

**RESEARCH ARTICLE**



# **Biophysical and biochemical parameters of Sorghum associated to shoot fly resistance**

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## **ARTICLE HISTORY**

Received: 23 September 2024 Accepted: 18 October 2024 Available online Version 1.0 : 06 November 2024

Check for updates

## **Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Mohana J, Murugan M, Srinivasan T, Kavithamani D, Meenakshi P, Prakash K. Biophysical and Biochemical Parameters of Sorghum Associated to Shoot Fly Resistance. Plant Science Today.2024;11(sp4):01-20. <https:/doi.org/10.14719/pst.5220>

# **Abstract**

The sorghum shoot fly, *Atherigona soccata* (Muscidae: Diptera), represents a significant biotic constraint to sorghum production, leading to considerable yield losses globally. This study aimed to systematically classify sorghum genotypes based on their resistance to *A. soccata* infestation. A total of 188 genotypes were subjected to rigorous evaluation employing standardized screening methodologies. The analysis revealed substantial variability in resistance levels across the genotypes. Based on damage assessments in field trials, 14 genotypes were selected for further investigation under controlled pot culture conditions. Comprehensive biochemical analyses were conducted on each genotype under both uninfested and infested scenarios. Among the evaluated genotypes, IS 10588 and IS 8380 exhibited high levels of resistance, IS 12787 demonstrated moderate resistance, while TNFS 230 was classified as moderately resistant to *A. soccata* infestation. Critical morphological and biochemical traits associated with resistance were identified, including trichome density, leaf glossiness, and enzyme activity levels of peroxidase (PO), polyphenol oxidase (PPO), tannins and phenolic compounds. The study concludes that these morpho-physiological and biochemical characteristics contribute significantly to the resistance mechanisms in sorghum against *A. soccata*. Thus, these identified genotypes may serve as valuable genetic resources for breeding programs aimed at enhancing resistance to *A. soccata* in sorghum.

## **Keywords**

sorghum; shoot fly; resistance; biophysical; biochemical

# **Introduction**

Sorghum (*Sorghum bicolor*) is an ancient cereal grain cultivated across diverse climatic regions, recognized for its nutritional profile and adaptability in various culinary applications. It is notably rich in phenolic compounds, many of which exhibit antioxidant properties, rendering it particularly beneficial for individuals with gluten intolerance or celiac disease. The intricate starch composition of sorghum results in slow digestibility, which is advantageous for managing certain health conditions. Additionally, sorghum's reduced requirement for water and fertilizers relative to other cereal crops positions it as a critical element in sustainable agricultural practices [\(https://](https://sorghumgrowers.com) [sorghumgrowers.com\)](https://sorghumgrowers.com). While sorghum is cultivated globally, Sudan, Nigeria, India and the USA collectively account for approximately 57% of the

worldwide sorghum cultivation area and 45% of its total production.

Despite its agricultural significance, sorghum is susceptible to various pests, including stem borers, shoot flies, green bugs, midges, leaf beetles, aphids and earhead bugs, leading to an estimated annual yield loss exceeding US\$1 billion (1). Among these pests, the sorghum shoot fly, *Atherigona soccata*, poses a particularly grave threat, contributing to economic losses estimated at over US\$274 million annually (2). The adult shoot fly oviposits eggs on the abaxial surface of sorghum leaves, typically near the midrib (3, 4). Following a 2-4 day incubation period, larvae (maggots) emerge and migrate to the leaf's adaxial surface, subsequently penetrating the stem at the growing point. The larvae feed on the senescent tissues of the plant, resulting in the characteristic "deadheart" symptom, which severely impairs plant vigor. Upon completion of feeding, the larvae pupate within the stem or at the soil surface, with the pupal stage lasting approximately 7-10 days before adult emergence (5). The developmental cycle of the shoot fly, influenced by abiotic factors such as temperature and humidity, typically spans 21-28 days. This rapid lifecycle, characterized by high fecundity and multiple generations annually, is exacerbated by the pest's ability to utilize alternative host plants, including other cereals and weeds. Furthermore, the widespread cultivation of improved but susceptible sorghum varieties, continuous cropping practices, and reduced genetic variability within sorghum populations have collectively facilitated the establishment of the shoot fly as a predominant pest in sorghum agroecosystems (6).

Integrated pest management (IPM) strategies for controlling the sorghum shoot fly encompass practices such as timely scouting, adjusting planting dates, increasing seed densities, removing and disposing of infested seedlings, treating seeds with insecticides and employing cultural practices such as intercropping and crop rotation (7). However, several challenges hinder the effective implementation of these IPM strategies, including climate variability leading to unpredictable rainfall, the escalating costs of insecticides, the emergence of insecticide-resistant pest populations, environmental contamination from chemical inputs, and a shortage of labor for mechanical pest control (8). Host plant resistance (HPR) represents a foundational approach in sustainable crop protection, particularly for sorghum, where water scarcity frequently limits yields and farmers are often reluctant to invest in costly chemical inputs. HPR allows for the transmission of resistance traits to subsequent generations, providing a long-term pest management solution that can be augmented with other IPM strategies as necessary. This resistance is particularly beneficial for managing pests like the shoot fly, which predominantly target the vulnerable early seedling stages, complicating pest monitoring and control efforts. If left unmanaged, shoot fly infestations can lead to poor crop establishment and significant resource wastage due to insufficient plant cover.

