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Screening of cotton germplasm for their reaction against leafhopper, *Amrasca biguttula biguttula* Ishida (Homoptera: Cicadellidae)

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ABSTRACT: Preliminary screening of twenty-nine cotton genotypes (*Gossypium hirsutum*) against the leafhopper *Amrasca biguttula biguttula* Ishida undertaken, during August 2019 to February 2020, revealed fifteen genotypes viz., TCH 357, TCH 1809, TCH 1828, TCH 1895, TCH 1897, TCH 1941, TSH 383, TVH 002, TVH 003, TKH 0762, TKH 1225, SVPR 6, CO 15, KC3 and Suraj, as moderately resistant. Selected preliminary screening entries subjected to advanced screening revealed TCH 357, TCH 1809, TCH 1895, TCH 1897, TCH 1941, TCH 1828, TSH 383, TVH 002, TVH 003, TKH 0762, SVPR 6 and CO 15 as moderately resistant with population range of 2.75 to 4.42 numbers per three leaves and KC3 resistant. In the artificial screening, the resistant cultivar KC3 had least leafhoppers (2.67 per plant) and it had 56 trichomes per 300 μm^2, which was higher than resistant check NDLH 1938. The resistant genotype KC3 had the maximum phenol (4.3 mg g^{-1}), amino acid (136 mg g^{-1}) and tannin (167 mg g^{-1}), while the susceptible genotype DCH 32 had the lowest amount of total phenol (1.2 mg g^{-1}), amino acids (18 mg g^{-1}) and tannin (40 mg g^{-1}).

KEYWORD: Genotypes, resistant cultivars, trichomes, phenols, tannin

INTRODUCTION

Amrasca biguttula biguttula Ishida (Homoptera: Cicadellidae), has been abundant in cotton in recent years, from the vegetative through reproductive stages of crop growth. Pesticides used to manage pests in the cotton ecosystem in India account for 45 per cent of all pesticides used. India, which has one third of the world's cotton farmers, account for 54 per cent of all pesticides used annually in cotton, despite occupying just 5 per cent of land under crops (Environmental Justice Foundation, 2007; Aktar *et al.,* 2009). As a result, developing a resistant/ tolerant cultivar is critical to reduce the three "R's" in the environment: resistance, resurgence, and residues. With this background, studies on refinement of screening methodology, identification of host plant phenotypic and genotypic features that contributes to resistance to leafhopper, using scanning electron microscope and biochemical characterisation were undertaken.

MATERIALS AND METHODS

Preliminary screening under natural condition

Twenty-nine cotton genotypes including NDLH – 1938 (Resistant check) and DCH 32 (Susceptible check), were included in the screening experiment (Table 1). Standard check was sown in the middle and ends of two sides of the genotypes with a spacing of 75cm row x 45 cm plant, in the 20 m^2 plots.

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Genotypes were sown in four rows in a randomised block design with three replications. Between each genotype, bhendi was sown as an infester crop and the bhendi plants in the susceptible check were clipped after a substantial increase of leafhopper population (>2 nos./3 leaf) at 30 days after sowing (DAS). If the adult leafhopper population in susceptible check (>8 nos./3 leaf) was not reached even after 75 days, the experiment was repeated until the population was sufficient in natural conditions. From sowing to harvest, no pesticides were used. Standard agronomic cultivation practices were followed. Screening experiment was undertaken in the fields of the Department of Cotton in Coimbatore during August 2019 – February 2020.

Population assessment of leafhopper under natural condition

Nymphs population of leafhoppers was recorded on ten randomly selected plants in each replication for all the genotypes, including standard check at 30, 45 and 60 days after sowing. In each plant three leaves from top, middle and bottom were observed and mean population per three leaves was recorded.

Damage assessment of leafhopper under natural condition

Hopper burn injury was assessed as per the methodology enumerated by Indian Central Cotton Committee (1960). A visual rating of hopper injury on each genotype was recorded on 30, 45 and 60 days after sowing and leafhopper injury grade index was calculated.

