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Research Article

Biochemical analysis of metabolites in cotton (*Gossypium hirsutum* L.) conferring resistance to Leaf hopper *Amrasca biguttula biguttula* (Ishida)

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Abstract
The cotton leafhopper *Amrasca biguttula biguttula* (Ishida) has emerged as a major threat, causing significant yield losses (40-60%). To address this, we aimed to identify cotton genotypes with superior biochemical traits for leafhopper resistance. Various biochemical parameters and elemental composition by EDAX-spectroscopy were analyzed. Field and polyhouse screenings were conducted for host plant resistance (HPR) study. The ANOVA, correlation, and stepwise regression analyses were conducted to assess the traits relationship and their impact on leafhopper resistance. Results showed significant genotype variations ($p < 0.05$) were observed in biochemical traits and leafhopper injury index. Two verities NDLH1938 and Suraksha and three F_1 hybrids derived from crosses *viz*., TVH002 × Suraksha, NDLH1938 × Suraksha, and TVH002 × NDLH1938 were showing significant resistance by recording lower leafhopper population (2.28-5.17), injury index (0.69-0.97), susceptibility index (1.58-5.00) and host preference survival rate (2.21-2.46). The HPR study suggests that lines are less preferred by the leafhoppers to survive which indicates factors influencing antibiosis. Biochemical profiling revealed that gossypol ($r = -0.75$), tannins ($r = -0.69$), and calcium ($r = -0.78$) were negatively correlated with pest density. It indicates the genotypes with higher levels of gossypol, tannins, and calcium, and lower levels of reducing sugars, total proteins, free amino acids, and chlorophyll exhibited resistance to the leafhopper. Stepwise regression analysis indicated the combined effect of these biochemical traits ($R^2 = 0.93$) in contributing to a high-level of resistance rather than individual traits. Our findings highlight the importance of biochemical traits in influencing leafhopper resistance and the need for comprehensive trait combinations in breeding resistant cotton varieties.

Keywords: Breeding, cotton, correlation, gossypol, injury index, leafhopper resistance, tannins, regression.

INTRODUCTION

In India, cotton (*Gossypium hirsutum* L.) cultivation primarily relies on rainfed conditions, and the successful incorporation of the Bt gene has resulted in an impressive coverage of 96% of cotton-growing regions with Bt hybrids. However, despite these advancements, cotton productivity in India remains below the global average, with an annual yield of only 36.21 million bales (COCPC, 2022). Various factors contribute to this optimal production, posing risks to the farmers, notably the ever-increasing costs of pesticides due to the relentless pressure exerted by cosmopolitan pests (Kranthi, 2017). Among these pests, the cotton leafhopper, scientifically known as *Amrasca biguttula biguttula* (Ishida), has emerged as a severe problem in recent years. Both adult and nymph leafhoppers pierce and suck the plant sap, resulting in leaves to curling downward and exhibiting hopper burn symptoms that impede crop photosynthesis and yield losses ranging from 40% to 60% (Manivannan *et al*., 2021; Kranthi *et al*., 2018; Kranthi, 2017). Additionally, the honeydew excreted by leafhoppers facilitates the growth of black mold, further impairing photosynthesis.

In the pursuit of developing cotton genotypes resistance to leafhoppers, key factors considered in reducing the pest preference for food and fitness (antibiosis) (Painter, 1968). Cotton plants possess unique biochemical components, such as gossypol, tannins, total proteins, reducing sugars, free amino acids, chlorophyll content, and elements, which disrupt insect metabolism and hinder their growth, development, and reproduction (Manivannan *et al*., 2021; Sandhi *et al*., 2017; Khalil *et al*., 2017; Shinde *et al*., 2014). On contrary certain plant substances may act as feeding incitants and stimulants, promoting insect feeding (Bhoge *et al*., 2019). Indiscriminate insecticide use and cultivation of susceptible cotton hybrids have caused insecticidal resistance and led to worsening leafhopper infestations. Therefore, breeding cotton plants with detrimental biochemical traits is vital for influencing insect host preference (host plant resistance-HPR).

Our study aimed to select cotton genotypes with superior biochemical traits for leafhopper resistance. Field and polyhouse screenings, along with correlation and regression analyses, were conducted to assess and analyze the traits. The results will guide the breeders in the selection of resistant plants and their suitability for hybrid cotton production.

MATERIALS AND METHODS

The experiments were conducted at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, India, located at coordinates 11.0122° N, 76.9354° E, with an elevation of 432.0 m. The study involved five cotton genotypes selected as parents (**Table 1**), and a half-diallel crossing scheme was employed to generate 10 F1 hybrids. Field experiments were established in *Kharif* 2022, utilizing a randomized block design (RBD) with three replications. Pre-treated seeds of the parents and hybrids, along with three checks (KC3 as resistant, Suraj as moderately resistant, and DCH32 as susceptible), were sown in two rows on the furrows of 6m long ridges with a spacing of 90 × 30 cm. The cultivation followed the recommended package of practices outlined in the TNAU Crop Production Guide. To increase the leafhopper pest load, okra-CO4 hybrid (*Abelmoshus esculentus*) seeds were planted intermittently, one row for every five rows of cotton, serving as infestor rows.

Table 1. Selected genotypes including their pedigree and main characteristics.

Variety	Pedigree	Origin/source	Selection Character
TVH002	Suraj × TCH1819	CRS-Veppanthattai	High yielding, compact, short duration, medium staple fibre (MSF), and moderately leafhopper resistant (MLR)
CO17	Khandwa 2× LH220	DC-Coimbatore	Compact, early maturity, MSF, and susceptible to leafhopper
NDLH1938	NDLH 1797 X NDLH 1325	RARS-Nandyal	High yielding, resistant to leafhopper (LR), and MSF
Suraksha	Surabhi × (MCU5 × Z2)	CICR-Coimbatore	High yielding semi compact variety, excellent fibre quality, medium size bolls, LR, and high ginning %
Nano	CCH 526612 X VNWH-1	CICR-Coimbatore	MLR, MSF, high yield, and short plant stature

CRS, cotton research station; **DC**, department of cotton; **RARS**, regional agricultural research station; **CICR**, Central Institute for Cotton Research.

