Field Crops Research 261 (2021) 108029

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Morpho-physiological traits and leaf surface chemicals as markers conferring resistance to sorghum shoot fly (*Atherigona soccata* Rondani)

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ARTICLE INFO

Keywords:
Shoot fly
Atherigona soccata
Sorghum bicolor
Shoot fly deadhearts
Phenols
Proteins
Tannins
Soluble sugars
HPLC
GC–MS
Leaf surface chemicals

ABSTRACT

Sorghum shoot fly, *Atherigona soccata,*causes substantial economic losses in sorghum globally. Cultural practices and host plant resistance are effective measures for mitigating the losses caused by sorghum shoot fly. Therefore, we evaluated 32 sorghum genotypes consisting of a set of 10 restorer lines, 10 CMS (cytoplasmic male-sterile) lines and their respective maintainers exhibiting resistance/susceptibility to shoot fly along with resistant and susceptible checks under field conditions. The traits such as leaf glossiness, leaf sheath pigmentation, percentage plants with shoot fly deadhearts and number of shoot fly eggs per plant were used as morphological markers for selecting genotypes with resistance to shoot fly during the rainy and post rainy seasons of 2016 and 2017. The test material was also subjected to biochemical analysis (total soluble sugars, protein and tannin contents), while the leaf surface chemicals were analysed by GC–MS to identify the compounds associated with resistance/susceptibility to shoot fly. The genotypes differed significantly for all the traits, except percentage plants with shoot fly deadhearts during the 2016 rainy season. The genotypes ICSB 458, ICSA/B 467, ICSA/B 487, ICSA/B 14037, IS 18551 and ICSV 93046 exhibited moderate to high levels of resistance to shoot fly based on number of plants with shoot fly deadhearts, plants with shoot fly eggs and total number of shoot fly eggs. The shoot fly resistant genotypes ICSB 84, ICSA/B 467, ICSB 487, ICSB 14024, and IS 18551 had low shoot fly deadheart incidence, higher amounts of condensed tannins, soluble sugars, phenols and lower protein content as compared to the susceptible genotypes. Thirteen unique compounds were identified from leaf surface extracts by GC–MS which were associated with shoot fly resistance/susceptibility. While HPLC analysis revealed that Protocatechuic and coumaric acids were present in most of the sorghum genotypes, but their amounts were significantly greater in resistant as compared to the susceptible ones. The findings of the study highlight the importance of various morphological and biochemical traits conferring resistance to sorghum shoot fly, and these traits can be used as markers to identify shoot fly resistant genotypes for use in breeding programs.

1. Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop globally after wheat, rice, maize and barley. It is cultivated worldwide for food, feed, and fodder/forage. India is one of the largest producers of sorghum, popularly known as '*jowar*', with an area of around 5.02 million ha, production of 4.80 million tons and average productivity of 956 kg/ha (Anonymous, 2018). Sorghum has gained greater importance recently as a replacement to other nutrient exhaustive crops being utilized for biofuel production. Sorghum grain is a staple food for millions of people in the semi-arid regions of Africa and Asia where it is used to make food products such as tortillas, breads, cakes, noodles, couscous,

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https://doi.org/10.1016/j.fcr.2020.108029
Received 2 July 2020; Received in revised form 2 December 2020; Accepted 3 December 2020

beer and porridge (Rooney and Waniska, 2000). Owing to its quick growth, high yield potential and quality of biomass, sorghum serves as a versatile crop for meeting the needs of growing human population, especially in the mixed crop-livestock systems. Sorghum is damaged by over 150 insect species, of which sorghum shoot fly, *Atherigona soccata* (Rondani) is a major pest in Asia, Africa and the Mediterranean Europe (Sharma, 1993). In spite of the development of several elite varieties, the grain and forage yields of sorghum are quite low (Sharma et al., 2003). Therefore, there is a need to produce high yielding hybrids with resistance to shoot fly.

Shoot fly damage starts after 7–10 days of seedling emergence, the typical symptom of damage is drying of the central shoot, due to cutting of the growing point by shoot fly maggot (Dhillon et al., 2006; Padmaja et al., 2010) leading to the formation of a deadheart (Fig. 1). If there is enough moisture and nutrient availability, the shoot fly damaged plants may produce axial tillers (Fig. 2), which may also be attacked if there is subsequent shoot fly damage. The tillers at times lead to production of productive tillers, and contribute to grain yield. These are often one month late than the main plants, and at times are attacked by panicle feeding insects such as sorghum midge, *Stenodiplosis sorghicola* Coq. and the head bug, *Calocoris angustatus* Leth. (Sharma, 1993). Sorghum shoot fly causes substantial damage in the late-sown crops, affecting both grain and fodder yields. Inappropriate cultural practices (mono-cropping and ratooning) and irrational use of pesticides to combat shoot fly damage has led to large scale cultivation of shoot fly susceptible varieties and hybrids (Sharma et al., 2003). Various control measures have been used to check the shoot fly infestation, of which seed treatment with systemic insecticides being the most effective. However, the resource-poor farmers of semi-arid tropics cannot afford costly chemicals, and additionally, the short sowing window does not allow them to go for early planting to avoid shoot fly damage (Sharma, 1985). Host plant resistance and use of cultural practices are the most economic and effective measures to reduce the extent of losses due to shoot fly damage by keeping the infestation below economic threshold levels (ETL) (Sharma and Nwanze, 1997). Host plant resistance is a complex trait, and is the final outcome of interactions among the component traits (morphological and biochemical) imparting resistance to insect pests (Dhillon and Sharma, 2004).

Several genotypes with resistance to shoot fly have been identified, which can be used in resistance breeding programs (Pradhan and Jotwani, 1978; Taneja and Leuschner, 1985; Sharma et al., 2003). To broaden the genetic base for shoot fly resistance, there is a need for gaining an understanding of different mechanisms of resistance to this insect in a wide array of shoot fly resistant/susceptible genotypes. In spite of substantial genetic variability in sorghum germplasm, linkage drag for lower yield, poor nutritional quality and cross incompatibility has limited the use of landraces in sorghum breeding programs. Additionally, cytoplasmic male sterile lines have shown greater susceptibility to shoot fly damage; which further complicates the inheritance of resistance or susceptibility to shoot fly by cytoplasmic and nuclear genes (Dhillon et al., 2006). Integrated pest management practices involving genetic and agronomic approaches can be more effective in overcoming the constraints in breeding for shoot fly resistance.

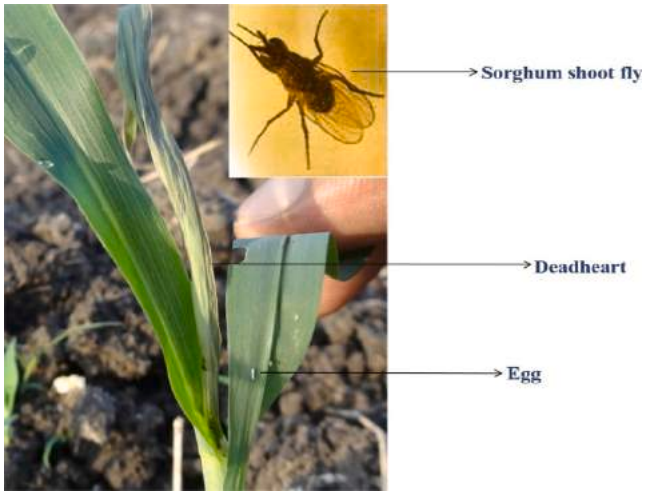

Fig. 1. Sorghum seedling with shoot fly egg and deadheart (Inset: sorghum shoot fly).

Fig. 2. Sorghum seedling with axial tillers.

In sorghum, the presence of certain nutritional and anti-nutritional compounds might be associated with the developmental biology of insects, influencing the expression of resistance to sorghum shoot fly (Raina, 1984). Resistance to sorghum shoot fly is also an outcome of interactions between various morphological and biochemical factors (Ogwaro, 1978; Delobel, 1982; Raina, 1982). Several phenolic compounds influence the shoot fly resistance/susceptibility, based on the presence or absence and, even the concentrations of these compounds (Pandey et al., 2005). Therefore, there is a need to gain an understanding of the morphological and biochemical components in sorghum which might be associated with expression of resistance to shoot fly. For instance, trichome density, leaf sheath pigmentation and leaf glossiness are negatively correlated with the number of shoot fly eggs and deadhearts. Similarly, 3,4-Dihydroxy benzoic acid is present in high concentrations during the seedling stage. It is a by-product of dhurrin hydrolysis and influences the expression of resistance to shoot fly damage (Chamarthi et al., 2011). Therefore, the present studies were designed to evaluate a set of 32 diverse sorghum genotypes for morphological and physico-chemical traits to identify parents with diverse mechanisms of resistance to produce sorghum hybrids with shoot fly resistance for cultivation by the farmers.

