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Research Article

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Biochemical and Morphological screening of cotton (*Gossypium hirsutum* L.) hybrids for Jassid resistance (*Amrasca biguttula*)

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Abstract

The cotton hybrids of seven lines (BSH 18, MCU 5, CO 14, TCH 1828, CO 17, TCH 11199 and Suraj) and five testers (KC2, KC3, GISV 323, GTHV 15-34 and RHC 1409) were raised in RBD for obtaining 35 hybrids. The hybrids along with their parents were studied for traits viz., mean number of Jassids per plant, injury grade, total phenol content, total reducing sugars, total soluble proteins, trichome length, trichome density and chlorophyll index along with the seed cotton yield per plant. Higher phenolic content, lower reducing sugar, lower soluble proteins, greater chlorophyll index, and higher trichome density would result in fewer jassids per plant. MCU 5 X KC3 hybrid had the lowest injury grade (0.13), highest total phenol content (3.84 mg/g), lowest total reducing sugars (1.14 mg/g), highest chlorophyll index (58.33), lowest trichome length (0.43), highest trichome density (271.42 numbers per centimetre square) and highest yield of 150.74 g/plant. Hence this F₁ hybrid confers resistance against jassids. Hence, higher phenolic content along with higher trichome density offers resistance to jassids in cotton plants. This cross could be further forwarded to F₂ to identify the jassid segregants for further screening. Thus, knowledge into these biochemical analyses and trichome density of the cultivar might be used to breed for jassid resistance, which would assist minimise the usage of pesticides and pest loads from the fields, thereby increasing the population's productivity.

Key words : Jassid, cotton, biochemical analysis, phenol, reducing sugar, soluble proteins, trichome length, trichome density

Introduction

Cotton (*Gossypium hirsutum* L.), a profitable crop, is a globally important industrial commodity. It is the second-oldest and most significant commercial crop after food grains. India is responsible for 33% (10.7 mha) of global cotton acreage and 22% (5.4 mt) of global cotton

production. In India, hybrids cover around 70% of the land, upland types 20%, and diploid cultivars 10%(Anon, 2012).

Cotton is attacked by a variety of insect pests from the time it is planted until it is harvested. Sucker pests like as aphids, thrips, leaf hoppers, and white flies create major problems in the early stages, leading in a drop in cotton output and quality. Cotton yields are reduced by 22.58 percent due to sucking bugs (Satpute *et al.*, 1990). Non-target pests with piercing-sucking mouthparts, such as leaf bugs, cotton spider mites, cotton aphids, and whiteflies, survive better and occasionally become problem as broad-spectrum pesticides are reduced in cotton due to the usage of insect resistant, genetically engineered (GE) genotypes (Xu *et al.*, 2008).

As a result, using morphological and biochemical HPR features as a cotton plant defence mechanism offers a different management technique for controlling these insect pests. Cotton cultivars with high pesticide resistance would require fewer pesticide treatments, lowering costs and dangers connected with pesticide resistance while also benefiting insect populations. Researchers have hitherto concentrated on morphological HPR features in cotton, with just a few findings on biochemical HPR(phenolics). We employed a significantly larger variety of hybrids in comparison to these experiments, spanning morphobiochemical HPR characteristics ranging from harmful to enticing insects, such as increased soluble protein and sugar levels(Nishanth *et al.* 2016).

Given the current scenario of piercing sucking pest outbreaks on cotton, there is an immediate need to produce sucking pest resistance varieties or hybrids. As a result, the current study was done to identify sucking pest resistant or tolerant hybrid lines based on biochemical findings.

Materials and Methods

The BSH 18, MCU 5, CO 14, TCH 1828, CO 17, TCH 11199 and Suraj parents of *Gossypium hirsutum* L. were used as lines while KC2, KC3, GISV 323, GTHV 15-34 and RHC 1409 were used as testers during the summer of 2021 (testers parent for jassid resistance and lines selected had good quality for lint). Dock's approach involved crossing each of the four lines with four tests independently using manual emasculation and pollination (Doak, 1934). In 2021, 35 F₁ hybrid, along with their 12 parents and a continuous check, were created (SVPR 2). The genotypes were then grown using Randomized Block Design in two replications (RBD) at the Tamil Nadu Agricultural University in Coimbatore.

