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## Plant biochemicals induced resistance against cotton leafhopper, *Amrasca biguttula biguttula* (Ishida)

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### Abstract

Cotton is one of the world's most important fibre crops. The bollworm complex and sucking pests are the most devastating of the biotic and abiotic stresses limiting cotton production. Cotton leafhoppers are devastating sucking pests, causing both quantitative and qualitative losses. The bollworm complex was stopped by the introduction of Bt cotton. But the sucking pest complex, especially leafhoppers, has flourished, gradually aggravating economic damage. The widespread use of insecticides to control sucking pests has resulted in pest resistance to insecticides of various modes of action. At this point, the host plant resistance (HPR) mechanism (natural pest management method) would be helpful for this situation's handling. In these aspects, biochemicals are an important mechanism of resistance in HPR. Tested for biochemical activity were protein, phenol, proline, free amino acid, and total soluble sugar. From January to April 2022, 41 genotypes were studied for leafhopper resistance against biochemicals. Strong resistance is linked to protein and phenol content, while susceptibility is linked to total soluble sugar content. Strong resistance is linked to protein and phenol content, while susceptibility is linked to total soluble sugar content. The highest protein content was found in TVH/JR/2021-22-3 (57.96 mg/g), and the lowest in TCH 2026 (2.07 mg/g). TCH 2021 had the most proline (145.04 µmol/g) and C14xGSHB 5-3-6-1 had the least (24.22 µmol/g). Phenol content was found to be high in JR/AKH/2021-22 9631 (94.64 µg/g) and low in C14xPP 21-3-1-3 (13.70 µg/g). JR/AKH/2021-22 9637 (32.64 mg/g) had high levels of free amino acids. C14 × C 27 5- 2-1-5 had the highest total leaf carbohydrate (7.20 mg/g).

**Keywords:** Cotton, Leafhopper, Biochemicals, Protein, Phenol, Carbohydrate

### 1. Introduction

Cotton has been cultivated all over the world for over 5000 years. India is the world's largest cotton producer, accounting for approximately 22% of global cotton production. India contributes approximately 37% of the world's cotton cultivation area. The Cotton Corporation of India says that in 2020–21, there were 371.00 lakh bales of cotton grown on 129.57 lakh hectares, with an average yield of 487 kg per hectare. Cotton is infested by more than 326 insect species all over the world. The Indian cotton environment is being attacked by 162 insect pests. Among them, 12 of them are responsible for key pests of cotton (Senguttuvan, 2019) [11]. The leafhopper is one of the sucking pests that have a huge impact on cotton. Both adults and nymphs are sucking plant sap and introducing salivary toxins (Hormechan *et al.*, 2001) [5]. The symptoms are expressed as young leaves turn yellow and reddening develops along the margins. Finally, leaves show downward cupping that is called the "hopper burn symptom." Cotton young boll dropping reduces yield due to extreme damage caused by leafhoppers (Panwar *et al.*, 2014) [7]. This study identified resistance sources among the 41 genotypes as per the host plant resistance protocol. Host plant resistance is a cheap and safe way to avoid of leafhoppers (Devi *et al.*, 2018) [3].

### 2. Materials and Methods

The present study was conducted at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, from January to April 2022. In this study, 41 genotypes were studied for leafhopper resistance against bio-chemicals along with a resistance (NDLH 1938) and susceptible (DCH 32) standard check.

#### 2.1 Population and damage assessment of leafhopper under protected condition

Nymphs' population of leafhoppers was recorded on randomly selected plants in each replication for all the genotypes, including standard check at 30, 45 and 60 days after sowing.

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In each plant three leaves from top, middle and bottom were observed and mean population per three leaves was recorded. 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 and
4	Extreme curling, yellowing, bronzing and browning, drying of leaves and defoliation, 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 and P represented the number of leafhopper population of same plant under the each entry. Grouping of injury index to categories of resistance was as follows.

LIGI	Category
0.0 > ≤ 1.0	Resistant
1.0 > ≤ 2.0	Moderately Resistant
2.0 > ≤ 3.0	Susceptible
3.0 > ≤ 4.0	Highly Susceptible

### Biochemical analysis

The cotton leaves were collected 50 days after planting from potted plants under net house conditions at TNAU, Coimbatore. In this experiment, the biochemical components of each cotton culture were recorded. This was done by following the given procedure.