2. Material & methods

2.1. Experimental material

A set of 32 genotypes were chosen from a collection of diverse material available at the Gene Bank, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for this study on the basis of their reaction (susceptible or resistant) against sorghum shoot fly. The test

genotypes comprised of a set of 10 restorers, 10 cytoplasmic male-sterile (CMS) lines and their respective maintainers, along with resistant (IS 18551) and susceptible (Swarna) checks (Table 1). The experiments were conducted for two seasons (Rainy and Post rainy seasons) over two years. The test material was planted in a randomized complete block design (RCBD). There were three replications of the test material sown in two rows of 2 m each, with a spacing of 75 cm between the rows in black Vertisols under field conditions at ICRISAT (latitude 17.53 °N, 78.27 °E, at an altitude of 545 m above sea level), Patancheru, Telangana, India. The plant to plant distance was maintained at 10 cm by thinning at 5 days after seedling emergence (DAE). The interlard fish meal technique (Taneja and Leuschner, 1985; Sharma et al., 1992) was used to ensure uniform and high shoot fly incidence under field conditions (Fig. 3). The recommended agronomic practices were followed for raising the crop (Riyazaddin et al., 2015).

2.2. Observations on oviposition and deadheart formation

Leaf glossiness (GS) was visually rated at 10–12 DAE in the early morning hours when the expression of this trait is most apparent, and when there was maximum reflection of light from the leaf surface as described by (Sharma et al., 1997). Leaf glossiness (1 = highly glossy, light yellow shining and erect leaves, and 5 = non-glossy, dull green drooping leaves) (Fig. 4). The leaf sheath pigmentation (LSP) was recorded visually on a scale of 1–5 (1 = pigmented plumule with dark leaf sheath; 5 = non-pigmented plumule with green leaf sheath) (Fig. 5). The density of trichomes (Fig. 6) on the abaxial and adaxial surface of the leaves was recorded at 12 DAE by taking a 2.5 cm^2 portion from the centre of the fifth leaf. For this purpose, the leaf samples were collected from three plants at random and placed in stoppered glass vials (10 mL capacity) containing acetic acid and alcohol (2 : 1) for 24 h to clear the chlorophyll, and subsequently transferred into lactic acid (90 %) as a preservative. The percent plants with shoot fly deadhearts (SFDH%), number of plants with SF eggs (PWSFE) and total number of SF eggs

Table 1
List of the test genotypes with their pedigree and (A-, B- and R-lines).

A- and B- lines		
Sr. No	Genotype	Pedigree
1	ICSA/B 14013	(ICSB 38 x SSV 84-6-3)-1-2
2	ICSA/B 14024	(ICSB 344 x SSV 84-5-1)-2-1-1
3	ICSA/B 14027	(ICSV 93046 x ICSB 88010)-1-3-2-1-1-1-1-1
4	ICSA/B 487	(ICSB 102 x ICSV 700) 5-2-4-2-1-2
5	ICSA/B 458	(ISS 18432 x ICSB 6) 11-1-1-2-2
6	ICSA/B 323	(IS 29016 x ICSB 26) 2
7	ICSA/B 14037	([[SPV 462 x (ICSB 102 x PS 28060-2)-1-2-2-1-5-3] x 296 B]-2-1-1-1-1-1 x ICSB 451)-3-1-2-3-1-1-1
8	ICSA/B 467	[((ICSB 11 x ICSV 700) XPS 19349 B) x ICSB 13] 4-1
9	ICSA/B 84	296 B
10	ICSA/B 52	Ind. Syn. 422-1
R- lines/restorers		
1	ICSV 25280	(ICSV 93046 x SSV84)-7-2-1-3
2	ICSV 25303	(IS 19273 x SSV 84)-2-2-1-1-2-1-1
3	ICSV 25337	(ICSR 93034 x SSV 74-5-1)-1
4	ICSV 93046	[((ICSV 700 x ICSV 714)-20-1-1-1-1-1-1 x UChV2 x Bulk Y-55)-1-5-1)-5-2-5-1-1)]-9-1-3-1-1-
5	SSV 74	Selection from 23558 (PAB 74)- Zera-zera landrace, Ethopia)
6	ICSV 700	(IS 1082 x SC 108-3)-1-1-1-1-1
7	BTx 623	BTx 3197/SC170 –6-4
8	S 35	IS 36556
9	ICSV 25316	(DSV 4 x SSV 84)-1-2-2-3-2-3-4
10	ICSV 25292	(DSV 4 x SSV 84)-1-1-1-1-2-1-2
11	IS 18551	Resistant check
12	Swarna	Susceptible check

Fig. 3. Interlard fishmeal technique.

(NSFE) were recorded at 14 and 28 DAE. Shoot fly deposits small (2 mm) white, cigar-shaped eggs, singly on under surface of the leaf. After hatching, maggot enters the plant through the whorl and destroys the growing point, eventually leading to drying up of the central leaf called deadheart. The percentage of plants with deadhearts were calculated by dividing the number of deadhearts by the total number of seedlings in each plot [(seedlings with deadhearts/total number of plants) × 100)].

2.3. Biochemical analysis

For biochemical analysis the infested leaf samples were collected at 5^{th} leaf stage from five plants at 12–14 DAE and freeze-dried in a lyophilizer (Modulyo D, Thermo Savant, Japan) at −45 °C, and stored in the deep freeze at −20 °C. The lyophilized samples were grounded to form a fine powder, which was used for estimation of total soluble sugars, proteins, condensed tannins and total phenols. The total soluble sugars were estimated by Anthrone reagent method (Hedge and Hofreiter, 1962), while the estimation of proteins was done using Lowry's method (Lowry et al., 1951). The total phenols were estimated by using the method defined by (Bray and Thorpe, 1954), and Vanillin hydrochloride method (Burns, 1971) was employed for the estimation of condensed tannins.

2.4. GC–MS and HPLC analysis

The leaf samples were collected from the field at 10–12 DAE during the 2017 rainy and post rainy seasons. Phenolic compounds from different sorghum genotypes were extracted and analysed by the method described by (Hahn et al., 1983), with a few modifications. Available standards such as gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids were prepared at 100 ppm concentrations and filtered through 0.22 µm (PVDF) Millipore filter.

2.4.1. High performance liquid chromatography (HPLC) of phenolic compounds

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®) having a PCM 11 reciprocating piston pump, and a 2996 photodiode array detector (in the range of 190−800 nm) was used for obtaining fingerprints 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

Fig. 4. Leaf glossiness in sorghum seedlings.

Fig. 5. Leaf sheath pigmentation.

followed by a 5 min linear gradient to 5 % of solvent B. Flow rate was 1 mL/min. There were three replicates for each genotype and the experiment was conducted in a completely randomized design. The chromatographic data were recorded and processed by Empower 3 software. Phenols were identified and quantified by comparing the peak area obtained at similar retention times with known concentrations of the standards.

2.4.2. GC–MS

The third leaf was collected in centrifuge tube (25 mL capacity) containing 10 mL of HPLC grade hexane at 10 DAE. The tube was vortexed for extracting the leaf surface compounds in hexane. The extract was concentrated to 0.5 mL under a stream of nitrogen, and analysed with GCMS QP 2010 Ultra (Shimadzu, Kyoto, Japan), equipped with an autosampler AOC 20 *i* series. The following instrument run conditions were used: ion source temp. 240 °C; column CBP 5, 25 m × 0.2 mm i.d., 0.25 μm film thickness column; carrier gas helium at a constant flow rate of 1 mL min^{-1}; temperature program: 50 °C (2 min), 50−280 °C (10 min), 280 °C (10 min); injection temperature: 250 °C, interface temperature: 280 °C, solvent cut time 3 min, splitless injection, mass range of *m/z* 20 to *m/z* 600. Data acquisition and processing was done with GC Solutions 4.1. The compounds were identified by comparing the spectral data with the library (NIST and WILEY) of standard compounds.