The leafhopper population was recorded on three leaves representing top, middle and bottom canopies on ten randomly selected plants. The plants were also visually graded for injury as per Indian Central Cotton Committee (ICCC) (**Table 2**). The leafhopper injury index was calculated as per Nageswara, 1973. The leafhopper susceptibility index was worked out on an individual plant basis by multiplying the population with the respective injury index (Mahal, 1993).

Laboratory experiments were conducted for leafhopper resistance in polyhouse at DC, TNAU. The delinted seeds were planted in mud pots (30 x 28 cm) filled with alfisols + farm yard manure potting material in July, 2022. All genotypes received the same watering and polyfeed® fertilizer application (19:19:19 N-P-K). Each seedling was covered with 50 micron paint filter mesh clothe cages. When the plants reached the leaf area and canopy development stage (50-60DAS), 10 plants of uniform size

Table 2. Cotton leafhopper injury index, grades, and pubescence ratings categorizes genotypes into different resistance levels

Leafhopper injury Index	Leafhopper injury grade (Per cent intensity)	Resistance rating
0	Excellent (0%)	Immune plant and/or Resistant
0.1 – 1.0	I (1-10%)	
1.1 – 2.0	II (10.1-25%)	Moderately resistant
2.1 – 3.0	III (25.1-50%)	Susceptible
3.1 – 4.0	IV (>50%)	Highly susceptible

I = slight yellowing, II = yellowing and necrosis, III = intense yellowing and necrosis, IV = complete necrosis.

$$LII = \frac{G_1P_1+G_2P_2+G_3P_3+G_4P_4}{P_1+P_2+P_3+P_4},$$

Where P_1 to P_4 is number of population falls under the grades (G_1-G_4) for each entry of ICCC.

and vigour were selected for each genotype. Leafhopper survival on different entries was studied by releasing individually caged plant with ten pairs of young adult leafhoppers collected from leafhopper colony established on susceptible cotton genotype, DCH32 (in open field raised) using a HS01 insect aspirator (Harpal and Sons™, Ambala, Haryana) (Kaur *et al*., 2022). The leafhopper numbers were recorded after 7 days of release, by slight disturbance of plant and monitoring adult movement inside the cages.

Biochemical profiling was performed at 60 days after sowing (DAS) at the Department of Biochemistry, TNAU. Five healthy plants of each genotype, protected by mesh cloth cages, were selected from the polyhouse. The third and fourth fully opened leaves from the top of each plant were detached using a razor and stored in an icebox to preserve their freshness. Later, small randomly sampled quantities of leaf tissue were obtained from the stored leaves for various biochemical analyses.

Gossypol content was determined in a one gram leaf sample using the Sadasivam and Manickam procedure (Sadasivam and Manickam, 1992). The Ninhydrin technique was employed to estimate the total free amino acids in a one gram leaf sample (Moore and Stein, 1948). Total protein analysis was conducted on a 50 milligram leaf sample using the Lowry method (Lowry *et al*., 1951). Tannins were quantified in a 0.25 gram leaf sample using the Folin-Denis method (Cl and Indira, 2016). Total reducing sugars were measured in a 100 milligram leaf sample using the dinitrosalicylic acid method (Dubois *et al.,* 1956). Total chlorophyll content was estimated using multi-wavelength double beam UV-VIS spectrophotometry (Systonic®, Panchkula, Haryana) on 50 milligram leaf samples extracted with 80% acetone (Arnon, 1949). A series of respective standards were run and plotted on graphs for calculations of components (**Fig. 1**). Elemental analysis was performed using energy dispersive X-ray spectroscopy (EDS) (TESCAN MIRA3 XMU with EDAX, Carl Zeiss Microscopy, Germany) with specific parameters (Girao *et al.,* 2017). The analysis was conducted at the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Statistical analysis included analysis of variance (ANOVA) and least significant differences (LSD) test, performed using the “Agricolae” R package (v.4.2.3) (De Mendiburu and De Mendiburu, 2019), with significance set at p > 0.05. Correlation and stepwise regression analyses were conducted at a significance level of 0.05 using the “Corrplot” (Wei *et al*., 2017) and “leaps” (Lumley and Miller, 2020) R-packages, respectively, to examine the relationship and factors influencing leafhopper resistance. Scatter plots were created using Microsoft Excel (Microsoft 27 Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

The mean squares of ANOVA revealed that statistically significant genotypes differences ($p < 0.05$), reflecting substantial trait variability. Genotypes variability accounted for the largest portion of this variability (**Table 3 and 4**). The genotypes exhibited varying mean squares across traits, ranging from 0.37 for Chlorophyll ‘a’ to 629.77 for Leafhopper susceptibility index (LSI), these findings implying the potential for improved selection outcomes in the future (Bourgou *et al*., 2022). These findings align with Manivannan *et al*. (2021) and Sandhi *et al*. (2017) study when screening genotypes against cotton leafhopper resistance. To provide a comprehensive understanding of the results, the individual genotypes means on traits basis with significant groups, standard errors difference (SEd), coefficients of variation (CV %), and LSD values are presented in **Table 5**.

