Constitutive and Inducible Resistance to *Atherigona soccata* (Diptera: Muscidae) in *Sorghum bicolor*

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ABSTRACT Host plant resistance is one of the important components for minimizing the losses because of sorghum shoot fly, *Atherigona soccata* (Diptera: Muscidae) attack. Therefore, we studied the constitutive and inducible biochemical mechanisms of resistance to *A. soccata* in a diverse array of sorghum genotypes to identify lines with diverse mechanisms of resistance to this insect. Fifteen sorghum genotypes with different levels of resistance to *A. soccata* were evaluated. Methanol extracts of 10-d old damaged and undamaged sorghum seedlings were subjected to high-performance liquid chromatography analysis. Association between peak areas of the identified and unidentified compounds with parameters measuring *A. soccata* resistance was determined through correlation analysis. Amounts of *p*-hydroxy benzaldehyde and the unidentified compounds at RTs 24.38 and 3.70 min were associated with susceptibility to *A. soccata*. Genotypes exhibiting resistance to *A. soccata* were placed in four groups, and the lines showing constitutive and/or induced resistance to *A. soccata* with different combinations of biochemical factors potentially could be used for increasing the levels of resistance to *A. soccata* in sorghum.

KEY WORDS *Sorghum bicolor*, *Atherigona soccata*, induced resistance, flavonoids, biochemical mechanisms of resistance

Sorghum, *Sorghum bicolor* (L.) Moench, is an important cereal crop in Asia, Africa, Australia, and the Americas. It is cultivated on ≈44 million hectares worldwide, 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 $1,000 million annually in the semi-arid tropics (SAT) (ICRISAT 1992). Nearly 150 insect species damage the sorghum crop, of which *Atherigona soccata* (Rondani) (Diptera: Muscidae) is an important pest in Asia, Africa, and the Mediterranean Europe (Sharma 1993). The *A. soccata* females lay white, elongated, cigar-shaped eggs singly on the abaxial leaf surface of sorghum seedlings between 7–30 d after seedling emergence. After egg hatching, the neonate larvae crawl to the plant whorl and move downward between the folds of the young leaves. After reaching the growing point, it cuts the growing tip resulting in drying of the central leaf known as a 'deadheart.'

Timely planting, manipulation of cultural practices, resistant varieties, and need-based application of insecticides can be used for minimizing the losses because of *A. soccata.* Most of the planting times in the SAT are dictated by the onset of rains, while chemical insecticides are beyond the reach of resource-poor farmers. To overcome these problems, it is important to identify and develop sorghum cultivars with stable resistance to this pest. A number of genotypes with low to moderate levels of resistance to *A. soccata* have been identified in sorghum germplasm (Taneja and Leuschner 1985, Sharma et al. 2003), and resistance to *A. soccata* is expressed in terms of oviposition nonpreference, antibiosis, and tolerance (Sharma and Nwanze 1997, Dhillon et al. 2005, Sivakumar et al. 2008).

Plant resistance to *A. soccata* is mediated by a number of morphological and biochemical factors (Sharma 1993, Chamarthi et al. 2011). Phenolic compounds, such as 3-deoxyanthocyanidins, *p*-hydroxybenzoates, *p*-coumarates, and flavonols are involved in host plant resistance to biotic stresses (Lo et al. 1999, Weston et al. 1999, Weir et al. 2004). Resistance to *A. soccata* is associated with morphological traits such as leaf glossiness and trichomes (Sharma and Nwanze 1997), biochemical components such as low amounts of protein (Mote et al. 1979, Kamatar et al. 2002, Chamarthi et al. 2011), polyphenol oxidase and peroxidase activity (Bhise et al. 1996), and chlorophyll content (Singh and Jotwani 1980). High amounts of lignins (Blum 1963), amino acids (Khurana and Verma 1982), phenolics (Khurana and Verma 1983, Kumar and Singh 1998), tannins, and total sugars (Kamatar et al. 2002) are associated with resistance to *A. soccata* damage. The amino acid lysine has been reported to be absent in the leaf tissues of sorghum genotypes exhibiting resistance to *A. soccata* (Singh and Jotwani 1980).