#### 2.2 Estimation of Total Soluble Protein

Total soluble protein was estimated by the Lowry *et al.*, (1951) method. A 500 mg leaf sample was taken from each of the 41 genotypes. Then the samples were centrifuged and extracted with buffer. The sample (0.2 ml) extract was pipetted out into different test tubes. Working standard solutions of 0.4, 0.8, 1.2, 1.6, and 2.0 ml were pipetted out into a series of test tubes. The volume of all the tubes added up to 1 ml. A tube with 1 ml of water served as the blank. 5 ml of alkaline copper solution was added to the tubes, mixed well, and incubated at room temperature for 10 min. Folin-Ciocalteu Reagent (0.5 ml) was added to the tubes, mixed well immediately and incubated at room temperature in the dark for 30 min. The absorbance was read at 660 nm against the blank. A standard graph was drawn and the amount of protein in the sample was calculated and expressed as mg/g.

#### 2.3 Estimation of Total Phenol Content

Total phenol was estimated by the Bray and Thorpe method (1954). For each of the 41 genotypes, exactly 500mg of the sample was weighed and ground with a pestle and mortar with

80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was saved. The residue was re-extracted five times at a volume of 80% ethanol, centrifuged, and then pooled with the supernatant, and the supernatant evaporated to dryness. The residue dissolved in a known volume of distilled water (5 ml). The different aliquots (0.2 to 2 ml) were pipetted out into test tubes and the volume was made up to 6 ml in each test tube with distilled water. Folin-Ciocalteu reagent (0.5 ml) was added. After 3 minutes, 2 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for exactly one minute, cooled, and then absorbance at 650nm was measured against a reagent blank. A standard curve was prepared using different concentrations of catechol. The concentration of phenols in the test sample was shown on the standard curve as micrograms per gramme (µg/g) of leaf material.

#### 2.4 Estimation of Total Free Amino acids

Moore and Stein's (1948) method for total free amino acid extraction and estimation 0.5 ml of sample extracts was taken from different test tubes. One ml of ninhydrin reagent was added to the test tubes and mixed well. The volume was made up to 1 ml with water. Four ml of the ninhydrin-citrate-glycerol were added and mixed well. After 15 minutes of boiling, the tubes cooled under running water and the absorbance of the purple colour was observed at 570 nm (green filter) against a reagent blank. The amount of total free amino acids was calculated using a standard curve prepared from leucine by pipetting out 0.1-1.0 ml (10-100 mg range) of the working standard solution. The results are expressed as mg/g of sample.

#### 2.5 Estimation of Proline

The Bates *et al.* (1973) method was used to calculate proline. Leaf samples (500mg) from the 41 genotypes was homogenised in a pestle and mortar with 10 ml of 3% aqueous sulphosalicylic acid and filtered through Whatman No. 2 filter paper. The extraction process was repeated, and the filtrates were combined. 2 ml of filtrate was mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin. The reaction was stopped after 1 hour in a boiling water bath by placing it in an ice bath. Toluene (4 ml) was added and vigorously mixed for 20-30 seconds. The toluene chromophore layer was aspirated and warmed to room temperature. At 520 nm, the absorbance of red was compared to that of a reagent blank. The amount of proline in the sample was determined by comparing it to a pure proline standard curve. The proline content was expressed in micromoles per gramme (µmol/g) of leaf.

#### 2.6 Estimation of Total Leaf Carbohydrate

The Hedge and Hofreiter (1962) method were used to calculate total leaf carbohydrate. A 500 mg of sample was weighed and ground with 80 percent ethanol in a pestle and mortar. In each test tube, 0.5 ml of the sample was taken, and the volume was increased to 1 ml in test tubes with distilled water and 4 ml of anthrone reagent was added to each test tube. The test tubes were heated in a boiling water bath for 8 minutes before being rapidly cooled. Finally, at 630 nm, the absorbance was measured and result was expressed as mg/g.

### 3. Statistical analysis

A completely randomised design was used to conduct

statistical analysis on the protected net house conditions. The total numbers of nymphal population and laboratory biochemical parameters were analyzed using OP stat software.