Concerning the leafhopper infestation, the entries differed significantly in injury index (0.69-3.25), susceptibility index (1.58-56.37) and number of leafhopper per three

(a)

$$\text{Gossypol\%} = \frac{\text{Mean concentration} \times \text{Net weight}}{10 \times \text{Sample content} \times \text{Initial weight}}$$

$$\text{Where: Mean concentration} = \text{OD values},$$
$$\text{Net weight} = \text{Final weight} - \text{Initial weight}$$

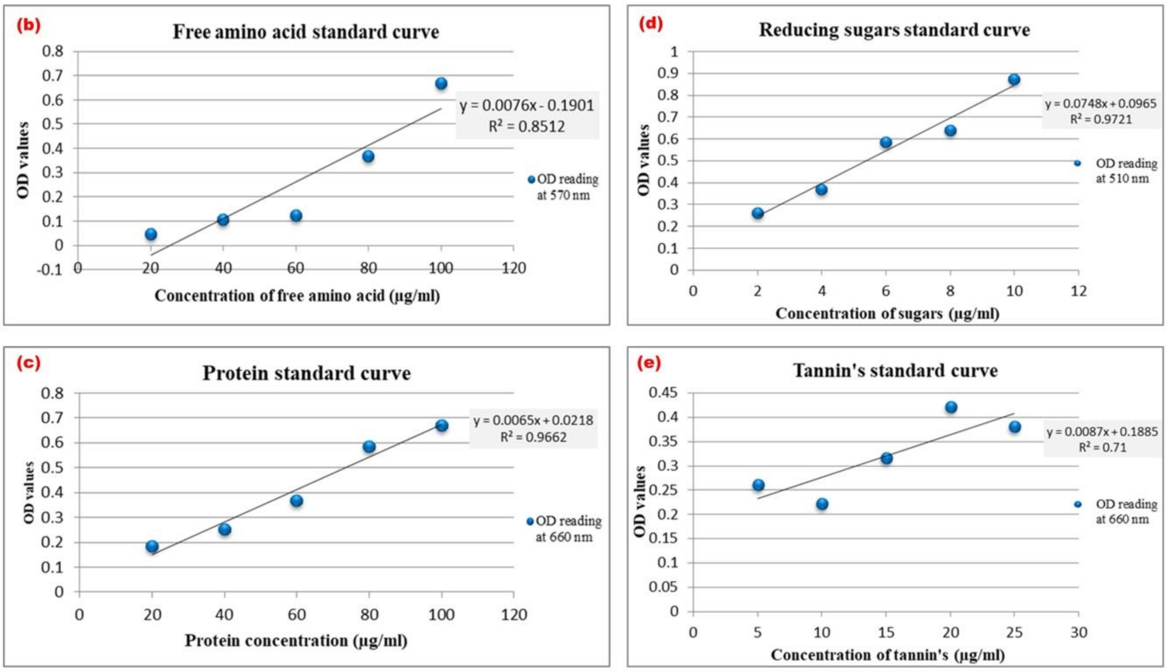

Fig 1. Formulae for gossypol percent estimation (a). A series of respective standards were run and plotted on graphs for calculations of free amino acids (b), total protein (c), reducing sugars (d) and tannins (e). The intensity of colors analysed at different OD values using a double beam UV-VIS Spectrophotometer (a, b & e) and a kanad photon-393 digital calorimeter (c & d).

Table 3. ANOVA mean squares of leafhopper population and related traits.

Source	Df	NLTP	LII	LSI	HPSP (nos.)	HPS (%)	LPI (%)
Replication	2	0.10	0.01	0.21	0.01	0.01	0.08
Genotype	17	47.67**	1.92**	629.77**	1.99**	49.78**	282.40**
Error	34	0.08	0.01	0.30	0.01	0.43	0.31
Total	53						

**, Significant differences at 0.01 levels of probability; Df, Degrees of freedom. NLTP, Number of leafhopper three leaves per plant; LII, Leafhopper injury index; LSI, Leafhopper susceptibility index; HPSP, Host preference survival population numbers; HPS, Host preference survival per cent; LPI, Leafhopper per cent intensity.

Table 4. ANOVA mean squares of different biochemical traits

Source	Df	G (%)	FAA (mg/g)	TP (mg/g)	TC (mg/g)	RS (mg/g)	Chl.a (mg/g)	To. Chl, (mg/g)
Replication	2	0.02	0.16	2.50	0.34	0.54	0.34	0.12
Genotype	17	1.45**	5.71**	57.21**	127.53**	16.32**	0.37**	0.96**
Error	34	0.01	0.04	0.48	0.79	0.09	0.02	0.04
Total	53							

G, Gossypol%; FAA, Free amino acids; TP, Total protein; T, Tannins content; RS, Reducing sugars; Chl.a, chlorophyll a; To.Chl, Total chlorophyll.

