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K Keerthivarman
Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Subashnini
Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Aravind
Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
K Keerthivarman
Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Morphological and biochemical screening of upland cotton (*Gossypium hirsutum* L.) hybrids for jassid (*Amrasca devastans* Dist.) resistance

K Keerthivarman, S Subashnini and K Aravind

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Abstract
The current experiment was carried out in the field in a Randomized Block Design (RBD) for parents *viz.*, MCU5, MCU7, CO14, and CO17, which were utilized as lines (high yield) and KC2, KC3, NDLH1755 and NDLH1938, which were used as testers throughout the summer season of 2021. From the mean values among all parents calculated, KC 2 had lowest jassid population of mean 4.13 numbers per leaf, highest amount of phenol (3.72 mg/g), low amount of reducing sugars (1.22 mg/g) and high chlorophyll index (58.73). Among other hybrids the hybrid MCU 5 × KC 3 outperformed for highest phenol content (3.72 mg/g), highest chlorophyll index (58.73) with lowest Jassid count (4.13 numbers/ plant). Hence from the correlation results, number of jassids per plant would be lower for high phenolic content, low reducing sugar, lower soluble proteins, higher chlorophyll index and higher trichome density. Hence breeding could be made for resistance to jassids by insight into this biochemical analysis and trichome density of the cultivar which would help reduce use of insecticides and pest load from the fields thereby enhancing the yield of the population.

Keywords: Jassid screening, biochemical, phenol, reducing sugar, trichome length, trichome density, soluble proteins

Introduction

Cotton (*Gossypium hirsutum* L.), a valuable crop is a major industrial commodity across the world. It is the second-oldest and most important commercial crop. India accounts for 33% (10.7 million hectares) of world cotton acreage and 22% (5.4 million tonnes) of global cotton production. The unsystematic application of chemical pesticides to control major pests on cotton led to development of pesticide resistance in both targeted and non-targeted pests, disruption of the natural balance of pests and their natural enemies, resurgence of minor pests, pollution of the crop ecosystem, health and economic risks and development of sucking pest resistance (Pawar and Kadam 1995) [17]. Furthermore, conventional pesticides do not provide the intended control of sucking pests and leafhoppers and whitefly outbreaks on cotton are common (Ahmad *et al.* 2002) [1].

Eco-friendly pest management is required in an integrated approach to ensure a sustainable production. Despite the availability of effective insecticides and the drawbacks of chemical insecticide use, cultivating resistant cotton cultivars is the cheapest and most harmless strategy to managing sucking pest infestations in an integrated pest management programme. Breeders can use the resistant cultivar in their breeding programme to produce resistant genotypes that are environmentally sustainable, socially stable, and economically viable. As a result, resistant cotton cultivars play an important role in a long-term agricultural system. Expertise is required to collect data on specific biochemical features and their relationship to sucking pest infestation (Rizwan *et al.* 2021) [19]. The number of sucking pests varies from cultivar to cultivar due to physical characteristics of cotton plants, particularly on leaves. Biochemical components such as protein, nitrogen, phosphorus, potassium, phenol, and sugar are among the most well-known characteristics associated with resistance. Potential biotic stressors are expressed by insects to their host plants. Plants that are attacked by insects respond by changing the composition and characteristics of their cell walls, as well as the content of their nutrients (secondary metabolite biosynthesis) (Hopkins and Huner, 2004) [9]. Modifications in plant protein profiles and oxidative enzymes have been shown to be among a plant's earliest responses to insect herbivores (Rafie *et al.* 1996; Chaman *et al.* 2001 and Ni *et al.* 2001) [5, 16].

As a result, the current research will focus on the many biochemical (phytochemical) compounds found in plants that are important for conferring resistance/susceptibility to sucking pest in chosen cotton cultivars.

Materials and Methods

The current experiment was carried out in the field in a Randomized Block Design (RBD) for parents, MCU5, MCU7, CO14, and CO17, which were utilized as lines (high yield) and KC2, KC3, NDLH1755 and NDLH1938, which were used as testers throughout the summer season of 2021(testers parent for jassid resistance and lines selected had good quality for lint). Using Dock's method, each of the four lines was crossed with four testers individually utilizing manual emasculation and pollination (Doak, 1934) [6]. In 2021, 16 hybrid F_1 seeds were raised, along with their 8 parents and a standard check (MCU 5). The genotypes were then raised in Department of cotton, Tamil Nadu Agricultural University, Coimbatore (2020-2021).