Cotton genotypes were tested for Jassid resistance:

The populations to be screened for resistance might be generated in fields under natural infestation, either in endemic areas or by using strategies to increase field infection. If not, do not use scoring or screening processes since there should be enough insects to infest the plant damage equally. Standard inspections should be kept for comparing resistant and vulnerable individuals.

Jassid population and damage assessment

Jassid injury was examined using the techniques of the Indian Central Cotton Committee (ICCC, 1960) and based on infestation symptoms. On the 15th, 30th, 45th, 60th, and 75th days after sowing, a visual evaluation of hopper injury and number of jassid per plant on each entry was recorded, and a mean injury index (grade index) was determined. The following were the injury grades for jassid.

Grades	Symptoms
1.	Leaves free from crinkling or with no yellowing, bronzing and drying
2.	Few leaves on lower portions of the plant curling, crinkling and slight yellowing
3.	Crinkling and curling all over, yellowing, bronzing and browning in the middle and lower portion, plant growth hampered
4.	Extreme curling, yellowing, bronzing and browning, drying of leaves and defoliation, stunted growth

A leafhopper resistance index was calculated as proposed by Nageswara Rao (1973).

$$\frac{G_1 \times P_1 + G_2 \times P_2 + G_3 \times P_3 + G_4 \times P_4}{P_1 + P_2 + P_3 + P_4}$$

Where G represented the number of the grade of ICCC and P the number of plants under the each entry. Grouping of injury index to categories of resistance was as follows.

Grade index	Category
0.0 – 1.0	Resistant
1.1 – 2.0	Moderately resistant
2.1 – 3.0	Susceptible
3.1 – 4.0	Highly susceptible

Total phenols:

The total phenols ($\mu\text{g/gm}$) were determined using Malick and Singh's method (1980). Healthy and Jassid-infested cotton plant shoots (5000 mg) were grinded in 5ml ethanol (80%) for 10 minutes in a water bath at 70°C . After chilling and macerating with 80 percent ethanol, centrifuged at 10,000 rpm for 5 minutes. A water bath was used to dry the supernatant. To dissolve the residue, 1 mL pure water was utilized. 2 ml of 20 percent Na_2CO_3 solution and 1 ml of sample folins ciocalteau reagent were added to this. With distilled water, the total volume of the reaction mixture was increased to 10 ml, and the absorbance was measured at 650 nm. A typical curve with 10-100 g of catechol were created. The standard curve was used to quantify the quantities of phenols in the unknown samples. The standard is prepared using catechol. 1000ppm catechol stock solution: 100mg diluted in 100ml The colour intensity of the standards is measured at 650nm using this 200ppm, 400ppm, 600ppm, and 800ppm.

Total Phenol (%) =

$$\frac{\text{Conc.}(\mu\text{g})}{\text{OD of standard}} \times \frac{\text{OD of sample}}{1 \text{ ml}} \times \frac{V (5\text{ml})}{W (500\text{mg})} \times 100 (\mu\text{g/gm}) \text{ phenol}$$

Total reducing sugars:

The total reducing sugars were calculated using the Miller *et al.* (1972) method, with a few modifications. Fresh shoots from jassid-infested and healthy cotton plants were extracted twice with 5 ml of hot 80 percent ethanol, and the supernatant was collected and evaporated over a water bath. The sugars were completely dissolved after 10 mL of distilled water was added. This generated 1.5 mL of alcohol-free extract, which was then raised to 3 mL by adding distilled water. 3 mL dinitrosalicylic acid reagent was gently mixed into this. In a hot water bath, the reaction mixture was allowed to develop colour for 5 minutes. The components were combined, chilled, and the absorbance was measured at 510 nm after adding 1 mL Rochelle salts solution (40 percent). Using a standard graph of glucose formed by known glucose concentrations (100-500 g), the total reducing sugars of the unknown samples were determined.

