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Morphological and Biochemical resistance in sorghum genotypes against Sorghum shoot fly, *Atherigona soccata* (Rondani) (Muscidae: Diptera)

Manimegalai, S[1], P. Anandhi, [2*]P. R. Nithya[1], S. Elamathi[2]

[1]Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India, 641003
[2]Department of Tamil Nadu Rice Research Institute, Aduthurai Tamil Nadu Agricultural University, Coimbatore, India, 641003

ABSTRACT

Sorghum shoot fly, Atherigona soccata (Rondani) is an important pest of sorghum distributed in almost all sorghum growing areas of India, attacking the crop at the seedling stage wherein the absence of appropriate management measures may result in heavy yield loss and host plant resistance is a major component in managing this pest. Utilization of resistance as a control strategy has very much practical relevance and hence identification of resistant sources for shootfly through morphological and physicochemical characteristics was carried out in pre-release sorghum genotypes of Tamil Nadu Agricultural University. Leaf glossiness, trichome density, trichome length, trichome width, and seedling vigor were associated with resistance and had a major bearing on the expression of resistance to shoot fly. Among the genotypes tested, TNS 671 and TNS 665 showed better performance in terms of dead heart, percentage of plants with eggs, and recovery resistance compared to others which were then subjected to biochemical estimation and GCMS analysis. Leaf biochemical characters viz., total phenol, cellulose, total amino acids, silica, tannin, and lignin were found to be negatively correlated and total sugar positively correlated with resistance. GC-MSanalysis of the sorghum genotypes revealedthe presence of compounds, viz., carboxylic acids, heptadecene, and hentriacontane only in the resistant entry, IS18551. An interesting finding of this study was the presence of hentriacontanein resistant cultivar, IS18551 which is reported to have kairomonal activity. The characters associated with resistance or susceptibility can be used in further resistant breeding programmes.

Keywords: *Sorghum, Sorghum bicolour, shoot fly, Atherigona soccata, physical- chemical characters, GC – MS, Resistance.*

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench is cultivated in 86 countries covering an area of about 42.60million hectares with an annual production of 59.81million tonnes. In India, it is the thirdmost important cereal crop cultivated after rice and is currently grown in4.90mha with an annual production of 4.7 million tonnes [10]. Shoot fly, *Atherigona soccata* (Rondani) is a major constraint in sorghum production especially in Asia, Africa, and Mediterranean Europe. It causes an average loss of 50 percent in Tamil Nadu [2 and 1], but the infestations at times may be over by 90per cent [17], and hence the pest need to be necessarily managed by adopting different methods.

Different granular and sprayable insecticides have been found effective against sorghum shoot flies. However, insecticides are expensive, uneconomical, and beyond the reach of resource-poor farmers. Sorghum is normally cultivated in Tamil Nadu under rainfed conditions, especially by farmers of poor economic status. Increasing emphasis has been given during the last couple of decades to the host plant resistance approach.

Corresponding Author:* ***P. Anandhi*
Email Address: ***anandhi.aaidu@gmail.com***

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Crop resistance to sorghum shoot flies is an amalgamation of morphological, anatomical, and biochemical traits of plants. Keeping the above points in view, the present investigations were carried out to assess the morphological and biochemical characteristics of some pre-release cultures of TNAU sorghum genotypes for use in developing shootfly resistance for sustainable crop production.

MATERIALS AND METHODS

The reaction of sorghum genotypes against *A. soccata* under field condition

The experiments were conducted at Tamil Nadu Agricultural University (TNAU), Coimbatore, and Agricultural Research Station, Kovilpatti, Tamil Nadu, India. The experimental material consisted of eleven genotypes (TNS 667, TNS 665, TNS 671, TNS 648, TNS 669, TNS, 623, TNS 664, TNS 668, TNS 670, TNS 672, TNS 666) along with resistant (IS 18551) and susceptible (DJ 6514) checks and was screened under natural conditions for their reaction to *A. Soccata* in terms of number of eggs/ 5 plants, number of plants with eggs (%), dead hearts (%) at 21 days after emergence (DAE), recovery resistance at 28 DAE (percentage tillers with dead hearts, and total number of tillers, numbers of tillers having panicles with grains as percentage productive tillers at crop maturity time).

Each genotype was sown in four row plots of 2m row length and rows were 75 cm apart. Three replications were maintained for each genotype in a randomized block design (RBD). The seeds were sown manually at a depth of 5 cm below the soil surface.

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Irrigation was done after sowing. Thinning was done one week after seedling emergence and spacing of 10 cm was maintainedbetween the plants. Optimum infestation of shoot flies was ensured by the use of a fish meal trap. All the agronomic practices were carried out based on the recommendations of the Tamil Nadu Agricultural University Crop Production Guide, 2018.

