_1	A_2	A_3	A_4	B	Mean	A_1	A_2	A_3	A_4	B	Mean	A_1	A_2	A_3	A_4	B	Mean
N 122	34.67	35.68	40.71	37.52	35.46	36.81	48.66	48.38	56.65	45	50.27	49.79	69.84	77.55	69.15	62.31	69.73	69.72
KS 57	28.86	35.75	39.4	30.25	36.19	34.09	40.75	46.72	50.01	45.86	50.97	46.86	71.47	74.14	72.8	60.94	73.87	70.64
Tx 3042	37.8	44.44	39.39	34.03	34.34	38	54.77	55.67	51.8	49.18	50.44	52.37	80.43	77.3	77.51	64.24	68.03	73.5
Wheatland	43.27	41.79	40.05	34.58	35.84	39.11	52.55	50.81	48.89	46.81	47.13	49.24	78.05	77.25	71.91	58.59	72.16	71.59
Mean	36.15	39.41	39.89	34.09	35.46		49.18	50.4	51.84	46.71	49.7		74.95	76.56	72.84	61.52	70.95	
Checks								23.47						40.19				
IS 18551 (R)		15.72						49.66						78.31				
DJ 6514 (S)		31.88																
For comparing	LSD	*F*-prob					LSD	*F*-prob					LSD	*F*-prob				
Genotypes	2.72	0					3.29	0.02					2.19	0.01				
Cytoplasms	3.05	0					3.68	0.09					2.45	0				
C × G	6.09	0.02					7.36	0.1					4.9	0				

LSD at 5%.
DAE, days after seedling emergence; LSD, Least significant difference.

Table 2: Reaction of different cytoplasmic and maintainer lines to shoot fly in terms of deadheart (%) (Postrainy season of 2006–07 and 2007–08)

	14 DAE						21 DAE						28 DAE					
S. No.	A_1	A_2	A_3	A_4	B	Mean	A_1	A_2	A_3	A_4	B	Mean	A_1	A_2	A_3	A_4	B	Mean
N 122	30.59	31.98	32.62	25.96	26.37	29.5	37.49	37.11	38.64	29.52	34.42	35.43	48.8	43.55	43.1	31.78	39.33	41.31
KS 57	32.31	27.62	32.21	24.15	32.29	29.72	38.6	34.91	39.83	30.85	37.28	36.29	43.98	40.34	45.12	34.98	42.74	41.43
Tx 3042	35.69	40.36	36.6	29.22	31.5	34.67	45.15	46.86	44.68	38.15	39.58	42.89	52.11	63.59	60.59	44.24	45.54	53.21
Wheatland	36.68	34.59	34.29	27.73	29.06	32.47	42.61	43.28	43.28	35.48	34.78	39.88	50.13	51.3	49.79	38.85	38.46	45.7
Mean	33.81	33.64	33.93	26.76	29.8		40.96	40.54	41.61	33.5	36.51		48.75	49.7	49.65	37.46	41.52	
Checks																		
IS 18551 (R)		6.732						13.8						17.97				
DJ 6514 (S)		38.47						49.82						56.27				
For comparing	LSD	*F*-prob					LSD	*F*-prob					LSD	*F*-prob				
Genotypes	1.87	0					2.04	0					2.83	0				
Cytoplasms	2.1	0					2.28	0					3.17	0				
C × G	4.19	0.01					4.57	0.22					6.34	0				

LSD at 5%.
DAE, days after seedling emergence; LSD, Least significant difference.