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Physiological and biochemical changes in terms of micronutrients and secondary metabolites during seedling development also affect the expression of resistance to *A. soccata* in sorghum (Sharma and Nwanze 1997, Singh et al. 2004, Chamarthi et al. 2011).

Induced resistance also plays an important role in resistance to insects (Underwood et al. 2005). Therefore, the present studies were conducted to quantify qualitative and quantitative differences in biochemical profiles of damaged and undamaged seedlings of a diverse array of sorghum genotypes with different levels of resistance or susceptibility to *A. soccata* to pin-point constitutive and inducible components of resistance to *A. soccata.* Genotypes exhibiting constitutive and/or induced resistance to *A. soccata*, and with different combinations of biochemical factors associated with *A. soccata* resistance, can be used for increasing the levels of resistance to this insect in sorghum.

Materials and Methods

The experimental material consisted of 15 diverse sorghum genotypes comprising of seven germplasm accessions (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, and IS 2205), three improved lines (SFCR 125, SFCR 151, and ICSV 700) identified earlier to be resistant to *A. soccata*, and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) susceptible to *A. soccata* (Sharma et al. 1992, 2003). Of these, IS 18551 and Swarna served as resistant and susceptible checks, respectively, based on their reaction to *A. soccata* damage under field conditions (Taneja and Leushner 1985, Sharma et al. 2005). The experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, under field, greenhouse, and laboratory conditions during 2004 to 2005.

Expression of Resistance to *A. soccata.* The test material was planted in the field during the rainy (July to November) and postrainy (October to March) cropping seasons, 2004 to 2005. Each genotype was sown in two rows of 2 m length, between row spacing of 75 cm, and plant to plant distance of 10 cm. There were three replications in a randomized complete block design. *A. soccata* infestation was optimized through the use of the interlard fish-meal technique (Soto 1974, Sharma et al. 1992). Thinning was carried out at 7 d after seedling emergence (DAE) (before egg laying by the *A. soccata*). Data were recorded on numbers of eggs, seedlings with eggs, and seedlings with deadhearts at 14 DAE. Data on numbers of eggs were expressed as numbers per 10 seedlings, and seedlings with eggs and deadhearts were expressed as percentages.

HPLC Fingerprints of Isoflavonoids in Damaged and Undamaged Seedlings of Different Sorghum Genotypes. The sorghum genotypes grown in the greenhouse (28 ± 2°C and 75 ± 5% RH), after 9-d of seedling emergence, the test genotypes were confined with mated *A. soccata* females (16 flies seedlings^{-40}) collected from the field in a cage ($40 \times 30 \times 14$ cm) (Dhillon et al. 2005, Sivakumar et al. 2008). Three days after infestation, the deadhearts (central leaf whorl that dried up as a result of *A. soccata* damage) were collected, and the larvae removed from the deadhearts. Central whorl leaves from uninfested plants were similarly collected as controls. The samples were freeze-dried in a lyophylizer at −45°C for 3 d. After freeze drying, the samples were ground in a mortar and pestle, and kept in sealed packets in desiccators till analysis.

Phenolic compounds from different sorghum genotypes were extracted and analyzed by the method described by Hahn et al. (1983), with a few modifications. Lyophilized sorghum leaf powder (100 mg) was extracted in 5 ml of 100% methanol by sonication for 30 min, and centrifuged at 5,000 rpm for 10 min. The supernatant was collected and partitioned with 5 ml of hexane in a separation funnel until the two phases separated clearly, and the process was repeated three times. Methanol extracts from different separations were combined and reduced to near dryness in a vacuum roto-evaporator, and redissolved in 3 ml of high-performance liquid chromatography (HPLC) grade methanol. The samples were filtered through 0.45 μm pore size Millipore filter. Available standards such as gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids were prepared at 100 ppm concentrations, and filtered as described above.