Grade	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, and stunted growth

Leafhopper injury grade Index (LIGI)

A leafhopper injury grade index was calculated as proposed by Nageswara Rao (1973),

$$LHRI = \frac{G1 \times P1 + G2 \times P2 + G3 \times P3 + G4 \times P4}{P1 + P2 + P3 + P4}$$

Where G represented the number of the grade of ICCC (now ICAR-CICR) and P represented the number of leafhopper population of same plant under the each entry. Grade index with ≤ 1.0 grouped as resistant, $1.0 > \leq 2.0$ as moderately resistant, $2.0 > \leq 3.0$ as susceptible and $3.0 > \leq 4.0$ as highly susceptible.

Advanced screening under artificial condition

Artificial screening for leafhopper resistance was conducted at the net house, Department of Cotton, TNAU, Coimbatore. Cotton seeds of genotypes along with standard check for resistant and susceptible to leafhopper were sown during September 2020 in pots and for each entry three plants were maintained. The genotypes in the potted plants were placed at random but equidistantly apart in a circle inside the hopper net. Each pot represented a replication. Three replications were maintained for each genotype. Three hundred field collected adults were released in the middle and the top of the set up. Caging was used for screening. The leafhopper was released on 15 day old plants and counts of population and hopper burn injury were taken up to 60 days after sowing.

Trichome density by Scanning Electron Microscope

A fresh leaf sample was taken from a 6-week-old plant in a pot culture. Fixation was done using glutaraldehyde and formaldehyde, followed by osmium tetroxide postfixation. By critical point drying, the fixed tissue was dehydrated by liquid carbon dioxide. For observation under the microscope, the dried specimen was mounted on a specimen stub using an adhesive such as epoxy glue and sputter-coated with gold alloy. A microtome was used to segment the samples. Images taken with a scanning electron microscope (SEM) at 1000x magnification were captured. In a 300μm^2 region, SEM images of trichome density were gathered.

Table 1. Preliminary screening of genotypes under natural condition against leaf hopper

No.	Genotype	No./ 3 leaves	grade index	Rating	SCY/pl (g)
1	TCH 357	2.24(1.94)^{d}	1.82	MR	375(13.62)^{n}
2	TCH 1764	3.46(1.32)^{i}	2.35	S	320(13.44)^{ij}
3	TCH 1772	4.87(1.80)^{lm}	2.57	S	355(18.32)^{l}
4	TCH 1807	4.25(1.66)^{k}	2.85	S	380(18.18)^{n}
5	TCH 1809	2.76(1.66)^{f}	1.46	MR	300(18.40)^{gh}
6	TCH 1811	3.73(1.41)^{j}	2.28	S	350(16.45)^{kl}
7	TCH 1828	3.47(2.40)^{i}	1.34	MR	300(12.06)^{gh}
8	TCH 1895	1.75(0.71)^{c}	1.49	MR	255(0.71)^{d}
9	TCH 1897	3.42(1.56)^{i}	1.38	MR	435(3.76)^{o}
10	TCH13/22	4.97(1.35)^{m}	3.42	HS	385(3.73)^{n}
11	TCH13/24	4.72(1.52)^{l}	2.67	S	475(4.34)^{p}
12	TCH 1941	2.50(1.47)^{e}	1.56	MR	325(4.32)^{hi}
13	TSH 383	1.75(1.47)^{c}	1.37	MR	310(4.31)^{i}
14	TSH 387	3.00(1.38)^{g}	2.86	S	355(4.12)^{l}
15	TVH 002	1.25(1.70)^{a}	1.27	MR	285(3.54)^{fg}
16	TVH 003	2.25(1.10)^{d}	1.32	MR	280(1.10)^{df}
17	TVH 007	3.25(1.44)^{h}	2.67	S	260(2.06)^{de}
18	TKH 0762	2.25(1.36)^{d}	1.33	MR	365(2.06)^{l}
19	TKH 1225	1.25(1.42)^{a}	1.21	MR	265(2.20)^{e}
20	SVPR 6	1.75(1.40)^{c}	1.23	MR	215(2.20)^{c}
21	CO 14	3.25(1.40)^{h}	2.42	S	310(2.19)^{hi}
22	CO 15	1.75(1.40)^{c}	1.24	MR	385(2.15)^{n}
23	CO 17	3.25(1.48)^{h}	2.45	S	185(2.01)^{b}
24	KC3	1.25(1.26)^{a}	1.12	MR	180(1.26)^{b}
25	RCH 659	2.75(1.39)^{f}	2.34	S	335(1.60)^{jk}
26	BGDS 1063	2.25(1.36)^{d}	2.46	S	330(1.60)^{j}
27	Suraj (check)	2.25(1.39)^{d}	1.38	MR	325(1.64)^{ij}
28	NDLH – 1938 (RC)	1.50(1.38)^{b}	0.84	R	270(1.64)^{ef}
29	DCH 32 (SC)	5.25(1.38)^{n}	3.29	HS	145(1.64)^{a}
	SE	0.0175		0.2258	
	CD	0.0350		0.4523	