In light of the benefits of employing HPR to manage *A. soccata*, ongoing efforts are focused on identifying resistant genotypes and elucidating the mechanisms underlying their resistance. This study aimed to evaluate 188 sorghum genotypes for resistance to *A. soccata* infestation, with findings contributing to the understanding of resistance mechanisms and aiding future breeding programs.

# **Materials and Methods**

## **Plant Material**

During the late Rabi season (December to April) of 2023- 2024, a total of 188 sorghum genotypes maintained by the Department of Millets at Tamil Nadu Agricultural University, Coimbatore, India, were systematically screened for resistance against the sorghum shoot fly (*Atherigona soccata*). Alongside these genotypes, two resistant controls (IS18551 and IS2205) and two susceptible controls (DJ6514 and Swarna) were incorporated into the evaluation to provide benchmarks for resistance assessment.

## **Field Screening**

The field screening experiment was conducted utilizing a randomized complete block design (RCBD) with three replications. Each genotype was planted in a single row on 10-meter-long ridges, maintaining a spacing of 45 cm between rows and 15 cm between plants. The experiment was executed under standard agronomic practices, without the application of insecticides, to ensure an accurate assessment of natural resistance.

## **Phenotypic Data Collection on Biophysical Factors**

**Leaf Glossiness**: Leaf glossiness was visually assessed 14 days after seedling emergence (DAE) during the early morning hours. A scoring scale from 1 to 5 was employed, where 1 represented non-glossy leaves (characterized by dark green, dull, broad, and drooping leaves) and 5 denoted highly glossy leaves (identified as light green, shiny, narrow, and erect) (9).

**Seedling Vigor**: Seedling vigor was evaluated at 10 DAE using a 1 to 5 rating scale. A score of 1 indicated poor vigor (weak plants with stunted growth), 2 represented low vigor (shorter plants with limited leaf expansion), 3 indicated moderate vigor (average plant height and leaf development), 4 signified good vigor (healthy plants with optimal leaf expansion) and 5 denoted high vigor (maximum height and robust growth) (10).

**Dead heart Percentage (DH%)**: The dead heart percentage was recorded at 15, 21 and 28 DAE by assessing plant populations and counting the number of plants exhibiting dead heart symptoms during these observation periods. The formula employed for calculation was:

Dead heart (%)=(Number of plants with dead heart)/ (Plant population)×100

Based on infestation rates, the genotypes were categorized into five resistance groups: Highly Resistant (1 -5%), Resistant (5-25%), Moderately Resistant (25-50%), Susceptible (50-85%) and Highly Susceptible (>85%) (11).

**Trichome Density**: Trichome density was assessed at 21

DAE on both the abaxial (lower) and adaxial (upper) leaf surfaces. The central sections of the third or fifth leaf from the base were examined using a random sample of five seedlings per genotype  $(10)$ . Leaf segments  $(2 \text{ cm}^2)$  were subjected to a chlorophyll-clearing procedure utilizing a glacial acetic acid and 70% ethanol solution (2:1 ratio) for 24 hours. Following this, samples were transferred to lactic acid in small vials for a duration of up to five days. The cleared leaf bits were mounted on slides and examined under a phase contrast microscope (Euromex iScope, Eu2160058®, The Netherlands) at 10x magnification. Trichomes were enumerated on both leaf surfaces across three randomly selected microscopic fields, with trichome density expressed as the number of trichomes per mm².

**Oviposition Percentage**: Oviposition data were recorded at 28 DAE by assessing the number of eggs and the number of seedlings with eggs to calculate the oviposition percentage. The formula used was:

Oviposition Percentage=(Number of seedlings with eggs)/ (Plant population)×100

Genotypes were subsequently categorized into five groups based on oviposition percentage: Highly Resistant (1 -5%), Resistant (5-25%), Moderately Resistant (25-50%), Susceptible (50-85%) and Highly Susceptible (>85%) (11).

## **Plant Preparation and Sampling for Biochemical Analyses**

Seeds from selected genotypes were sown in plastic trays (15 cm height x 30 cm diameter) filled with a potting mix comprising equal parts sand, red earth and vermicompost. These trays were placed within insect-proof cages (60 x 60 x 60 cm). Following germination, seedlings were thinned to one per pot at 7 days after sowing (DAS). Uniform fertilization was applied post-thinning and watering was performed regularly. At 10 DAS, plants exhibiting uniform growth were allocated to two groups: one for infested conditions and another for uninfested conditions, each protected within separate insect-proof cages (90  $\times$  90  $\times$  60 cm) (13).

Adult *A. soccata* flies were collected from the field utilizing fish meal-baited traps and subsequently separated by sex via brief exposure to refrigerated conditions (15 ºC). The flies were then introduced into the cages containing plants designated for the infested group. Leaf samples were collected from each genotype at 25 DAE using a sharp, sterile razor. The third and fourth fully expanded leaves were excised at their base, wrapped in aluminum foil, stored in ice boxes, and transported to the laboratory. The samples were preserved in a -20°C deep freezer until further analyses.