2.5. Statistical analysis

The data were subjected to analysis of variance (ANOVA) across seasons using GenStat 14th Edition. The treatments (test genotypes) were considered as fixed effects and the morphological traits associated with shoot fly resistance/susceptibility were used as random effects. Statistical significance for the differences among the genotypes was judged by F-test, the genotypic means were compared by the least significant difference (LSD) at F_p 5 %. Pearson correlation coefficients for the traits associated with shoot fly susceptibility/resistance were calculated using corrplot package in R studio (Wei et al., 2017). Principal Coordinate Analysis (PCA) was done using factoextra package in R

Fig. 6. Trichomed and non-trichomed leaves.

studio for depicting the association of shoot fly damage (shoot fly deadhearts, plants with shoot fly eggs) and biochemical components viz. total phenols, condensed tannins, total soluble sugars and protein content.

3. Results

3.1. Genotypic resistance to sorghum shoot fly, A. soccata

3.1.1. Shoot fly deadhearts, number of shoot fly eggs and plants with shoot fly eggs

There were significant differences among the sorghum genotypes in terms of shoot fly deadhearts (%), number of plants with shoot fly eggs and total number of shoot fly eggs (Table 2). The differences in percentage of plants with shoot fly deadhearts were non-significant at 28 DAE during the 2017 rainy season. However, the deadheart incidence at 28 DAE varied significantly across seasons. Similarly, significant variation was found for total number of shoot fly eggs and plants with shoot fly eggs at 14 and 28 DAE among the test genotypes during rainy and post rainy seasons in 2016, while it was significant in post rainy season of 2017. The interaction effects across years during the post rainy seasons were non-significant. The genotypes ICSA/B 84, ICSB 458, ICSA/B 467, ICSA/B 487, ICSA/B 14037, ICSV 700, ICSV 25337, ICSV 93046 and IS 18551 with lower deadheart incidence and lesser number of eggs exhibited moderate to high resistance against shoot fly.

3.1.2. Trichome density

In the present studies, the genotypes varied significantly for trichome density during both the rainy (2016, 2017) and post rainy (2016) seasons. The genotypes (ICSB 458, ICSA/B 487, ICSB 14027, ICSA/B 14037, IS 18551, ICSV 700, and ICSV 25337) that exhibited moderate to high resistance against shoot fly also recorded high trichome density on both the leaf surfaces. Whereas, the genotypes exhibiting moderate to low resistance to shoot fly recorded low density or absence of trichomes on both the leaf surfaces.

3.1.3. Leaf glossiness score (GS)

The ANOVA across the years revealed significant differences among the test genotypes for leaf glossiness. The genotypes IS 18551, ICSA/B 467, ICSA/B 487, ICSV 700 and ICSV 93046 were glossy (leaf glossiness score <3.0), whereas Swarna, SSV 74, BTx 623, ICSA 323, ICSA/B 52 and ICSV 25292 were non-glossy (leaf glossiness score >4.0). The genotypes with glossy leaves suffered significantly lower shoot fly damage than the non-glossy ones.

3.1.4. Leaf sheath pigmentation (LSP)

The genotypes with pigmented leaf sheath (IS 18551, ICSV 93046, ICSA/B 467, ICSA/B 487, ICSA/B 14037 and ICSB 458) exhibited

Table 2
ANOVA for rainy (R) and post rainy (PR) during 2016-17, ICRISAT Patancheru, India.

ANOVA	Range								σ^2g					
	2016 R		2016 PR		2017 R		2017 PR		2016			2017		
	Min	Max	Min	Max	Min	Max	Min	Max	R	PR	R x PR	R	PR	R x PR
GS	1.0	5.0	1.0	5.0	1.0	4.5	1.0	5.0	**9.63****	**11.26****	**1.360**	**6.71****	**15.38****	**2.4***
LSP	1.0	4.0	1.0	4.5	1.5	3.0	1.5	5.0	**6.77****	**4.07****	**0.540**	**2.99***	**8.9****	**2.73****
SFDH (%) at 14 DAE	13.7	97.8	5.2	75.4	37.8	96.9	6.1	66.9	**9.29****	**4.11****	**2.31***	**1.20**	**4.78****	**0.60**
PWSFE at 14 DAE	11.0	33.5	3.0	24.5	5.5	22.5	3.5	23.3	**2.48***	**6.1****	**2.12***	**1.30**	**3.91****	**2.79****
NSFE at 14 DAE	20.5	82.0	4.5	30.0	13.5	58.0	3.8	26.0	**4.1****	**6.57****	**3.93***	**1.52**	**6.42****	**1.72***
SFDH (%) at 28 DAE	78.6	100.0	19.3	86.8	65.6	100.0	26.8	92.6	**1.15**	**4.49****	**2.3***	**7.29****	**3.38****	**1.01**
PWSFE at 28 DAE	14.0	33.5	6.5	28.5	6.0	26.0	5.8	25.5	**1.94***	**2.71***	**1.55***	**1.79***	**4.93****	**3.46****
NSFE at 28 DAE	26.5	87.0	6.5	43.5	16.5	47.0	6.3	35.8	**5.03****	**4.71****	**3.74****	**1.05**	**4.79****	**1.78***
TD_{aba}	0.0	46.0	0.0	59.5	0.0	46.0	0.0	40.0	**10.44****	**18.79****	**0.520**	**10.44****	**1.00**	**0.62**
TD_{ada}	0.0	72.0	0.0	79.5	0.0	73.5	0.0	57.9	**20.38****	**13.12****	**0.450**	**27.36****	**1.27**	**1.33**

(GS: leaf glossiness score; LSP: leaf sheath pigmentation; SFDH: shoot fly deadhearts; PWSFE: plants with shoot fly eggs; NSFE: number of shoot fly eggs; TD_{aba}: trichome density on abaxial surface; TD_{ada}: trichome density on adaxial surface; R: rainy season; PR: post rainy season; σ^2g: variance).
**, and * Significant at Fp value 0.01 % and 0.05 % respectively.

moderate to high levels of resistance to shoot fly whereas, the non-pigmented genotypes (Swarna, SSV 74, BTx 623, ICSA/B 52, ICSA/B 323 and ICSA 14024) were found to be susceptible.

3.2. Association of morphological traits with expression of resistance to sorghum shoot fly, A. soccata

The leaf glossiness score (GS), leaf sheath pigmentation (LSP), percent shoot fly deadhearts (SFDH%), plants with shoot fly eggs (PWSFE), number of shoot fly eggs (NSFE) and trichome density on abaxial (TD_{aba}) and adaxial (TD_{ada}) surface are some of the important traits which have been found to be associated with the shoot fly resistance. In the present studies, SFDH (%) were recorded at 14 and 28 DAE and the genotypes with lower incidence of SFDH were found to be resistant than the genotypes with high incidence. SFDH (%) was positively correlated with the traits like PWSFE, NSFE both at 14 and 28 DAE and negatively correlated with TD_{aba} and TD_{ada} during post rainy season in 2016. Similar results were obtained during rainy and post rainy seasons in 2017 (Figs. 7–10).

In the present studies the leaf glossiness score was significantly and positively correlated with SFDH (%) in 2016 rainy season while with SFDH (%), PWSFE and NSFE during post rainy season. A significant and negative correlation of GS with TD_{aba} and TD_{ada} was found during the rainy season while with TD_{ada} (−0.420*) in 2016 post rainy season. Similarly, in 2017, GS was found to be positively correlated with LSP and SFDH (%), whereas, it was negatively correlated with TD_{ada} during the rainy season. However, it was significantly positively correlated with, SFDH (%), PWSFE and NSFE and negative correlation was observed with TD_{aba} and TD_{aba} in post rainy season.

The coloured plumule of the leaf has also been found to be associated with shoot fly resistance/susceptibility. In the present studies, the LSP exhibited positive correlation with SFDH (%), whereas, it was negatively correlated with TD_{aba} and TD_{ada} during 2016 rainy season. Similarly, LSP exhibited positive correlation with SFDH (%), PWSFE and NSFE, and negative correlation with TD_{ada} (−0.378*) during the 2016 post rainy season. A similar trend was observed during the rainy and post rainy seasons in 2017.

3.3. Biochemical composition of sorghum genotypes in relation to expression of resistance to shoot fly

3.3.1. Total soluble sugars (%) (TSS)

TSS concentration in sorghum genotypes was higher during the rainy season as compared to the post rainy season (Fig. 11). The percentage TSS content varied from 8.63 to 17.01 with an average value of 14.99 (SE ± 0.30) during the rainy season, 2017. The genotype ICSA 52 (17.01) recorded maximum TSS content, followed by ICSA 323 (16.99), ICSB 84 (16.86) and ICSV 25292 (16.72), while SSV 74 recorded minimum TSS (8.63). During the post rainy season, the TSS (%) ranged from 4.05 to 14.51, with a mean value of 6.53% (SE ± 0.35). The lowest TSS content was recorded in ICSA 467 (4.05), followed by ICSB 52 (4.11), ICSB 467 (4.26), ICSB 14024 (4.58) and ICSB 487 (4.70), the maximum TSS content was observed in ICSA 52 (14.51).