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Table 2. Advanced screening of genotypes under artificial condition

No.	Genotype	No. /3 leaves	Grade Index	Rating
1	TCH 357	4.00(1.94)[h]	1.9	MR
2	TCH 1809	2.75(2.04)[b]	2.0	MR
3	TCH 1828	3.83(2.00)[g]	1.9	MR
4	TCH 1895	3.50(1.93)[e]	1.8	MR
5	TCH 1897	3.67(1.78)[f]	2.0	MR
6	TCH 1941	3.75(1.58)[fg]	1.8	MR
7	TSH 383	4.42(2.71)[i]	1.8	MR
8	TVH 002	2.92(0.71)[c]	1.8	MR
9	TVH 003	3.17(1.56)[d]	1.9	MR
10	TKH 0762	3.67(1.59)[f]	1.7	MR
11	TKH 1225	3.27(1.58)[d]	2.5	S
12	SVPR 6	3.67(1.56)[fg]	2.0	MR
13	CO 15	3.50(1.51)[e]	2.0	MR
14	Suraj	3.24(1.44)[d]	2.6	S
15	KC3	2.67(1.79)[b]	1.0	R
16	NDLH – 1938 (RC)	2.00(1.10)[a]	1.0	R
17	DCH 32 (SC)	6.83(1.44)[j]	3.1	HS
	SE	0.0204		
	CD	0.0415		

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Estimation of biochemical parameters

Estimation of total phenol: Fresh leaf sample (200 mg) was extracted with 10 ml absolute methanol. One ml of Folin Ciocalteau reagent diluted with equal volume of distilled water before use was added to one ml of alcohol extract in a test tube followed by 2 ml of 20 per cent sodium carbonate. The mixture was heated on a boiling water bath for one minute. The blue colour was measured at 660 nm. Reagent blank was maintained with 80 per cent ethanol. Total phenols were calculated from catechol standard (Bray and Thorpe, 1954).

Estimation of tannin: A 200 mg sample of fresh cotton leaf was extracted with 10 ml absolute methanol. The extracted material was rotavated for 20 minutes with a screw cap culture tube. The mixture was centrifuged at 3000 rpm for 10 minutes, and the supernatant was utilised to conduct the analysis. Each extract is poured in one ml aliquots into a culture tube (each sample maintains with blank). Five ml of vanillin reagent (sample) and five ml of 4 percent HCL solution (blank) were added at 1.0 minute intervals, then maintained in a water bath for exactly 20 minutes. The absorbance at 500 nm was measured using a Spectrophotometer.

Table 3. Leaf trichome density of advanced genotypes

SNo.	Genotypes	No./ 3 leaves	Rating	Trichomes(300 µm²)		
				No.	Branch	Total
1	TCH 1897	3.67(1.92)^{f}	MR	4	4	16
2	TVH 002	2.92(2.04)^{c}	MR	2	2	4
3	TCH 1828	3.83(1.80)^{g}	MR	7	2	14
4	SVPR 6	3.67(2.00)^{f}	MR	9	2	18
5	TCH 1941	3.75(1.78)^{fg}	MR	5	2	10
6	TSH 383	4.42(1.58)^{i}	MR	6	2	12
7	TCH 1895	3.50(2.24)^{e}	MR	2	1	2
8	TCH 357	4.00(0.71)^{h}	MR	3	2	6
9	TVH 003	3.17(1.55)^{d}	MR	5	4	20
10	TKH 0762	3.67(1.59)^{f}	MR	3	8	24
11	TCH 1809	2.75(1.52)^{b}	MR	5	4	20
12	CO 15	3.50(1.58)^{e}	MR	6	3	18
13	KC3	2.67(1.51)^{b}	R	7	8	56
14	NDLH – 1938 (RC)	2.00(1.44)^{a}	R	4	4	16
15	DCH 32 (SC)	4.50(1.65)^{i}	HS	1	1	1
	SE	0.0187				
	CD	0.0382				