## **Biochemical Analyses**

**Peroxidase Enzyme (PO)**: Five grams of plant tissue were homogenized with phosphate buffer in a 1:5 ratio and centrifuged for 15 minutes at 3000 rpm. The supernatant was utilized as the enzyme source. In a test cuvette, 3 mL of pyrogallol, 0.1 mL of enzyme extract and 0.5 mL of 1%  $H<sub>2</sub>PO<sub>4</sub>$  were combined. Absorbance was measured every 30 seconds for 3 minutes at 430 nm using a spectrophotometer  $(14).$ 

**Protein Content**: Protein content in sorghum leaves was estimated using the Lowry method. A 0.5 g sample was ground with 10 mL of phosphate buffer, centrifuged and the supernatant was used for protein estimation. Bovine serum albumin served as the working standard, with varying volumes (0.2, 0.4, 0.6, 0.8 and 1 mL) prepared in different test tubes. Two test tubes containing 0.1 mL and 0.2 mL of enzyme extract were also included, with volumes adjusted to 1 mL. Subsequently, 5 mL of alkaline copper solution was added to each tube, followed by a 10-minute incubation. Afterward, 0.5 mL of Folin-Ciocalteu reagent was added, and the mixture was incubated in the dark for 30 minutes. The color developed was measured at 660 nm absorbance (14).

**Total Soluble Sugars**: A 0.5 g plant sample was homogenized with 10 mL of 80% ethanol at room temperature. Sugars were extracted by centrifugation, with the extraction process repeated thrice. A 0.1 mL portion of the extract was placed in test tubes, and the volume was adjusted to 1 mL using distilled water. Then, 4 mL of precooled anthrone reagent was added to the test tubes, followed by a 5-minute incubation in a water bath. After cooling, the absorbance of the resulting dark green color was measured at 630 nm (14).

**Amino Acids**: A 0.5 g plant sample was ground with 10 mL of 80% ethanol and centrifuged for 10 minutes at 3000 rpm. The residue underwent double extraction, and the supernatant was used to estimate total amino acids. In test tubes, 0.1 mL of the extract was mixed with 1 mL of ninhydrin, with the volume adjusted to 2 mL using distilled water. The tubes were placed in a water bath for 20 minutes. Afterward, a diluent mix of 5 mL was added and the intensity of the purple color was measured at 570 nm after 15 minutes using a colorimeter (14).

**Total Phenols**: A 0.5 g plant sample was extracted with 10 mL of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Pyrocatechol solution was used as the working standard in varying volumes (0.1, 0.2, 0.3, 0.4 and 0.5 mL) across different test tubes. A 0.5 mL extract was taken, and the volume was adjusted to 3.5 mL with distilled water. Subsequently, 0.5 mL of Folin-Ciocalteu reagent was added and allowed to react for 5 minutes, followed by the addition of 1 mL of 20%  $Na<sub>2</sub>CO<sub>3</sub>$ . Optical density was measured at 660 nm after a 30-minute incubation (14).

**Tannins**: The extraction method was identical to that used for phenol estimation. A 0.5 mL portion of the sample extract was diluted to 7.5 mL with water in a test tube. Following this, 0.5 mL of Folin-Denis reagent and 1 mL of  $Na<sub>2</sub>CO<sub>3</sub>$  were added. After a 30-minute incubation, absorbance was measured at 700 nm (14).

**Chlorophyll**: A 0.5 g plant sample was extracted with 25 mL of acetone, divided into five 5 mL aliquots. The mixture was centrifuged at 3000 rpm for 10 minutes and the supernatant was used for estimation. The final volume was adjusted to 25 mL with acetone. Absorbance readings were taken at wavelengths of 645 nm and 663 nm against a solvent blank  $(15).$ 

## **Statistical Analysis**

Field data from the RCBD experiment were subjected to one -way ANOVA. Differences were evaluated for significance using the Tukey HSD test and treatment means were compared using LSD at a 5% probability level. Genotypes were classified into distinct clusters based on phenotypic traits associated with shoot fly resistance, such as leaf glossiness, oviposition percentage, deadheart percentage, and trichome density, using cluster analysis. The Pearson correlation coefficient method was employed to calculate correlations among biophysical factors and between biochemical and biophysical factors. Principal component analysis (PCA) was performed to reduce dimensionality within large datasets. All statistical analyses were executed using R software version 4.4.0.

# **Results**

# **Field Screening of Sorghum Genotypes for Shoot Fly Infestation at Different Growth Stages through Morphological and Biochemical Attributes**

## **Morphological Attributes**

**Leaf Glossiness:** The assessment of leaf glossiness revealed a score range of 1.2 to 5.0 among the genotypes. Notably, the resistant checks, IS 18551 (4.5) and IS 2205 (4.7), exhibited the highest glossiness scores, indicating their potential resistance to infestation. In stark contrast, the susceptible checks, DJ 6514 (1.6) and Swarna (1.8), demonstrated the lowest scores (Fig. 2A, 2B; Table 2).

**Seedling Vigor:** Evaluation of seedling vigor across 188 genotypes utilized a scale from 1 to 5. Genotypes SOR 14083, SOR 14088, SOR 14105, SOR 14106, SOR 14110, SOR 14116, IS 158, IS 1859, IS 18088, IS 362, IS 4807, TNS 702 and TNS 704 showed maximum vigor and resistance to shoot fly. The resistant checks IS 18551 and IS 2205 also scored high on this metric, with ratings of 4.9 and 4.5, respectively. Conversely, the susceptible checks Swarna and DJ 6514 reflected lower vigor ratings of 2.1 and 2.6, respectively (Fig. 2C; Table 2).