Five plants were selected from each genotype for recording thedata on the number of eggs laid / 5 plants and plants with eggs and deadhearts (%) at 14and 21 days after emergence (DAE). Recovery resistance was assessed at 78 DAE in terms of the percentage of tillers with a deadheart. At crop maturity, data on the total number of tillers and the number of tillers having panicles were recorded. Recovery resistance was assessed on a scale of 1-9 based on productive tillers [7]

Evaluation of sorghum entries for morphological traits

The leaf glossiness was calculated at 10 DAE on the 1-5 scale [18& 19]. Trichome density was assessed by taking the central portion of the fifth leaf from three seedlings selected at random. The leaf pieces of $2cm^2$ were placed in an acid and alcohol solution (2:1) in a glassvial. The leaf pieces were kept in this solution for 24hand transferred into Lactic acid (90%).Leaf segments cleared of the chlorophyll content were observed for the trichome density. The leaf sections were mounted on a slide in a drop of lactic acid and observed under a microscope for the density of trichomes (number/mm^2), trichome length (µm), and trichome width (µm). The seedling vigor was also recorded on1-5 rating scale at 10 DAE.

Evaluation of sorghum entries for biochemical traits against shoot flies

Biochemical constituents of the above entries were estimated to know if there were any significant chemical compositions. Leaf samples for these studies were taken at the seedling stage (14 and 28 DAE) by randomly selecting five seedlings per net plot from each resistant and susceptible entry and washed under tap water after removing the root. The fresh plant samples were used for phenol, total soluble sugar and whereas the dried plant samples were used for the estimation of cellulose, total amino acid, silica, and tannin.Total phenol, total soluble sugar, tannin, and silica were determined by the standard methods described in [24].

GC- MS analysis of the compounds present in selected sorghum entries

For GC - MS analysis of the compounds on sorghum plants, the sorghum seeds were sown in the greenhouse under no-choice conditions [5 & 6]. Each genotype had four rows, and there were 40 seedlings in each tray. A sample of 0.5 g was taken from ten--day-old seedlings, immersed overnight in 10 ml of HPLC grade hexane in separate vials, and filtered through Whatman No.1 filter paper. 5 mg of anhydrous sodium sulphate was added to the filtrate and left for dehydration for 2 hours, again filtered, and then subjected to flash vacuum evaporation for complete removal of hexane. The leftover residue was collected by rinsing the container with 1 ml of HPLC-grade hexane. The sample was then filtered using a 2-micron nylon filterpaper and stored in separate vials in a deep freezer for GC-MS analysis [22]. Chromatographic separation was carried out using GCMS-QP plus equipped with a capillary column RXI-IMS (30 m x 0.25 mm, 0.25 mm 1D). Helium (99.99% pure) was used as a carrier gas with a flow rate of 0.98 ml/min. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10 to 200°C / min, then 5 to 280°C / min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. The injection was performed in splitless mode (10:1) and the volume used was 1µl. The mass spectra of compounds in samples were obtained by ionization voltage at 70 eV and the detector was operated in scan mode from 500 amu. The chemical constituents were identified by matching the mass spectra of reference compounds in the mass library of the National Institute of Standards and Technology (NIST) version 2.0. The relative amounts of individual components were expressed as percent peak areas relative to the total peak area.

Statistical analysis

Data were subjected to analysis of variance and the significance of differences between the genotypes was tested by F – tests, while the treatment means were compared by least significant difference (LSD) at P = 0.05.

RESULTS AND DISCUSSION

Response of different sorghum entries to shoot fly damageunder field condition

Under field conditions, the expression of shoot flydead hearts was significant (Table 1). The mean results from TNAU, Coimbatore, and ARS, Kovilpatti showed that the entries *viz*., TNS 665, TNS 667, and TNS 671 showed significantly lesser dead hearts at 14 DAE than the susceptible check, DJ 6514. Other entries *viz*., TNS 648, TNS 669, TNS 672, and TNS 666 showed resistant reactions against shoot fly. At 21 DAE, TNS 667, TNS 665, and TNS 671 were considered as resistant as they exhibited less than 20 percent dead heart compared to DJ 6514 (68.25% dead heart).

The genotypeswhich showed resistant reactions against shoot flies were again tested at TNAU, Coimbatore, and ARS, Kovilpatti. TNS 667 TNS 665 and TNS 671 showed resistant reactions when compared to the susceptible check, DJ 6514.

Ovipositional preference

The seedlings of sorghum genotypes TNS 668, TNS 664, TNS 670 and DJ 6514 were significantly more preferred for oviposition compared to resistant check, IS 18551 (9.09% plants with eggs and 2.75 eggs / 5 plants) on 21 DAE under field condition. IS 18551, TNS 665, TNS 671, and TNS 667 were significantly less preferred for egg laying (9.09 to 17.35% plants with eggs) at 21 DAE. TNS 669, TNS 648, TNS 672, and TNS 666 showed moderate levels of oviposition preference under differentconditions in the field (Table 2).

