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Leaf surface chemicals of sorghum seedlings influence the genotypic resistance to shoot fly, *Atherigona soccata*

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Abstract Sorghum shoot fly, *Atherigona soccata* is one of the serious constraints to sorghum production, and host plant resistance is an important component for controlling this pest. We studied the expression of resistance to *A. soccata* in a diverse array of sorghum genotypes in relation to composition of leaf surface chemicals during the seedling stage. The sorghum genotypes IS 1054, IS 1057, IS 2146, IS4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, and IS 18551 exhibited antixenosis, and less deadhearts of resistance to sorghum shoot fly, *A. soccata*. Compounds undecane 5- methyl, decane 4- methyl, hexane 2, 4- methyl, pentadecane 8- hexyl, and dodecane 2, 6, 11- trimethyl, present on the leaf surface of sorghum seedlings, were associated with susceptibility to shoot fly; while 4, 4- dimethyl cyclooctene was associated with resistance to shoot fly. The compounds associated with resistance/susceptibility to shoot fly, can be used as marker traits to select for resistance to this insect, and used as a basis for diversifying and increasing the levels of resistance to this pest. The role of biochemical compounds for developing sorghum varieties with resistance to shoot fly, *A. soccata* has been discussed.

Keywords Sorghum shoot fly . *Atherigona soccata* . Resistance mechanisms . Leaf surface chemistry . Biochemical components

Introduction

Sorghum is an important cereal crop in Asia, Africa, Australia, and the Americas. It is cultivated on approximately 44 million hectares world wide, and is the fifth major cereal 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 sorghum shoot fly, *Atherigona soccata* (Rondani) is one of the most important pests in Asia and Africa. The shoot fly females lay white, elongated, cigar- shaped eggs singly on the abaxial leaf surface of sorghum seedlings. On emergence, 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 'deadheart'.

Host plant resistance to insects is often mediated by chemicals produced by the host plant that acts as attractants, repellents, oviposition and feeding stimulants, feeding deterrents, and/or affect the development and survival of insects. Phenolic compounds such as, 3-deoxyanthocyanidins or allelochemicals (*p*-hydroxybenzoates, *p*-coumarates, and flavonols) are involved in sorghum plant resistance to various biotic stresses (Lo et al. 1999; Weston et al. 1999; Weir et al. 2004). The leaf surface constitutes an interface between the shoot fly and the host plant (Ogwaro 1978), and physiological and biological changes in terms of secondary metabolites during the seedling stage have a profound effect on sorghum plant interactions with shoot fly (Singh et al. 2004).

It has been observed that females of *A. soccata* are attracted to volatiles emitted by the seedlings of susceptible sorghum genotypes, and to phototactic (optical) stimuli that may facilitate orientation to its host for oviposition (Nwanze et al. 1998a). A smooth amorphous wax layer and sparse wax crystals characterize shoot fly resistant and moderately resistant genotypes, while susceptible genotypes possess a dense mesh of crystalline epicuticular wax (Nwanze et al. 1992). Highly waxy leaves also retain more water as droplets than a non-waxy leaves and vice-versa (Nwanze et al. 1990b; Sree et al. 1994). Chemicals present on the surface of sorghum seedlings play an important role in host plant resistance/susceptibility to insects (Sharma and Nwanze 1997). Therefore, the present studies were come out to understand the role of chemicals on the leaves of sorghum seedlings in host plant resistance/susceptibility to *A. soccata*.

Material 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 shoot fly, and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) susceptible to shoot fly (Sharma et al. 1992; 2005). Of these, IS 18551 and Swarna served as

resistant and susceptible checks, respectively. The Origin and pedigrees of the test genotypes are given in Table 1. The experiments were conducted at the research farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, under field and laboratory conditions during the 2004 - 2006 rainy (July-November) and post-rainy (October-March) seasons.

Evaluation of sorghum genotypes for different components of resistance to shoot fly, *Atherigona soccata*

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, and seedlings with deadhearts at 21 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.