#### 4. Results and Discussion

Mean leafhopper incidence ranged from 1.13 (NDLH 1938) to 4.78/3 leaves (DCH 32). Based on the resistance index, the fortyone genotypes were grouped into four categories viz., resistant, moderately resistant, susceptible and highly susceptible. Among these genotypes, seventeen genotypes were identified as moderately resistant viz., C14 × GSHB 5-3-6-3, C14 × GSHB 180 7-1-5-2, TCH 1608 × 1822-6-2-1-1, TCH 1608 × 1822-6-2-2-3, TCH 1608 × 1822-6-2-2-4, TCH 1608 × 1822-7-2-1-5, TVH/JR/2021-22 2, JR/AKH/2021-22 9631, VS9-S11-1×1608-8-1-2-3, C14 × GSHB 180 7-1-2-1, C14 × GSHB 180 7-1-2-2, C14 × GSHB 180 7-1-2-4, C14 × GSHB 180 7-1-2-3, JR/AKH/2021-22 9637, TVH/JR/2021-22 3 and TCH 1608 × 1822-6-2-1-2 and Suraj. Leafhopper population was comparatively low (1.48 – 2.35 nos./3 leaves) in these entries which was on par with standard check NDLH 1938. The remaining genotypes were recorded as susceptible (21 genotypes) and TCH 2024 was highly susceptible to leafhopper which was on par with the susceptible check, DCH 32 (Table 1).

#### 4.1 Total Soluble Protein

Among the 41 genotypes, the protein content is highest in NDLH 1938 (60 mg/g of leaf sample), followed by TVH/JR/2021-22-3 (57.96 mg/g of leaf sample), TCH 1608 ×1822-6-2-1-3 (56.62 mg/g of leaf sample) and VS9-S11-1 ×1608 8-1-2-3 (56.59 mg/g of leaf sample). Our results are in accordance with Ramani 2017 (294.68 mg/g). The protein content is low in C14 ×GSHB 180 7-1-2-4 (16.92 mg/g of leaf sample) followed by C14 × VS 7-1-9-1 (17.62 mg/g of leaf sample) and TCH2026 (20.74 mg/g of leaf sample).

#### 4.2 Total Phenol content

Among the 41 genotypes, the phenol content is highest in NDLH 1938 (95.30 µg/g of sample), followed by JR/AKH/2021-22 9631 (94.64 µg/g of sample), Suraj (93.98 µg/g of sample), and JR/AKH/2021-22 9637 (µg/g of sample). Similarly, other scientist Ramani *et al.* 2017 worked on 12 cotton varieties of *G. hirsutum*. They discovered that the highest concentration of phenol was found in the leaves of variety C-1622 (1029.75mg/g). The findings of this experiment are also in line with the results of Divya *et al.* 2017 [14] that resistant genotypes of cotton possess higher

content of phenol then susceptible ones. The Findings are consistent with those of other scientists Balakrishnan N 2006, Sushma Deb 2015 and Bhoge *et al.* 2019 [2]. The phenol content is low in C14 × PP 21-3-1-3 (13.70 µg/g of sample), followed by C14× GSHB 5-3-6-4 (18.50 µg/g of sample) TCH 2024 (19.64 µg/g of sample) and TCH 2021 (22.88 µg/g of sample).

#### 4.3 Total Free Amino acids

Among the 41 genotypes, the free amino acid content was high in JR/AKH/2021-22 9637 (32.64 mg/g) of the sample, followed by TCH 2029 (32.12 mg/g) and TVH/JR/2021-22 3 (30.68 mg/g). Similarly, other scientists Praveen 2013 and Onkara Naik 2015 found that resistant genotypes showed higher level of free amino acids. The free amino acid content was low in TCH 1608 ×1822-6-2-2-4 (7.96 mg/g), followed by TCH 1608× 1822-7-2-1-1 (8.1 mg/g) C14 × PP 21-3-1-3 (8.4 mg/g), C14 ×VS 7-1-9-1 (8.4 mg/g) and TCH 2024 (8.78 mg/g).

