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ORIGINAL ARTICLE

Leaf surface chemistry of sorghum seedlings influencing expression of resistance to sorghum 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, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, and IS 18551 exhibited antixenosis for oviposition, and suffered less deadhearts due 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 as well as 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.

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Abbreviations

GC-MS	Gas chromatography-mass spectrometry
CRD	Completely randomized design
DAE	Days after seedling emergence
ICSV	ICRISAT sorghum variety
LSD	Least significant difference
RCBD	Randomized complete block design
ANOVA	Analysis of variance
SAT	Semi arid tropics

Sorghum (*Sorghum bicolor* (L.) Moench) 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.

Host plant resistance to insects is often mediated by chemicals produced by the host plant that act 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;

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Weir et al. 2004). The leaf surface constituents are an interface between the shoot fly and the host plant (Ogwaro 1978, Chamarthi et al. 2010), 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; Chamarthi et al. 2010).

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. 1998). 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 non-waxy leaves and vice-versa (Nwanze et al. 1990; 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 undertaken to understand the role of biochemical components in host plant resistance/susceptibility to *A. soccata*.

Materials and methods

Plant material

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 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, each 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 (DAE) (before egg laying by the shoot fly). Data were recorded on number of eggs per seedling and seedlings with eggs, and seedlings with deadhearts at 21 DAE from all plants in the two row plots. Numbers of eggs were expressed as number of eggs per 10 seedlings; while 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 (Chamarthi et al. 2008, 2010). Each genotype had four rows, and there were 40 seedlings in each tray. For no-choice tests, only one genotype was planted in each tray. There were three replications for no-choice tests in a completely randomized design (CRD). 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). Oven temperature was 50°C for 2 min, then raised to 280°C at10°C/min, and held at this temperature for 5 min. The total run time was 30 min. Injection temperature was 250°C, and GC-MS interface temperature was 280°C. Solvent delayed for 3 min. 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 (Agilent Technologies). 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 1). 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 2). Of the 150 compounds detected, 10 compounds (Table 3) showed significant association with expression of resistance to *A. soccata*. Of major compounds detected, hexanal (RT 4.15 min) was present in both the resistant (IS 18551) and susceptible, (Swarna) checks, 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 cyclooctene (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 2).

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

Table 1 Shoot fly, *Atherigona soccata* oviposition and dead-heart 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

Table 2 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- dimethyI	4, 4- dimethyl cyclooctene	Eicosane	Decane 4- rnethyI	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

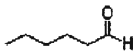
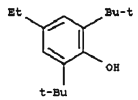
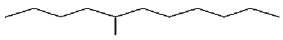
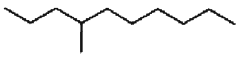
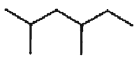
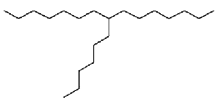
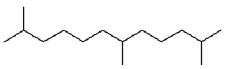
coefficients for undecane 5-methyl with oviposition were non-significant (Table 4). These compounds possibly acted as attractants/oviposition stimulants for the sorghum shoot fly, *A. soccata*. The compound 4, 4-dimethyl cyclooctene was significantly and negatively associated with deadhearts and eggs 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. 2005; Chamarthi et al. 2008, 2010). 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. 1998). 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 with 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

Table 3 Chemical formulas and structures of the identified compounds by GC-MS

Compound name	Formula	Structure
1. Eicosane	$C_{20}H_{42}$	
2. Tridecane	$C_{13}H_{28}$	
3. Hexanal	$C_6H_{12}O$	
4. Lonol 2	$C_{16}H_{22}O$	
5. Undecane 5- methyl	$C_{12}H_{26}$	
6. 4, 4- dimethylcyclooctene	$C_{10}H_{18}$	
7. Decane 4- methyl	$C_{11}H_{24}$	
8. Hexane 2, 4- dimethyl	C_8H_{18}	
9. Pentadecane 8- hexyl	$C_{21}H_{44}$	
10. Dodecane 2, 6, 11- trimethyl	$C_{15}H_{32}$	

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*.

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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