GC-MS analysis of the compounds on leaf surface of sorghum seedlings

To collect samples for GC-MS analysis of the compounds on leaf surface of sorghum seedlings, the sorghum seeds were sown in the greenhouse under no-choice conditions (Dhillon et al. 2005b). Ten days after seedling emergence, 3rd leaf of the sorghum seedlings (which is the preferred site for oviposition) was collected in a 25 ml centrifuge tube containing 10 ml HPLC grade hexane. After 1 min, the leaves were removed from the centrifuge tubes, and the leaf extract thus obtained was used for GC-MS analysis.

Compounds extracted in hexane from the leaf surface of different sorghum genotypes were concentrated to 0.5 ml under a stream of nitrogen, and analyzed by GC-MS (Agilent

Technologies 6890 NGC) with 5973 inert mass selective detector. One µl of the sample was injected through the autosampler to the HP-5MS capillary column (30 m length × 0.25 mm i.d × 0.25 µm film thickness). The oven program was: 50^0C (2 min) – 10^0C /min 280^0 C (5 min) - (total run time 30 min). Injection temperature was 250 ^{0}C, and GC-MS interface temperature was 280 ^{0}C. Solvent delayed for 3 minutes. MS scan range was 30-600 Da. Compounds were identified by comparing their spectral data with those of the library of the mass spectrometer.

Statistical analysis

Data were subjected to analysis of variance (ANOVA, GenStat version 10th), and significance of differences between the genotypes was tested by F-test, while the significance of differences between the genotypic means was judged by least significant differences (LSD) at $P \leq 0.05$. GC-MS data was recorded and processed by Chem Station Software. The relationship of compounds present on the leaf surface and expression of resistance to shoot fly was assessed through Pearson's correlations.

Results

Expressions of resistance to sorghum shoot fly, *Atherigona soccata*

There were significant differences in oviposition and deadheart formation among the genotypes tested (Table 2). The genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, and IS 18551 showed antixenosis for oviposition and had less deadhearts at 21 days after seedling emergence (DAE) under multi-choice field conditions.

GC-MS profiles of compounds on the leaf surface of sorghum seedlings in relation to expression of resistance to shoot fly, *Atherigona soccata*

There were significant differences in GC-MS profiles of the leaf surface chemicals among the genotypes tested (Table 3). Of the 150 compounds detected, 10 compounds showed significant association with expression of resistance to *A. soccata*. Of major compounds detected, hexanal at (RT 4.15 min) was present both in IS 18551 and Swarna, but the peak area was greater in the resistant check, IS 18551. Pentadecane, 8 - hexyl (RT 15.34 min), and lonol 2 (RT 15.8 min) were present only in the susceptible genotypes, Swarna and CK 60B, but absent in rest of the genotypes. Dodecane, 2, 6, 11- trimethyl (RT 13.37 min) was present only in the shoot fly susceptible genotypes CK 60B, ICSV 745, 296B, ICSV 112, and Swarna, but absent in resistant genotypes (except in genotype IS 1057). Compound 4, 4- dimethyl cyclo octene (RT 7.31 min) was present in the resistant genotypes IS 2146, IS 2312, and IS 18551, but absent in all other genotypes; while hexane 2, 4-dimethyl (RT 7.31 min) was absent in IS 2146, IS 2312, IS 18551 and IS 4664, but present in rest of the genotypes. Compound undecane 5-methyl (RT 8.83 min) was present in all the genotypes, except in IS 4664, IS 2205 and Swarna. Its amounts were greater in SFCR 125, ICSV 745, 296B, and ICSV 112. Compound eicosane (RT 14.91 min) was present in all genotypes, except in the susceptible check, Swarna. More amounts of eicosane were detected in IS 4664. Decane, 4-methyl (RT 8.08 min) was present in all genotypes, but had more peak area in SFCR 125, ICSV 700, CK 60B, ICSV 745, 296B, ICSV 112, and Swarna as compared to that on the resistant check, IS 18551 (Table 3).

Undecane 5- methyl; decane 4- methyl; hexane 2, 4- methyl; pentadecane 8- hexyl; and dodecane 2, 6, 11- trimethyl were significantly and positively associated with deadhearts and eggs per 10 seedlings, but the correlation coefficients for undecane 5-methyl with oviposition were non-significant (Table 4). These compounds possibly acted as attractants/oviposition stimulants for the sorghum shoot fly, *A. soccata*. The compound 4, 4-dimethyl cyclooctene was significantly and negatively associated with deadhearts and eggs per 10 seedlings, and imparted resistance to shoot fly. The compounds eicosane, tridecane, and hexanal showed a positive correlation, while lonol 2 showed a negative correlation with shoot fly damage, but the correlation coefficients were non-significant.