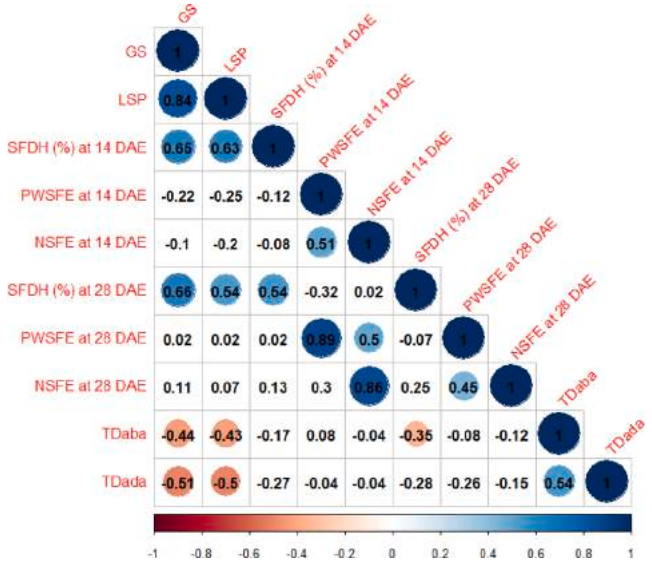

Fig. 7. The Pearson correlation matrix for rainy season 2016.

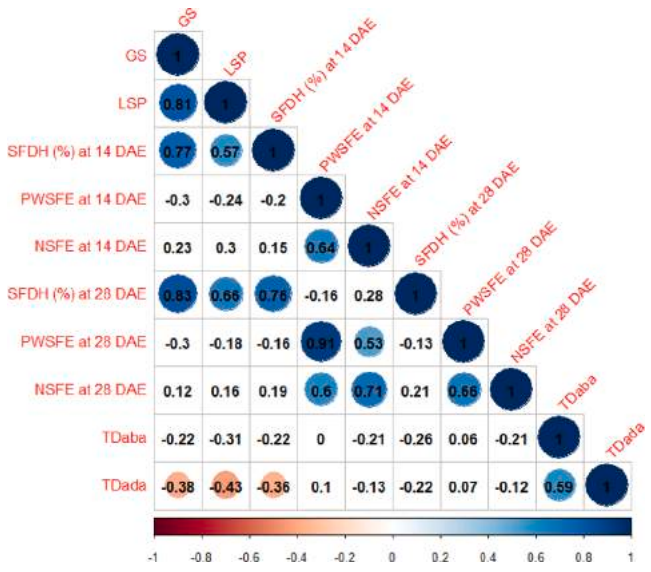

Fig. 8. The Pearson correlation matrix for rainy season 2017.
Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circles are proportional to the correlation coefficients.

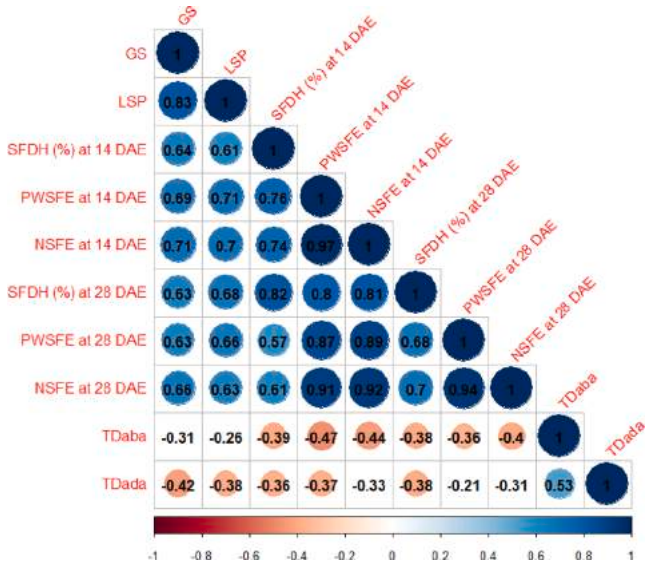

Fig. 9. The Pearson correlation matrix for post-rainy season 2016.
Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circles are proportional to the correlation coefficients.

3.3.2. Protein content (mg/g)

The protein content was higher in rainy season than in the post rainy season, but the differences were trivial (Fig. 12). In rainy season, the

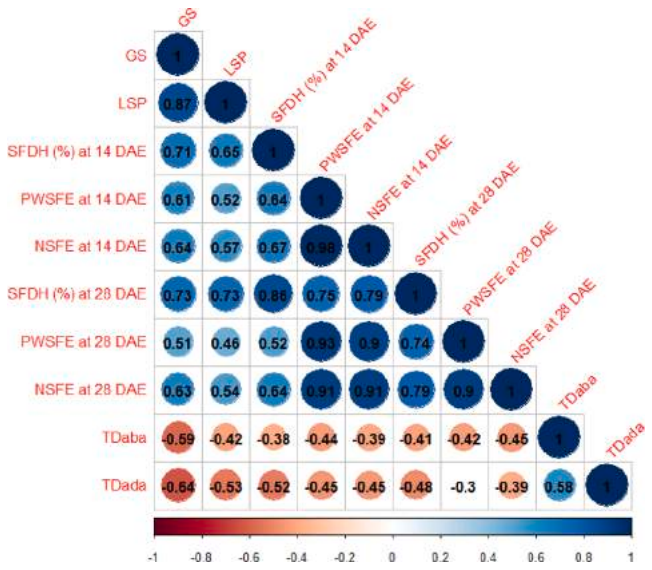

Fig. 10. The Pearson correlation matrix for post-rainy season 2017. Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circles are proportional to the correlation coefficients.

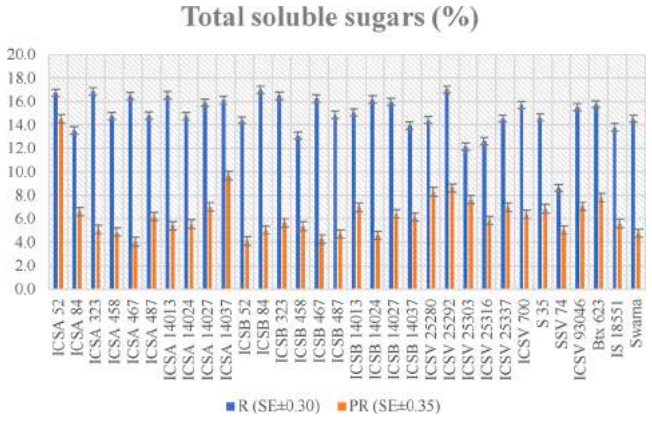

Fig. 11. Total soluble sugar percentage (TSS%) in test genotypes during rainy and post rainy seasons, 2017.

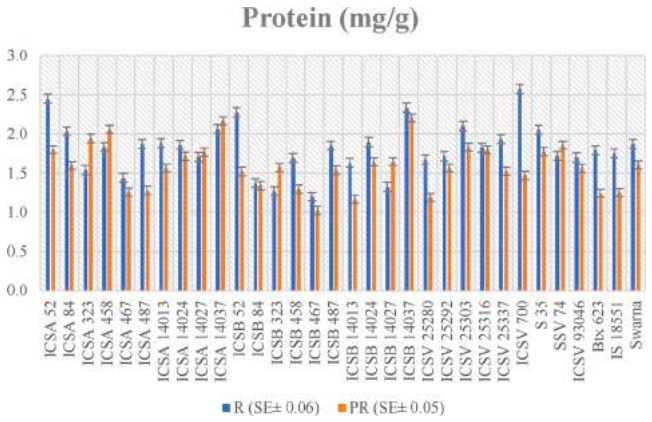

Fig. 12. Protein content in test genotypes during rainy and post rainy seasons, 2017.

protein content ranged from 1.20 to 2.58, with an average value of 1.82 (SE ± 0.06). ICSB 467 recorded lowest protein content (1.20), followed by ICSB 323 (1.27), ICSB 14027 (1.32), ICSB 84 (1.37) and ICSA 467 (1.44). While, the highest protein content was recorded in ICSV 700 (2.58). The shoot fly resistant genotypes ICSA/B 467, IS 18551, ICSB 487, ICSB 84, ICSB 14024 and ICSV 25292 had relatively lower protein content than the susceptible ones. During the post rainy season, the protein content ranged from 1.03 to 2.20, with a mean value of 1.59 (SE ± 0.05). The genotype ICSB 467 had minimum amount of proteins, followed by ICSB 14013, ICSV 25280, BTx 623 and IS 18551, the highest protein content was recorded in ICSB 14037 (2.20 mg/g of sample).