#### 4.4 Proline

Proline, an amino acid, acts as an important element in resistance to leafhoppers in cotton plants. Among the 41 genotypes, the proline content is highest in NDLH 1938 (147.11 µmol/g of tissue), followed by TCH 2021 (145.04 micro mole/g of tissue), TVH/JR/2021-22 2 (139.48 µmol/g of tissue), and TCH 1608× 1822-7-2-1-1 (125.19 µmol/g of tissue). The proline content was low in C14 × GSHB 5-3-6-1(24.22 µmol/g of tissue) followed by C14 ×GSHB 5-3-6-4 (25.03 µmol/g of tissue) and VS9-S11-1 × 1608-8-1-2-2 (33.47 µmol/g of tissue).

#### 4.5 Total leaf carbohydrate

The total leaf carbohydrate is high in C14 × C27 5-2-1-5 (7.20 mg/g of sample) followed by C14×GSHB 180 7-1-5-1 (7.03 mg/g of sample), TCH 1608 ×1822-6-2-2-3 (6.79 mg/g of sample) and C14×GSHB5-3-6-4 (6.44 mg/g sample). A similar observation also found with Sonalkar *et al* 2020 that the leafhopper population had significant positive correlation with total sugar (r=0.855). TCH 2024 (1.31 mg/g) has the lowest total leaf carbohydrate (1.31 mg/g), followed by TVH/JR/2021-22 (1.47 mg/g of sample), TCH 2021 (2.04 mg/g), and JR/AKH/2021-22 9637 (2.15 mg/g). These findings are in conformity with Ramandeep Kaur Sandhi 2017 [10], Vijaykumar N Ghante 2019 and Rizwan Muhammad 2021. Hence, I revealed that the higher amount of carbohydrate was negatively correlated with leafhopper resistance.

**Table 1:** Cotton leafhopper resistance scale and biochemical analysis of cotton leaf

S. No.	Cotton genotypes	Mean No. of Leafhopper Nymphal Population	Leafhopper Injury Grade Index	Resistance rating scale	Protein (mg/g)	Phenol (µg/g)	Free amino acid (mg/g)	Proline (µmol/g)	Total soluble sugars (mg/g)
1	NDLH 1938	1.13	1.0	R	60	95.30	23.15	147.11	3.50
2	DCH 32	4.78	4.0	HS	44.92	75.74	16.17	65.61	4.66
3	Suraj	1.55	2.0	MR	54.92	93.98	22.51	68.70	3.14
4	Jadoo	2.15	2.7	S	41.77	44.30	18.46	39.96	2.63
5	JR/AKH/2021-22 9631	1.48	1.8	MR	48.37	94.64	23.1	110.58	2.52
6	JR/AKH/2021-22 9637	1.69	1.4	MR	54.92	84.44	32.64	42.40	2.15
7	TVH/JR/2021-22 1	2.15	2.8	S	54.92	38.18	13.44	40.94	1.47
8	TVH/JR/2021-22 2	1.89	1.9	MR	48.62	58.94	30.46	139.48	3.66
9	TVH/JR/2021-22 3	1.69	1.4	MR	57.96	78.86	30.68	54.57	5.17
10	TCH 2021	2.76	2.7	S	40.66	22.88	10.2	145.04	2.04
11	TCH 2023	2.56	2.4	S	53.55	25.46	11.56	44.83	4.66



