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Physico-chemical mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum, *Sorghum bicolor*

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Keywords
Atherigona soccata, *Sorghum bicolor*, micronutrients, resistance mechanisms, secondary metabolites, shoot fly

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Abstract

Sorghum shoot fly, *Atherigona soccata* is an important pest of sorghum, and host plant resistance is one of the important components for minimizing the losses due to this pest. Therefore, we evaluated a diverse array of sorghum genotypes to identify physico-chemical characteristics conferring resistance to *A. soccata*. Susceptibility to shoot fly was associated with high amounts of soluble sugars, fats, leaf surface wetness and seedling vigour; while leaf glossiness, plumule and leaf sheath pigmentation, trichome density and high tannin, Mg and Zn showed resistance to shoot fly. Stepwise regression indicated that Mg, Zn, soluble sugars, tannins, fats, leaf glossiness, leaf sheath and plumule pigmentation and trichome density explained 99.8% of the variation in shoot fly damage. Path coefficient analysis suggested that leaf glossiness, trichome density, Mg and fat content and plant plumule pigmentation can be used as markers traits to select for shoot fly resistance in sorghum.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in Asia, Africa, Australia and the Americas. It is cultivated on approximately 44 million hectares world-wide, and is the fifth major cereal crop after wheat, rice, maize and barley. Insect pests are one of the major yield reducing-factors in sorghum, and result in losses of over $1000 million in grain and forage yield of sorghum worldwide (ICRISAT 1992, 2007). Nearly 150 insect species damage sorghum, and the sorghum shoot fly, *Atherigona soccata* (Rondani) (Diptera: Muscidae), is one of the most important pests in Asia and Africa. The adult female lays white, elongated, cigar-shaped eggs singly on the abaxial leaf blade of sorghum seedlings. On emergence, the neonate larva crawls to the plant whorl and continues to move downward between the folds of the young leaves. After reaching the base of the meristem, the larva cuts the growing point resulting in drying of the central leaf known as 'deadheart'.

Timely planting, manipulation of cultural practices, resistant varieties and need-based application of insecticides can be used for minimizing the losses due to shoot fly. However, planting times in the semi-arid tropics (SAT) are dictated by the onset of rains, while chemical insecticides are beyond the reach of resource poor farmers. Therefore, host plant resistance is one of the important components for shoot fly control when the sowings are delayed due to uneven rainfall during the rainy season (Sharma 1985). During the post-rainy season, the sorghum cultivars to be grown must have moderate to high levels of primary or recovery resistance to shoot fly (Sharma 1993). Efforts have been made to transfer shoot fly resistance into cytoplasmic male-sterile and restorer lines to produce shoot fly resistant hybrids (Sharma et al. 2005). The cultivars grown during the post-rainy season must have moderate levels of resistance to shoot fly. None of newly developed varieties or hybrids that are susceptible to shoot fly have been able to replace the landrace cultivars Maldandi (M 35-1), Phule, and

Yashoda, which have moderate levels of resistance to shoot fly (Sharma 1993). However, the level of resistance to shoot fly in the identified sources varies with insect density and across environments (Sharma and Nwanze 1997; Dhillon et al. 2005), and therefore, it is important to identify genotypes with diverse mechanisms of resistance to increase the level and diversify the basis of resistance to this insect for sustainable crop production.

Resistance to shoot fly is expressed in terms of oviposition non-preference, antibiosis and tolerance (Taneja and Leuschner 1985; Dhillon et al. 2005; Sivakumar et al. 2008). A number of physico-chemical traits have earlier been reported to be associated with resistance/susceptibility in sorghum to shoot fly (Sharma and Nwanze 1997). However, no in-depth studies have been carried out on different physico-chemical characteristics on the same set of genotypes, which will be useful for comparing resistant/susceptible genotypes for the reported and/or new physico-chemical traits associated with resistance to this pest. Therefore, the present study was aimed at characterizing a group of known resistant and susceptible genotypes for different physico-chemical characteristics to identify the factors responsible for resistance/susceptibility to shoot fly in sorghum. The objective was to identify the key physico-chemical characteristics conferring resistance to shoot fly, which could be used to select shoot fly-resistant lines from the segregating breeding materials for use in sorghum improvement.