The samples and standards (20 μl) were chromatographed singly and in mixtures on a Waters Sunfire C_{18} column (4.6×250 mm) with 5 μm pore size. A Waters High Performance Liquid Chromatography (HPLC) 2695 Separation Module (Alliance^{R}) having a PCM 11 reciprocating piston pump, and a 2996 photodiode array detector (in the range of 190–800 nm) was used for obtaining a fingerprint of the phenolic compounds in different sorghum genotypes. Multistep gradient solvent system of 2% acetic acid (A) and 2% acetic acid-acetonitrile (B) was used for separation. The separation was programmed isocratically: 5% of solvent B for 10 min, followed by a 7.5 min linear gradient to 15% of solvent B, which was run isocratically for 13.5 min, followed by a 10-min linear gradient to 50% of solvent B. This was run isocratically for 4 min, followed by a 5 min linear gradient to 15% of solvent B, and finally followed by a 5 min linear gradient to 5% of solvent B. Flow rate was 1 ml/min. The solvents were run at six curve (linear). There were three replicates for each genotype, and the experiment was conducted in a completely randomized design. The spectrum detection was made at 254 nm. The chromatographic data were recorded and processed by the Millennium^{32} software version 4.0. Phenols were identified and quantified by comparing the peak area obtained at similar retention times of the peak area with known concentrations of the standards.

Statistical Analysis. Field data on *A. soccata* damage parameters were subjected to analysis of variance (ANOVA) in a randomized complete block design using GenStat 10th version (GenStat 2008). Signifi-

Table 1. Oviposition response and deadhearts caused by *A. soccata* on 15 sorghum genotypes at 14 d after seedling emergence under field conditions (rainy and postrainy seasons, 2004–2005, ICRISAT, Patancheru, India)

Genotypes	Eggs seedlings^{-1}[a]	Seedlings with eggs (%)	Seedlings with deadhearts (%)
IS 1054	4.5	42.5	9.5
IS 1057	5.7	47.4	12.2
IS 2146	3.3	32.2	8.6
IS 4664	6.4	42.6	18.3
IS 2312	3.7	34.5	8.4
IS 2205	4.1	32.1	8.4
SFCR 125	4.9	42.0	14.7
SFCR 151	4.3	35.3	11.3
ICSV 700	5.8	42.2	12.9
CK 60B	13.9	80.3	35.6
ICSV 745	15.4	82.0	45.3
296B	12.6	72.2	32.6
ICSV 112	16.8	87.3	44.4
IS 18551 (R)	3.2	29.1	6.8
Swarna (S)	15.3	80.4	48.6
Fp	<0.001	<0.001	<0.001
Least significant difference ($P = 0.05$)	4.1	13.9	17.0

[a] Means of four seasons. R, resistant check; S, susceptible check.

cance 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 at $P \leq 0.05$. The HPLC fingerprints of phenolic compounds were recorded and processed by the Millennium[32] software version 4.0. Simple correlation analyses were performed using Pearson's correlations to understand the association between phenolic profiles and various parameters used to measure genotypic resistance (oviposition and deadhearts) to *A. soccata.* Diversity among the sorghum genotypes was assessed using Principal Component Analysis (PCA) based on the HPLC fingerprints of the undamaged seedlings.

Results

Relative Susceptibility of Sorghum Genotypes to *A. soccata.* There were significant differences in numbers of eggs per 10 seedlings ($F = 12.89$; df = 14, 28; $P = 0.001$ at 14 DAE); seedlings with eggs ($F = 19.39$; df = 14, 28; $P = 0.001$ at 14 DAE); and deadheart formation ($F = 6.69$; df = 14, 28; $P = 0.001$ at 14 DAE) among the genotypes tested (Table 1). The 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 number of eggs and percentage plants with deadhearts as compared with the susceptible check, Swarna.