**Deadheart Percentage (DH%):** At 14 days after emergence (DAE), the DH% due to shoot fly infestation ranged significantly from 1.03% to 33.4%. Notably, several genotypes demonstrated less than 5% DH, including IS 18551 (0.6%), IS 13803 (1.03%), IS 2205 (1.5%) and SOR 14088 (3.1%). By 21 DAE, DH% escalated to between 2.2% and 66.6%, with IS 9709 (2.2%) and IS 10558 (3.5%) maintaining less than 5% DH. The trend continued at 28 DAE, where DH% varied from 4.8% to 85.7%, with IS 10558 (4.8%) exhibiting robust resistance. Among the evaluated genotypes, 51 were classified as resistant (5-25%), 102 as moderately resistant (25-50%), and 34 as susceptible (50- 85%). SOR 14086 was notably highly susceptible at 85.7%. The resistant checks IS 18551 and IS 2205 showed DH percentages of 13.1% and 16.2%, while the susceptible checks Swarna and DJ 6514 recorded 71.4% and 68.4%, respectively (Table 1).

**Trichome Density:** A total of 188 genotypes were assessed for trichome density, revealing that 74 genotypes exhibited compared to the upper surface. The resistant checks, IS 18551 (308.4/mm² upper and 224.2/mm² lower) and IS 2205 (362.8/mm² upper and 310/mm² lower), demonstrated significantly higher densities. In contrast, the susceptible checks, DJ 6514 (255.7/mm<sup>2</sup> upper and 185.2/mm<sup>2</sup> lower) and Swarna  $(230/mm^2$  upper and  $148.5/mm^2$  lower), recorded considerably lower trichome densities (Fig. 2E, 2F; Table 2).

**Oviposition Percentage:** The oviposition percentages across the genotypes varied significantly, ranging from 1.26% (IS 9437) to 87.5% (SOR 14352). The resistant checks IS 2205 and IS 18551 recorded oviposition rates of 7.6% and 8.5%, respectively, whereas the susceptible checks, Swarna and DJ 6514, exhibited significantly higher rates of 50.8% and 61.6%. Among the 188 genotypes assessed, 5 showed oviposition percentages below 5%, indicating low preference for egg-laying. Additionally, 84 genotypes had oviposition percentages between 5-25%, 63 between 25- 50% and 36 between 50-85% (Table 1).

**Number of Eggs:** The deposition of eggs varied markedly among the genotypes. In the resistant checks, IS 18551 harbored 7 eggs on 6 plants, while IS 2205 contained 4 eggs on 4 plants. In contrast, the susceptible checks, Swarna and DJ 6514, recorded significantly higher numbers, with 30 eggs on 14 plants and 42 eggs on 31 plants, respectively. The highest egg count was observed in IS 1096 (43 eggs on 20 plants), while the lowest count was found in SOR 14088, SOR 14096, SOR 14097, SOR 14122, SOR 14339, IS 9437 and IS 9489, each with 1 egg on 1 plant.

**Correlation within Biophysical Factors:** The interrelations among biophysical factors are depicted in Figure 4A. A significant positive correlation was established between leaf glossiness and trichome density on the lower surface ( $r =$ 0.24). Conversely, it displayed significant negative correlations with DH% at 15 DAE ( $r = -0.16$ ) and a nonsignificant negative correlation with oviposition percentage  $(r = -0.11)$ , the number of eggs  $(r = -0.09)$ , trichome density on the upper surface ( $r = -0.09$ ), as well as DH% at 21 DAE ( $r =$ -0.12) and 28 DAE (r = -0.13). Seedling vigor was significantly negatively correlated with oviposition percentage (r = -0.31) and DH% at 28 DAE ( $r = -0.20$ ), while also positively correlated with the number of eggs  $(r = 0.17)$ . Nonsignificant negative correlations were observed with trichome density on the lower surface ( $r = -0.01$ ), DH% at 21 DAE ( $r = -0.05$ ) and leaf glossiness ( $r = -0.10$ ), alongside a non



**Fig 1.** Plant preparation for biochemical analysis



**Fig. 2** Biophysical factors A) Glossy leaves B) Non-glossy leaves C) Seedling vigor D) Deadheart E) Trichomed leaves E1) Upper Surface E2) Lower Surface F) Nontrichomed leaves F1) Upper Surface F2) Lower Surface

**Table 1:** Reactions of sorghum genotypes to shoot fly under field conditions



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60 SOR 14354  $11(3.37)^{m_u}$   $48.2(36.79)^{lm}$   $18.0(20.43)^{A-G}$   $56.3(41.41)^{bc}$   $61.6(46.09)^{e-h}$ 

Plant Science Today, ISSN 2348-1900 (online)



61 SOR 14355 6.3(2.60)<sup>w-H</sup> 45.4(43.21)<sup>n-p</sup> 9.2(20.28)<sup>7-9.</sup> 38.2(41.12)<sup>r-v</sup> 52.2(48.25)<sup>m-r</sup>

C.D 0.05% 0.761 13.92 14.089 10.89 11.36



In a column, means followed by similar alphabets superscipted are not significant different by LSD (p=0.05)

\*Mean of three replications



**Table 2**: Physical parameters of sorghum genotypes evaluated for resistance to shoot fly



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 $^{k}$  255.6(15.98)<sup>q-</sup>

 $^{c}$  294.9(17.18)<sup>g-k</sup>

 $4.1 (2.152)^{h}$ 

130 IS 15019 4.0 (2.064)<sup>i</sup>





\*Mean of three replications

Values in parantheses are square root transformed values

In a column, means followed by similar alphabets superscipted are not significant different by LSD (p=0.05)