Table 5. Mean performance of leafhopper and biochemical traits in cotton entries

Entries	No. per three leaves	Injury index	Suscepti-bility index	Host preference		Per cent Intensity (%)	Gossy-pol (%)	Free amino acids (mg/g)	Total protein (mg/g)	Tannins (mg/g)	Reducing sugars (mg/g)	Chlorophyll content (mg/g)	
				HPSP (nos.)	HPS (%)							Chl. a	Total Chl.
TVH002	8.04^{g}	1.81^{g}	14.52^{h}	3.57^{c}	17.85^{c}	20.41^{fg}	1.41^{d}	1.87^{g}	11.62^{e}	20.69^{d}	3.21^{gh}	1.24^{bc}	2.58^{a}
CO17	13.22^{b}	2.91^{b}	38.42^{b}	4.45^{a}	22.23^{a}	29.41^{c}	0.31^{j}	4.58^{b}	17.54^{a}	17.88^{ef}	6.71^{d}	1.71^{a}	1.70^{c}
NDLH1938	3.27^{l}	0.86^{k}	2.81^{m}	2.31^{f}	11.55^{f}	9.81^{ij}	2.10^{b}	1.07^{ijk}	10.26^{fg}	31.49^{a}	4.26^{f}	0.69^{f}	1.19^{de}
Suraksha	4.13^{k}	0.91^{jk}	3.74^{l}	2.46^{f}	12.30^{f}	9.09^{jk}	1.40^{d}	0.94^{jk}	6.83^{hij}	29.33^{b}	2.84^{hi}	0.70^{f}	1.20^{de}
Nano	8.56^{f}	1.83^{g}	15.64^{g}	3.31^{d}	16.53^{d}	19.05^{g}	0.70^{gh}	1.17^{hij}	10.23^{fg}	18.29^{e}	4.42^{f}	1.11^{bcd}	1.61^{c}
TVH002 × CO17	10.47^{d}	2.63^{d}	27.55^{d}	4.10^{b}	20.52^{b}	31.98^{b}	0.41^{ij}	4.35^{bc}	18.02^{a}	14.50^{g}	9.31^{a}	0.98^{de}	2.48^{ab}
TVH002 × NDLH1938	4.80^{ij}	0.95^{j}	4.58^{kl}	2.28^{f}	11.40^{f}	9.77^{ij}	2.18^{fg}	2.28^{f}	16.25^{b}	26.14^{c}	5.52^{e}	0.99^{de}	1.35^{cde}
TVH002 × Suraksha	2.28^{m}	0.69^{l}	1.58^{n}	2.23^{f}	11.15^{f}	8.93^{ij}	2.41^{a}	0.80^{k}	6.15^{ij}	31.34^{a}	4.44^{f}	1.32^{b}	1.04^{e}
TVH002 × Nano	9.14^{e}	1.86^{fg}	16.98^{f}	3.65^{c}	18.23^{c}	22.83^{e}	1.82^{c}	2.94^{e}	13.18^{d}	22.26^{d}	7.66^{c}	1.18^{bcd}	1.63^{c}
CO17 × NDLH1938	5.17^{i}	0.97^{j}	5.00^{k}	2.41^{f}	12.07^{f}	11.01^{i}	0.95^{ef}	1.53^{h}	7.15^{hi}	22.07^{d}	2.42^{i}	0.96^{de}	1.64^{c}
CO17 × Suraksha	6.42^{h}	1.56^{i}	9.99^{j}	3.32^{d}	16.60^{d}	17.13^{h}	1.30^{d}	1.48^{h}	7.73^{h}	13.71^{g}	3.55^{g}	1.05^{cde}	1.50^{cd}
CO17 × Nano	11.75^{c}	2.81^{c}	33.02^{c}	4.28^{ab}	21.40^{ab}	31.75^{b}	1.04^{e}	4.12^{c}	16.90^{ab}	13.49^{g}	8.65^{b}	1.74^{a}	2.60^{a}
NDLH1938 × Suraksha	3.15^{l}	0.73^{l}	2.30^{mn}	2.21^{f}	11.13^{f}	7.70^{k}	1.98^{bc}	2.27^{f}	5.61^{ij}	20.76^{d}	2.53^{i}	0.81^{ef}	1.05^{e}
NDLH1938 × Nano	7.85^{g}	1.64^{h}	12.88^{i}	2.81^{e}	14.05^{e}	21.75^{ef}	0.26^{j}	1.36^{hi}	9.65^{g}	14.61^{g}	4.26^{f}	1.73^{a}	1.51^{cd}
Suraksha × Nano	10.45^{d}	1.94^{e}	20.24^{e}	3.76^{c}	18.78^{c}	26.13^{d}	0.63^{gh}	1.49^{h}	14.48^{c}	16.41^{f}	7.19^{cd}	1.08^{bcd}	1.69^{c}
KC3	4.41^{jk}	0.94^{j}	4.17^{kl}	2.34^{f}	11.70^{f}	8.89^{jk}	1.84^{c}	2.52^{f}	6.56^{hij}	30.87^{a}	3.50^{g}	0.96^{bcd}	2.20^{b}
Suraj	9.25^{e}	1.91^{ef}	17.72^{f}	3.23^{d}	16.13^{d}	22.23^{e}	0.55^{hi}	3.53^{d}	11.04^{ef}	16.53^{f}	5.28^{e}	1.28^{bc}	1.06^{e}
DCH32	17.35^{a}	3.25^{a}	56.37^{a}	4.41^{a}	22.05^{a}	39.26^{a}	0.81^{fg}	5.13^{a}	17.18^{ab}	13.24^{g}	9.51^{a}	1.80^{a}	2.73^{a}
Mean	7.76	1.68	15.97	3.17	15.87	19.28	1.23	2.41	11.47	20.76	5.29	1.19	1.71
SEd	0.23	0.03	0.42	0.97	0.56	1.73	0.12	0.16	0.57	0.73	0.25	0.11	0.17
CV (%)	3.65	2.55	3.19	4.17	4.17	2.92	4.92	8.16	6.07	4.31	5.79	4.37	5.04
LSD (P = 0.05)	0.47	0.08	0.85	0.22	1.10	1.40	0.20	0.33	1.61	1.50	0.51	0.23	0.34

The different lowercase superscripts (a-n) in the same column represent significant differences.

leaves (2.28-17.25) as given in **Table 5**. The average values of NLTP were 7.76 (CV = 3.65%), LII was 1.68 (CV = 2.55%) and LSI 15.97 (CV = 3.19%). With respect to NLTP, LII, and LSI mean, the hybrid TVH002 × Suraksha was found to be highly resistant with the lowest leafhopper populations (2.28), injury index (0.69) and susceptibility index (1.58) and susceptible check hybrid DCH32 was considered as highly susceptible genotype with the highest values (17.35, 3.25, and 15.67). The F_1 hybrids derived from crosses *viz*., TVH002 × Suraksha (0.69), NDLH1938 × Suraksha (0.73), NDLH1938 (0.86) and the variety Suraksha (0.91) had lower injury index than resistant check KC3 (0.94). Whereas, the hybrids TVH002 × NDLH1938 (0.95) and CO17 × NDLH1938 (0.97) had on par injury index with KC3. The entries which showed a higher injury index and higher leafhopper population also showed a higher susceptibility index (**Table 5**).