HPLC Fingerprints of Phenolic Compounds From Damaged and Undamaged Seedlings of Sorghum in Relation to Expression of Resistance to *A. soccata.* There were considerable differences in the HPLC profiles of phenolic compounds in the seedlings of different sorghum genotypes, and between the damaged and undamaged sorghum seedlings (Table 2). The compound *p*-hydroxy benzoic acid (RT 18.63) was present in both damaged and undamaged seedlings of all the genotypes, except in SFCR 151, and its amounts were greater in the undamaged seedlings of *A. soccata*-resistant genotypes than in the susceptible ones. However, amounts of this compound were lower in damaged seedlings of *A. soccata*-resistant genotypes (IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 151, and ICSV 700). Amounts of *p*-hydroxy benzoic acid were greater in damaged seedlings of the *A. soccata*-susceptible than in the resistant genotypes. Its amounts were greater (0.35–0.80 mg/g) in the *A. soccata*-susceptible genotypes (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) than in the resistant check, IS 18551 (0.06 mg/g). Amounts of *p*-hydroxy benzoic acid in IS 4664 and ICSV 700 (that were moderately resistant to *A. soccata* damage) were on par with those of the resistant check, IS 18551.

p-hydroxy benzaldehyde (RT 23.41) was present in undamaged seedlings of most of the sorghum genotypes, except in IS 2312, SFCR 125, SFCR 151, and 296B. However, in the *A. soccata*-damaged seedlings, it was present only in the *A. soccata* susceptible genotypes. In the undamaged seedlings, *p*-hydroxy benzaldehyde amounts were greater (0.12–0.25 mg/g) in the *A. soccata*-susceptible genotypes (Swarna, CK 60B, ICSV 745, and ICSV 112) as compared with the resistant check, IS 18551 (0.08 mg/g). The results suggested that the amounts of *p*-hydroxy benzoic acid and *p*-hydroxy benzaldehyde were greater in the *A. soccata* susceptible genotypes, and their concentrations declined in the *A. soccata*-damaged seedlings, which are not preferred by the *A. soccata* females for egg laying under field conditions.

Low amounts of cinnamic acid (RT 43.24 min) were detected in damaged seedlings of IS 2146, IS 4664, and IS 2205. Low amounts of luteolin (RT 41.93 min) and apigenin (RT 43.58 min) were present in damaged and undamaged seedlings of most of the test genotypes. However, apigenin was present in IS 18551 (resistant), but absent in undamaged seedlings of Swarna (susceptible), and SFCR 125. Apigenin was not detected in damaged seedlings of IS 18551, SFCR 151, and 296B, but it was present in Swarna (Table 2).

The unidentified compounds with peaks at RTs 21.44 and 40.66 min were present in the undamaged seedlings of *A. soccata* resistant genotypes (except the unidentified compound at RT 21.44 min in IS 1054, and the unidentified compound at RT 40.66 min in SFCR 125), but absent in the susceptible genotypes (except in ICSV 745). The unidentified compound at peak RT 24.38 min was present only in undamaged seedlings of the susceptible genotypes, but absent in the resistant genotypes (except in IS 1057 and IS 4664). However, this unidentified compound was absent in damaged seedlings of all the genotypes.