12	TCH 2024	2.76	3.1	HS	26.03	19.64	8.78	94.83	1.31
13	TCH2026	1.89	2.2	S	20.74	63.62	27.40	105.71	5.01
14	TCH 2029	2.29	2.8	S	29.14	32.78	32.12	114.15	3.91
15	C14 × C 27 7-2-1-1	2.15	2.4	S	24.92	37.28	16.41	44.025	3.55
16	C14 × C 27 5- 2-1-5	2.70	2.5	S	18.18	23.42	18.08	42.88	7.20
17	VS9 -S11-1×1608 -8-1-2-1	2.09	2.1	S	51.22	41.42	25.11	50.51	3.94
18	VS9 -S11-1×1608 -8-1-2-2	2.49	2.4	S	42.88	28.22	13.14	33.47	3.01
19	VS9 -S11-1×1608 -8-1-2-3	1.89	1.7	MR	56.59	72.80	27.32	47.59	6.32
20	C14 × PP 21 -3-1-3	2.90	2.7	S	38.48	13.70	8.4	34.61	4.02
21	C14 × VS 7-1-8-2-2	1.89	2.3	S	31.03	47.90	27.73	43.21	4.83
22	C14 × VS 7-1-9-1-1	2.49	2.7	S	17.62	29.06	8.4	105.71	5.03
23	C14 × GSHB 5-3-6-1	2.49	2.3	S	40.37	27.86	29.53	24.22	4.94
24	C14 × GSHB 5-3-6-3	2.15	2.0	MR	38.1	38.00	14.72	125.19	4.15
25	C14 × GSHB 5-3-6-4	2.90	3.0	S	30.96	18.50	20.83	25.03	6.44
26	C14 × GSHB 180 5-5-2	2.49	2.7	S	33.44	29.54	15.19	70.81	4.55
27	C14 × GSHB 180 7-1-2-1	2.09	1.7	MR	39.70	41.90	22.5	53.44	5.03
28	C14 × GSHB 180 7-1-2-2	1.95	1.7	MR	22.81	47.18	20.37	54.90	4.8
29	C14 × GSHB 180 7-1-2-3	1.55	1.5	MR	35.81	74.24	17.53	44.18	6.36
30	C14 × GSHB 180 7-1-2-4	1.69	1.7	MR	16.92	73.70	11.56	51.00	3.02
31	C14 × GSHB 180 7-1-5-1	1.95	2.3	S	43.25	42.14	16.36	115.12	7.03
32	C14 × GSHB 180 7-1-5-2	2.15	2.0	MR	27.22	38.84	19.14	76.33	3.21
33	TCH 1608 × 1822-6-2-1-1	2.35	2.0	MR	34.18	31.64	14.94	97.27	4.61
34	TCH 1608 × 1822-6-2-1-2	1.89	1.3	MR	39.18	51.38	25.90	62.20	3.52
35	TCH 1608 × 1822-6-2-1-3	2.56	2.3	S	56.62	25.88	12.65	57.01	5.96
36	TCH 1608 × 1822-6-2-2-3	2.15	2.0	MR	52.11	40.70	20.78	110.90	6.79
37	TCH 1608 × 1822-6-2-2-4	2.29	2.0	MR	32.85	37.34	7.963	34.61	4.93
38	TCH 1608 × 1822-7-2-1-1	2.35	2.3	S	24.92	34.58	8.1	125.19	6.09
39	TCH 1608 × 1822-7-2-1-2	2.29	2.3	S	37.48	34.82	19.44	82.82	6.42
40	TCH 1608 × 1822-7-2-1-4	1.95	2.3	S	25.85	64.88	9.763	46.94	5.57
41	TCH 1608 × 1822-7-2-1-5	1.95	2.0	MR	28.33	44.06	27.00	54.90	3.55
C.D	-	0.065			1.272	2.289	0.753	3.620	0.155
SE(m)	-	0.023			0.451	0.812	0.267	1.284	0.055
SE(d)	-	0.033			0.638	1.148	0.378	1.815	0.078
C.V	-	1.812			2.031	3.003	2.433	3.113	2.158

## 5. Conclusion

The results of this experiment showed that genotypes with higher levels of protein, phenol, proline, and free amino acids are resistant to the leaf hopper, *Amrasca biguttula biguttula*. Leafhoppers are more vulnerable to genotypes with higher total soluble sugar content. The protein content of TVH/JR/2021-22-3 (57.96 mg/g) indicates that it is resistant to leafhopper with a nymph population of 1.69 nos./3 leaves. The higher concentration of phenol is present in JR/AKH/2021-22 9631 (94.64 mg/g) and JR/AKH/2021-22 9637 contains a higher concentration of free amino acid (32.64 mg/g). TCH 2021 contains a higher proline content of 145.04 µmol/g. Several biochemical elements present in cotton genotypes have a positive or negative impact on leafhopper populations. Insect resistant cotton varieties would help control pests. Cotton genotypes with biochemical host plant resistance traits have a lot of variation, which can be used to develop sucking insect pest resistant cultivars.

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