Materials and Methods

The experimental material consisted of a diverse array of 15 sorghum genotypes comprising of seven germplasm accessions (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312 and IS 2205), three breeding lines (SFCR 125, SFCR 151 and ICSV 700) identified earlier to be resistant to shoot fly (Sharma et al. 2006), and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B and ICSV 112) susceptible to shoot fly. The experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, Andhra Pradesh, India during the 2004–2005 rainy (July–November) and post-rainy (October–March) seasons.

Evaluation of sorghum genotypes for resistance to shoot fly

The test material was planted in the field during the 2004–2005 rainy (July–November) and post-rainy (October–March) cropping seasons. Each genotype was sown in two rows of 2 m length, with a row-row spacing of 75 cm, and plant to plant distance of 10 cm. There were three replications in a randomized complete block design (RCBD). Shoot fly infestation was optimized through the use of the interlard fish-meal technique (Soto 1974; Sharma et al. 1992). Thinning in the test material was carried out 7 days after seedling emergence (before egg laying by the shoot fly). Data were recorded on numbers of eggs per seedling and seedlings with eggs at 14 and 21 days after emergence (DAE), and plants with deadhearts at 14, 21 and 28 DAE from all plants in the two row plots. Data on numbers of eggs were expressed as number of eggs per 10 seedlings. Seedlings with eggs and deadhearts were expressed as percentages. Recovery resistance was assessed in terms of percentage tillers with deadhearts at 28 DAE.

Characterization of sorghum genotypes for morphological traits

Data were recorded on leaf glossiness, trichome density on abaxial and adaxial surfaces of the leaf blade, seedling vigour, plumule and leaf sheath pigmentation, days to 50% flowering, and plant height at maturity. Leaf glossiness was evaluated visually on a 1–5 scale at 10 DAE (fifth leaf stage, when the expression of this trait is most apparent) in the early morning hours when there was maximum reflection of light from the leaf surface (1 = highly glossy and 5 = non-glossy) (Sharma and Nwanze 1997). The presence and density of trichomes was measured at 10 DAE on the central portion of the fifth leaf blade taken from three randomly selected seedlings. For this purpose, leaf pieces (2 cm^2) taken from the central portion of the leaf were placed in acetic acid and alcohol (2 : 1) in stoppared glass vials (10 ml capacity) for 24 h to clear the chlorophyll, and subsequently transferred into lactic acid (90%) as a preservative (Maiti and Bidinger 1979). The leaf sections were mounted on a glass slide in a drop of lactic acid, and magnified at 10x under a stereomicroscope. The trichomes on abaxial and adaxial surfaces of the leaf blade were expressed as number of trichomes in a $10\times$ microscopic field. Seedling vigour was recorded at 10 DAE on a 1–5 rating scale (1 = highly vigorous and 5 = poor seedling vigour) (Sharma and Nwanze 1997). The leaf surface wetness on the central whorl leaf was recorded in the test material planted in plastic cups (10 cm diameter) and kept in the open outside the greenhouse. The

10-day-old seedlings were brought to the laboratory in the early morning hours (0430 to 0630 h), the central leaf whorl was pulled out and mounted on a slide, and observed under the microscope (10 × magnification), and the data were recorded on a 1–5 scale (1 = leaf blade without water droplets; and 5 = entire leaf blade densely covered with water droplets) (Sharma and Nwanze 1997). The moisture content of the 10-day-old seedlings was determined by recording the fresh weight, and then the dry weight after 3 days of drying at 55°C in an oven. The pink pigment on the plumule (embryonic shoot) and leaf sheath was assessed visually on a 1–5 rating scale at 5 DAE (Dhillon et al. 2006b).

Mineral and biochemical composition of sorghum genotypes

Sorghum seedlings at the 5-leaf stage were collected from the field and lyophilized at −45°C for 3 days. The lyophilized seedlings were then powdered in a Willey mill. The lyophilized seedling powder samples used for analysis of chemical composition of different sorghum genotypes. Nitrogen (N), Phosphorus (P) and Potassium (K) were determined by digesting the samples with sulphuric acid-selenium. N and P in the digests were analysed using an auto-analyser (Skalar Analytical B.V, Model SA2000/4000 segmented flow analyser, Netherlands), and K in digests was analysed using an atomic absorption spectrophotometer (Sahrawat et al. 2002a). Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) were determined in triacid digest using atomic absorption spectrophotometry (Sahrawat et al. 2002b). Protein was estimated by multiplying N content × 6.25. The results on sorghum plant analysis are the means of two independent analyses. Fat content was estimated by the Soxhlet extraction procedure (AOCS 1981), lignins by using the acid detergent dispersible lignin (ADDL) method given by Van Soest and Robertson (1985), soluble sugars by the phenol–sulphuric acid method (Dubois et al. 1956), polyphenols by the Folin Denis method (AOAC 1984) and hydrolysable tannins by the vanillin–hydrochloric acid method (Price et al. 1978).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) in a randomized complete block design (RCBD) using GenStat® 10th version (GenStat 2008). Significance of differences among the genotypes for each trait was tested by F-test. When the ANOVA showed significant genotypic differences, the significance of differences between the genotypic means was judged by least significant difference (LSD) at $P \leq 0.05$. Simple correlations, stepwise regression and path coefficient analyses were performed to understand the association between the morphological traits and various parameters used to measure resistance (oviposition and deadhearts) to sorghum shoot fly (Dhillon et al. 2005). Diversity among the sorghum genotypes with different combinations of characteristics associated with resistance/susceptibility to shoot fly was assessed through principle component analysis.