3.3.3. Condensed tannins (mg catechin/g)

During the rainy season, the tannin content (mg catechin/g) varied from 18.64 to 56.62, with an average value of 38.48 (SE ± 1.64) (Fig. 13). The genotypes BTx 623 and ICSA 458 recorded the maximum value of 56.62, followed by ICSV 25280 (51.22) and ICSV 25316 (48.61). Lowest amounts were recorded in ICSB 84 (18.64), followed by ICSA 323 (24.22), ICSB 323 (25.378), ICSA 14013 (27.35) and ICSB 14027 (27.53). The shoot fly resistant genotypes had more amounts of condensed tannins, although there were a few exceptions (BTx 623 and ICSA 458). During the post rainy season, maximum amount of condensed tannins was recorded in ICSB 52 (59.23), followed by ICSB 52 (47.91). The lowest amount of condensed tannins was recorded in ICSA 14037 (9.06), followed by the susceptible check, Swarna (12.54), ICSB 84 (13.07), ICSV 25337 (16.72) and ICSV 25292 (16.90). The tannin content was higher in the post rainy season compared to the rainy season.

3.3.4. Total phenols (mg/g)

There were no significant differences in phenol content of the leaves across seasons (Fig. 14). During the rainy season, the phenol content ranged from 6.57 to 11.30 (mg/g sample), with an average value of 9.06 (SE ± 0.20). The genotype BTx 623 recorded highest phenol content (11.30) followed by ICSA 14037 (11.23), ICSB 52 (10.50) and ICSB 458 (10.44). ICSB 323 exhibited lowest value of 6.57, followed by ICSB 14027 (7.03), ICSV 25280 (7.68), ICSV 25337 (7.80) and ICSB 467 (7.85). During the post rainy season, the amounts of phenols in test genotypes ranged from 7.62 to 11.68 mg/g, with an average of 10.73 (SE ± 0.17). The genotypes ICSV 25316 (11.68) and ICSA 14037 (11.66) recorded maximum phenol content, followed by S-35 (11.54), ICSB 458 (9.86), ICSA 467 (9.58), ICSB 14013 (9.34) and ICSB 84 (8.18). The lowest phenol content was recorded in ICSB 467 (7.62).

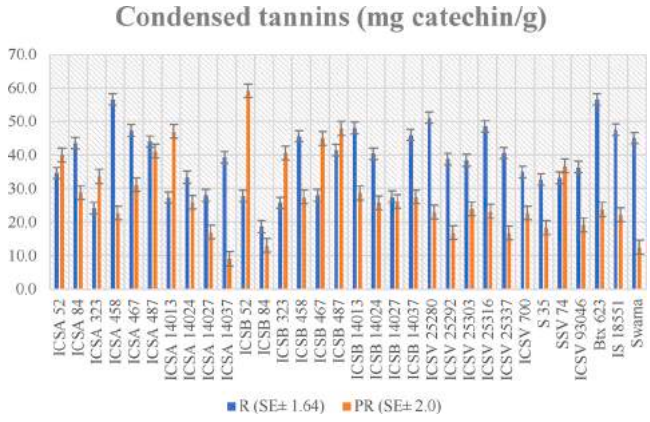

Fig. 13. Concentration of condensed tannins in test genotypes during rainy and postrainy seasons, 2017.

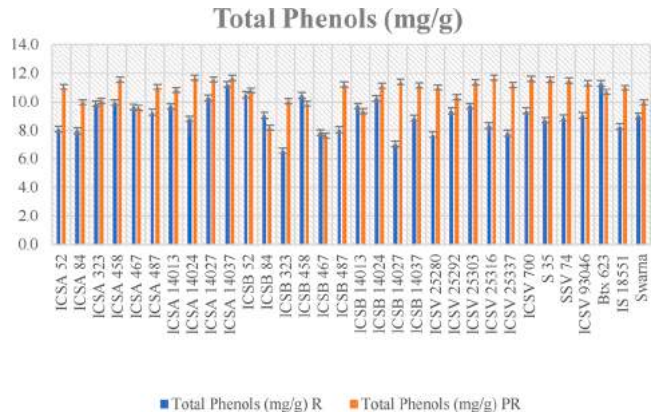

Fig. 14. Phenol content in test genotypes during rainy and post rainy seasons, 2017.

3.4. Association of biochemical components with expression of resistance to sorghum shoot fly, A. soccata using Principal coordinate analysis

The association of shoot fly damage in terms of shoot fly eggs and deadhearts was measured using Principal coordinate analysis. The susceptible genotypes formed distinct clusters (Fig. 15) exhibiting higher association with shoot fly deadheart (%), number of shoot fly eggs at 14 and 28 DAE. Similarly, the resistant genotypes also formed distinct clusters exhibiting positive association with condensed tannins and negative correlation with total number of eggs and deadheart incidence at 14 and 28 DAE.

3.5. Metabolite profiling using HPLC and GC-MS analysis

3.5.1. HPLC fingerprinting

The HPLC fingerprinting of methanol extracts of leaf samples yielded 25 different peaks in total with varying peak areas among the genotypes tested. From these, a total of 12 compounds were identified (matching with the reference set library) and quantified (Tables 3–5). The phenolic constituents and their concentrations varied during the rainy and post rainy seasons. Many common peaks were observed between retention time 2.89–13.96 min. The resistant check, IS 18551 recorded 13 peaks in total, with four known compounds (3,7,4- Trihydroxy flavone, Coumaric acid, Gentisic acid and Protocatechuic acid), while the susceptible check, Swarna recorded 12 peaks in total with four known compounds (3,7,4- Trihydroxy flavone, Gentisic acid, Protocatechuic acid and Naringenin). These genotypes had 7 peaks in common, with three common compounds in varying concentrations. The concentration of different compounds differed between the rainy and post rainy seasons. Swarna showed the presence of Naringenin in the post rainy season only, but it was absent in IS 18551 in both the seasons. Coumaric acid was found only in IS 18551. Protocatechuic acid and 3,7,4-Trihydroxy flavone were present in higher concentrations in Swarna in both the seasons, while Protocatechuic acid was absent in IS 18551 during the rainy season. The HPLC chromatograms for resistant and susceptible checks along with few other genotypes are shown in Fig. 16(A–H)

3.5.2. Leaf surface chemistry using GCMS

While studying the leaf surface chemicals using GC–MS analysis, several compounds were identified in resistant and susceptible genotypes, and there were significant differences among the genotypes tested in terms of presence or absence and their concentration. The spectrum of the compounds detected in the samples matched >90 % with the compounds in the library (HIT 1). A total of 150 compounds were identified, of which 13 unique compounds were associated with shoot fly resistance or susceptibility. Of the major compounds detected, hexanal was present in both the resistant (IS 18551) and susceptible, (Swarna) checks (Table 6), but the peak areas were greater in the resistant check, IS 18551 (Fig. 17).