Total soluble proteins:

Jassid-infested and healthy cotton plant shoots of 50mg were macerated in enzyme assay buffer and centrifuged for 10 minutes at 10000 rpm. The supernatant (0.4 mL) was mixed with 4 mL of alkaline copper sulphate solution and permitted to stand at room temperature for 10 minutes. The Folins-ciocalteau phenol reagent (0.4 mL) was added to the above reaction mixture and incubated for 30 minutes at room temperature before being measured at 660 nm. A standard curve

was built using a known concentration of bovine serum albumin. A standard calibrated curve with known bovine protein concentrations was used to estimate protein content in both types of samples (Lowrey *et al.* 1951).

Bovine Serum Albumin Standards:

Weigh accurately 50mg of Standard Bovine Serum Albumin (BSA) were added to 0.1N NaOH mixed well make up to 50 ml in a standard flask. The working standard made by diluting 10ml of above BSA stock to 50ml 0.1N NaOH in a standard flask 1ml of this solution contains 200µg protein.

$$\text{Total soluble proteins} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}(200\mu\text{g /ml})$$

Trichome density and Trichome length analysis

The MI-DC1300, MI-DC3000, and MI-DC5000 are digital cameras that provide crisp images with excellent contrast and colour when used with compound microscopes. The photographs were exhibited on the slides for the leaf samples that were newly gathered at 50 DAS and sliced to the size of one centimetre square, then visualized on the computer screen through ScopePhoto software. The images were then saved for analysis.

Chlorophyll index

It was determined using a KONICA MINOLTA SPAD – 502 machine. The SPAD metre is a handy instrument for quickly and non-destructively determining chlorophyll index.

SPAD metre handling

The instrument had already been turned on. Without collecting a sample, the measuring head was pushed shut with a finger until a beep was heard. The display displayed 0.00 after the beep, indicating that the calibration was complete. The blooming stage measurement sample was placed in the measuring head and pressed down with a finger. The thick portions of the leaf, such as veins, were not measured by the measuring head (Fiebo *et al* 1998). It was held in place until a beep was heard and the displayed value was presented. The measurement was automatically recorded to memory. The results of the SPAD metre are proportional to the amount of chlorophyll in the leaf and were estimated by measuring the leaf absorbance in red and near-infrared regions (Uddling *et al.*, 2007).

Seed cotton yield per plant

Five plants were randomly selected from each genotype in two replications raised in RBD design along with their parents were used for the analysis. Hence from the mean of five random plants taken, the yield of the cotton is calculated in grams per plant.

Result and discussion

Mean of parents and hybrids

From the mean table 1. of lines and testers, BSH 18 showed high mean number of Jassids per plant (9.32 numbers per leaf) with minimum chlorophyll index (39.13). CO 17 had maximum chlorophyll index of 54.17. Highest yield was contributed by the parent CO 14 (150.35 g/plant). The line TCH 1199 had minimum injury grade with 0.21 and KC 2 had maximum injury grade (1.38). The tester parent KC 2 had minimum phenol content with 2.40 mg/g, high reducing sugar of 2.63 mg/g, highest total soluble proteins of 33.63 mg/g, highest trichome length of 1.93 mm and lowest trichome density of 82.77 numbers per centimetre square. Contrastly the parent KC3 had minimum mean number of Jassid per plant, highest total phenols among all parents (3.31 mg/g), lowest soluble proteins (27.49 mg/g). lowest trichome length (1.50 mm) and highest trichome density (183.38 numbers per centimetre square). The parent RC 1409 had lowest total reducing sugars (1.36 mg/g).