-significant positive correlation with trichome density on the upper surface ( $r = 0.03$ ) and DH% at 15 DAE ( $r = 0.10$ ). At 21 DAE, DH% was significantly positively correlated with oviposition percentage ( $r = 0.33$ ), the number of eggs ( $r =$ 0.15) and DH% at 15 DAE ( $r = 0.62$ ). It exhibited nonsignificant positive correlations with trichome density on both upper and lower surfaces  $(r = 0.08$  and 0.01, respectively). At 28 DAE, DH% demonstrated significant positive correlations with oviposition percentage  $(r = 0.26)$ , as well as DH% at 15 DAE ( $r = 0.37$ ) and 21 DAE ( $r = 0.79$ ). It showed a non-significant negative correlation with the number of eggs ( $r = -0.06$ ) and non-significant positive correlations with trichome density on both lower and upper surfaces (r = 0.09 and 0.02, respectively). Notably, trichome density on the lower surface exhibited a non-significant negative correlation with oviposition percentage ( $r = -0.03$ ) and a non-significant positive correlation with the number of eggs ( $r = 0.03$ ), but it was significantly positively correlated with trichome density on the upper surface ( $r =$ 0.70). Trichome density on the upper surface demonstrated a non-significant positive correlation with both oviposition percentage ( $r = 0.03$ ) and the number of eggs ( $r = 0.08$ ). Furthermore, the number of eggs was significantly positively correlated with oviposition percentage ( $r = 0.50$ ) (Fig. 3, 4A, 5A).

## **Biochemical Attributes**

A thorough phenotypic assessment of 188 genotypes against the shoot fly resulted in the selection of 14 genotypes for detailed biochemical evaluation. This selection included both resistant and susceptible checks, as illustrated in Figures 6 and 7. Notably, the activity of polyphenol oxidase (PO) demonstrated a significant 10% increase in the shoot flyresistant checks IS 18551 and IS 2205 under infested conditions compared to their uninfested counterparts. Conversely, the susceptible checks, Swarna and DJ 6514, exhibited minimal increases of 1.97% and 3.18%, respectively. Among the assessed genotypes, those with heightened PO activity under infestation included IS 7071 (17.43-18.97 ΔO.D./g), IS 12787 (16.2 ΔO.D./g-17.65 ΔO.D./g), SOR 14351 (15.4-16.84 ΔO.D./g) and IS 9437 (12.93 ΔO.D./g-14.21 ΔO.D./g). In contrast, genotypes such as IS 10558 (11.91 ΔO.D./g-12.07 ΔO.D./g), IS 859 (7.73 ΔO.D./g-8.13 ΔO.D./g), SOR 1410 (6.43 ΔO.D./g-7.11 ΔO.D./g), TNFS 230 (6.08 ΔO.D./g -7.45 ΔO.D./g), IS 2228 (5.61 ΔO.D./g-6.14 ΔO.D./g) and IS 8380 (4.98 ΔO.D./g-5.09 ΔO.D./g) revealed lower PO activity. Similarly, the activity of polyphenol oxidase (PPO) exhibited notable increases in specific genotypes: SOR 14351 (18.01 ΔO.D./g - 19.43 ΔO.D./g), IS 12787 (15.46 ΔO.D./g - 16.51 ΔO.D./g), IS 9437 (13.73 ΔO.D./g - 14.58 ΔO.D./g) and IS 7071 (13.42 ΔO.D./g - 13.74 ΔO.D./g). Additionally, resistant checks IS 18551 (18.56 ΔO.D./g - 19.43 ΔO.D./g) and IS 2205 (17.03 ΔO.D./g - 18.81 ΔO.D./g) displayed elevated PPO activity. In stark contrast, the susceptible genotypes Swarna (10.45  $ΔO.D. /g - 10.76 ΔO.D. /g)$  and DJ 6514 (9.41  $ΔO.D./g - 9.58$ ΔO.D./g) revealed significantly lower PPO levels.

Total phenolic content varied across genotypes, with higher concentrations noted in SOR 14351 (6.61%), IS 9437 (5.51%) and IS 12787 (4.57%), which were comparable to the resistant checks IS 18551 (6.8%) and IS 2205 (8.08%) after infestation by the shoot fly. Conversely, lower phenolic content was observed in genotypes such as IS 10558 (0.41%), SOR 1410 (3.26%), IS 7071 (3.4%), IS 2228 (3.8%), IS 8380 (3.01%) and IS 859 (3.60%), akin to the susceptible checks Swarna (2.24%) and DJ 6514 (1.92%). Tannin content was notably elevated in resistant genotypes IS 18551 (17.84%) and IS 2205 (20.44%), while it was markedly lower in the susceptible genotypes Swarna (9.50%) and DJ 6514 (8.13%). The amino acid content was similarly higher in the resistant checks IS 18551 (3.2%) and IS 2205 (2%), contrasting with the lower levels found in the susceptible checks Swarna (0.58%) and DJ 6514 (0.78%). Furthermore, total soluble protein and soluble sugars measured from the leaves indicated increased levels at 28 days after emergence (DAE) in the susceptible checks Swarna and DJ 6514 compared to the resistant checks IS 18551 and IS 2205 post-shoot fly infestation. In contrast, total chlorophyll content from the leaves declined at 28 DAE following shoot fly infestation, as shown in Figures 4B, 5B and Table 3.