Recovery resistance

Genotypes TNS 671and TNS 665 showed better recovery resistance (recovery resistance score (of 4.0, 4.70), higher recovery resistance (48.33 %, 43.75 %), and were next to the resistant entry, IS 18551(3.85, 51.25 %) against 8.85 and 6.25 percent in susceptible entry, DJ 6514. All tested genotypes recorded higher resistance scores compared to susceptible entries (Table 3).

Morphological characteristics of sorghum genotypes associated with resistance /susceptibility to sorghum shoot fly, *A. soccata*

Significant variations in trichome density (4.00no./mm^2 to 6.08no./mm^2), trichome length (34.35 µm to 43.26 µm),and trichome width (9.23 to 10.67 µm) were observed between the

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sorghum genotypes in a 10X microscopic field (Table 4). The resistant entry IS 2205 had more trichome density (15.33 No. / mm^2) and trichome length (53.98 µm) followed by another resistant entry IS 18551 with moderate trichome density (4.25 no. /mm^2) and more trichome length (47 µm) and width (7.49 µm). DJ 6514 also had trichome density of 4.00 no. / mm^2 with trichome length of 31.01 µm and width of 5.09 µm. The identified TNS resistant entries *viz*., 667, 665, and 671 had less trichome density (2.00, 1.66, and 1.91 µm) and more trichome length (43.26, 35.47, and 34.37 µm) compared to the resistant entries. However, the trichome width was found to be higher (9.23, 10.67, and 10.62 µm) compared to 7.18 and 7.49 µm in IS 2205 and IS 18554, respectively.

The genotypes, *viz*., IS 2205 and IS 18551 showed maximum height, leaf expansion, and robustness at the seedling stage (12 DAE). TNS 667, TNS 665, and TNS 671 also showed maximum seedling vigor (grade 1.00 to 1.67). However, the susceptible check showed poor growth, low leaf expansion, and poor adaptation (Grade 4.50) (Table 4).

The seedling glossiness of two resistant (IS 2205 and IS 18551) and one susceptible (DJ 6514) showed different grades. Genotypes IS 2205 and IS 18551 resulted in grade 1 (Pale green, Shiny, narrow leaves pointed upward) and susceptible check DJ 6514 showed Grade 3 4.00 (broad, dull green, and drooping leaves). The tested TNS entries *viz*., 667, 665 and 671 also recorded glossiness in the range of 2.00 to 2.67 (Table 4).

Bio-chemical composition concerning the concerning expression of resistance to shoot fly

Biochemical analysis of sorghum entries showed the highest phenol content in resistant check IS 18551 (15.02 mg/g) and was on par with the genotype, TNS 671 (14.82 mg/g), followed by TNS 665 (13.60 mg/g). Susceptible check, DJ 6514 was found to have lowest total phenol content (12.20 mg/g) and negatively correlated with the shoot fly incidence. The maximum percentage of sugar was recorded in the susceptible check, DJ 6514 (10.00) followed by TNS 665 (7.94) and TNS (8.54). Least sugar content was found in resistant IS 18551 (9.36 %). TNS 661 was on par with IS 18551 for cellulose (33.40 and 34.60 %), silica (5.20 and 5.72 %) and lignin (8.00 and 8.50 mg/g) content with the least values recorded in the susceptible check DJ 6514 (27.80 %, 3.82 %, 12.00 mg/g and 6.22 mg/g). Maximum amino acids were present in TNS 671 (12.26 %) followed by IS 18551 (11.84 %) which was in turn on par with TNS 665 (10.00 %). Amino acids content was found lowest in the susceptible check (7.30 %) (Table 5).

GC - MS Profiles of compounds on the sorghum seedlings concerning expression of resistance to shoot fly, *A. soccata*. Among the genotypes tested with resistant and susceptible check, there is significant differences in GC - MS profiles of leaf surface (Table 6, Fig 1-4)Ten compounds were commonly detected in all the resistant (IS 18551), tested genotypes (TNS 671 and TNS 665) and susceptible (DJ 6514).Of the major compounds detected, 2-(acetoxymethyl)-3-(methoxycarbonyl) biphenylenewas the component present in resistant and bothtested genotypes. Higher quantity of ethanone was present in resistant IS 18551 and TNS 671. The compounds present in tested entry TNS 671 were benzene di carboxylic acid, bis (2-methyl propyl) ester, 13-methyl-Z-14 nanacosene, nonacosane, 2-(acetoxymethyl)-3-methoxy carbonyl) biphenylene and 4-(4-hydroxyphenyl)-4-methyl-2-pentanone. TNS 665 had one component N-(aminocarbonyl)-2-chloroacetamide. The compounds present in both TNS 671 and susceptible DJ 6514 were metaraminol, arsenous acid, glutaric acid, di(2-psopropoxyphenyl) ester, and H-indole,1-methyl-2-phenyl. These compounds possibly might have acted as attractants/oviposition stimulants for the sorghum shoot fly.