The hybrid BSH 18 X KC 2 shown in table 2.had highest injury grade of 3.85. BSH 18 X GISV 323 had maximum total soluble proteins of 39.56 mg/g. MCU 5 X KC3 had lowest injury grade (0.13), highest total phenol content (3.84 mg/g), lowest total reducing sugars (1.14 mg/g), highest chlorophyll index (58.33), lowest trichome length (0.43), highest trichome density (271.42 numbers per centimetre square) and highest yield of 150.74 g/plant. The hybrids of MCU 5 X RHC 1409 and CO 17 X KC 3 had lowest chlorophyll index (21.86) and total soluble proteins (27.49 mg/g) respectively. The hybrid TCH 1199 X GISV 323 had minimum mean number of jassids per plant (4.16)The hybrid Suraj X RHC 1409 had maximum jassids mean population per leaf (9.84 numbers), lowest total phenol content (1.12 mg/g), highest total reducing sugars (3.85 mg/g), lowest chlorophyll index (28.76) and lowest yield of 90.26 g/plant.

Correlation

From the correlation table 3. The number of jassids per plants showed positive significant correlation with amount of reducing sugars (0.720), total soluble protein (0.392) and trichome length (0.563) but negatively correlated with the amount of phenol (-0.593) and trichome density (-0.728). Negative correlation of number of jassids with trichome density was also reported by

Ahmad *et al.* (2005), Ashfaq *et al.* (2010), Rustamani *et al.* (2014), Gonde *et al.* (2015), Kanher *et al.* (2016), Sankeshwar *et al.* (2016) and Amin *et al.* (2017). Number of jassids per plants exhibited non significant negative correlation with injury grade (0.089). Injury grade showed highly negative correlation with amount of phenol (-0.431) and trichome density (-0.477) of the plant. The phenol content present in the plant was highly negatively correlated with amount of reducing sugars (-0.793), amount of soluble proteins (-0.361) present but positively significant with seed cotton yield per plant (0.301) thereby indirectly effecting the jassid resistance to the plant. Amount of reducing sugar is positively correlated with the total soluble proteins (0.713) as reported by Bonde *et al.* (2016) but negatively correlated with the chlorophyll index (-0.361). Seed cotton yield of the plant (-0.129) had negative non-significant correlation with number of jassids per plant.

Discussion

As a result of the aforesaid findings, high phenolic content, low reducing sugar, lower soluble proteins, greater chlorophyll index, and higher trichome density would result in fewer jassids per plant. Similar findings for high phenolic content with lower jassid incidence was reported by Shinde *et al.* (2014). Rohini *et al.* (2011) reported higher phenolic content along with higher trichome density offers resistance to jassid in cotton plants which was in accordance with the result obtained. Manivannan *et al.* (2021) also reported that phenol content in cotton plants acts as the feeding deterrent hence the population of jassid feeding that respective plant would be lower. He also reported that higher the protein more would be the infestation by jassids, which was also in agreement with the results obtained. This result was in accordance with the present study. Singh *et al.* (1988) also reported higher the amount of reducing sugar more susceptible the cultivar would be.

Result

Among other hybrids, MCU 5 X KC3 hybrid had the lowest injury grade, highest total phenol content, lowest total reducing sugars, highest chlorophyll index, lowest trichome length, highest trichome density and highest yield of 150.74 g/plant. Hence this F₁ hybrid confers resistance against jassids. Hence, higher phenolic content along with higher trichome density offers resistance to jassids in cotton plants. This hybrid could be further forwarded to F₂ to identify the jassid segregants for further screening. Thus, knowledge into these biochemical analyses and trichome density of the cultivar might be used to breed for jassid resistance, which would assist minimise the usage of pesticides and pest loads from the fields, thereby increasing the population's productivity.