The unidentified compound at RT 4.15 min was absent in undamaged seedlings of all the genotypes, but present in the damaged seedlings (except in IS 18551, IS 2312, and SFCR 151), suggesting that this unidentified compound was produced as a result of *A. soccata* damage to the sorghum seedlings. Unidentified compounds with peaks at RTs 2.13, 36.51, and 38.88

Table 2. Qualitative and quantitative estimation of phenolics by HPLC in the damaged and undamaged seedlings of 15 sorghum genotypes for resistance to *A. soccata* (ICRISAT, Patancheru, India)

	Peak area (%)														
	UK	UK	UK	UK	p-hbac[a]	UK	p-hbal[a]	UK	UK	UK	UK	UK	Lu[a]	Ca[a]	Ap[a]
Genotype/RT	2.13 min	2.76 min	3.70 min	4.15 min	18.63 min	21.44 min	23.41 min	24.38 min	36.51 min	37.08 min	38.88 min	40.66 min	41.93 min	43.24 min	43.58 min
IS 1054 D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IS 1054 UD	0.50	3.16	0.69	*	0.04	*	0.08	*	*	69.28	3.16	0.26	0.02	*	0.03
IS 1057 D	*	12.15	4.77	0.76	0.19	*	*	*	*	18.62	1.97	*	0.02	*	0.04
IS 1057 UD	0.30	2.55	0.81	*	0.30	0.33	0.08	0.46	*	64.90	2.37	0.10	0.02	*	0.02
IS 2146 D	*	14.40	5.33	0.48	0.11	*	*	*	*	34.64	2.80	*	0.01	0.01	0.02
IS 2146 UD	0.31	3.09	1.11	*	0.31	2.56	0.08	*	*	61.14	2.79	0.21	0.01	*	0.02
IS 18551 D (R)	*	11.18	4.66	*	0.06	*	*	*	*	38.60	3.28	*	0.01	*	*
IS 18551 UD(R)	0.13	2.18	0.81	*	0.56	3.03	0.08	*	0.33	60.31	2.98	0.15	0.02	*	0.03
IS 4664 D	*	11.90	4.49	0.39	0.37	*	*	*	*	25.61	1.49	*	0.02	0.01	0.03
IS 4664 UD	0.18	2.02	0.79	*	0.32	2.25	0.18	0.61	0.66	65.50	2.98	0.10	0.02	*	0.03
IS 2312 D	*	9.48	4.09	*	0.11	*	*	*	*	42.28	5.96	*	0.02	*	0.02
IS 2312 UD	0.21	2.53	0.99	*	0.43	2.62	*	*	0.39	61.67	2.45	0.18	0.01	*	0.03
IS 2205 D	*	*	5.33	0.80	0.23	*	*	*	*	22.17	*	*	0.03	0.01	0.03
IS 2205 UD	0.35	2.38	1.01	*	0.23	1.87	0.06	*	0.53	66.74	2.68	0.15	0.02	*	0.02
SFCR 125 D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SFCR 125 UD	*	2.93	1.42	*	0.33	3.60	*	*	0.38	50.44	2.92	*	0.01	*	*
SFCR 151 D	*	11.61	5.08	*	*	*	*	*	*	37.06	*	*	0.01	*	*
SFCR 151 UD	0.28	2.97	1.18	*	0.39	4.17	0.25	*	*	56.93	3.96	0.23	0.01	*	0.02
ICSV 700 D	*	11.44	4.72	0.83	0.68	*	*	*	*	21.07	*	*	0.03	*	0.03
ICSV 700 UD	0.08	2.59	1.11	*	0.35	2.66	0.12	0	0	65.88	1.67	0.12	0.02	*	0.02
Swarna D (S)	*	14.93	5.95	0.75	0.36	*	0.17	*	*	15.64	*	*	0.01	*	0.02
Swarna UD S)	0.14	3.44	1.56	*	0.34	*	0.16	1.95	0.46	52.27	0.89	*	0.01	*	*
CK 60B D	*	12.91	6.33	0.54	0.35	*	0.10	*	*	22.23	0.36	*	0.02	*	0.01
CK 60B UD	0.18	3.85	1.44	*	0.33	*	0.18	3.77	0.66	58.47	3.52	*	0.02	*	0.01
ICSV 745 D	*	11.22	5.54	1.28	0.80	*	0.14	*	*	3.87	0.42	*	0.01	*	0.01
ICSV 745 UD	0.05	3.57	1.61	*	0.42	*	0.12	166	0.78	47.41	1.80	0.16	0.02	*	0.03
296B D	*	*	5.08	1.49	0.60	*	0.11	*	*	19.05	0.77	*	0.03	*	*
296B UD	0	3.74	1.73	*	0.22	*	*	2.11	0.97	61.64	1.59	*	0.02	*	0.02
ICSV 112 D	*	15.67	6.21	1.56	0.37	*	0.06	*	*	16.84	1.05	*	0.02	*	0.02
ICSV 112 UD	0	2.29	1.05	*	0.74	*	0.25	1.54	0.97	58.29	1.22	*	0.02	*	0.02