DISCUSSION

Non - preference, antibiosis and tolerance are the major components involved in the mechanism of resistance to sorghum shoot fly[9& 8]. In the present study, TNS 671and TNS 665 exhibited ovipositional non-preference leading to a lesser dead heart, a high percentage of productive tillers (or) percentage of recovery resistance. It was understood from the present study that theresistant genotypes produced more numbers of uniform productive tillers than the susceptible ones and yield more undershoot fly infestation. Tested genotypes, TNS 667, TNS 665, and TNS 671 had glossy leaves with trichomes measuring more in terms of length and width than susceptible ones. These plant characteristics make the plant relatively less susceptible to shoot fly damage which correlates with [9 & 8]. More of glossiness might result in more reflection of light from the leaf surface, which may influence the oviposition behavior of shoot-fly females. Seedling vigor of TNS 667, TNS 665 and TNS 671 were positively associated with resistance to shoot fly and this result falls in line with [23]. However, [9] showed negative association results of glossiness with shoot fly incidence. Hence from the present study, it can be inferred that morphological traits such as leaf glossiness, vigor, leaf trichome density, trichome length and width, have a major rolein the expression of resistance to shoot fly.

A negative association of total phenols, cellulose, total amino acids, silica, tannin and lignin with resistance was observed in the present study. However, total sugar was found to have a positive correlation with shoot fly incidence. As observed in the present studies, total sugar has earlier been reported to be positively associated with susceptibility to shoot flywhich is in conformation with reports of [12] and [21].

More number of considerable genetic variationswas recorded in resistant, moderately resistant and susceptible genotypes based on shoot fly damage, morphological traits and biochemical composition. From the results of present study, it can be concluded that the contribution by biochemical factors was comparatively less when compared to the morphological factors such as leaf glossiness and trichome density. These studies provided additional information on some of the bio-chemical characteristics that have not been earlier reported to be associated with shoot fly resistance. The physico - chemical traits that are identified from this study should be further studied in depth by using either iso-lines RILS or back cross populations to study the cause-and-effect phenomena.

The compounds present in resistant (IS 18551) tested genotypes (TNS 671 and TNS 665) and susceptible genotype (DJ 6514) against sorghum shoot fly were determined using GCMS. The heat map showing the compounds detected from each of the four genotypes highlighted variations in content of metabolites between genotypes (Fig.1).

The chemical compounds present in susceptible alone were amino ethyl phosphonate, 3-heptadecene, 1-heptacosanol, octacosanol, indolizine, and 2 -(4-methyl phenyl) responsible for susceptibility to shoot fly. These compounds might have possibly acted as an oviposition stimulants to the sorghum shoot fly. The compounds ethanone and 2-(acetoxymethyl)-3-(methoxycarbonyl) biphenylyne might be negatively associated with oviposition and dead heart incidence and this might be a

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repellent. Further studies are needed to assess the relative contribution of different characters for shoot fly resistance and the use of such characters for sustainable sorghum production.

Higher amount of 2,4-dihydroxybenzoic acid and 2,6-dihydroxybenzoic acid were reported in resistant entry IS 18551 is in confirmation with the findings of [22] who reported higher levels of p-hydroxybenzoic acid in IS 18551, IS 4664 and ICSr700.Benzoic acid and carboxylic acids were reported to have insect repellant properties [11]. Lower levels or absence of such carboxylic acids add to the susceptible nature of entry DH6514 towards insects.

Cuticular plant wax acts as the primary interface between the plant andits environment playing a key role in maintainingthe plant's integrity within an inherently hostile environment. Nonacosane and its derivatives constitute plant cuticular waxes [20] and IS18551 was found to have high levels of nonacosane. Hentriacontane is a wax precursor found in plants and is attributed to drought resistance [13 and 3] and glaucousness [25 and15]. [16] Reported the absence of hentriacontane in susceptible Triticale cultivars to grain aphid and high content in resistant cultivars, supporting its role in resistance against insect pests.

Heptadecane plays a major role in host finding cue for biocontrol agents [4 and 14] which in turn substantiate the resistant nature of this accession towards the pest.

Secondary metabolites are known to play a crucial role in determining the level of susceptibility as well as resistance against pests and diseases. The variation in levels of metabolites among the sorghum genotypes, detected *via* GCMS analysis in study could thus form a platform for breeding programs for improving resistance against sorghum shoot flies.

CONCLUSIONS

The sorghum genotypes TNS 665, TNS 671 and TNS 667 showed more glossiness, trichome characters *viz.,* trichome density, length and width and this was substantiated by lesser preference in egg laying, lesser deadheart, and more of productive tillers. The biochemical parameters also contributed to a smaller extent to crop behavior which added strength to morphological characters in relation to plant resistance to *A. soccata.* GCMS analysis showed the presence of few compounds common to both resistant check and tested genotypes.These genotypes can be effectively utilized as parents for developing high-yielding varieties with resistance or tolerance to shoot fly.