Nickel carbonyl (Ni $(CO)_4$), 4-t-Butyl-2-methoxypiperidine was

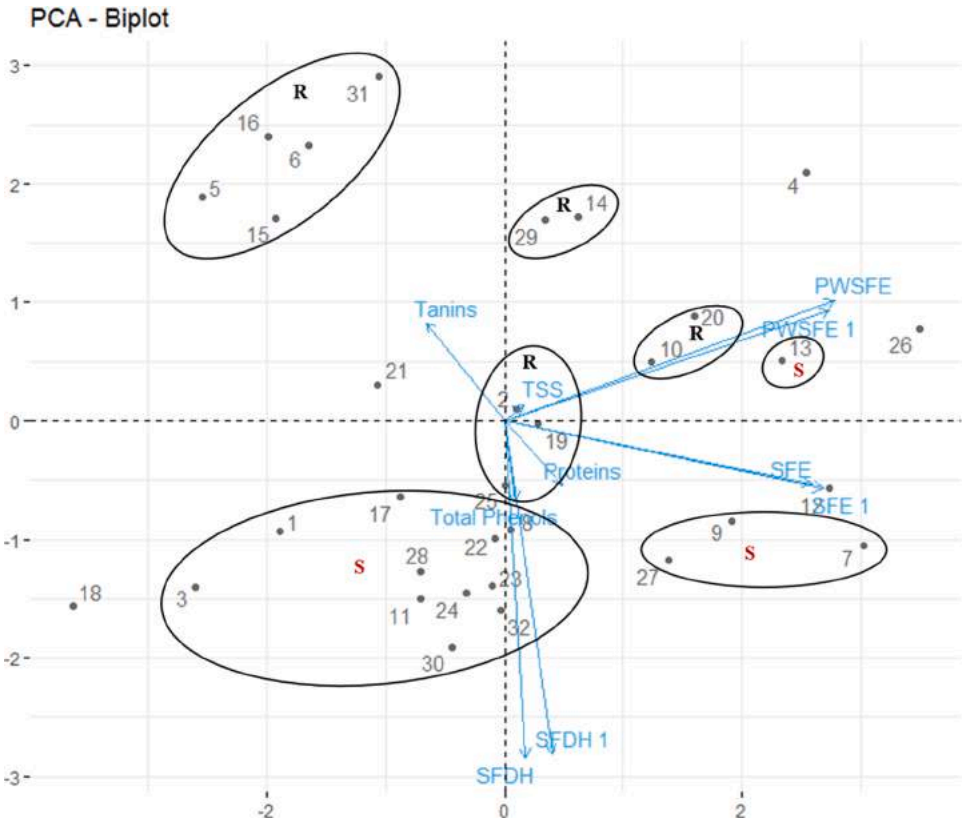

Fig. 15. Principal Coordinate Analysis (PCA) based on shoot fly damage.
(SFDH and SFDH 1: shoot fly deadhearts at 14 days and 28 DAE respectively) and shoot fly eggs along with (SFE and SFE 1: shoot fly eggs at 14 and 28 days after emergence; PWSFE and PWSFE 1: plants with shoot fly eggs at 14 and 28 days after emergence) with biochemical traits viz. Phenol content, Total soluble sugars, Condensed tannins and Protein content (1 = ICSA 52, 2 = ICSA 84, 3 = ICSA 323, 4 = ICSA 458, 5 = ICSA 467, 6 = ICSA 487, 7 = ICSA 14013, 8 = ICSA 14024, 9 = ICSA 14027, 10 = ICSA 14037, 11 = ICSB 52, 12 = ICSB 84, 13 = ICSB 323, 14 = ICSB 458, 15 = ICSB 467, 16 = ICSB 487, 17 = ICSB 14013, 18 = ICSB 14024, 19 = ICSB 14027, 20 = ICSB 14037, 21 = ICSV 25280, 22 = ICSV 25292, 23 = ICSV 25303, 24 = ICSV 25316, 25 = ICSV 25337, 26 = ICSV 700, 27 = S 35, 28 = SSV 74, 29 = ICSV 93046, 30=BTx 623, 31 = IS 18551, 32=Swarna).
R corresponds to moderate to highly resistant while S corresponds to moderate to highly susceptible genotypes.

Table 3
Flavonoids detected in male sterile (A) lines during rainy (R) and post rainy season (PR).

Flavonoids (µg/100 µg)	Season	ICSA 52	ICSA 84	ICSA 323	ICSA 458	ICSA 467	ICSA 487	ICSA 14013	ICSA 14024	ICSA 14027	ICSA 14037
3,4 Dihydroxy Benzoic acid	R	0.30	1.39	0.36	0.23	0.42	–	0.91	–	–	0.53
	PR	0.25	0.31	0.31	0.04	–	0.23	–	0.27	0.52	0.53
Protocatechuic acid	R	–	–	–	–	–	–	0.90	0.71	0.63	0.67
	PR	–	–	–	–	–	–	0.90	0.74	0.36	0.54
Chlorogenic acid	R	4.70		3.16	2.47	2.84	2.49	–	–	–	–
	PR	4.96	3.92	5.07	3.00	1.39	2.72	–	–	–	–
Fisetin	R	1.03	–	–	–		–	–	–	–	–
	PR	5.04	–	–	–		–	–	–	–	–
3,7,4 Trihydroxy flavone	R	–	–	–	–	0.07	–	–	–	2.09	4.70
	PR	1.41	–	0.08	–	–	–	10.74	–	1.53	0.05
Gentisic acid	R	10.59	15.15	8.42	10.27	6.63	9.42	7.72	3.90	8.29	12.95
	PR	17.30	11.81	6.57	10.27	4.61	9.42	5.53	3.67	9.69	12.95
Vanillic acid	R	–	–	1.14	–	–	–	–	–	–	–
	PR	–	–	–	1.16	–	–	–	–	–	0.91
Coumaric acid	R	0.82	–	–	2.48	3.51	2.42	0.26	–	0.21	1.12
	PR	1.99	–	1.40	2.60				–	2.43	1.12
Ferulic acid	R	–	–	–	–	–	–	–	–		0.84
	PR	–	–	–	–	–	–	–	–	0.28	0.67
Formononetin	R	1.45	–	–	–	–	–	–	–	–	10.95
	PR	1.45	–	–	–	–	–	–	–	0.44	–
Naringenin	R	–	–	–	–	0.50	0.49	–	–	1.07	–
	PR	–	–	–	0.43	–	0.15	0.05	–	–	–

Table 4
Flavonoids detected in maintainer (B) lines during rainy (R) and post rainy season (PR).

Flavonoids (µg/100 µg)	Replication	ICSB 52	ICSB 84	ICSB 323	ICSB 458	ICSB 467	ICSB 487	ICSB 14013	ICSB 14024	ICSB 14027	ICSB 14037
3,4 Dihydroxy Benzoic acid	R	0.29	–	–	–	–	–	–			
	PR	–	–	–	–	–	–	–	0.55	0.47	32.29
Naringin	R	–	–	–	–	–	–	–	–	–	
	PR	–	–	–	–	–	–	–	–	–	21.61
Gallic acid	R	–	–	–	–	–	–	–	–	–	
	PR	–	–	–	–	–	–	–	–	–	0.29
Protocatechuic acid	R	0.95	1.00	0.87	0.03	0.73	0.52	0.69	1.05	1.15	1.00
	PR	1.01	1.02	0.49	0.68	0.59	0.97	0.72	0.71	0.63	–
3,7,4 Trihydroxy flavone	R	–	–	5.14	0.23	0.78	1.61	10.83	2.97	2.87	6.59
	PR	0.02	5.63	1.72	7.92	5.57	0.38	2.38	1.53	1.46	5.77
Gentisic acid	R	9.40	6.58	2.88	0.60	6.45	5.75	6.62	5.48	6.04	5.58
	PR	8.84	6.21	–	5.37	5.30	10.20	10.05	16.93	12.72	20.65
Vanillic acid	R	–	–	–	–	–	–	–	–	–	–
	PR	–	–	–	–	–	–	–	–	0.04	0.79
Coumaric acid	R	1.08	2.69	–	0.35	–	–	0.27	–	1.86	–
	PR	1.40	1.67	–	1.13	–	2.90	0.15	1.99	–	–
Ferulic acid	R	–	–	–	–	–	–	0.33	–	0.42	0.53
	PR	–	–	–	–	–	0.86	0.66	0.62	0.97	0.70
Naringenin	R	–	0.90	–	–	–	–	–	–	–	–
	PR	–		–	–	–	1.21	–	0.91	1.17	2.93

Table 5
Flavonoids detected in restorer (R) lines during rainy (R) and post rainy season (PR).

Flavonoids (µg/100 µg)	Season	ICSV 25280	ICSV 25292	ICSV 25303	ICSV 25316	ICSV 25337	ICSV 700	S 35	SSV 74	ICSV 93046	Btx 623	IS 18551	Swarna
3,4 Dihydroxy Benzoic acid	R	0.60	0.62	–	–	–	–	–	–	–	–	–	–
	PR	–	–	–	–	0.40	–	–	–	–	–	–	–
Protocatechuic acid	R	–	–	0.72	0.61	0.80	1.25	0.88	0.53	0.63	0.58	–	0.61
	PR	–	0.16	1.12	0.90	1.65	0.66	–	0.74	1.04	1.01	0.70	0.93
3,7,4 Trihydroxy flavone	R	1.70	2.17	4.01	2.58	2.03	3.53	5.33	1.31	3.27	2.36	1.31	2.59
	PR	–	1.00	2.55	3.24	5.08	7.23	–	3.45	6.41	10.02	4.35	10.42
Gentisic acid	R	–	–	4.40	5.48	5.00	10.77	5.04	3.48	3.28	–	6.03	2.47
	PR	7.95	1.28	4.43	8.73	10.79	7.07	–	5.67	5.20	–	8.54	5.66
Coumaric acid	R	–	–	0.21	1.17	1.36	2.19	–	0.86	1.00	–	1.67	–
	PR	–	–	1.15	1.67	–	0.91	–	0.99	–	–	1.30	–
Ferulic acid	R	–	–	–	–	–	–	–	–	–	–	–	–
	PR	–		1.22	0.88	–	–	–	–	–	–	–	–
Naringenin	R	–	0.50	–	–	–	1.66	–	–	–	–	–	0.76
	PR	–	0.50	–	–	–	1.66	–	–	–	–	–	0.76