UK, unknown; *p*-hbac, *p*-hydroxy benzoic acid; *p*-hbal, *p*-hydroxy benzaldehyde; Lu, Luteolin; Ca, Cinnamic acid; Ap, Apigenin.

[a] Concentrations of identified compounds calculated by comparing the mean peak area of samples with peak area of standards at known concentrations. RT, retention times; D, damaged seedlings; UD, undamaged seedlings; —, not studied; *, absence of compound.

Table 3. Association of phenolic compounds with expression of resistance to *A. soccata* (ICRISAT, Patancheru, India)

RT (min)	Phenolic compound	Deadhearts (%)	Seedlings with eggs (%)	Eggs seedlings per 10 seedlings
2.13	Unknown	−0.32	−0.30	−0.33
2.76	Unknown	0.39	0.39	0.38
3.70	Unknown	0.50[a]	0.50[a]	0.52[a]
4.15	Unknown	0.37	0.38	0.39
18.63	*p*-hydroxybenzoic acid	0.32	0.33	0.33
21.44	Unknown	−0.42	−0.47	−0.45
23.41	*p*-hydroxybenzaldehyde	0.52[a]	0.49[a]	0.51[a]
24.38	Unknown	0.49[a]	0.53[a]	0.52[a]
37.08	Unknown	−0.11	−0.09	−0.09
36.51	Unknown	0.36	0.36	0.38
38.88	Unknown	−0.30	−0.28	−0.30
40.66	Unknown	−0.29	−0.31	−0.31
41.93	Luteolin	−0.05	0.04	0.04
43.24	Cinnamic acid	−0.22	−0.27	−0.24
43.58	Apigenin	−0.23	−0.19	−0.20

[a] Correlation coefficients significant at $P = 0.05$. RT, retention time.

min were present in undamaged seedlings of all the genotypes, but absent in the damaged seedlings, although, there were a few exceptions, indicating that these were either intermediate metabolites of the phenylpropanoid biosynthetic pathway or degraded products of the compounds in the undamaged seedlings.

Unidentified compounds with peaks at RTs 2.76 and 3.70 min were present in greater concentrations in damaged seedlings than in the undamaged seedlings, and there were significant differences in the amounts of these unidentified compounds between *A. soccata*-resistant and susceptible genotypes. The unidentified compound at RT 2.76 min was absent in damaged seedlings of IS 2205 and 296B. Greater amounts of the unidentified compound at RT 37.08 min were recorded in undamaged seedlings as compared with damaged seedlings of different genotypes, and its amounts were greater in the *A. soccata*-resistant genotypes than in the susceptible check, Swarna (Table 2).

Association of Phenolic Compounds With Expression of Resistance to *A. soccata*. *p*-hydroxy benzaldehyde and the unidentified compounds at RTs 24.38 and 3.70 min were significantly and positively associated with percentage deadhearts, seedlings with eggs, and eggs per 10 seedlings at 14 DAE (Table 3). There were no significant correlations between *A. soccata* damage and other compounds. The results suggested that *p*-hydroxybenzaldehyde, and the unidentified compounds at RTs 24.38 and 3.70 min were associated with susceptibility to *A. soccata.*