Total soluble proteins

Total soluble protein in cotton leaves was estimated using Lowrey *et al.* 1951 [13]. Using a known concentration of bovine serum albumin, a standard curve was created. Protein content in both types of samples was estimated using a standard calibrated curve with known bovine protein values.

Total reducing sugars: With a few adjustments, the total reducing sugars were computed using the Miller *et al.* (1972) approach. The total reducing sugars of the hybrids and parents were estimated using a standard graph of glucose created by known glucose concentrations (100-500 g).

Total phenols

Malick and Singh's (1980) [11] technique was used to determine total phenols (µg/gM). The hybrids and parents were determined using the standard curve. Catechol is used to make the standard. Catechol stock solution, 1000ppm: 100mg in 100ml dilution The standards' colour intensity is measured at 650nm using this 200ppm, 400ppm, 600ppm, and 800ppm scale.

Cotton genotypes were tested for Jassid resistance:

The population to be examined for resistance was raised in field conditions. For comparing resistant and vulnerable individuals, standard inspections should be made.

Jassid population and damage assessment

Jassid injury was examined using the technique of the Indian Central Cotton Committee (ICCC, 1960) [10] 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.

Table 1: Show the symptoms

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; and
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).

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

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

Trichome density and Trichome length analysis

The MI-DC1300, MI-DC3000, and MI-DC5000 are compound microscope digital cameras that provide crisp images with excellent contrast and colour. The photos for the leaf samples that were newly harvested at 50 DAS and sliced to the size of one centimetre square were presented on the slides using the Scope Photo software. Then still shots were taken, and the analysis performed.

Chlorophyll index

A KONICA MINOLTA SPAD – 502 machine was used to determine it. The SPAD metre is a useful tool for measuring chlorophyll index fast and non-destructively. The measurement was saved to memory automatically. The SPAD meter results were proportional to the quantity of chlorophyll in the leaf, and they were calculated by measuring the leaf absorbance in the red and near-infrared bands (Uddling *et al.*, 2007) [24].

Result and Discussion

Mean performance of parents and hybrids

The parents MCU 5, MCU 7, CO 14 and CO 17 were used as lines with good fibre quality traits; KC 2 KC3, NDLH 1755 and NDLH 1938 were used as testers with resistance to Jassid. MCU 5 had highest yield of 117.69 g/plant among all parents. Among the lines, CO 17 had maximum mean jassid population per leaf (9.69) with lowest amount of phenol (1.25 mg/g), highest amount of reducing sugar (3.87 mg/g) and lowest chlorophyll index (22.43). KC 2 had lowest jassid population of mean 4.13 numbers per leaf, highest amount of phenol (3.72 mg/g), low amount of reducing sugars (1.22 mg/g) and high chlorophyll index (58.73). MCU 7 among the lines had maximum injury grade of 2.40 reported as moderately resistant and maximum trichome length of 1.70 mm per square centimetre. The tester NDLH 1938 had lowest injury grade of 0.36 with the lowest trichome length of 0.49 mm per square centimetre. The tester KC3 had highest trichome length of 1.73 mm per square centimetre and lowest total soluble protein (27.46 mg/g). The tester NDLH 1755 had highest trichome density of 290.66 numbers per square centimeter with highest total soluble proteins (37.32 mg/g). Lowest trichome density of 115.15 numbers per square centimeter was recorded for MCU 5.

The hybrid MCU 5 X KC 3 noted for highest phenol content (3.72 mg/g), highest chlorophyll index (58.73) with lowest Jassid count (4.13 numbers/ plant). The hybrid MCU 5 X NDLH 1755 had lowest soluble protein content per plant hence next best lowest jassid count of 4.14 numbers per plant. The F_1 of the cross MCU 5 X KC 2 found to have susceptible injury grade of more than three with lowest phenol content of 1.38 mg per gram of the leaf. The hybrid of the cross CO 14 X NDLH 1938 had high trichome density (290.66 mean number of hairs per cm^2) with resistant injury grade of less than one and highest seed cotton yield per plant (155.32 g/ plant) indicating trichome density indirectly paves way for jassid resitance thereby increasing seed cotton yield of the plant. The hybrid MCU 7 X NDLH 1755 had lowest mean of seed cotton yield per plant (82.78 g/ plant) among the hybrids with susceptible grade. The F_1 of the cross CO 17 X KC 2 had highest trichome length of 2.19 mm with lowest trichome density (129.30 numbers) mean value. CO 17 x NDLH 1938 $F_{1,}$ had highest total reducing sugars (3.49 mg/g) with highest mean jassid per plant of 8.9 numbers per plant. CO 17 x NDLH 1938 F_1 had the lowest injury grade (0.36) among all hybrids.