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Authors' contributions

All the authors contributed to the study conception and design. Subhashini S executed the experiment and analysed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

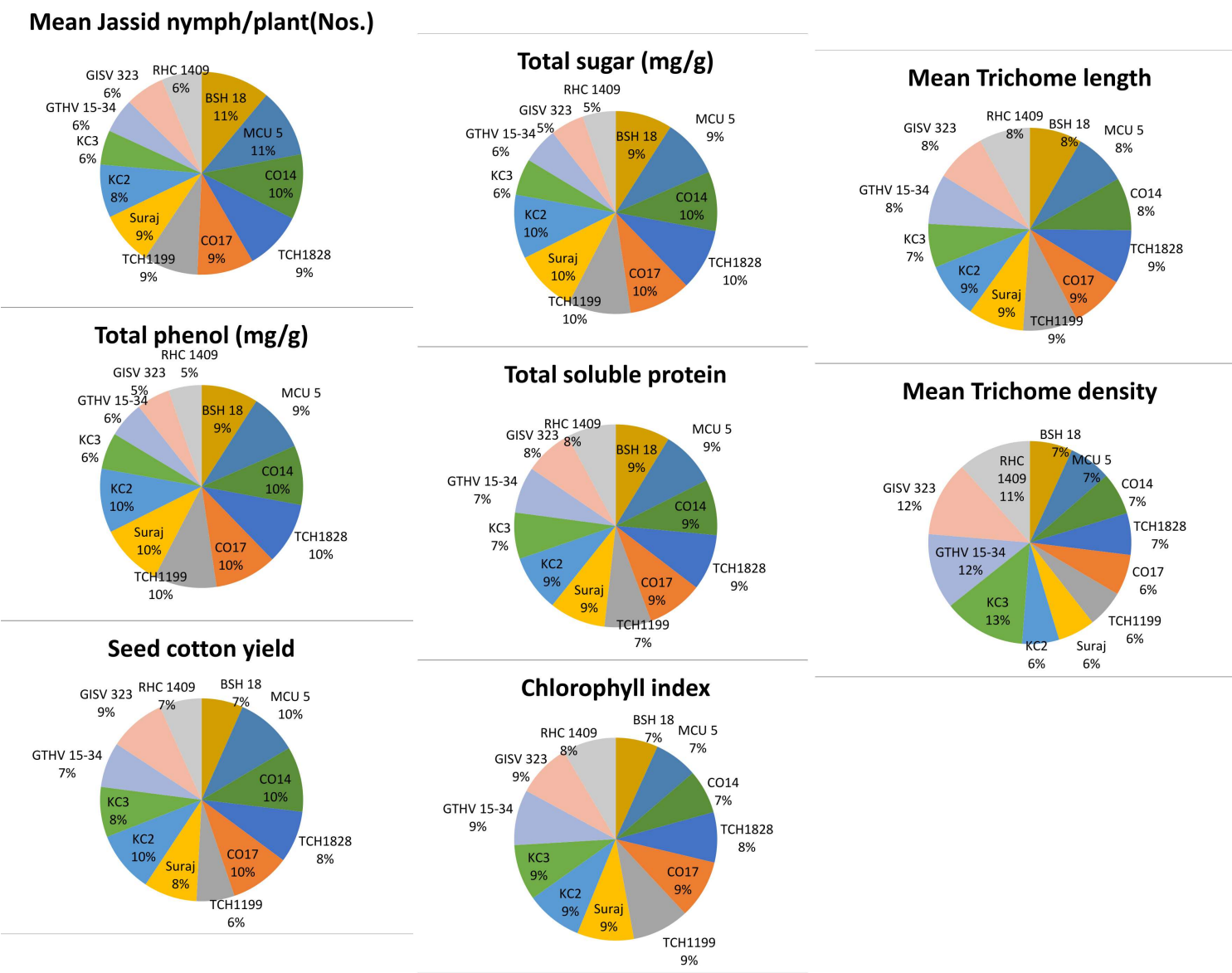


Figure 1

Mean performance of parents for Jassid population biochemical and trichome studies along with the yield parameter

Supplementary Files

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- [Table1.docx](#)