Future scope of the study

The results from the study showed that Sorghum genotypes TNS 665, TNS 671 and TNS 667 can be further used in shoot fly resistance breeding programme and can be developed as a sorghum shootfly resistant variety.

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Author contributions

SM and PA conceived and designed research. SM and PA conducted experiments. SM, PA, and PRN analyzed data. SM, PA, SE and PRN wrote the manuscript. All authors read and approved the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Table 1 Ovipositional preference and damage by sorghum shoot fly, A.Soccata on different sorghum genotypes evaluated for resistance (TNAU, Coimbatore)

Genotypes	Eggs/5 plants (Nos.)		Plants with eggs (%)		Dead heart (%)		**Resistant* Scoring**
	14 DAE	21 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
IS18551(R)	3.20	2.75	9.09	9.09	9.09	9.09	HR
DJ 6514 (S)	9.50	12.25	50.83	60.17	50.83	68.25	S
TNS 667	4.75	5.50	12.70	17.35	16.20	19.10	R
TNS 665	4.00	4.50	10.83	13.83	14.58	16.27	R
TNS 671	4.70	4.25	11.00	11.00	16.00	18.33	R
TNS 648	5.50	6.25	15.91	19.64	24.50	28.86	MR
TNS 669	5.20	6.20	16.29	18.74	23.70	29.25	MR
TNS 623	7.50	7.75	26.70	30.17	34.68	36.70	MS
TNS 664	6.50	7.25	25.64	37.40	38.00	40.00	MS
TNS 668	7.50	8.00	28.50	29.75	35.52	37.15	MS
TNS 670	7.25	7.50	25.74	29.50	23.64	35.60	MS
TNS 672	5.25	5.25	22.73	24.46	25.91	27.27	MR
TNS666	5.25	6.25	18.75	21.29	26.42	26.61	MR

DAE – Days after seedling emergence Values are the mean of two locations (TNAU, Coimbatore, and ARS, Kovilpatti)
1-9 scale (1=<10%; 2=11-20%; 3=21-30%; 4=31-40%;5=41-50%;6=51-60%;7=61-70%;8=71-80%; 9=>80%)
HR - Highly Resistant, MR - Moderately Resistant, MR- Moderately Susceptible, S - Susceptible)

Table 2 Ovipositional preference and damage by sorghum shoot fly, A. Soccata on different sorghum genotypes and resistant scoring. (TNAU, Coimbatore)

Genotypes	Eggs/5 plants (Nos.)		Plants with eggs (%)		Dead heart (%)		**Resistant* Scoring**
	14 DAE	21 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
IS 18551	2.00	3.50	8.75	9.25	8.50	9.75	HR
DJ 6514	8.50	10.25	46.83	59.75	46.72	53.96	S

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TNS 667	3.25	4.75	12.92	13.25	15.00	18.61	R
TNS 665	3.50	4.25	14.20	15.60	17.28	19.10	R
TNS 671	3.25	4.00	11.39	14.20	16.74	17.83	R

Values are the mean of two locations (TNAU, Coimbatore and ARS, Kovilpatti)
-9 scale (1=<10%; 2=11-20%; 3=21-30%; 4=31-40%;5=41-50%;6=51-60%;7=61-70%;8=71-80%; 9=>80%)
HR - Highly Resistant, MR - Moderately Resistant, MR- Moderately Susceptible, S - Susceptible)

Table 3 Recovery resistance of different genotypes of sorghum evaluated for their resistance to sorghum shoot fly, A. Soccata (TNAU, Coimbatore)

Sorghum genotypes	Recovery resistance(%)	Recovery resistance score
IS 18551	51.25	3.85
DJ 6514	6.25	8.85
TNS 667	33.33	6.20
TNS 665	43.75	4.70
TNS 671	48.33	4.00
TNS 648	44.58	5.05
TNS 669	32.33	6.05
TNS 623	16.25	7.75
TNS 664	34.00	5.60
TNS 668	25.00	6.60
TNS 670	15.83	7.70
TNS 672	29.58	6.20
TNS 666	14.17	8.40
SE(d)	2.2015	0.4056
CD(0.05)	4.7966	0.8837

Values are the mean of two locations (TNAU, Coimbatore and ARS, Kovilpatti).
Scores: 1(>60 % plant with UPT); 2(55 – 60 % plant with UPT); 3(50 – 55 % plant with UPT); 4(45 – 50 % plant with UPT); 5(40 - 45 % plant with UPT); 6(35 – 40 % plant with UPT); 7(30 – 35 % plant with UPT); 8(25 – 30 % plant with UPT); 9(< 25 % plant with UPT).
UPT: Uniform Productive Tillers.