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

Estimation of total free amino acids: Phosphate buffer pH 7.0 was used to extract 200 mg of cotton leaves. In test tubes, 1.0 ml of extract was collected. The leaf extract was then combined with 1.0 ml of 10% pyridine and 1.0 ml of 2.0% ninhydrine solution in each tube. The tubes were then heated in a boiling water bath for half an hour, and the volume was increased to 15.0 ml with distilled water to dilute the solution. The coloured solutions' absorbance was measured at 570 nm (Hamilton and Van Slyke, 1943).

Statistical analysis

The total number of insects in the population was transformed to get square root values. The results of the laboratory biochemical analysis were translated into arcsine transformed numbers. Duncan's Multiple Range Test was used to differentiate the mean values of the treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Preliminary screening

Mean leafhopper incidence ranged from 1.25 (KC 3) to 5.25/3 leaves (DCH 32). Based on the resistance index, the twenty nine genotypes were grouped into four categories *viz*., resistant, moderately resistant, susceptible and highly susceptible. Among these genotypes, fifteen genotypes were identified as moderately resistant *viz*., TCH 357, TCH 1809, TCH 1828, TCH 1895, TCH 1897, TCH 1941, TSH 383, TVH 002, TVH 003, TKH 0762, TKH 1225, SVPR 6, CO 15, KC3 and Suraj. Leafhopper population was comparatively low in these entries with leafhopper injury grade II which was on par with standard check NDLH 1938. The remaining genotypes were recorded as susceptible (11 genotypes) and TCH13/22 was highly susceptible to leafhopper which was on par with

Table 4. Biochemical analysis of genotypes

No	Genotypes	Rating	Total phenol (µg/g)	Amino acid (µg/g)	Tannin (µg/g)
1	TCH 357	MR	3.3(1.94)^{e}	81(9.92)^{d}	152(12.55)^{h}
2	TCH 1809	MR	3.7(2.00)^{g}	84(8.22)^{de}	116(11.73)^{c}
3	TCH 1828	MR	2.8(1.82)^{bc}	86(9.72)^{ef}	99(11.98)^{b}
4	TCH 1895	MR	3.1(2.07)^{d}	92(8.69)^{i}	127(9.92)^{d}
5	TCH 1897	MR	2.7(2.19)^{b}	76(11.68)^{c}	135(12.94)^{e}
6	TCH 1941	MR	2.9(2.12)^{c}	83(9.51)^{de}	140(12.27)^{ef}
7	TSH 383	MR	2.9(1.30)^{c}	95(4.30)^{ij}	138(6.36)^{ef}
8	TVH 002	MR	2.7(0.37)^{b}	87(0.71)^{fg}	138(0.71)^{ef}
9	TVH 003	MR	3.4(1.57)^{ef}	98(3.23)^{j}	157(3.61)^{h}
10	TKH 0762	MR	3.5(1.58)^{f}	67(2.95)^{b}	137(3.50)^{ef}
11	SVPR 6	MR	2.8(1.52)^{bc}	94(3.20)^{i}	143(3.53)^{fg}
12	CO 15	MR	3.8(1.60)^{g}	75(3.03)^{c}	98(3.23)^{b}
13	KC3	R	4.3(1.64)^{i}	136(3.49)^{k}	167(3.67)^{i}
14	NDLH 1938 (RC)	R	4.0(1.62)^{h}	90(3.16)^{g}	150(3.57)^{gh}
15	DCH 32 (SC)	HS	1.2(1.34)^{a}	18(2.19)^{a}	40(2.62)^{a}
	SE		0.0164	0.0827	0.1549
	CD		0.0336	0.1693	0.3174

R – Resistant; MR – Moderately Resistant; S – Susceptible; HS – Highly Susceptible; RC – Resistant Check; SC- Susceptible Check. Figures in the parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT.

the susceptible check, DCH 32 (Table 1). Insect resistant crop varieties have the unique advantage of providing inherent insect control which is compatible with other methods of insect control and provides more practical approach in leafhopper management. Several workers reported on the varietal susceptibility in cotton to *Amrasca devastans* and accessions resistant to leafhopper (Balasubramanian *et al.,* 1978; Ambekar and Kalbhor, 1981; Chandramani *et al*., 2004). Manivannan *et al.,* 2017).