Results

Relative susceptibility of sorghum genotypes to shoot fly

There were significant differences for numbers of eggs per 10 seedlings ($F_{14,28} = 12.89$, $P < 0.001$ at 14 DAE; $F_{14,28} = 11.98$; $P < 0.001$ at 21 DAE); seedlings with eggs ($F_{14,28} = 19.39$, $P < 0.001$ at 14 DAE; $F_{14,28} = 7.14$, $P < 0.001$ at 21 DAE); and deadheart formation ($F_{14,28} = 6.69$, $P < 0.001$ at 14 DAE; $F_{14,28} = 16.42$, $P < 0.001$ at 21 DAE; and $F_{14,28} = 10.18$, $P < 0.001$ at 28 DAE) among the genotypes tested. Genotypes IS 1054, IS 2146, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700 and IS 18551 had significantly lower number of eggs and percentage plants with eggs, and seedlings with deadhearts as compared to the susceptible check, Swarna (table 1). Tiller deadhearts among the test genotypes also varied significantly ($F_{14,28} = 6.02$, $P < 0.001$).

Morphological characteristics of different sorghum genotypes in relation to expression of resistance to shoot fly

There was a significant variation in the leaf surface wetness ($F_{14,28} = 121.09$, $P < 0.001$), leaf glossiness ($F_{14,28} = 34.27$, $P < 0.001$), trichomes (abaxial = $F_{14,28} = 30.92$, $P < 0.001$; and adaxial = $F_{14,28} = 38.20$, $P < 0.001$), seedling vigour ($F_{14,28} = 2.68$, $P < 0.007$) and pigmentation (plumule = $F_{14,28} = 44.58$, $P < 0.001$; and leaf sheath = $F_{14,28} = 18.36$, $P < 0.001$) among the test genotypes. Genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700 and IS 18551 had significantly lower leaf surface wetness, more numbers of trichomes, high leaf glossiness intensity, and more pigmentation in the plumule and leaf sheath as compared to the

Table 1 Oviposition and deadheart formation due to sorghum shoot fly on 15 sorghum genotypes under field conditions

	Eggs seedlings $^{-10}$		Seedlings with eggs (%)		Plants with deadhearts (%)		Tillers with deadhearts (%)	
Genotype	14 DAE	21 DAE	14 DAE	21 DAE	14 DAE	21 DAE	28 DAE	28 DAE
IS 1054	4.5	6.5	42.5	63.1	9.5	39.3	48.5	21.1
IS 1057	5.7	8.7	47.4	68.3	12.2	44.0	54.6	25.1
IS 2146	3.3	5.6	32.2	55.5	8.6	32.5	42.2	20.6
IS 4664	6.4	9.9	42.6	77.4	18.3	51.2	64.4	26.2
IS 2312	3.7	5.7	34.5	53.9	8.4	29.6	42.8	27.5
IS 2205	4.1	6.0	32.1	54.2	8.4	31.1	45.5	25.6
SFCR 125	4.9	9.0	42.0	67.3	14.7	47.9	60.6	30.5
SFCR 151	4.3	8.5	35.3	66.8	11.3	42.8	54.1	27.0
ICSV 700	5.8	8.3	42.2	68.5	12.9	45.1	57.2	26.2
CK 60B	13.9	13.9	80.3	92.8	35.6	76.6	85.9	41.5
ICSV 745	15.4	13.4	82.0	94.3	45.3	84.1	91.7	45.7
296B	12.6	13.2	72.2	92.9	32.6	73.5	83.5	39.2
ICSV 112	16.8	14.0	87.3	93.8	44.4	78.9	81.8	51.7
IS 18551 (R)	3.2	5.4	29.1	57.6	6.8	33.1	43.0	30.7
Swarna (S)	15.3	15.1	80.4	96.8	48.6	80.1	92.6	37.3
LSD (P = 0.05)	4.1	2.9	13.9	17.7	17.0	14.2	16.8	10.8