Diversity Among Sorghum Genotypes Based on HPLC Fingerprints. Principal component analysis placed the sorghum genotypes in six groups. *A. soccata*-resistant genotypes were placed in four groups (group A: IS 2205, IS 4664, SFCR 151—lines with moderate levels of resistance to both *A. soccata* and stem borer; group B: IS 2146, IS 2312, and IS 18551—lines with high levels of resistance to *A. soccata;* group C: ICSV 700 and SFCR 125—improved *A. soccata* resistant genotypes; and group D: IS 1054 and IS 1057—lines with adaptation to postrainy season). The *A. soccata* susceptible lines were placed in two groups (group E: 296B, ICSV 112, and CK 60B; and group F: ICSV 745 and Swarna—improved high yielding varieties) (Fig. 1).

Discussion

Oviposition nonpreference (antixenosis), antibiosis, and recovery are the major components of resistance to *A. soccata* (Doggett et al. 1970, Raina et al. 1981, Sharma and Nwanze 1997, Dhillon et al. 2005, Sivakumar et al. 2008). Besides morphological factors, biochemical factors also play a significant role in resistance to *A. soccata* (Ogwaro 1978, Delobel 1982, Raina 1982). Mixtures of hydroxyl benzoic and cinnamic acids have earlier been reported to be present in sorghum seedlings (Woodhead and Bernays 1978). *p*-hydroxy benzoic acid occurred in highest concentration, followed by caffeic, ferulic, *p*-coumaric, and *o*-coumaric acids. Gentisic, vanillic, protocatechuic, and γ-resorcylic acids were present in low concentrations. This suggests that *p*-hydroxy benzaldehyde was probably produced as a result of hydrolysis of *dhurrin.* Phenolic acids (gallic, protoctechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids) have earlier been reported to be associated with resistance to fungal diseases in sorghum (Hahn et al. 1983). Six phenolic acids (protoctechuic, *p*-hydroxy benzoic, vanillic, syringic, *p*-coumaric, and ferulic acids) have been reported to be associated with *A. soccata* damage (Panday et al. 2005). In the present studies, we observed that *p*-hydroxy benzoic acid, *p*-hydroxy benzaldehyde, cinnamic acid, luteolin, apigenin, and some unidentified compounds from damaged and undamaged seedlings of sorghum were associated with expression of resistance or susceptibility to *A. soccata.*

The phenolic compounds are present in the undamaged plant tissues largely in the form of esters, and when the plant cells are ruptured, esterases release the free phenolic acids (Woodhead and Cooper-Driver 1979). Mixtures of phenolic acids and their esters reduce feeding on artificial media when presented to insects at concentrations similar to those occurring in

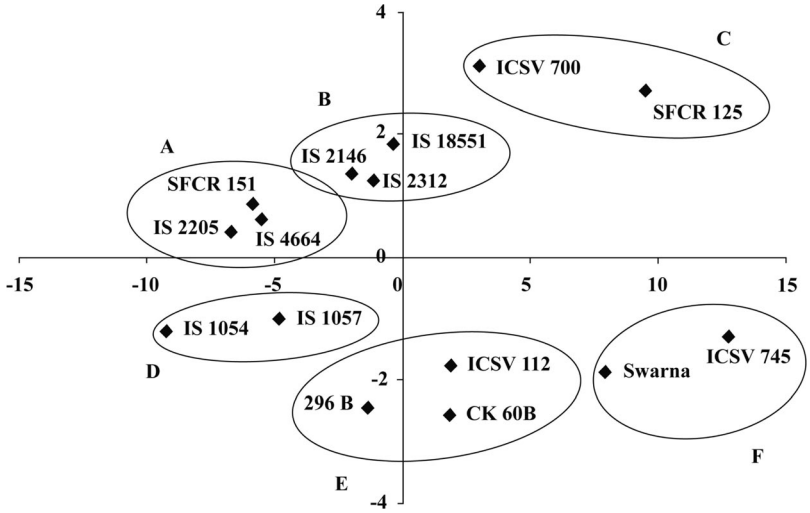

Fig. 1. Principal component analysis based on HPLC fingerprints of methanol extracts of the seedlings of 15 sorghum genotypes.