## **Correlation Between Biophysical and Biochemical Factors**

The correlations within the biochemical parameters are illustrated in Figure 4B, while the relationships between biophysical and biochemical factors are shown in Figure 8. The correlation analysis revealed that the oviposition percentage was significantly negatively correlated with seedling vigor ( $r = -0.67$ ) and significantly positively correlated with deadheart percentage (DH%) at 15 days after emergence (DAE) ( $r = 0.55$ ). Additionally, the number of eggs was significantly negatively correlated with amino acid content under both infested ( $r = -0.66$ ) and uninfested ( $r = -0.66$ ) 0.63) conditions, while being significantly positively correlated with DH% at 15 ( $r = 0.67$ ), 21 ( $r = 0.60$ ) and 28 ( $r =$ 0.65) DAE. In uninfested conditions, protein content showed a significant positive correlation with chlorophyll content under both infested ( $r = 0.57$ ) and uninfested ( $r = 0.50$ ) conditions, as well as with soluble sugars in both infested (r  $= 0.55$ ) and uninfested ( $r = 0.54$ ) conditions. In infested conditions, protein was significantly positively correlated with chlorophyll content ( $r = 0.73$ ) and soluble sugars ( $r =$ 0.69), demonstrating consistency across both conditions. However, it exhibited a significant negative correlation with phenol content in uninfested conditions (r = -0.59).

Soluble sugars were significantly negatively correlated with phenol content in both infested  $(r = -0.63)$ and uninfested ( $r = -0.57$ ) conditions, as well as with tannin content under both conditions (infested:  $r = -0.57$ ; uninfested:  $r = -0.56$ ). Conversely, soluble sugars were significantly positively correlated with chlorophyll content in both infested ( $r = 0.96$ ) and uninfested ( $r = 0.96$ ) conditions, and with DH% at 15 ( $r = 0.58$ ), 21 ( $r = 0.68$ ) and 28 (r = 0.72) DAE. In infested conditions, soluble sugars displayed a perfect correlation with soluble sugars ( $r = 1.00$ ). In infested conditions, soluble sugars were significantly negatively correlated with phenol content ( $r = -0.60$ ) and tannin content ( $r = -0.55$ ) in both infested and uninfested conditions. However, they exhibited significant positive correlations with chlorophyll content (r = 0.94 in infested



**Fig 3.** Heatmap representing biophysical factors recorded from 188 sorghum genotypes screened for resistance against shoot fly (*A. soccata*). Color gradient red shows higher level of incidence and the color decreased to light yellow indicates lower levels of shoot fly incidence in sorghum. DH1- Deadheart percentage at 15DAE; DH2- Deadheart percentage at 21DAE; DH3- Deadheart percentage at 28DAE; glosi- leaf glossiness; Vig- seedling vigor; TDupper- Trichome density upper surface; TDlower- Trichome density lower surface.



**Fig. 4.** Correlation among factors recorded in sorghum genotypes screened for resistance against shoot fly (*A. soccata*): a) Biophysical (188 genotypes); Trichome density (US- Upper Surface, LS- Lower Surface); DH1- Deadheart percentage at 15DAE; DH2- Deadheart percentage at 21DAE; DH3- Deadheart percentage at 28DAE; glosi- leaf glossiness; Vigo- seedling vigor; b) Biochemical (14 genotypes)



**Fig. 5.** Heatmap for factors recorded in selected 14 sorghum genotypes screened for resistance against shoot fly (A. soccata): a) Biophysical; b) Biochemical



Fig. 6. Cluster Analysis: The clustering of 188 sorghum genotypes forming four clusters depicting resistance categories against shoot fly (*A. soccata*) based on field data collected for leaf glossiness, seedling vigor, trichome density, deadheart and oviposition percentages.

Fig. 7. Selection Index of genotypes. 10 genotypes were selected based on the biophysical parameters along with two resistant checks and two susceptible checks.

## **Table 3:** Biochemical analysis for selected sorghum genotypes



\*Mean of two replications

In a column, means followed by similar alphabets superscipted are not significant different by LSD (p=0.05)

and  $r = 0.95$  in uninfested conditions) and with DH% at 15 (r  $= 0.58$ ), 21 (r = 0.69) and 28 (r = 0.72) DAE.