Correlation

Number of jassids per plants showed highly positive significance with amount of reducing sugars (0.914) but negatively correlated with trichome density (-0.835), amount of phenols (-0.857) present in the plant and chlorophyll index (-0.871). Ahmad *et al.* (2005), Ashfaq *et al.* (2010) [3], Rustamani *et al.* (2014) [21], Gonde *et al.* (2015) [8], Kanher *et al.* (2016) [12], Sankeshwar *et al.* (2016) [22] and Amin *et al.* (2017) [2] also reported negative correlation of number of jassids with trichome density. Trichome length was found to have non significant positive correlation with number of jassids which is not in agreement with Batti *et al.* (2015) [4]. Injury grade showed high negative correlation with amount of phenol (-0.652) and chlorophyll index of the plant (-0.490). The phenol content present in the plant was highly negatively correlated with amount of reducing sugars (-0.849), amount of soluble proteins present (-0.432). Amount of reducing sugar is highly negatively correlated with the chlorophyll index (-0.914). Seed cotton yield of the plant had negative non-significant correlation with number of jassids per plant (-0.021) and pest index score (-0.023).

Hence from the above results, number of jassids per plant would be lower for high phenolic content, low reducing sugar, lower soluble proteins, higher chlorophyll index and higher trichome density. Shinde *et al*. (2014) [23] also reported high phenolics provide resitance to plants from Jasssids. Higher phenolic content along with higher trichome density in cotton plant would result in a highly resistant plant was also suggested by Rohini *et al.* (2011) [20]. As phenol content in cotton plants acts as the feeding deterrant the population of jassid feeding that respective plant would be lower. This result was in accordance with Manivannan *et al.* (2021) [15]. Similar result of higher protein favouring higher number of jassid population was also reported by him. Higher amount of reducing sugar would result in susceptible genotypes for jassid. Hence breeding could be made for resistance to jassids by insight into this biochemical analysis and trichome density of the cultivar which would help reduce use of insecticides and pest load from the fields thereby enhancing the yield of the population.

Table 2: Mean performance of parents for Jassid population biochemical and trichome studies along with the yield parameter

Crosses	Mean Jassid nymph/plant (Nos.)	Jassid injury grade	Trichome length (mm/sq.cm)	Trichome density (nos./sq.cm)	Total phenol (mg/g)	Total reducing sugar (mg/g)	Total soluble protein (mg/g)	Chlorophyll index	Seed cotton yield per plant (g/plant)
Lines									
MCU5	9.28 ab	1.31 cde	1.58 defg	115.15 l	1.38 e	3.62 a	36.42 bcd	28.10 hijk	102.83 fgh
MCU7	9.49 a	2.40 b	1.70 cdef	154.89 ijk	1.32 e	3.64 a	32.79 ef	25.78 ijk	90.53 ghij
CO14	9.49 a	1.75 bc	1.44 efghi	148.91 ijkl	1.32 e	3.79 a	35.51 cd	23.74 jk	89.05 hij
CO17	9.69 a	1.29 cde	1.23 hijk	145.60 jkl	1.25 e	3.87 a	30.15 gh	22.43 k	83.22 ij
Testers									
KC2	4.13 i	0.73 defg	0.92 kl	273.96 abc	3.72 a	1.22 g	32.71 ef	58.73 a	79.24 j
KC3	4.14 i	0.69 defg	1.73 bcde	262.31 bcd	3.65 a	1.30 g	27.46 i	57.47 a	94.67 fghi
NDLH 1755	4.14 i	0.74 defg	1.26 ghij	290.66 ab	3.47 a	1.42 g	37.32 abc	56.20 a	88.51 hij
NDLH 1938	4.54 hi	0.36 g	0.49 m	238.15 cde	3.45 a	1.43 g	32.12 fg	54.66 ab	90.17 ghij
Mean	6.86	1.16	1.30	203.70	2.45	2.54	33.06	40.89	103.42
Max	9.69	2.40	1.73	290.66	3.72	3.87	37.32	58.73	117.69
Min	4.13	0.36	0.49	115.15	1.25	1.22	27.46	22.43	89.82

* Same alphabetical letters indicate denotes they were non- significant

Table 3: Mean performance of 16 hybrids for Jassid population, biochemical and trichome studies along with the yield parameter