Host preference (host plant resistance) study revealed that the numbers of leafhopper survived (nos.) after 7 days and their survival percent were shown in **Table 5**. Except, for one entry CO17 (4.45 nos. and 22.23%), all other showed lesser survival when compared with susceptible check DCH32 (4.41 nos. and 22.05%). The six entries were superior to resistant check KC3 (2.34 nos. and 11.70%) in terms of HPSP (nos.) and HPS (%). The remaining entries were on par or ranges between moderately resistant check Suraj (3.23 nos. and 16.13%) and resistant check KC3. The mean leafhopper per cent intensity was depicted in **Table 5**. The HPR and screening certainly helped in selecting the stable resistant lines (Khalil *et al*., 2017; Painter, 1968). This HPR study indicated that higher number of leafhopper survivals was observed on susceptible lines than on resistant lines (Gangopadhyay *et al*., 2017). Our study suggests that lines are less attractive for the leafhoppers to survive, which would indicate a higher level of resistance (Sandhi *et al*., 2017; Iqbal *et al*., 2011; Mahal *et al*., 1993).

The study presented data on several biochemical parameters in various cotton genotypes (**Table 5**) were differed significantly. Plant biochemical constituents provide a natural defense against herbivores. These chemicals can act as feeding stimulants, physiological inhibitors, or cause nutrient deficiencies in insects. Phenolic compounds (Gossypol) can inhibit insect growth and reduce survival rates (Halder *et al*., 2016; War *et al*., 2012; Bernards and Båstrup-Spohr, 2008). In our study, we found gossypol per cent ranging from 0.26 (NDLH1938 × Nano) to 2.41% (TVH002 × Suraksha), with an average of 1.23% (CV = 4.92%). The hybrids *viz*., TVH002 × Suraksha, TVH002 × NDLH1938 (2.18%), NDLH1938 × Suraksha (1.98%), and variety NDLH1938 (2.10%) recorded higher gossypol content than the resistant check KC3 (1.84%). The correlation **(Fig. 2A**) between gossypol and leafhopper population was significantly negative (r = -0.75), this indicates high gossypol content of the hybrids is directly linked with leafhopper resistance (Rohini *et al*., 2011; Balakrishnan, 2006). The trend observed for gossypol content was significantly lower in susceptible entries, but not all susceptible entries had low gossypol some fall in medium ranges also. DCH 32 is a susceptible check which recorded 0.81%, which is higher than moderately resistant entries. Our results are in accordance with (Bhoge *et al*., 2019; Khalil *et al*., 2017; Sandhi *et al*., 2017), who reported that less amount of gossypol in susceptible entries.

The study also found significant differences among entries concerning free amino acid (FAA), with levels ranging from 0.80 (TVH002 × Suraksha) to 5.13 mg/g (DCH32). The average amount of FAA was 2.41 mg/g (CV = 8.16%). Higher levels of FAA content were found in susceptible genotypes DCH32, CO17 (4.58 mg/g), and hybrid TVH002 × CO17 (4.35 mg/g) and which showed a highly significant positive correlation with leafhopper pest density (r = 0.82). A similar trend was observed for total protein (TP) content, with susceptible entries having higher levels than resistant entries, ranging from 5.61 (NDLH1938 × Suraksha) to 18.02 mg/g (TVH002 × CO17). The average TP content of 11.47 mg/g (CV = 6.07) and recorded a positive correlation with the pest densities (r = 0.78). This result indicated that both protein and amino acids act as feeding stimulants for leafhoppers. Certain FAA is essential for the growth and development of leafhoppers, making them particularly attractive as a food source (Febvay *et al*., 1999). Therefore, leafhoppers are attracted to plants that have high levels FAA, and can be deterred by plants that have low levels of them. This finding confirmatory with studies of Manivannan *et al*. (2021), Bhoge *et al*. (2019) and Khalil *et al*. (2017) were reported that higher levels of FAA and TP in most of the susceptible entries. The scatter plots for highly positive (FAA) and negative correlated (G %) traits with leafhopper for better understanding is given in **Fig. 2C and 2D**.

Tannin levels also varied significantly among entries, ranging from 13.24 to 31.49 mg/g and average of 20.76 mg/g (CV = 4.31%). The negative correlation (r = -0.69) was seen between tannins and leafhopper densities. Higher tannin levels were found in the high field resistant entries TVH002 × Suraksha (31.34 mg/g), NDLH1938 (31.49 mg/g), and KC3 (30.87 mg/g). In contrast, significantly lower tannin contents were noted in susceptible hybrids CO17 × Nano (13.49 mg/g) and TVH002 × CO17 (14.50 mg/g). These results are supports the findings of Sandhi *et al*. (2017) and Bhat *et al*. (1981) who also reported that resistant genotypes had significantly higher tannin content with low pest density. Whereas, it oppose the studies of Rizwan *et al*. (2021) and Shinde *et al* (2014) were reported a significant positive correlation between tannin and leafhopper density (i.e. susceptible entries recorded with higher tannins).