Chlorophyll content in uninfested conditions showed a significant negative correlation with polyphenol oxidase (PPO) levels (r = -0.58 in both infested and uninfested conditions) and with phenol content ( $r = -0.74$  in infested and  $r = -0.72$  in uninfested conditions) and tannin levels ( $r = -1$ 0.63 in both conditions). Conversely, chlorophyll content was significantly positively correlated with DH% at 15 ( $r =$ 0.67), 21 (r = 0.68), and 28 (r = 0.75) DAE and with itself in infested conditions ( $r = 0.99$ ). Chlorophyll content in infested conditions exhibited significant negative correlations with PPO levels  $(r = -0.61)$  and with phenol content ( $r = -0.72$  in infested and  $r = -0.74$  in uninfested conditions) and tannin levels ( $r = -0.56$  in both conditions). It was significantly positively correlated with DH% at 15 ( $r =$ 0.65), 21 (r = 0.66) and 28 (r = 0.73) DAE. The DH% at 15 DAE was significantly negatively correlated with PPO levels (r = - 0.58 in both conditions) and with phenol content ( $r = -0.66$  in infested and  $r = -0.64$  in uninfested conditions) and amino acid levels ( $r = -0.62$  in infested and  $r = -0.63$  in uninfested conditions). Conversely, DH% at 15 DAE was significantly positively correlated with DH% at 21 DAE (r = 0.88) and 28 DAE (r = 0.87). DH% at 21 DAE was significantly negatively correlated with amino acid levels ( $r = -0.55$  in both conditions). Additionally, it exhibited a significant positive correlation with DH% at 28 DAE ( $r = 0.95$ ). DH% at 28 DAE was significantly negatively correlated with tannin levels (r = -0.54 in infested and r = -0.55 in uninfested conditions). Leaf glossiness was significantly positively correlated with trichome density on the lower surface  $(r = 0.60)$ . Furthermore, trichome density on the upper surface was significantly positively correlated with trichome density on the lower surface ( $r = 0.52$ ). Trichome density on the lower surface also exhibited significant positive correlations with phenol levels in both infested ( $r = 0.59$ ) and uninfested ( $r = 0.59$ ) 0.58) conditions, as well as with trichome density on the upper surface ( $r = 0.70$ ). In uninfested conditions, tannin levels were significantly positively correlated with phenol levels in infested conditions ( $r = 0.71$ ) and with tannin levels in infested conditions ( $r = 1.00$ ). In infested conditions, tannin was significantly positively correlated with phenol levels (r = 0.70). Phenol levels in infested conditions showed significant positive correlations with polyphenol oxidase (PO) levels ( $r = 0.58$ ), as well as with PPO levels in both uninfested ( $r = 0.80$ ) and infested ( $r = 0.79$ ) conditions. In uninfested conditions, phenol levels were significantly positively correlated with PO levels (infested:  $r = 0.72$ ; uninfested:  $r = 0.69$ ), with PPO levels in both infested ( $r =$ 0.87) and uninfested ( $r = 0.86$ ) conditions, and with phenol levels in infested conditions ( $r = 0.93$ ). Furthermore, PPO was significantly positively correlated with PO enzyme levels ( $r = 0.83$ ) (Fig. 8).

# **Principal Component Analysis (PCA)**

The PCA revealed that the first two principal components (Dim 1 and Dim 2) together accounted for 59.9% of the total variance in the dataset. Dim 1, which explained 44.5% of the variance, was strongly linked to reproductive traits such as oviposition rates and egg numbers, highlighting their significance in distinguishing the genotypes. Dim 2, explaining 15.4% of the variance, was primarily associated with biochemical responses, including chlorophyll content and soluble sugars, indicating the plants' defense mechanisms against infestation. Genotypes positioned on the positive side of Dim 1 are likely to support higher reproductive success of the shoot fly, whereas those on the negative side indicate lower reproductive success. Genotypes higher on Dim 2 exhibit stronger biochemical defenses, while those lower on Dim 2 suggest weaker defenses. For instance, the genotypes Swarna and DJ 6514 are associated with reproductive traits and their position along Dim 1 suggests they may favor shoot fly reproduction. In contrast, genotype IS 8380, positioned positively on both Dim 1 and Dim 2, shows higher reproductive success alongside strong biochemical defenses.

Moreover, genotypes positioned high on Dim 3 show variability in secondary biochemical compounds such as protein and amino acid content. Dim 4 captures additional, less dominant variability but still plays a role in understanding the full range of differences between genotypes. Although it explains a smaller percentage of the variance, Dim 4 provides insight into specific physiological or biochemical traits that may not be fully represented in the earlier components. The clustering observed in the PCA plot highlighted distinct groupings based on these traits, suggesting that both reproductive performance and biochemical defenses are key drivers of variability among the genotypes. These findings emphasize the importance of integrating both biophysical and biochemical parameters to better understand plant-pest interactions and inform crop improvement strategies (Fig. 9).

#### **Discussion**

The Host Plant Resistance (HPR) technique, when combined with cultural practices, represents the most economical and effective strategy for mitigating losses due to shoot fly damage while maintaining infestations below economic threshold levels (ETL) (10). Although advancing the sowing date can help reduce the impact of shoot flies on crop stands, this cultural method is often impractical in specific regions due to prevailing agro-climatic conditions (16). In semi-arid regions, the short window for sowing significantly limits the ability to implement early planting practices aimed at avoiding shoot fly damage (17). Moreover, occasional heavy rain showers during typically dry seasons can lead to shoot fly infestations, even in early sown crops (18). While seed treatment with systemic insecticides is viewed as the most efficient approach to combat shoot fly infestations, resource-poor farmers in semi-arid tropics often struggle to afford these costly insecticides. Additionally, challenges related to the timely availability of treatments and the application process further complicate their use. Consequently, the recommended cultural practices and insecticidal interventions for shoot fly management often remain impractical due to time and resource constraints. Generally, a 1% increase in shoot fly damage (deadheart percentage, DH %) correlates with a grain yield loss of approximately 143 kg/ha. Under favorable



**Fig. 8.** Correlation between biophysical and biochemical factors recorded in selected 14 sorghum genotypes screened for resistance against shoot fly (*A. soccata*); DH1- Deadheart percentage at 15DAE; DH2- Deadheart percentage at 21DAE; DH3- Deadheart percentage at 28DAE; Glosi- leaf glossiness; Vig- seedling vigor; Tdup- Trichome density upper surface; Tdlo- Trichome density lower surface; grp- group.



Fig. 9. Principal Component Analysis (PCA): PCA of biophysical and biochemical factors recorded in selected 14 sorghum genotypes screened for resistance against shoot fly (A. soccata): The direction of the arrows shows the contributions of variables to principal components. Variables pointing in the same direction are positively correlated, while those pointing in opposite directions are negatively correlated.

conditions for shoot fly infestations, delayed sowing can lead to total crop losses of 90-100% (19, 20, 21).