DAE = days after emergence; R = resistant check; S = susceptible check.

susceptible check, Swarna, and were resistant to shoot fly (table 2). Leaf surface wetness and seedling vigour were positively and significantly associated with susceptibility to shoot fly; while leaf glossiness, trichome density and leaf sheath and plumule pigmentation were significantly and negatively associated with deadheart incidence, seedlings with eggs and percent tillers with deadhearts (table 5).

Mineral and biochemical composition of sorghum genotypes in relation to expression of resistance to shoot fly

The amounts of micronutrients such as N ($F_{14,28}$ = 61.47, $P < 0.001$), P ($F_{14,28}$ = 48.77, $P < 0.001$), K ($F_{14,28}$ = 58.02, $P < 0.001$), Mg ($F_{14,28}$ = 27.98, $P < 0.001$), Ca ($F_{14,28}$ = 7.13, $P < 0.001$), Zn ($F_{14,28}$ = 114.93, $P < 0.001$), Fe ($F_{14,28}$ = 336.42, $P < 0.001$) and Mn ($F_{14,28}$ = 164.41, $P < 0.001$) in the sorghum genotypes varied significantly. However, the differences in Cu content were non-significant ($F_{14,28}$ = 1.57, P = 0.151). The Zn content in the shoot fly-resistant genotypes IS 1054, IS 1057, IS 2146 and IS 18551 was significantly higher, and Fe content lower than the susceptible check, Swarna (table 3).

There were significant differences in protein ($F_{14,28}$ = 61.47, $P < 0.001$), fat ($F_{14,28}$ = 41.1, $P < 0.001$), total soluble sugars ($F_{14,28}$ = 21.52, $P < 0.001$), tannins ($F_{14,28}$ = 5.21, $P < 0.001$), lignins ($F_{14,28}$ = 4.20; $P < 0.001$), and moisture content ($F_{14,28}$ = 2.75, P = 0.011) in the seedling of the sorghum genotypes tested. However, the differences in polyphenols were non-significant ($F_{14,28}$ = 1.67, P = 0.132). The moisture content and tannins were significantly greater, and fats and soluble sugars lower in some of the shoot fly-resistant genotypes as compared to the susceptible check, Swarna (table 4). Total soluble sugars and fat contents were positively and significantly associated with susceptibility to shoot fly, while tannins, Mg and Zn were associated with resistance to this pest (table 5). Stepwise regression analysis indicated that leaf glossiness, leaf sheath and plumule pigmentation, trichomes, leaf surface wetness, Mg, soluble sugars, tannins, Zn and fats explained 99.8% variation for deadhearts. Furthermore, path coefficient analysis revealed that correlation coefficients and direct effects of leaf glossiness, plumule pigmentation, trichomes on adaxial leaf surface, Mg and fat contents were in the same direction, and these traits can be used to select sorghum genotypes with resistance to shoot fly (table 6).

Diversity among the sorghum genotypes and expression of resistance to shoot fly

Based on the deadheart incidence, and morphological, nutritional and biochemical traits of the sorghum genotypes, principle component analysis placed the test genotypes into three clusters. The

Table 2 Morphological characteristics of 15 sorghum genotypes evaluated for resistance to sorghum shoot fly

Genotype	Leaf surface wetness	Leaf glossiness	Trichome density		Seedling vigour	Pigmentation score	
			Abaxial	Adaxial		Plumule	Leaf sheath
IS 1054-R	1.0	2.3	118.9	67.7	2.2	1.0	4.3
IS 1057-R	1.1	2.8	112.2	68.9	1.8	1.3	2.0
IS 2146-R	1.0	1.6	149.0	104.7	2.3	1.0	2.8
IS 4664-R	1.0	2.7	102.6	75.9	2.9	1.7	2.3
IS 2312-R	1.5	2.0	118.2	77.5	1.6	2.2	2.8
IS 2205-R	1.2	1.5	150.7	102.7	2.5	1.3	2.2
SFCR 125-MR	1.2	2.1	178.0	124.2	2.8	1.5	2.5
SFCR 151-MR	1.3	2.2	138.0	96.2	2.8	1.7	2.3
ICSV 700-MR	1.1	2.1	174.6	102.0	2.9	1.0	2.2
CK 60B-S	3.2	4.8	3.1	0.8	3.2	5.0	5.0
ICSV 745-S	3.8	4.8	1.0	0.4	3.2	5.0	5.0
296B-S	4.2	4.7	1.3	0.4	4.0	2.0	3.2
ICSV 112-S	4.5	4.6	1.4	0.6	2.3	5.0	5.0
IS 18551-R	1.2	1.5	159.6	104.7	2.3	1.2	2.0
Swarna-S	4.8	4.8	24.7	14.2	2.9	1.0	3.5
LSD (P = 0.05)	0.38	0.7	35.4	21.3	1.1	0.7	0.8