Table 4 Morphological characteristics of sorghum genotypes associated with resistance/ susceptibility to sorghum shootfly, A. soccata

Sorghum genotypes	Trichome density (No./mm^2)	Trichome length (µm)*	Trichome width (µm)*	Seedling vigor (1-5) at 12 DAE*	Seedling glossiness (1-5) at 12 DAE*
IS 2205 (R)	15.33^{a}	53.98^{a}	7.18^{c}	1.00	1.00
IS18551(R)	4.25^{c}	47.00^{b}	7.49^{c}	1.00	1.33
DJ 6514 (S)	4.00^{c}	31.01^{e}	5.09^{d}	4.50	4.00
TNS 667	5.75^{bc}	43.26^{c}	9.23^{b}	1.50	2.00
TNS 665	4.00^{b}	35.47^{d}	10.67^{a}	2.00	2.67
TNS 671	6.08^{b}	34.35^{d}	10.62^{a}	1.67	2.00
SE (d)	2.25	1.56	0.56	1.69	1.04
CD (P=0.05)	2.16	3.17	1.14	0.71	0.89

Seedling vigor: 1=Plants showing maximum height, leaf expansion, and robustness: 5= Plants showing poor growth, low leaf expansion, poor adaption
Glossiness (1-5): 1= Lines with pale green, shiny, narrow leaves pointed upward; 5= Lines with broad, dull green, drooping leaves.
Values are the mean of two seasons and two locations (TNAU, Coimbatore, and ARS, Kovilpatti)
Means followed by common letter(s) are not significantly different at the 5% level by LSD.

Table5 Composition of biochemical and nutritional factors in resistant and susceptible sorghum genotypes against A.soccata

Sorghum genotypes	Total phenols (mg/g)	Total sugars (%)	Cellulose (%)	Total amino acids (%)	Silica (%)	Tannin (mg/g)	Lignin (mg/g)
IS 18551	15.02 ^{a}	9.36^{d} (17.82)	34.60^{a} (35.11)	11.84^{b} (20.1)	5.72^{a} (13.83)	17.98^{a}	8.5 ^{a}

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TNS 671	14.82 [a]	8.54[c] (16.99)	33.40[a] (34.38)	12.26[a] (20.5)	5.20[a] (13.18)	14.08[c]	8.0 [ab]
TNS 665	13.60 [b]	7.94[b] (16.37)	31.60[a] (33.24)	10.00[c] (18.43)	4.60[b] (12.37)	14.94[b]	7.26 [b]
DJ 6514	12.20 [c]	10.00[a] (18.43)	27. 80[b] (30.83)	7.30[d] (15.70)	3.82[c] (11.26)	12.00[d]	6.22 [c]
SE (d)	0.30	0.16	0.97	0.690	0.30	0.30	0.34
	0.65	0.34	2.06	0.327	0.63	0.63	0.72

Values are the mean of two seasons and two locations (TNAU, Coimbatore, and ARS, Kovilpatti)
Means followed by common letter(s) are not significantly different at the 5% level by LSD.