young plants (Fisk 1980). Occurrence of *p*-hydroxy benzaldehyde was suspected to act as an oviposition stimulant for adults and/or feeding activator for the maggots of *A. soccata* (Alborn et al. 1992). *p*-hydroxy benzaldehyde has been found to be a species-specific attractant to the females of *A. soccata* (H.C.S., unpublished data). Nicholson et al. (1987) demonstrated that sorghum mesocotyl accumulates a mixture of phenols in response to fungal infection, the major components were 3-deoxyanthocyanidins, apigeninidin, and luteolinidin. In the present studies, small quantities of luteolin and apigenin were detected in almost all the sorghum genotypes. Apigenin was present in the resistant check, IS 18551, but absent in the susceptible check, Swarna in the undamaged seedlings, while the reverse was true in case of *A. soccata*-damaged seedlings.

In general, the main feeding deterrent factors are only produced at the time of feeding. This is true of HCN, which is stored as glycoside-dhurrin, while the phenolic acids are stored as esters. As a result of damage to the plant tissue, these substrates in contact with enzymes produce the active compounds. The substrates themselves are not deterrents, but contain phenolic esters and glycosides that may have adverse effects on insect feeding and development (Woodhead and Bernays 1978). The unidentified compounds at RTs 21.44 and 40.66 min were present in the undamaged seedlings of the *A. soccata*-resistant genotypes, while the unidentified compound with a peak at RT 24.38 min was present in the undamaged seedlings of susceptible genotypes. However, these peaks were absent in damaged seedlings of all the test genotypes. The unidentified compound at RT 4.15 min was absent in undamaged seedlings, but present in damaged seedlings, while the unidentified compounds with peaks at RTs 2.13, 36.51, and 38.88 min were present in undamaged seedlings, but absent in damaged seedlings, suggesting that these compounds accumulate or degrade in response to *A. soccata* damage. The amounts of unidentified compounds at RTs 2.76 and 3.70 min were greater in the damaged than in the undamaged seedlings, and there were significant differences between the resistant and susceptible genotypes. The amounts of the unidentified compound at RT 37.08 min were greater in the resistant genotypes than in the susceptible check, Swarna. The *A. soccata* damaged seedlings had greater amounts of this compound than the undamaged ones.

Pandey et al. (2005) observed that amounts of protocatechuic, syringic, and *p*-coumaric acids were correlated negatively, whereas *p*-hydroxy benzoic, vanillic, and ferulic acids were correlated positively with *A. soccata* damage. The amounts of *p*-hydroxy benzoic acid and *p*-hydroxy benzaldehyde were greater in the *A. soccata*-susceptible genotypes, but their amounts decreased in the shoot-fly-damaged seedlings. This may be one of the reasons for nonpreference of shoot-fly-damaged seedlings for oviposition by the females of *A. soccata.* Based on diversity analysis, sorghum genotypes having different combination of biochemical characteristics, both in terms of constitutive and inducible resistance, can be used in sorghum improvement to increase the levels and diversify the basis of resistance to *A. soccata.* There is a need to undertake further studies to establish a cause and effect relationship for most of the compounds that showed a strong relationship with expression of resistance to *A. soccata*, and identify the genes or compounds, that are up or down regulated as a result of *A. soccata* damage to sorghum seedlings. The compounds showing a relationship with expression of resistance to *A. soccata* potentially could be used as marker traits to screen and breed for resistance to *A. soccata.* Genotypes exhibiting resistance to *A. soccata* were placed in four groups, and the lines showing constitutive and/or induced resistance to *A. soccata* with different combinations of

biochemical factors potentially could be used for increasing the levels resistance to *A. soccata* in sorghum.

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