R = resistant; MR = moderately resistant; S = susceptible.

Table 3 Micronutrient profile of 15 sorghum genotypes evaluated for resistance to sorghum shoot fly

Genotypes	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
IS 1054-R	3.85	0.41	3.12	0.38	0.45	53.50	1984.00	13.50	113.50
IS 1057-R	4.01	0.55	3.15	0.42	0.66	55.67	1866.00	14.33	130.00
IS 2146-R	3.88	0.51	3.23	0.35	0.52	64.33	1972.00	14.50	126.30
IS 4664-R	3.87	0.49	3.37	0.34	0.52	44.83	2653.00	15.00	137.70
IS 2312-R	3.80	0.49	3.69	0.38	0.53	39.50	2180.00	15.00	106.00
IS 2205-R	3.72	0.45	2.94	0.28	0.49	38.00	2257.00	14.50	115.00
SFCR 125-MR	3.74	0.46	3.32	0.32	0.50	38.50	2113.00	15.00	155.50
SFCR 151-MR	3.70	0.44	3.21	0.31	0.52	41.50	2110.00	12.50	108.50
ICSV 700-MR	3.71	0.47	3.03	0.33	0.55	39.17	2313.00	15.67	138.30
CK 60B-S	3.74	0.46	2.86	0.30	0.43	35.67	2934.00	15.67	137.30
ICSV 745-S	3.92	0.49	3.41	0.28	0.51	31.50	2443.00	15.00	137.50
296B-S	4.07	0.50	3.32	0.32	0.54	29.00	2248.00	10.00	115.50
ICSV 112-S	3.93	0.45	3.54	0.27	0.53	31.50	2055.00	14.00	117.00
IS 18551-R	3.84	0.46	3.02	0.32	0.51	45.50	1556.00	14.00	118.00
Swarna-S	3.72	0.37	2.83	0.24	0.42	39.00	1994.00	12.67	104.70
LSD (P = 0.05)	0.04	0.02	0.09	0.03	0.06	2.62	52.02	NS	3.35

R = resistant; MR = moderately resistant; S = susceptible.

genotypes showing susceptible reaction to shoot fly were placed in cluster C (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112), while those with moderate levels of resistance to shoot fly were placed in clusters A (SFCR 125, ICSV 700), and those with high and/or stable resistance to shoot fly were placed in cluster B (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205 and SFCR 151) (fig. 1).

Discussion

Oviposition non-preference (antixenosis), antibiosis and tolerance are the major components of resistance in sorghum to shoot fly (Doggett et al. 1970; Raina et al. 1981; Sharma and Nwanze 1997; Dhillon et al. 2005, 2006a; Sivakumar et al. 2008). As a result of shoot fly damage to the main shoot, more numbers of tillers are produced, depending on the

Table 4 Biochemical composition of 15 sorghum genotypes evaluated for resistance to sorghum shoot fly