HPR encompasses complex plant characteristics resulting from the interactions between insects and various plant traits, including both morphological and biochemical factors that confer resistance (22, 23, 24). Several sorghum genotypes resistant to shoot flies have been identified and are utilized in breeding programs aimed at enhancing resistance (25, 26, 27). To broaden the genetic base for effective shoot fly resistance, researchers must first comprehend the mechanisms of resistance present in both resistant and susceptible genotypes. In this field-based study of 188 genotypes, only a limited number exhibited minimal shoot fly infestation. These resistant genotypes possessed specific morphological and biochemical traits that adversely affected shoot fly oviposition and larval performance. Trichome density emerged as a significant factor in reducing damage and infestation by obstructing the oviposition, movement and survival of first-instar maggots. Resistant lines demonstrated higher trichome density on both upper and lower leaf surfaces, while susceptible lines exhibited lower densities. Additionally, leaf glossiness was identified as an important morphological trait contributing to shoot fly resistance. Genotypes with high leaf glossiness displayed greater resistance to shoot flies (2, 13). A negative correlation between leaf glossiness and both shoot fly oviposition and DH % was consistently observed (13, 31) (30). Seedling vigor was also greater in resistant genotypes compared to susceptible ones. The relationship between seedling vigor and the ability to escape shoot fly damage was evident, as resistant cultivars with significantly higher seedling vigor outgrew and spent less time in the vulnerable seedling stage than slowergrowing susceptible cultivars (32). However, it was noted that resistant checks demonstrated the lowest seedling vigor, while susceptible checks exhibited higher seedling vigor (28 ).

From the evaluated genotypes, 14 were selected based on their superior performance in terms of the lowest DH % at 28 days after emergence (DAE) and high leaf glossiness at 14 DAE, subsequently analyzed for their biochemical composition. Genotypes IS 10588 and IS 8380 exhibited higher levels of phenolic compounds, tannins, amino acids, and enzyme activities (polyphenol oxidase [PO] and polyphenol oxidase [PPO]), while showing lower levels of soluble sugars, proteins, and chlorophyll content. Notably, IS 10588 exhibited high leaf glossiness, low DH % at 28 DAE, high trichome density on the lower leaf surface, and the fewest shoot fly eggs per plant, establishing it as highly resistant to shoot flies. Similarly, IS 8380, characterized by medium glossiness and a DH % of 10-15 at 28 DAE, also displayed resistance. Genotypes with the fewest eggs per plant and the lowest incidence of dead heart were determined to be more resistant compared to others. In contrast, the highest number of eggs per plant was observed in susceptible genotypes, rather than resistant ones. The number of eggs per plant and per seedling emerged as critical traits for screening sorghum for shoot fly resistance, with susceptible genotypes showing significantly higher shoot fly oviposition (40). Genotypes characterized by low levels of soluble proteins, chlorophyll and soluble sugars, combined with high PO and PPO enzyme activity (37, 39, 41), were identified as shoot fly resistant, yielding lower DH % values. Higher phenol and tannin contents enhance plant resistance to shoot flies by disrupting their biology and colonization, contributing significantly to antibiosis (39, 42, 43). Typically, resistant genotypes exhibit lower soluble protein content compared to susceptible ones (36, 44). Following shoot fly infestation, chlorophyll content diminishes, with susceptible genotypes experiencing the highest rate of decline compared to resistant types (41, 45). Generally, plants with lower chlorophyll content are less susceptible to shoot fly damage (46, 13, 47).

Correlation studies revealed that the number of eggs per plant and DH % were significantly positively correlated with chlorophyll content and negatively correlated with phenol content, trichome density and leaf glossiness. Trichome density showed a significant positive correlation with seedling vigor and leaf glossiness (48). Additionally, trichome density, leaf glossiness and seedling vigor exhibited negative correlations with shoot fly damage parameters, such as oviposition % and DH % (40, 49, 50, 48). Total soluble sugars and protein content in sorghum seedlings demonstrated a significant positive correlation with DH % (44, 49). Conversely, tannin content was significantly negatively correlated with shoot fly damage (44). Leaf glossiness and seedling vigor were also negatively correlated with tannin and soluble sugar content (49). The oviposition % was significantly positively correlated with DH %, while the DH % at 21 DAE showed a significant positive correlation with DH % at 28 DAE (48).

## **Conclusion**

This study successfully classifies sorghum genotypes based on their resistance to shoot fly, providing a valuable resource for breeding programs and crop improvement strategies. Among the evaluated genotypes, **IS 10588** and **IS 8380** exhibited high resistance to shoot fly damage, while **IS 12787** demonstrated notable resistance, and **TNFS 230** was identified as moderately resistant. These genotypes present significant potential for incorporation into breeding programs, including Marker-Assisted Selection (MAS) and Quantitative Trait Locus (QTL) mapping, aimed at developing sorghum varieties with enhanced resistance to shoot fly. The implications of these findings are substantial for improving sorghum production, bolstering food security, and supporting the livelihoods of farmers. By utilizing these resistant genotypes, future breeding efforts can enhance the resilience of sorghum against pest infestations, contributing to more sustainable agricultural practices.

# **Acknowledgements**

The authors acknowledge the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore for granting permission for this study and offering support during the collection and analysis of insect samples.

# **Authors' contributions**

JM&MM- Wrote the manuscript. TS&DK- Designed the article and helped with revisions of the article. PM&KP analysed the data. All authors read and approved the final manuscript.

# **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interests to declare.

## **Ethical issues:** None

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