Discussion

Oviposition non-preference (antixenosis), antibiosis, and recovery are the major components of resistance in sorghum to shoot fly, *A. soccata* (Doggett et al. 1970; Raina et al. 1981; Sharma and Nwanze 1997; Dhillon et al 2005b). However, plants produce several chemical compounds in different quantities and proportions, which affect the host selection behavior of phytophagous insects (Painter 1958; Beck 1965; Schoonhoven 1968). These compounds act as attractants (oviposition and feeding stimulants), repellents (oviposition and feeding deterrents), or result in antibiosis (reduced survival and poor growth). The females of *A. soccata* are attracted to volatiles emitted by the seedlings of susceptible sorghum genotypes which may influence the orientation of shoot fly females to its host for oviposition (Nwanze et al. 1998a). Undecane 5-methyl; decane 4-methyl; hexane 2, 4-methyl; pentadecane 8-hexyl; and dodecane 2, 6, 11- trimethyl were associated with susceptibility to shoot fly. These compounds possibly acted as attractants and/ or oviposition stimulants for the sorghum shoot fly, *A. soccata*. The compound 4, 4- dimethyl cyclooctene was negatively associated oviposition and deadheart incidence, and this might act as repellent and/or oviposition suppressant. The compounds eicosane, tridecane, and hexanal showed a positive, while lonol 2 showed a negative association with shoot fly damage, but the correlation coefficients were non-significant. Their role in host plant selection, and expression of resistance to shoot fly needs to be studied further.

Volatiles in the leaf are produced from linolenic and linoleic acids through the lipoxygenase pathway (Pare and Tumlinson 1996). They are liberated from the cell membrane as a result of insect damage and, by the action of a lipoxygenase enzyme that produces hydroperoxides initially. A hydroperoxide lyase enzyme then converts the hydroperoxides to hexanal (from linoleic acid) and (E)-2-hexenal (from linolenic acid), which undergo further reactions to give other C6 aldehydes, alcohols, and esters (Hatanaka 1993; Bate and Rothstein 1998). The present study showed that hexane extracts of 3rd leaf of sorghum seedlings had aldehydes, alcohols, and esters. Thirty six volatile compounds were identified from plum by continuous vacuum steam distillation/ hexane extraction, of which the major compounds were hexenal, butyl acetate, (E)-2-hexenal, butyl butyrate, hexyl acetate, linalool, γ-decalactone, and γ-dodecalactone (Robert et al. 1992). In the present study 10 major compounds were detected in hexane extract of leaf surface of sorghum seedlings that

were significantly associated with expression of resistance/susceptibility to *A. soccata.* Further studies are needed to establish the dosage to damage response curves for these compounds to confirm their role in host plant resistance to *A. soccata*.

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Table 1 Origin and pedigrees of 15 sorghum genotypes (ICRISAT, Patancheru, India)

Genotypes	Pedigree
Shoot fly- resistant lines	
IS 1054	Maldandi 35-1, PI 248264 (Landrace, India)
IS 1057	Bird resistant, PI 248267 (Landrace, India)
IS 2146	Kaura, PI 221569 (Landrace, Nigeria)
IS 18551	Jijwejere 935 (Landrace, Ethiopia)
IS 4664	Dagri dahere (Landrace, India)
IS 2312	Safra shahadasal Q2-2-88 (Landrace, Sudan)
IS 2205	Jaglur (Landrace, India)
SFCR 125	(ICSV 705 × YT-3-47)-7-1-1-2
SFCR 151	(1011 E No 23-2 (PM 12645 × IS 2205))-5-1-2-2
ICSV 700	(IS 1082 × SC 108-3)-1-1-1-1-1
Shoot fly- susceptible lines	
Swarna	Selection from IS 3924
CK 60 B	Day milo × Black hull kafir
ICSV 745	((IS 3443 × DJ 6514)-1-1-1-1-1) × (E35-1× US/B 487)-2-1-4-1-1-3)-4-1-1-1
296 B	IS 3922 × Karad local
ICSV 112	[(IS 12622C × 555) × ((IS 3612C × 2219 B)-5-1 × E 35-1)]-5-2