Genotype	Moisture content (%)	Soluble polyphenols (mg/g)	Lignins (%)	Tannins (%)	Fats (%)	Soluble sugars (%)	Proteins (%)
IS 1054-R	91.93	34.64	1.27	0.19	5.49	2.86	24.08
IS 1057-R	91.73	32.77	1.18	0.21	5.64	*	25.06
IS 2146-R	91.47	29.40	1.65	0.21	4.64	2.82	24.23
IS 4664-R	92.04	31.19	1.41	0.20	5.10	2.80	24.17
IS 2312-R	91.31	30.74	1.13	0.18	5.83	2.88	23.75
IS 2205-R	91.28	33.29	1.20	0.18	5.17	2.83	23.22
SFCR 125-MR	92.43	33.70	1.25	0.17	5.40	2.80	23.38
SFCR 151-MR	91.72	36.11	1.19	0.16	4.82	2.90	23.14
ICSV 700-MR	91.39	35.64	1.21	0.14	5.29	2.99	23.19
CK 60 B-S	91.17	35.48	1.42	0.10	5.55	2.97	23.40
ICSV 745-S	91.18	26.06	1.19	0.10	6.44	2.99	24.51
296 B-S	91.58	36.15	1.10	0.18	6.89	3.16	25.44
ICSV 112-S	91.22	32.50	1.23	0.10	7.30	3.14	24.58
IS 18551-R	91.57	32.09	1.67	0.16	5.76	2.70	23.99
Swarna-S	91.04	31.82	1.43	0.08	6.40	3.10	23.24
LSD (P = 0.05)	0.67	NS	0.25	0.06	0.34	0.09	0.27

*Missing value.

R = resistant; MR = moderately resistant; S = susceptible.

level of primary resistance and shoot fly abundance (Doggett et al. 1970; Raina 1985). The shoot fly-resistant genotypes produce more numbers of uniform productive tillers than the susceptible ones, and yield more under shoot fly infestation (Sharma and Nwanze 1997).

Genotypes with glossy and trichomed leaves are relatively less susceptible to shoot fly damage (Maiti and Gibson 1983; Sharma and Nwanze 1997; Dhillon et al. 2005, 2006b), while leaf surface wetness is associated with susceptibility to shoot fly in sorghum (Nwanze et al. 1992; Dhillon et al. 2005). The plumule and leaf sheaths of the shoot fly-resistant genotypes have deeper pink pigment, while the susceptible genotypes were green coloured (Dhillon et al. 2006b). Light pink-pigmented plants with low chlorophyll content are less susceptible to shoot fly damage (Singh et al. 1981; Kamatar et al. 2003; Dhillon 2004; Dhillon et al. 2005). Possibly because of their effect on reflection of light from the leaf surface, which influence the oviposition behavior of shoot fly females (Sharma and Nwanze 1997). The results suggested that sorghum genotypes exhibiting leaf glossiness trait, trichomes, pigmented plumule and leaf sheath were highly resistant to shoot fly. Seedling vigour, earlier reported to be positively associated with resistance to shoot fly (Taneja and Leuschner 1985), showed a negative association with shoot fly resistance in the present studies, as reported by Dhillon et al. (2005).

The deficiency of plant nutrients or the presence of anti-nutritional factors in sorghum genotypes might adversely affect the development and survival of shoot fly larvae (Raina 1985). Although, there were significant differences among the test genotypes for moisture content, there was no apparent association between moisture content and the expression of resistance to shoot fly. Singh et al. (2004) reported that there is no relationship between moisture content of sorghum seedlings and shoot fly resistance. However, seedlings of maize genotypes with low moisture content have been reported to be resistant to spotted stem borer, *Chilo partellus* (Swin.) (Rao and Panwar 2002). Plant phenolics provide resistance to aphid, *Rhopalosiphum padi* (L.) in wheat (Juan et al. 2001), and to stem borer, *C. partellus* in maize (Kabre and Ghorpade 1998). However, in case of shoot fly resistance, no significant association was observed. This could be due to low phenol content of these sorghum genotypes and/or masking of these effects by the morphological traits such as leaf glossiness and leaf trichome density, which have a major bearing on the expression of resistance to shoot fly.

Positive association of N and P with oviposition by the shoot fly females during the seedling stage may be due to their association with production and release of chemical cues influencing the oviposition behavior of sorghum shoot fly (Singh and Jotwani 1980; Khurana and Verma 1983; Chavan et al.

Table 5 Association of morphological traits, biochemical constituents and plant nutrients with expression of resistance to sorghum shoot fly in 15 sorghum genotypes at 14, 21 and 28 days after seedling emergence (DAE)