Advanced screening

Seventeen entries including standard checks *viz*., NDLH 1938 and DCH 32 were selected from the preliminary screening for advanced screening experiment under protected net house. Advanced screening experiment revealed 12 entries *viz.,* TCH 357, TCH 1809, TCH 1895, TCH 1897, TCH 1941, TCH 1828, TSH 383, TVH 002, TVH 003, TKH 0762, SVPR 6 and CO 15 as moderately resistant with population ranging from 2.75 to 4.42 numbers per three leaves (Table 2). KC3 was reordered as resistant entry to the leafhopper which was on par with the resistant standard check NDLH 1938. The remaining two genotypes (TKH 1225 & Suraj) were recorded as susceptible and DCH 32 highly susceptible (Table 2). Adult leafhoppers were seen settling in more numbers on highly susceptible and susceptible plants indicating more sustained feeding on susceptible plants. Mohankumar (1996) and Manish (1998) reported similar results. Barroga and Bernardo (1993) observed more preferential settling of *A. devastans* on susceptible cotton. Considering the morphological mechanism of leafhopper resistance, Uthamasamy (1985) reported that morphological characters such

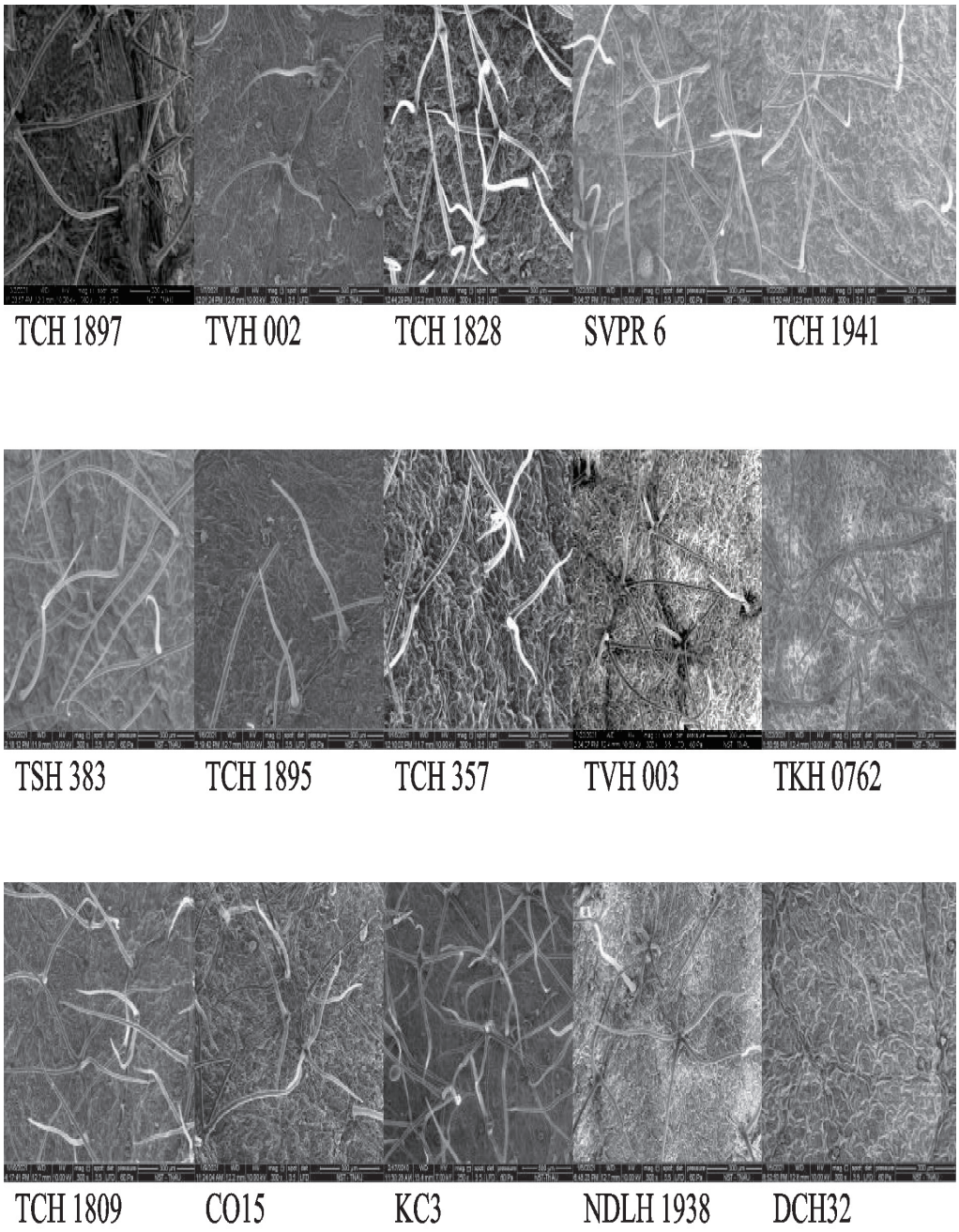

Fig 1. Trichomes density photograph by Scanning Electron Microscope

as hairiness of leaves, toughness of leaf veins, thickness of leaf lamina, length of the hair and angle of insertion are associated with the resistance to leafhoppers. H-1316, GISV-216 and CPD 1019 were reported as resistant (Rao *et al.,* 2011). Nine entries were categorized as highly resistant to leaf hopper with injury grade 1 with a population range of 0.57 to 3.57 per 3 leaves per plant (Sasikumar and Rathika, 2020).

Morphological parameters

Among the fifteen entries, nine genotypes were observed with maximum number of trichomes (12 to 56/ 300μm^2). It is concluded that trichome density play a major role in resistance and found to have significant negative correlation with the leafhopper population and damage (Fig. 1 & Table 3). The plant leaf characters, *i.e.,* leaf length, trichome density and trichome size are essential features to minimize jassid populations in cotton crop (Kanher *et al.,* 2016). The incidence of leafhoppers was also reported to be lower in high hairy varieties and higher in low hairy genotypes, indicating that trichome density plays a substantial role in sucking pest resistance (Manivannan *et al*., 2017).