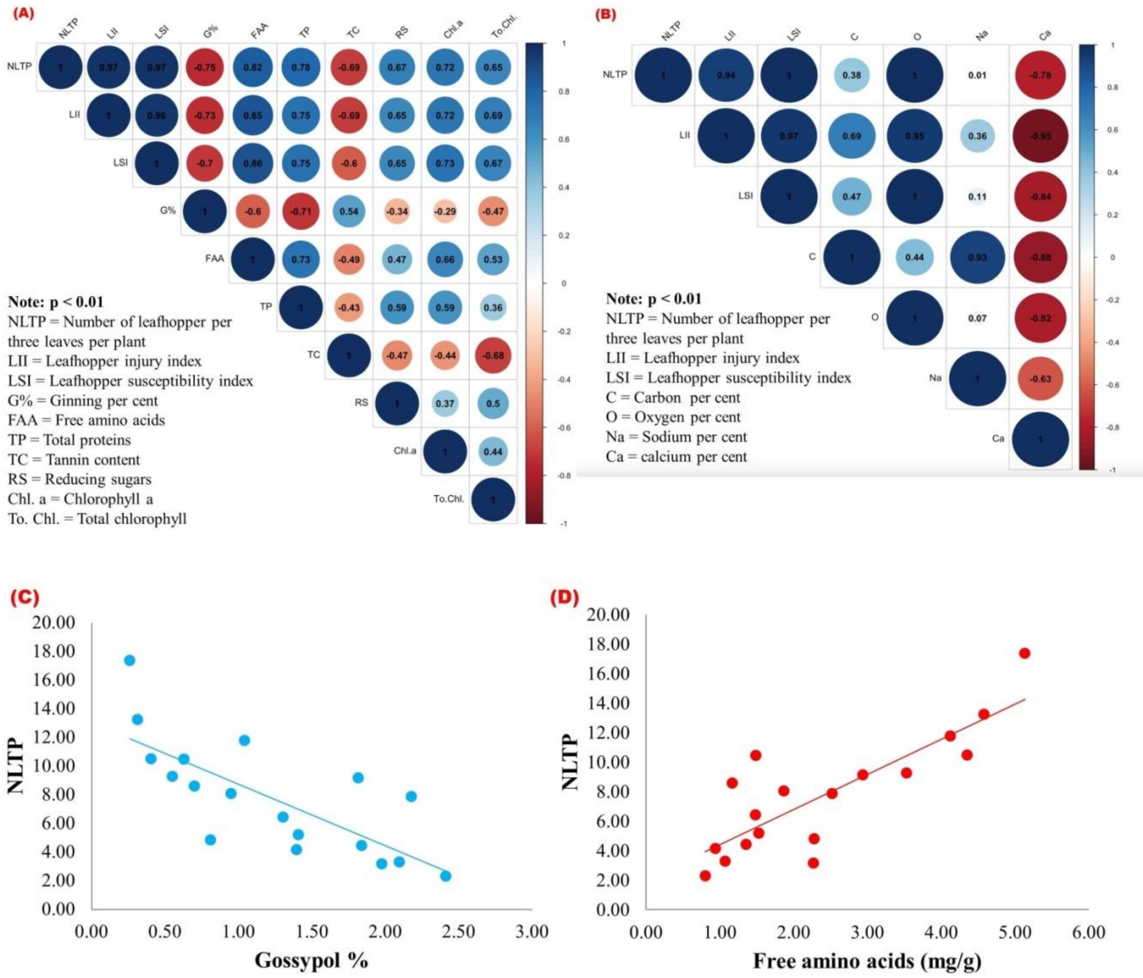

Fig. 2. Correlation matrixes (A and B). Correlation coefficients (r) between traits were calculated using Spearman's method in 'R'. The range of numbers (-1 to 1) represents the Spearman's rank between traits on the axes. Circle color and size reflect the correlation strength. Correlation scatter plots (C & D). Significant at $p < 0.05$.

Reducing sugars (RS) content ranged from 2.42 to 9.51 mg/g, with significantly lower RS content in highly resistant hybrids CO17 × NDLH1938 (2.42 mg/g) and NDLH1938 × Suraksha (2.53 mg/g). The average amount of RS was 5.29 mg/g (CV = 5.79%). Moderate levels of RS were observed in moderately field-resistant genotypes TVH002, Nano, and Suraj (3.21, 4.42 and 5.28 mg/g respectively), while significantly higher RS was noticed in susceptible hybrids TVH002 × CO17 and CO17 × Nano (9.31 and 8.65 mg/g respectively). Fewer resistant entries recorded more RS than moderate-resistant genotypes. Susceptible entries recorded higher RS than resistant entries and possessed significant positive correlation with population (r = 0.67), which line the findings of (Manivannan *et al*., 2021; Bhoge *et al*., 2019; Khalil *et al*., 2017; Sandhi *et al*., 2017; Bhat *et al*., 1981). In the case of chlorophyll 'a', significant content differences were recorded and it ranged from 0.69 (NDLH1938) to 1.74 mg/g (CO17 × Nano), with overall mean of 1.19 mg/g (CV = 4.37%). Susceptible check DCH 32 showed higher chlorophyll a (1.80 mg/g) whereas; all other entries had less content. Total chlorophyll content ranged from 1.04 (TVH002 × Suraksha) to 2.73 mg/g (DCH 32) and average of 1.71 mg/g (CV = 5.04%). chlorophyll content was observed higher in susceptible checks as compared with entries and followed a similar trend as chlorophyll 'a'. A positive correlation was observed between leafhopper population with both chlorophyll 'a' (r = 0.72) and total chlorophyll content (r = 0.65). This result indicates that variety with high chlorophyll content generally has more photosynthetic efficiency and yields better biomass and preferred by sucking pests especially for their succulent nature (Manivannan *et al*., 2021).