Traits	Plants with deadhearts (%)			Seedlings with eggs (%)		Eggs per 10 seedlings		Tiller deadhearts (%)
	14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
Morphological traits								
Leaf glossiness	0.96**	0.98**	0.97**	0.98**	0.97**	0.98**	0.96**	0.86**
Bottom leaf pigmentation	0.64**	0.65**	0.61*	0.70**	0.59*	0.64**	0.61*	0.61*
Leaf sheath pigmentation	0.72**	0.72**	0.66**	0.81**	0.67**	0.76**	0.64**	0.69**
Plumule pigmentation	0.66**	0.68**	0.63*	0.73**	0.63*	0.72**	0.62*	0.83**
Seedling vigour	0.50	0.63*	0.67**	0.51*	0.66**	0.51*	0.64**	0.39
Adaxial trichome density	−0.90**	−0.90**	−0.87**	−0.94**	−0.89**	−0.93**	−0.87**	−0.83**
Abaxial trichome density	−0.91**	−0.90**	−0.88**	−0.94**	−0.90**	−0.93**	−0.88**	−0.84**
Biochemical traits								
Moisture content	−0.50	−0.38	−0.37	−0.48	−0.35	−0.53*	−0.36	−0.46
Protein content	0.21	0.22	0.17	0.23	0.24	0.23	0.15	0.22
Total soluble polyphenols	−0.33	−0.25	−0.24	−0.23	−0.17	−0.27	−0.14	−0.28
Tannins	−0.87**	−0.84**	−0.85**	−0.84**	−0.82**	−0.86**	−0.86**	−0.75**
Total soluble sugars	0.76**	0.74**	0.72**	0.80**	0.73**	0.80**	0.74**	0.72**
Fat content	0.80**	0.78**	0.75**	0.82**	0.78**	0.83**	0.76**	0.81**
Lignins	−0.06	−0.09	−0.08	−0.14	−0.04	−0.12	−0.07	−0.27
Leaf surface wetness	0.95**	0.91**	0.90**	0.94**	0.89**	0.95**	0.90**	0.88**
Nutrients								
Nitrogen	0.21	0.22	0.17	0.23	0.24	0.23	0.15	0.22
Phosphorus	−0.28	−0.21	−0.23	−0.24	−0.22	−0.23	−0.26	−0.08
Potassium	0.12	0.11	0.09	0.12	0.12	0.12	0.12	0.19
Calcium	0.03	0.08	0.03	0.18	0.06	0.13	0.03	0.18
Magnesium	−0.77**	−0.73**	−0.69**	−0.75**	−0.69**	−0.76**	−0.71**	−0.73**
Manganese	−0.07	0.05	0.06	−0.03	0.04	−0.06	0.03	−0.06
Copper	−0.31	−0.33	−0.33	−0.33	−0.36	−0.32	−0.36	−0.25
Iron	−0.14	−0.03	0.00	−0.09	0.01	−0.09	−0.02	−0.10
Zinc	−0.63*	−0.67**	−0.70**	−0.66**	−0.66**	−0.67**	−0.70**	−0.77**
Stepwise regression equation								
Deadherats (%) = 108.7–108.5 Mg - 37.88 TSS + 23.2 T + 1.817 F + 24.689 GS - 3.784 BLP + 1.612 PP - 0.1778 TD + 0.3152 TA (R^2 = 99.8%).								

Mg, magnesium; TSS, total soluble sugars; T, tannins; F, fat content; GS, leaf glossiness; BLP, bottom leaf pigmentation; PP, plumule pigmentation; TD, trichomes on adaxial leaf surface; TA, trichomes on abaxial leaf surface. Correlation coefficients significant at P ≤ 0.05 (*) and 0.01 (**), respectively.

1990; Bhise et al. 1997; Singh et al. 2004). However, the correlation coefficients were non-significant. High amounts of Si and Ca (Chavan et al. 1990) lignins and phenols (Khurana and Verma 1983; Kumar and Singh 1998) have earlier been reported to be associated with shoot fly resistance. While no significant association of Ca, Cu, lignins, or total polyphenolics was observed with shoot fly resistance or susceptibility in the present study, plant Mg and Zn contents were significantly greater in some shoot fly resistant genotypes as compared to the susceptible check, Swarna. The results suggested that Mg and Zn are putative factors in shoot fly resistance in sorghum.

Tannin content of the immature sorghum grain has earlier been reported to be negatively associated with susceptibility to sorghum midge, *Stenodiplosis sorghicola* (Coq.) (Sharma et al. 1990, 1993a; b; Mohan et al. 1997). The present studies also showed a significant and negative correlation between tannin content and shoot fly damage. As observed in the present studies, sugar and protein contents have earlier been reported to be positively associated with susceptibility to stem borer (Kabre and Ghorpade 1999), midge (Sharma et al. 1990, 1993a; Mohan et al. 1997) and shoot fly (Kamatar et al. 2003; Singh et al. 2004).