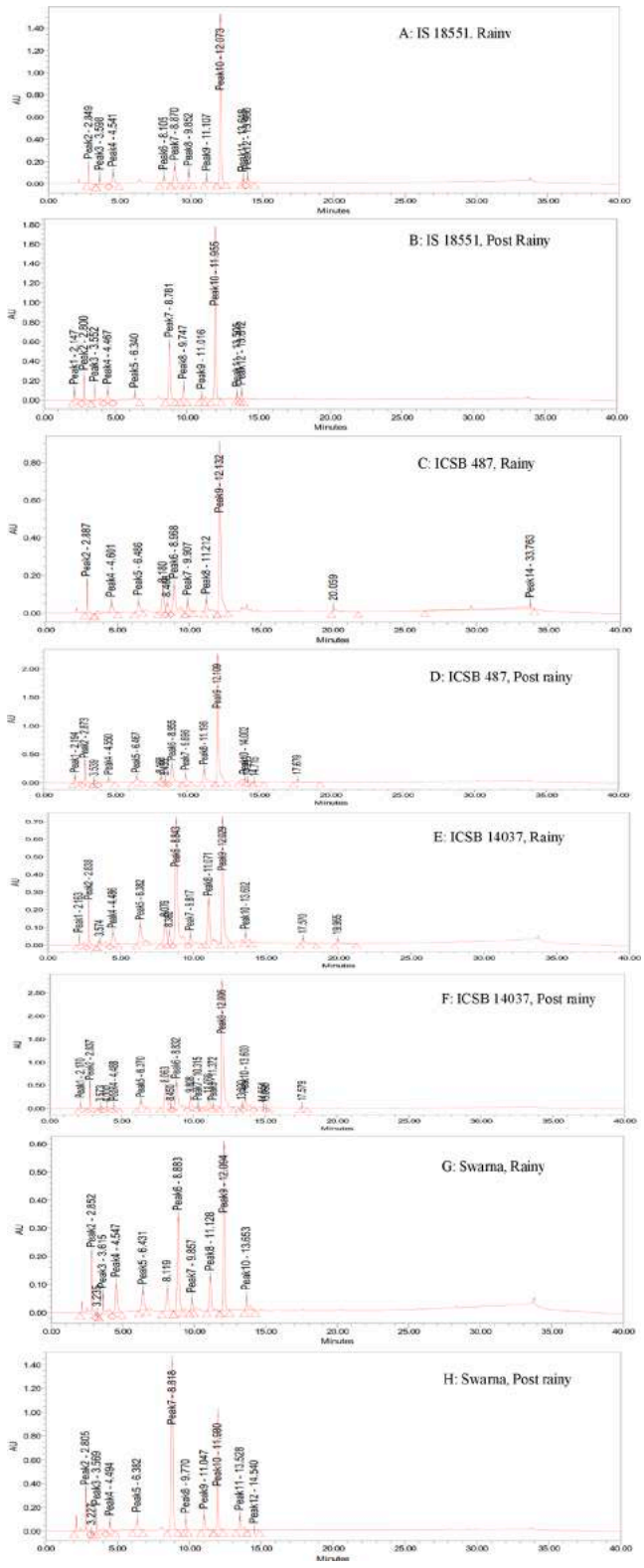

Fig. 16. (A-H) HPLC chromatographic profiles of phenolic compounds for checks and selected test genotypes.

present in Swarna and some other susceptible genotypes, but was absent in the resistant check, IS 18551 (Table 6). Furan, pentacosane and dotriacontane were present in IS 18551. Similarly, Pentadecane, 8 - hexy and lonol 2 were present only in the susceptible genotypes, Swarna and BTx 623, but absent in rest of the genotypes. Dodecane, 2, 6, 11- trimethyl was present only in the shoot fly susceptible genotypes ICSA 52, ICSA 323, ICSV 25303, ICSV 25316, SSV 74, BTx 623 and Swarna, but absent in resistant genotypes (except ICSB 14037 with smaller peak area). Compound 4, 4 dimethyl cyclooctene was present in the resistant genotypes IS 18551, ICSV 25337, and ICSV 700, but absent in all other genotypes; while hexane 2, 4-dimethyl was absent in resistant genotypes, but present in rest of the genotypes. Compound undecane, 5-methyl was present in all the genotypes, except in ICSV 25292, ICSV 25303 and Swarna. Compound eicosane was present in all genotypes, except in BTx 623. Higher amount of eicosane was present in ICSB 467 and ICSB 487. 3-Hexanone (CAS) Hexan-3-on was present in all genotypes with varying peak areas. Similarly, the compounds like lonol, 2, Furan,2,3-dihydro, Benzene, 1,3-bis (1,1-dimethylethyl)-(CAS) 1,3-Di-tert-butylbenzene$$ m-Di-tert-butylbenzene $$ Benzene, m-di-tert-butyl-$$ and Tetra deca methyl cyclo hepta siloxane, Cyclo-heptasiloxane, tetradecamethyl-(CAS) were found to be associated with the resistance to shoot fly in terms of less damage as compared to the susceptible genotypes.

Table 6
Compounds present/absent in resistant and susceptible checks based on GC-MS fingerprinting.

Compound	Resistant Check (IS 18551)	Susceptible Check (Swarna)
Benzene, 1,3-dimethyl-(CAS) m-Xylene $$ m-Xylol $$ 1,3-Xylene $$ 2,4-Xylene $$ m-Dimethylbenzene $$ 1,3-Dimethylbenzene $$ m-Methylbenzene $$	–	+
Cyclohexane, 1,3-dimethyl-, cis- (CAS) cis-1,3-Dimethylcyclohexane $$ cyclohexane, 1,3-dimethyl-, (Z)- $$ 1, cis-3-Dimethylcyclohexane	+	–
7- Hydroxyheptene-1 (CAS)	+	–
3-Hexen-1-ol, (Z)- (CAS) cis-3-Hexene-1-ol $$ Z-3-Hexenol $$ Leaf alcohol $$ 3-(Z)-Hexenol $$ cis-3-Hexenol $$ Blatteralkohol $$ cis-3-Hexanol $$	+	–
Benzene, 1,2,3-trimethyl-(CAS) 1,2,3-Trimethylbenzene $$ 1,2,3 TRIMETHYLBENZENE $$ Hemimellitene $$ Hemellitol $$	–	+
Compound undecane, 5-methyl	+	+
Compound 4, 4 dimethyl cyclooctene	+	–
Pentadecane, 8 - hexy and lonol 2	–	+
Nickel carbonyl (Ni $(CO)_4$), 4-t-Butyl-2-methoxypiperidine	–	+

4. Discussion

In view of the huge economic losses caused by sorghum shoot fly, it is important to develop varieties with resistance to this pest. The results of the present studies for assessing the diversity of mechanisms of resistance to shoot fly indicated that several morphological and biochemical traits contribute to shoot fly resistance. Sorghum genotypes with lesser number of plants with deadhearts are resistant to shoot fly, and this trait has been used to identify sorghum genotypes with resistance to shoot fly damage (Dhillon et al., 2005). Plants with low oviposition exhibit lower incidence of deadhearts (Gomashe et al., 2010), suggesting that oviposition non-preference is the principal component of resistance to shoot fly damage in sorghum.