Table 2 Shoot fly, *Atherigona soccata* oviposition and deadheart incidence in 15 sorghum genotypes at 21 days after seedling emergence under field conditions (ICRISAT, Patancheru, 2004-05)

Genotypes	Shoot fly damage parameters at 21 DAE		
	Eggs per 10 seedlings	Seedlings with eggs (%)	Deadhearts (%)
IS 1054	6.5	63.1	39.3
IS 1057	8.7	68.3	44.0
IS 2146	5.6	55.5	32.5
IS 4664	9.9	77.4	51.2
IS 2312	5.7	53.9	29.6
IS 2205	6.0	54.2	31.1
SFCR 125	9.0	67.3	47.9
SFCR 151	8.5	66.8	42.8
ICSV 700	8.3	68.5	45.1
CK 60B	13.9	92.8	76.6
ICSV 745	13.4	94.3	84.1
296B	13.2	92.9	73.5
ICSV 112	14.0	93.8	78.9
IS 18551 (R)	5.4	57.6	33.1
Swarna (S)	15.1	96.8	80.1
F. probability	< 0.001	< 0.001	< 0.001
LSD ($P < 0.05$)	2.9	17.7	14.2

DAE = Days after seedling emergence. R = Resistant check. S = Susceptible check. LSD = Least significant difference. Data are means of four seasons.

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Table 3 Biochemical constituents detected in hexane extracts from the leaf surface of different sorghum genotypes (GC-MS profiles) (ICRISAT, Patancheru, India)

Genotype	Peak area (%)									
	Hexanal	Hexane 2, 4-dimethyl	4, 4- dimethyl cyclooctene	Eicosane	Decane 4-rnethyl	Undecane 5- rnethyl	Tridecane	Dodecane 2, 6, 11-trimethyl	Pentadecane 8- hexyl	Lonol 2
IS 1054	*	0.56	*	3.04	1.12	0.76	1.65	*	*	*
IS 1057	*	0.54	*	2.74	1.21	0.73	1.29	0.67	*	*
IS 2146	*	*	0.45	3.47	0.94	0.69	1.88	*	*	*
IS 4664	*	*	*	4.88	0.78	*	0.77	*	*	*
IS 2312	*	*	0.49	2.78	1.01	0.66	*	*	*	*
IS 2205	*	0.49	*	2.76	1.1	*	*	*	*	*
SFCR 125	*	0.57	*	3.01	1.33	3.36	1.42	*	*	*
SFCR 151	*	0.49	*	2.9	1.14	0.67	1.38	*	*	*
ICSV 700	*	0.59	*	3.08	1.35	0.93	1.44	*	*	*
CK 60B	*	0.55	*	1.45	1.28	0.89	0.61	0.88	0.67	*
ICSV 745	*	0.66	*	3.18	1.51	3.82	*	0.9	*	*
296B	*	0.56	*	3.32	1.31	3.39	1.54	0.9	*	*
ICSV 112	*	0.54	*	3.21	1.26	3.24	1.48	0.9	*	*
IS 18551 (R)	0.75	*	0.58	3.18	1.15	0.81	1.75	*	*	*
Swarna (S)	0.47	0.56	*	*	1.24	*	*	0.77	0.61	0.39

* = Absence of compound. R = Resistant check. S = Susceptible check.

Table 4 Association of biochemical constituents on the leaf surface of sorghum seedlings with expression of resistance to sorghum shoot fly, *Atherigona soccata* (ICRISAT, Patancheru, India)

Compound Name	Deadhearts (%)	Eggs per 10 seedlings
Eicosane	-0.36	-0.40
Tridecane	-0.26	-0.28
Hexanal	-0.03	-0.04
Lonol 2	0.38	0.44
Undecane 5- methyl	0.54*	0.45
4, 4- dimethylcyclooctene	-0.53*	-0.59*
Decane 4- methyl	0.59*	0.52*
Hexane 2, 4- dimethyl	0.56*	0.56*
Pentadecane 8- hexyl	0.52*	0.57*
Dodecane 2, 6, 11- trimethyl	0.89**	0.88**

*, ** Correlation coefficients significant at $P < 0.05$ and 0.01, respectively.

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