There was considerable genetic diversity among the shoot fly-resistant genotypes based on shoot fly damage, morphological traits and biochemical composition. The contribution of nutritional and biochemical factors was comparatively lower than that of the morphological factors such as leaf glossiness

Table 6 Direct and indirect effects of physico-chemical traits on deadheart incidence in 15 sorghum genotypes caused by shoot fly

Trait	X1	X2	X3	X4	X5	X6	X7	X8	X9	r
Leaf glossiness (X1)	**1.75**	0.08	−0.09	−1.13	0.45	0.13	0.03	−0.06	−0.19	0.96**
Plumule pigmentation (X2)	1.19	**0.12**	−0.09	−0.86	0.33	0.10	0.02	−0.05	−0.10	0.67**
Bottom leaf pigmentation (X3)	1.07	0.07	**−0.15**	−0.70	0.27	0.14	0.02	−0.05	−0.11	0.56*
Trichome (abaxial) (X4)	−1.68	−0.08	0.09	**1.17**	−0.47	−0.12	−0.03	0.06	0.18	−0.89**
Trichome (adaxial) (X5)	−1.68	−0.08	0.09	1.16	**−0.47**	−0.12	−0.03	0.06	0.19	−0.88**
Magnesium (X6)	−1.18	−0.06	0.11	0.74	−0.29	**−0.19**	−0.02	0.06	0.15	−0.69**
Fat (X7)	1.41	0.06	−0.07	−0.95	0.39	0.11	**0.04**	−0.05	−0.18	0.77**
Tannin (X8)	−1.34	−0.07	0.09	0.79	−0.32	−0.13	−0.02	**0.08**	0.14	−0.77**
Total soluble sugars (X9)	1.38	0.05	−0.07	−0.87	0.37	0.12	0.03	−0.05	**−0.24**	0.72**

Path coefficients equation: Deadhearts (%) = 83.87 + 1.75 X1 + 0.12 X2 - 0.15 X3 + 1.17 X4 - 0.47 X5 - 0.19 X6 + 0.04 X7 + 0.08 X8 - 0.24 X9 (residual variance = 0.02).

*,**Correlation coefficients (r) significant at $P \leq 0.05$ and 0.01, respectively. Figures in bold are direct effects of different traits.

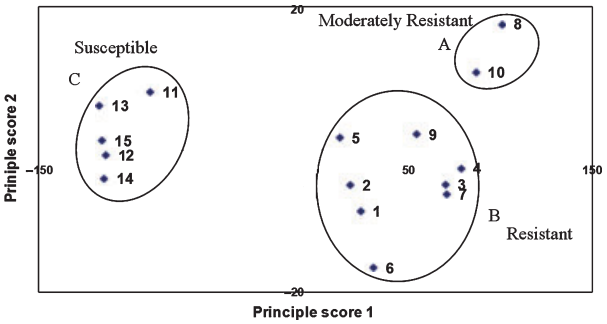

Fig. 1 Diversity among the sorghum genotypes based on number of deadhearts, morphological traits and biochemical and nutrient composition of seedlings (1 = IS 1054, 2 = IS 1057, 3 = IS 2146, 4 = IS 18551, 5 = IS 4664, 6 = IS 2312, 7 = IS 2205, 8 = SFCR 125, 9 = SFCR 151, 10 = ICSV 700, 11 = Swarna, 12 = CK 60B, 13 = ICSV 745, 14 = 296B, and 15 = ICSV 112).

and trichome density. To develop cultivars with stable resistance to shoot fly, there is need to use sorghum genotypes with different combinations of factors associated with shoot fly resistance. Therefore, we need to have a comprehensive understanding of the biochemical constituents that influence the expression of resistance to shoot fly for gene pyramiding in improved varieties and hybrids. The present studies based on a diverse array of sorghum genotypes with different levels of resistance to shoot fly provided a rational comparison of the contribution of different traits associated with shoot fly resistance, and pinpoint those that can be used as markers to screen and breed for resistance to this insect. These studies provided additional information on some of the biochemical traits that have not been earlier reported to be associated with shoot fly resistance. Also, some of the traits that were earlier thought to be contributing to shoot fly resistance based on a limited range of the materials, were in fact not really contributing to host plant resistance to shoot fly. The physico-chemical traits that linked to shoot fly resistance need to be studied in greater detail using either iso-lines, RILs, or backcross populations to study the cause and effect phenomena, and assess relative contribution of different traits for shoot fly resistance, and use of such traits for sorghum improvement for sustainable crop production.

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