Crosses	Mean Jassid nymph/ plant (Nos.)	Jassid injury grade	Trichome length (mm/sq.cm)	Trichome density (nos./sq.cm)	Total phenol (mg/g)	Total reducing sugar (mg/g)	Total soluble protein (mg/g)	Chlorophyll index	Seed cotton yield per plant (g/plant)
MCU5 X KC 3	4.65 hi	1.63 c	1.38 fghi	304.80 a	3.42 a	1.50 g	30.45 fgh	52.53 abc	106.28 fg
MCU5 X NDLH 1755	5.15 hi	1.16 cdef	0.54 m	262.10 bcd	3.39 a	1.76 fg	27.34 i	48.61 bcd	144.56 ab
MCU5 X KC 2	7.85 cdef	3.85 a	1.17 hijkl	271.42 abc	1.38 e	2.30 de	31.19 fg	44.48 def	124.10 de
MCU5 X NDLH 1938	7.62 defg	3.52 a	2.16 a	200.94 fgh	1.52 e	2.31 de	36.89 bcd	29.88 hij	106.58 fg
MCU7 X KC 2	7.35 efg	3.37 a	1.28 ghij	183.38 ghi	1.59 e	3.32 ab	35.47 cd	30.10 hij	124.96 cde
MCU7 X KC 3	7.20 efg	3.34 a	1.01 jkl	153.17 ijk	1.73 de	2.90 bc	39.56 a	32.72 ghi	128.60 bcd
MCU7 X NDLH 1755	7.15 efg	3.17 a	1.96 abc	184.88 ghi	1.77 cde	2.82 bcd	36.62 bcd	33.22 gh	82.78 ij
MCU7 X NDLH 1938	6.88 fg	3.12 a	1.92 abc	208.36 efg	2.21 bcd	2.71 cd	37.51 abc	38.59 fg	110.51 ef
CO14 X KC 2	6.78 fg	1.77 bc	1.50 defgh	227.06 def	2.32 bc	2.66 cde	38.63 ab	39.13 efg	136.90 bcd
CO14 X KC 3	6.71 fg	1.40 cd	2.04 ab	219.31 efg	2.35 b	2.65 cde	37.43 abc	40.33 ef	141.09 abc
CO14 X NDLH 1755	6.55 g	1.35 cde	2.03 ab	239.96 cde	2.46 b	2.64 cde	28.66 hi	40.57 ef	152.90 a
CO14 X NDLH 1938	5.17 hi	0.27 g	1.23 hijk	204.55 efgh	3.38 a	1.77 fg	32.80 ef	46.49 cde	155.32 a
CO17 X KC 2	8.12 bcde	0.61 efg	2.19 a	129.30 kl	2.56 b	2.49 cde	31.48 fg	40.79 ef	134.28 bcd
CO17 X KC 3	8.56 abcd	0.40 fg	1.13 ijkl	169.02 hij	2.68 b	2.32 de	32.30 efg	41.52 def	97.14 fghi
CO17 X NDLH 1938	8.90 abc	0.37 g	1.82 bcd	168.93 hij	2.69 b	3.49 a	34.74 de	42.88 def	131.34 bcd
CO17 X NDLH 1755	5.37 h	1.90 bc	0.87 l	275.35 abc	3.37 a	2.14 ef	27.46 i	45.70 cdef	92.44 ghij
Mean	6.88	1.95	1.51	212.66	2.43	2.49	33.66	40.47	123.11
Max	8.90	3.85	2.19	304.80	3.42	3.49	39.56	52.53	155.32
Min	4.65	0.27	0.54	129.30	1.38	1.50	27.34	29.88	82.78

Table 4: Simple correlation of Jassid population, biochemical and trichome analysis along with the yield parameter

	NJP	IG	TL	TD	P	RS	SP	CI	SCY
NJP	1								
IG	0.273	1							
TL	0.375	0.191	1						
TD	-.835**	-0.069	-0.341	1					
P	-.857**	-.652**	-0.37	.679**	1				
RS	.914**	0.324	0.353	-.799**	-.849**	1			
SP	0.269	0.366	0.298	-0.374	-.432*	0.342	1		
CI	-.871**	-.490*	-0.353	.792**	.921**	-.914**	-0.377	1	
SCY	-0.021	-0.023	0.181	-0.013	0.043	0.014	0.018	0.03	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

NJP - Mean number of Jassids per leaf, S - Amount of reducing sugars (mg/g), TL - Trichome length (mm), IG - Pest Injury Grade, SP - Total soluble proteins (mg/g), TD - Trichome Density (nos./cm^{-2}), P - Total amount of phenol (mg/g), CI - Chlorophyll index, SCY - Seed cotton yield per plant

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