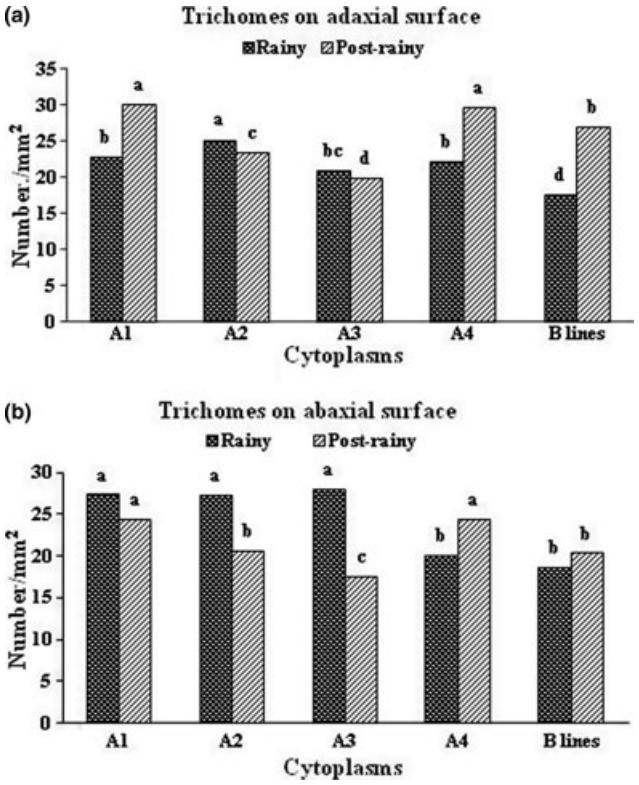

Fig. 2: Trichome density in different cytoplasmic male-sterile systems of sorghum. (a) Adaxial surface; (b) Abaxial surface. Bars with the same letters indicate that the cytoplasms are not significantly different at P = 0.05

and reduce the risk of disease epidemics directly related to cytoplasmic-genetic male-sterility systems (Ross and Kofoid 1979, Lenz and Atkins 1981, Kishan and Borikar 1988, Secrist and Atkins 1989). The ovipositional preference in the plants of different CMS and their maintainers was higher during rainy season compared with postrainy season, and oviposition increased from 14 DAE to 21 DAE. A_4 cytoplasm recorded significantly lower mean number of eggs during rainy and postrainy seasons and was less preferred for oviposition indicating antixenosis. Among the cytoplasms tested, A_4M was comparatively less preferred for oviposition (Dhillon et al. 2005). Plants of N122 recorded significantly lower number of eggs per plant compared to other genetic backgrounds such as Tx 3042 and Wheatland at 21 DAE across rainy and postrainy seasons. The range of deadhearts at different stages was less in postrainy season compared with rainy season, while it was more in CMS lines compared with maintainer lines during both the seasons. Dhillon et al. (2005) observed that the isogenic lines converted into different CMS backgrounds were susceptible to shoot fly and ovipositional preference and deadheart incidence were higher on the CMS than on the maintainer lines under multichoice conditions in the field and dual and multichoice tests in the greenhouse. Higher susceptibility of CMS lines than the corresponding maintainer lines has earlier been reported in case of sorghum shoot fly, midge, shoot bugs (*Peregrinus maidis* Ashmead) and aphids (*Melanaphis sacchari* Zehnter) (Sharma et al. 2004) and sorghum midge (*Stenodiplosis sorghicola* Coquillett) (Sharma et al. 1994, Sharma 2001). The Kansas male-sterility-based CMS lines such as KSA 34 to KSA 39 and Combine Kafir-based

Table 3: Reaction of different cytoplasmic and maintainer lines to shoot fly in terms of leaf glossiness and seedling vigour (Rainy and postrainy seasons of 2006–07 and 2007–08 combined)

S. No.	Glossiness						Seedling vigour					
	A_1	A_2	A_3	A_4	B	Mean	A_1	A_2	A_3	A_4	B	Mean
N 122	3.4	3.4	3	3	3	3.2	4	4	4	3.6	3.5	3.8
KS 57	3.5	3.5	3	3	3	3.2	4	4	3.7	3.6	3.6	3.8
Tx 3042	3.5	3.5	3	3	3	3.2	4	4	3.7	3.6	3.7	3.8
Wheatland	4	4	3	3	3	3.4	4	4	3.7	3.5	4	3.8
Mean	3.5	3.6	3	3	3		4	4	3.8	3.6	3.7	
Checks												
IS 18551 (R)		1.4						2				
DJ 6514 (S)		4						4				
For comparing	LSD	*F*-prob					LSD	*F*-prob				
Genotypes	0.02	0					0.04	0.06				
Cytoplasms	0.03	0					0.04	0				
C × G	0.05	0					0.09	0				

Least significant difference (LSD) at 5%.