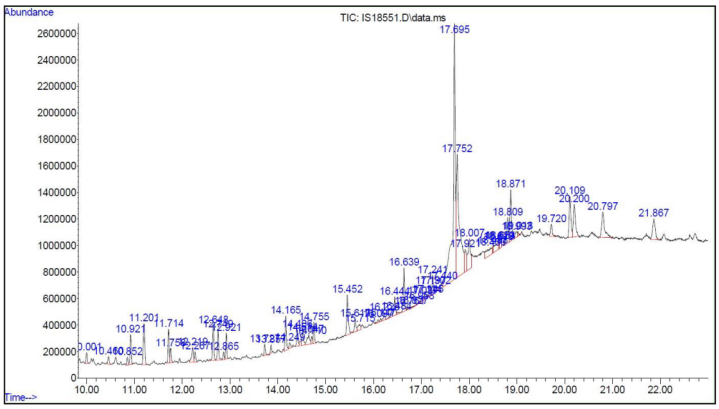

Fig.1 Chromatogram of resistant sorghum genotype, IS 18551

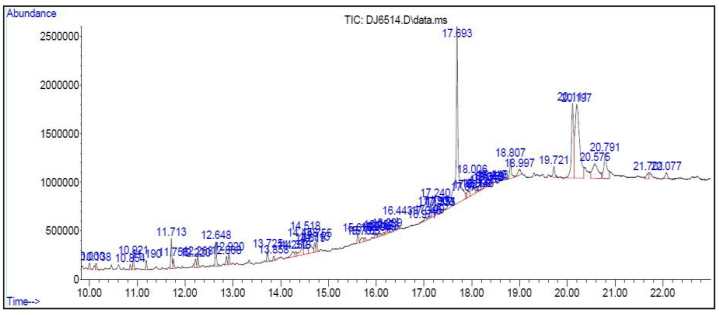

Fig. 2 Chromatogram of susceptible sorghum genotype, DJ 6514

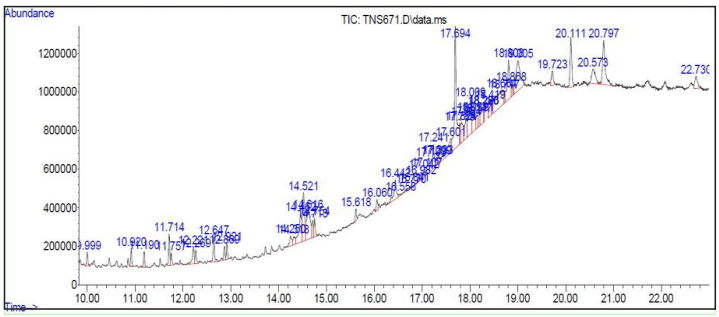

Fig. 3 Chromatogram of tested sorghum genotype, TNS 671

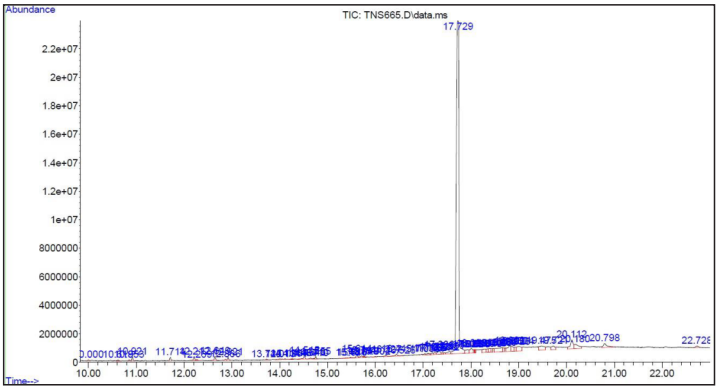

Fig. 4 Chromatogram of tested sorghum genotype, TNS 665

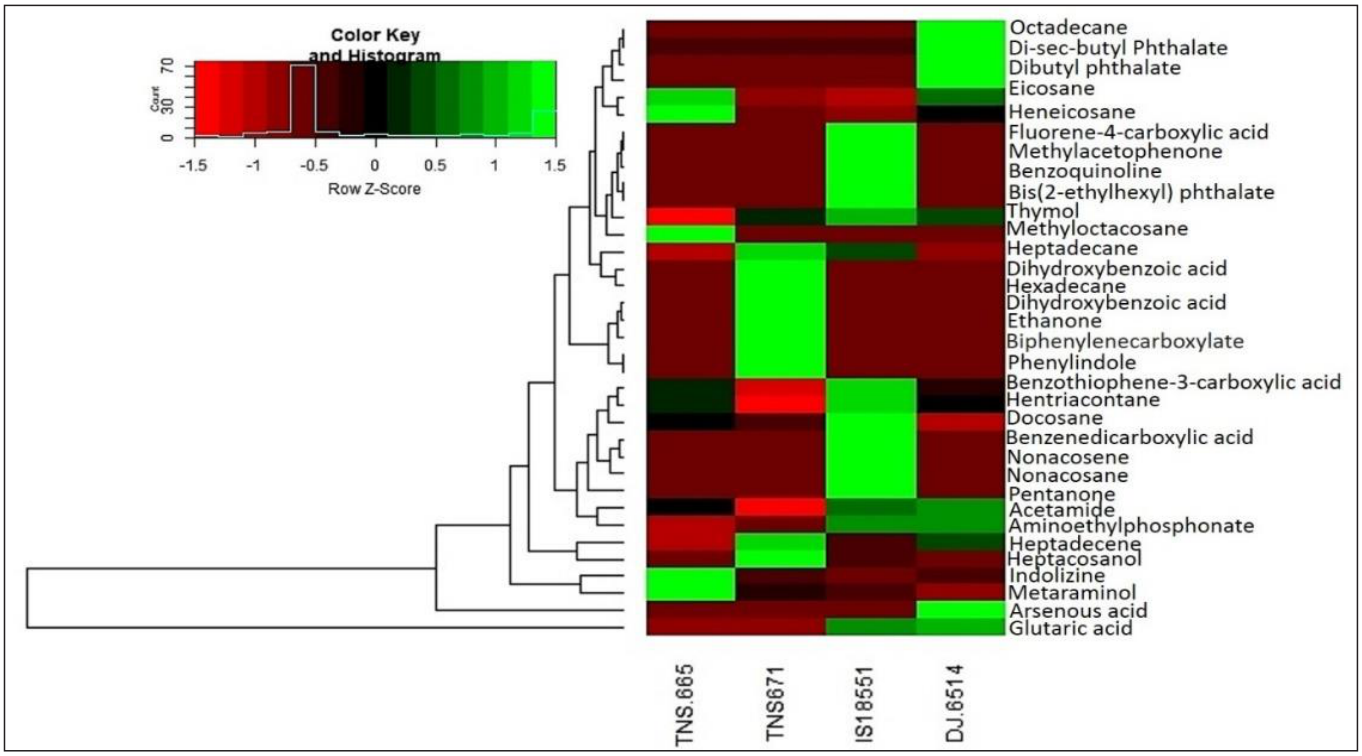

Fig. 5 Hierarchically clustered heat map showing levels of metabolites in different genotypes of sorghum