A combined graph of area spectra (EDS) provides constituent elements weight percentages (**Fig. 3**). In hybrid TVH002 × Suraksha, five major elements (carbon (C), oxygen (O), sodium (Na), phosphorus (P),

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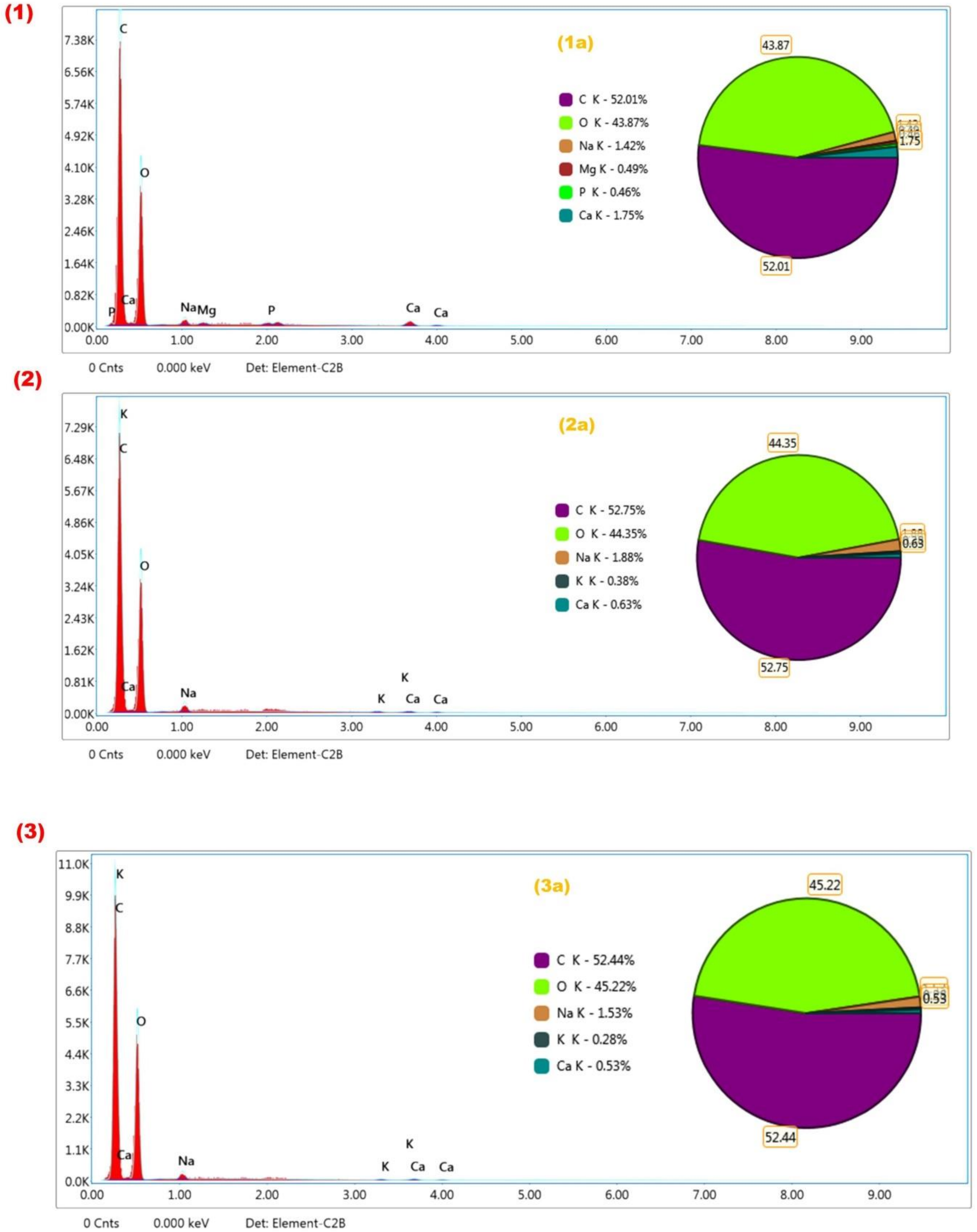

0 Cnts	0.000KeV	Det: Element-C2B	KeV: 15	Takeoff: 34.6	Live Time(s): 81.9
Amp Time(μs): 3.84		Resolution (eV): 127.4		Mag: 167 (fig.1), 179 (fig.2) & 192 (fig.3)	

Fig. 3. Area EDS spectrum of resistant hybrids TVH002 × Suraksha (1); NDLH1938 × Suraksha (2) and TVH002 × NDLH1938 (3). Colored pie charts indicate the weight % of corresponding EDS elemental overplay (1c, 2c & 3c). Each area spectrum peaks represents the amount of individual element quantity (conc.). The bottom table depicts instrument properties set as per the working sample.