CMS lines are equally susceptible to greenbug *Schizaphis graminum* (Rondani) (Ross and Kofoid 1979). Thakur et al. (1992) observed higher smut infection on hybrids on A lines than on hybrids based on B lines in pearl millet. On the contrary, Kanaka Durga et al.'s study (2008) on the influence of cytoplasm on the occurrence of leaf blight (*Exserohilum turcicum* (pass.) in sorghum found that considering area of the lesion, genotypes with sterile cytoplasm recorded significantly less leaf area damage than genotypes with fertile cytoplasm and both were significantly different from each other, and consequently, male-sterile cytoplasm contributed towards resistance. In a study on the expression of susceptibility to Fusarium head blight and grain mould in A_1 and A_2 cytoplasms of *Sorghum bicolor*, cytoplasm had no effect on head blight incidence or severity or on grain mould severity but had a significant effect on grain mould incidence, with A_1 exhibiting slightly lower incidence than A_2 (Stack and Pedersen 2003).

Among the cytoplasms tested during rainy and postrainy seasons, A_4 cytoplasm had recorded its superiority for lower deadheart formation over the other cytoplasms and maintainers. The A_4M (*maldandi*) cytoplasm had lower deadheart incidence than the other cytoplasms tested (Dhillon et al. 2005). N 122 was a promising genotype with lower deadheart formation in both the seasons.

During rainy season, significantly more number of trichomes on adaxial surface in A_2 and abaxial surface in A_3 cytoplasms were observed. On the contrary, during postrainy season, A_1 and A_4 cytoplasms recorded more number of trichomes on adaxial and abaxial surfaces. Pooled analysis over seasons and years indicated maximum number of trichomes on adaxial and abaxial surfaces in A_1 cytoplasms. The plants of N122 were superior for this trait in all four cytoplasms including maintainers for both upper and lower trichomes. Trichome density is the important trait contributing to genetic divergence (Aruna and Padmaja 2009), and the inheritance of trichome density was reported to be complex and it differed with the type of parents involved and with seasons (Jayanthi et al. 1999).

Plants of A_4 cytoplasm followed by A_3 cytoplasm were glossier compared with other cytoplasms and were on par to the maintainer lines. N122 appears to be less susceptible to shoot fly being more glossy compared with other genotypes across seasons and was promising for trichome density and low oviposition. However, the genotypes tested were almost as susceptible as the susceptible check. The level of resistance to shoot fly was higher when both glossy and trichome traits occurred together (Agrawal and House 1982, Dhillon et al. 2005). A_4 cytoplasms followed by A_3 cytoplasm recorded significantly higher seedling vigour.

In conclusion, the A_4 cytoplasm was found to be least susceptible to sorghum shoot fly as it was comparatively less preferred for oviposition and had lower deadheart incidence (37.46% in postrainy season and 61.52% in rainy season) than the other cytoplasms tested and thus can be exploited for developing shoot fly-resistant hybrids. The identified shoot fly-resistant lines may be sterilized into A_4 system and efforts should be made to increase the nuclear diversity for restoration. Combinations of shoot fly-resistant male-sterile lines and resistant restorers can be used to produce shoot fly-resistant hybrids because for obtaining shoot fly-resistant hybrids, resistance is required in both male and female parents (Jayanthi et al. 2000, Dhillon et al. 2006). The maximum content of chlorophylls and carotenoids in flag leaves was observed in hybrids with sterility type A_4 compared with hybrids on other cytoplasms (Kibal'nik and El'konin 2009), and this trait can also be exploited apart from resistance to shoot fly for increasing the productivity of sorghum.

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