The genotypes with diverse morphological and biochemical traits can be used as donors in sorghum breeding programs. Low levels of damage by shoot fly are associated with non-preference for oviposition (Gomashe et al., 2010), glossy leaves, and leaf sheath pigmentation, which can be used as morphological markers for selecting the genotypes exhibiting resistance to shoot fly at the seedling stage itself (Dhillon et al., 2005). Trichome density on the adaxial surface of leaf is associated with expression of resistance to shoot fly as the trichomes possibly hinder the movement of shoot fly maggot on the underside of leaf,

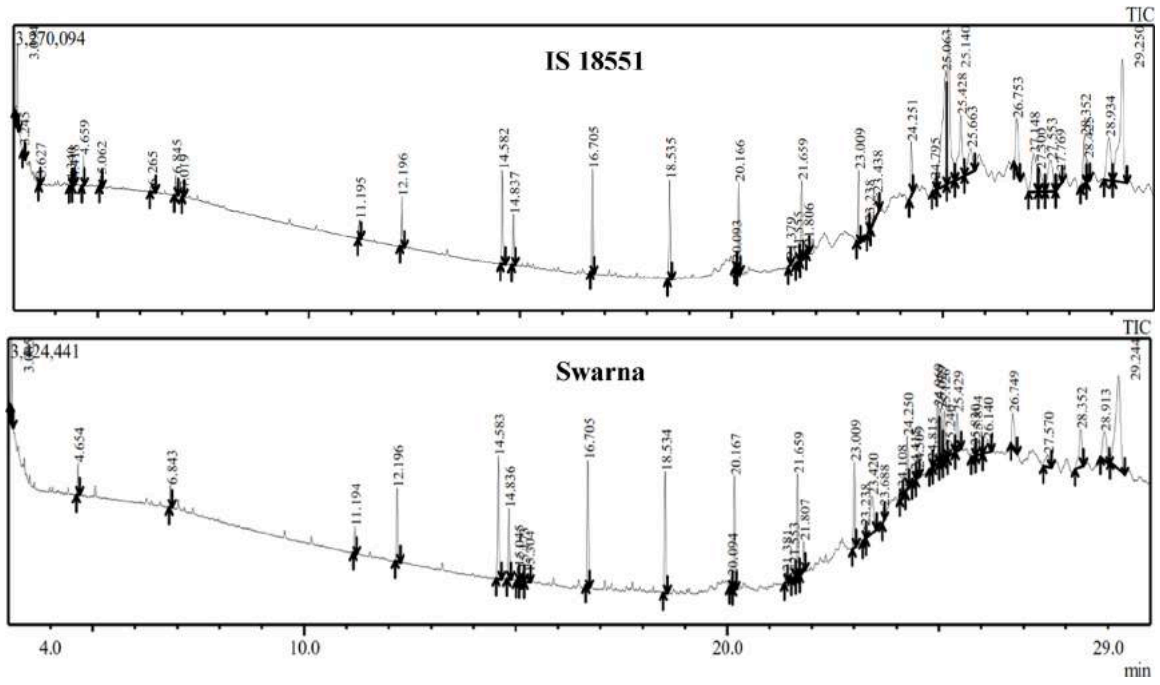

Fig. 17. GCMS chromatograms of resistant (IS 18551) and susceptible (Swarna) checks.

thereby, restricting it to reach the growing point (Sharma and Nwanze, 1997; Dhillon et al., 2005; Gomashe et al., 2010). The present studies suggested that trichome density on both the leaf surfaces was associated with genotypic resistance to sorghum shoot fly.

Expression of host plant resistance to insects is governed by a wide array of complex metabolic processes, which are involved in production of secondary metabolites (Chamarthi et al., 2011). The plants lacking such mechanisms are prone to damage by insect pests in terms of plant growth and grain yield. Micronutrients and biochemical constituents such as total soluble sugars, proteins, condensed tannins and phenols are associated with the resistance or susceptibility to sorghum shoot fly (Chamarthi et al., 2011; Riyazaddin et al., 2015). The results of present studies suggested that biochemical components influence the expression of susceptibility/resistance to shoot fly. The genotypes (ICSA 84 and ICSA 467, ICSB 52, ICSB 458 and ICSB 14024) exhibiting resistance to shoot fly damage had lower amounts of soluble sugars (during post rainy season), but had high amounts of condensed tannins, although there were a few exceptions (Kamatar et al., 2003 and Chamarthi et al., 2011). Similarly, the higher protein content has been associated with shoot fly susceptibility (Kamatar et al., 2003 and Singh et al., 2004). The resistant genotypes (ICSA/B 467, ICSB 84, ICSB 487, ICSB 14024 and IS 18551 had lower protein content than the susceptible genotypes. Phenolic compounds impart resistance to wheat aphid (Juan et al., 2001), and maize stem borer (Kabre and Ghorpade, 1999). Among the phenolic compounds, Gentisic acid was present in almost all the genotypes, with few exceptions in the present study. Vanillic acid, which is associated with susceptibility to shoot fly (Pandey et al., 2005), was present only in one susceptible genotype (ICSA 323) during the rainy season, However, vanillic acid was present in a few genotypes (ICSB 458, ICSB 14027 and ICSA/B 14037) exhibiting moderate to high levels of resistance to shoot fly damage. 3,4-Dihydroxy benzoic acid which is associated with oviposition preference to shoot fly (Alborn et al., 1992), was present in all A lines, (except ICSA 487, 14024 and 14027), but absent in B and R lines with a few exceptions (ICSB 52 and ICSV 25280, ICSV 25292). Protocatechuic and coumaric acids have also been associated with resistance to shoot fly. Although, both these compounds were present in most of the sorghum genotypes, but their amounts were greater in the shoot fly resistant genotypes (ICSB 84, ICSA 467, ICSA 487 and ICSV 700) than in the susceptible genotypes (Swarna). Certain compounds were present in a few genotypes only, suggesting their limited contribution towards resistance to shoot fly, like Formononetin was present in ICSA 52, ICSA 14037, and Fisetin, only in ICSA 52. Chlorogenic acid was also present in few genotypes, with higher concentration among the seedlings of the susceptible genotypes.

The oviposition preference of shoot fly is influenced by the volatiles secreted by seedlings (Nwanze, 1997). In present study, several chemical compounds such as Undecane 5-methyl; decane 4-methyl; hexane 2, 4-methyl; pentadecane 8hexyl and dodecane 2, 6, 11- trimethyl were putatively linked with shoot fly susceptibility, and there is a need for studying the exact mechanisms underlying this phenomenon (Chamarthi et al., 2011).

There is also a need to study the biochemical pathways of the identified compounds and understand their role in expression of resistance/susceptibility to sorghum shoot fly. There were a large number of compounds with unidentified HPLC/ GC–MS peaks, which could be associated with the resistance or susceptibility to shoot fly.

5. Conclusions

The current study was planned to understand the mechanisms of resistance to shoot fly in a diverse array of sorghum genotypes. The results suggested that morphological traits such as leaf glossiness, leaf sheath pigmentation, and presence of trichomes on the upper and lower surface of leaves can be used as markers in screening and breeding for resistance to sorghum shoot fly, *A. soccata*. The results of the present investigations also indicated that biochemical constituents have a considerable bearing on genotypic resistance or susceptibility to shoot fly damage. These morphological and biochemical components can be used as markers to develop shoot fly resistant genotypes for sustainable crop production. Based on the morphological and biochemical traits studied, the genotypes used in the current studies were classified as shoot fly resistant or susceptible. (Table 7).

Author contributions

Idea and project conceived: JJ, AAK, RSS HCS. Experiment carried

Table 7
Classification of test genotypes based on their reaction to shoot fly damage.

Response to shoot fly damage	Genotypes
Resistant (R)	ICSA/B 467, ICSA/B 487, ICSV 93046, IS 18551
Moderately resistant (MR)	ICSA/B 84, ICSB 458, ICSB 14024, ICSB 14027, ICSA/B 14037, ICSV 700, ICSV 25337
Susceptible (S)	ICSA/B 52, ICSA 458, ICSA 14024, ICSA 14027, ICSV 25280, ICSV 25292, ICSV 25303, ICSV 25316, S-35
Highly susceptible (HS)	ICSA/B 323, ICSA/B 14013, BTx 623, SSV 74, Swarna

out: NA, SPM and JB. Data analysis: NA, SPM and RN. Manuscript writing: NA, SPM, RN, JJ, JB and AAK.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The funding for this research was provided by Asia-Pacific Association of Agricultural Research Institution (AAPARI) Bangkok, 10100, Thailand through the CRP Dryland Cereals Scholarship Program and Sorghum Breeding, ICRISAT. First author expresses sincere thanks to these funding agencies for providing financial assistance, support for conducting research and other activities at ICRISAT. ICRISAT is a part of CGIAR consortium. Dr Naveen Arora, Ms. Joorie Bhattacharya and Mr. Rahul Nitnavare extend their warm gratitude and appreciation to Dr John Foulkes (Associate Professor, University of Nottingham) for his generous and invaluable feedback on the manuscript. Authors also express their sincere thanks to Mr. P Venkata Ramana, Mrs. P Ponnamma, Dr G Siva Kumar, Dr. Naresh Kumar, Dr Sumit Vashisth; Mr. Dhruva, Mr. Ranga Reddy, Mr. Veera Shetty and Mr. K Rajendra Prasad.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2020.108029.

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