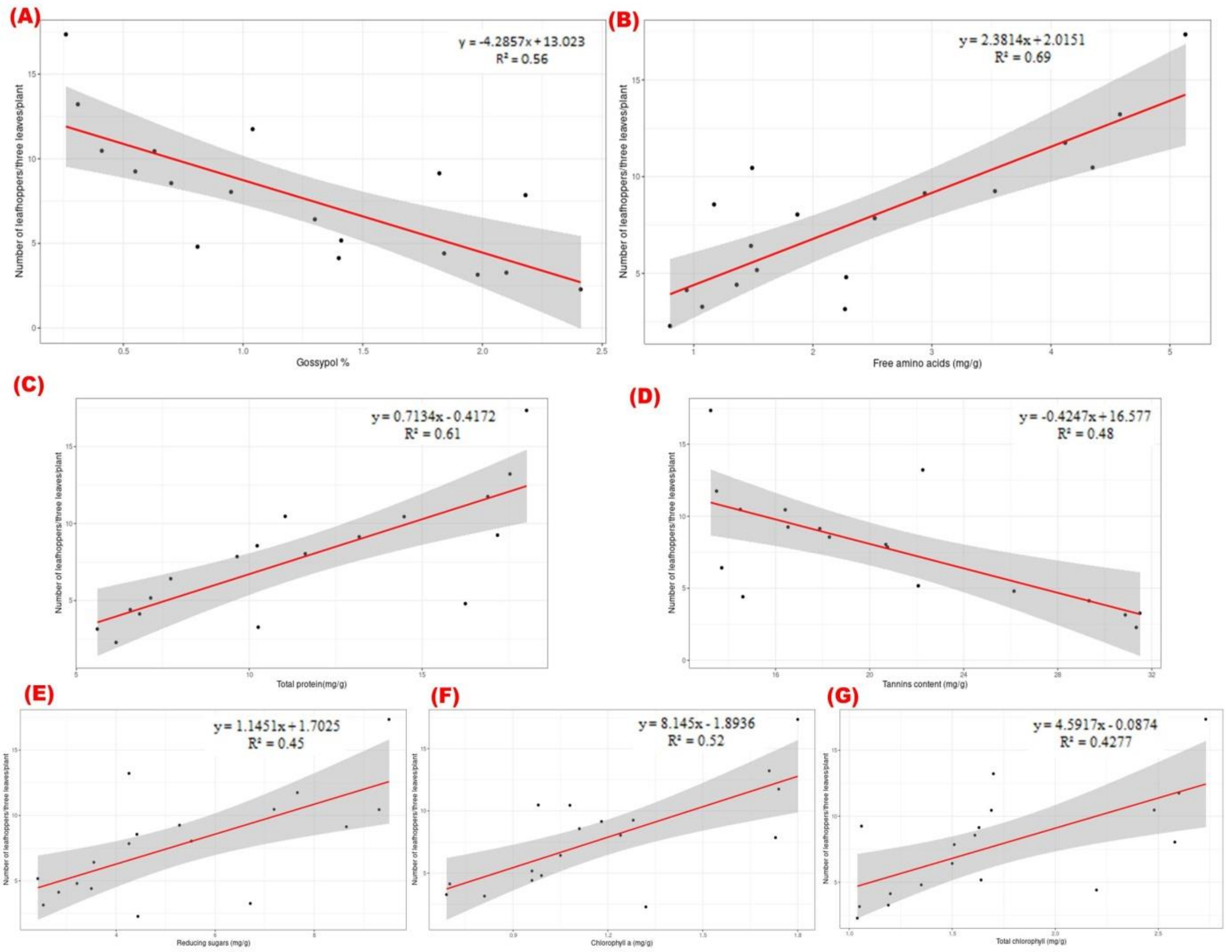

Fig. 4. Regression plots of different biochemical traits against leafhopper population. X-axis in A = Gossypol%, B = Free amino acids C = Total protein, D = Tannins content, E = Reducing sugars, F = Chlorophyll a, G = Total chlorophyll and Y-axis = Number of leafhopper three leaves per plant

magnesium (Mg) and calcium (Ca)) were detected. Whereas in the hybrids *viz*., TVH002 × NDLH1938 and NDLH1938 × Suraksha the elements *viz*., C, O, Na, K and Ca were mapped. The correlations between detected elements with leafhopper density among these three field resistant hybrids are presented in **Fig. 2B**. This result indicates that the plant with lesser quantity of elements *viz*., C, O, P, Na, Mg, and K and higher 'Ca' had capacity of more resistance and less preference for pest feeding. The results are in confirmatory with Manivannan *et al*. (2021) and Sandhi *et al*. (2017) were identified higher amount of Nitrogen (N), Ca, and Silicon (Si) was negative associated with leafhopper populations. Screening of genotypes especially for these biochemical profiles and elements has to be given importance, since genotypes with lesser profiles may not be preferred by the insects. So, it is evident that the genotype with higher gossypol, tannins and element 'Ca' and lower levels of RS, TP, FAA and chlorophyll exhibited resistance to the leafhopper. The biochemical traits *viz*., G%, FAA, TP, TC, RS, Chl.a and To.Chl were found significant factors for NLTP and selected for further stepwise regression analysis. When evaluated individually, their R^2 was 56%, 69%, 61%, 48%, 45%, 52%, and 42%, respectively (**Fig. 4**). This result indicates that the traits FAA followed by TP were identified as most beneficial factor for the leafhopper population (Sandhi *et al*., 2017). The trait G% was a highly significant factor influencing plant defense against insects. However, when traits are combined step-wise (**equation 1-6**) their adjusted R^2 values increased greatly (**Table 6**) indicating that these characteristics in combination contribute to leafhopper resistance. When all the factors were analyzed together, the R^2 was 0.93 (**equation 7**). This suggests that no single biochemical parameter alone is sufficient to provide resistance to leafhopper, but rather a combination of these characteristics is more effective.

Table 6. Stepwise regression equations of different traits to study their strengths.

S.No	Stepwise regression equations	R^2	Adjusted R^2	Residual SE
1	Y = 13.01 -4.28X1	0.56	0.53	2.72
2	Y = 6.46 -2.27X1-1.69X2	0.78	0.75	1.98
3	Y = 4.45 -1.78X1 +1.42X2 +0.18X3	0.79	0.75	1.99
4	Y = 7.63 -1.03X1 +1.15X2 +0.21X3 -0.18X4	0.85	0.81	1.74
5	Y = 6.54 -1.58X1 +1.15X2 +0.04X3 -0.12X4 +0.45X5	0.89	0.84	1.55
6	Y = 4.22 -2.80X1 +0.62X2 -0.17X3 -0.04X4 +0.58X5 +4.49X6	0.95	0.93	1.02
7	Y = 4.22 -2.90X1 +0.65X2 -0.20X3 -0.05X4 +0.60X5 +4.60X6 -0.29X7	0.96	0.93	1.06

Y = NLTP, Number of leafhopper three leaves per plant; X1 = gossypol%; X2, free amino acids; X3, total protein; X4, tannins; X5, reducing sugars; X6, chlorophyll a; X7, total chlorophyll. R2 = coefficient of determination; SE = standard error; significant at $p < 0.05$.

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