There are reports that leafhopper injury grade having negative association with plant height, inter nodal length, leaf hair density, hair length, hair density on mid vein, total chlorophyll, chlorophyll a and chlorophyll b and positive association with the moisture content (Ambekar and Kalbhor, 1981, Uthamasamy, 1985; Sivasubramanian *et al.,* 1991; Mohankumar, 1996; Murugesan and Kavitha, 2010; Venkatesha, 2014; Bhatti *et al.,* 2015; Amin *et al.,* 2017; Khalil *et al.,* 2017) of which the contribution of leaf hair density to the leafhopper resistance was made vivid in the present study.

Biochemical parameters

Phenol (4.3 μg g^{-1}), amino acid (136 μg g^{-1}) and tannin (167 μg g^{-1}) was maximum in the resistant variety (KC3) which was higher than the standard check (NDLH 1938). Remaining eleven moderately resistance entries showed total phenol content ranging between 2.7 and 3.8 μg g^{-1}. The susceptible culture DCH 32 recorded the lowest phenol (1.2 μg g^{-1}), amino acids (18 μg g^{-1}) and tannin (40 μg g^{-1}). All three biochemical parameters were on the higher side in the resistant varieties but lower side in the susceptible culture (Table 4). Lower leafhopper damage injury index was reported with higher quantity of biochemical components like tannins, phenols and gossypol (Rohini *et al.,* 2011; Shinde *et al.,* 2014; Venkatesha, 2014; Harijan *et al.,* 2017). Biochemical profiles revealed that higher level of chlorophyll, nitrogen, protein, amino acids and reducing sugars favors the leafhopper infestation. In contrast, phenol compound act as feeding deterrent, as most of the resistant genotypes showed higher level of phenol (Venkatesha, 2014; Manivannan *et al.,* 2021). Total phenols showed significant and negative correlation with jassid incidence in genotypes of cotton. Highly susceptible entries are preferred for settling and feeding whereas varieties less preferred for settling are less preferred for oviposition (Bhatti *et al.,* 2015; Bhoge *et al.,* 2019).

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