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Table 6 Compounds identified through GCMS in sorghum genotypes tested for their reaction to sorghum shoot fly, A.soccata

COMPOUNDS COMMONLY PRESENT IN RESISTANT, SUSCEPTIBLE AND TEST GENOTYPES						
	Compound name	Retention Time (Min.)	Peak Area(%)			
			Resistant (IS 18551)	Test genotypes		Susceptible (DJ 6514)
				TNS 671	TNS 665	
1	Octadecane	10.921	1.7	0.41	1.16	0.93
2	Di-sec-butyl Phthalate	11.714	2.00	0.31	1.58	2.23
3	Dibutyl phthalate 1,2 Benzenedicarboxylic acid	12.648	2.27	0.57	-	2.26
4	Eicosane	12.921	1.42	0.35	1.02	0.92
5	Heneicosane	14.514	1.20	4.43	-	2.66
6	9H-Fluorene-4-carboxylic acid	15.617	0.6	0.39	-	0.44
7	2'-Hydroxy-5'-methylacetophenone	16.444	1.91	0.80	1.08	0.30
8	Benzo[h]quinoline	17.037	0.14	0.23	0.85	0.44
9	Bis(2-ethylhexyl) phthalate	17.695	26.11	0.72		27.29
10	Thymol	17.921	1.01	1.52	0.17	0.30

COMPOUNDS PRESENT IN RESISTANT GENOTYPE ALONE						
	Compound name	Retention Time(Min.)	Resistant (IS 18551)	Test genotypes		Susceptible (DJ 6514)
				TNS 671	TNS 665	
1	2-methyloctacosane	10.001	0.67	--	-	-
2	Heptadecane,	12.219	0.96	-	-	-
3	2,4--Dihydroxybenzoic acid	14.165	2.02	-		-
4	Hexadecane	14.755	1.26	-	-	-
5	2,6-Dihydroxybenzoic acid	15.452	3.06	-	-	--
6	ethanone,	15.715	0.30	5.29	-	-
7	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylyne	16.226	0.58	0.66	2.73	
8	2-Methyl-7-phenylindole	17.921	1.01	-	-	-
9	9Benzothiophene-3-carboxylic acid	19.720	1.00	-	-	-
10	Hentriacontane	12.921	1.42	-	-	-
11	Docosane	10.001	0.67	-	-	-

COMPOUNDS PRESENT IN TEST GENOTYPES ALONE						
		RT(Min.)	Resistant(IS 18551)	TNS 671	TNS 665	Susceptible(DJ 6514)
1	Benzenedicarboxylic acid, bis(2-methyl propyl) ester	12.647	-	2.16	-	
2	13-Methyl-Z-14-nonconsent	14.462	-	2.12	-	-
3	Nonacosane	14.616	-	3.00	-	-
4.	2-(Acetoxymethyl)-3-methoxy carbonyl)	16.442	-	2.73	-	-
5	Biphenylene	18.301	-	0.66	-	-
5	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone	17.824	-	2.57	-	-
6	Acetamide, N-(aminocarbonyl)-2-chloro	14.114	-	-	0.11	-

COMPOUNDS PRESENT IN SUSCEPTIBLE GENOTYPE ALONE						
		RT(Min.)	Resistant(IS 18551)	TNS 671	TNS 665	Susceptible(DJ 6514)
1	Aminoethylphosphonate	11.190	-	-	-	0.76
2	3-Heptadecene	11.756	-	-	-	0.79
3	1-Heptacosanol	13.725	-	-	-	1.00
4	Octacosanol	14.458	-	-	-	1.42
5	Indolizine, 2-(4-methylphenyl)	20.111				10.61

COMPOUNDS PRESENT IN TEST GENOTYPES AND SUSCEPTIBLE GENOTYPES						
	Compound name	RT(Min.)	Resistant(IS 18551)	Test genotypes		Susceptible(DJ 6514)
				TNS 671	TNS 665	
1	Metaraminol	12.866	-	-	0.12	0.72
2	Arsenous acid	17.788	-	-	1.12	0.40
3	Glutaric acid, di(2-isopropoxy phenyl) ester	17.551	-	-	0.42	0.29
4	H-Indole, 1-methyl-2-phenyl	18.051	-	-	3.08	0.19

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