



# Biochemical Basis of Phytoimmunity in Cotton Genotypes against Leafhoppers

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**Abstract:** The research was carried out at Haradanahalli Farm, Chamarajnagar, University of Agricultural Sciences, Bangalore, Karnataka during 2020-21 to understand role of biochemical constituents and minerals in plant resistance against leafhoppers in cotton. Fifteen cotton genotypes representing each resistant category from the results of field screening were analyzed for various biochemical and mineral constituents. Higher amount of phenols, tannins, phosphorus and potassium were observed in highly resistant genotypes and total soluble sugars, total reducing sugars, crude proteins, total free amino acids and nitrogen were higher in highly susceptible genotypes. Total phenols, tannins, phosphorus and potassium had significant negative relationship with incidence of leafhoppers while, total soluble and reducing sugars, total free amino acids, crude proteins and nitrogen had significant positive association with incidence of leafhoppers.

**Keywords:** Genotypes, Resistant and Biochemical constituents

Cotton is the most important fiber crop in many regions of the world. Traditionally cotton is known as the backbone of non-food crops of agricultural economy of India (Sharma 2015). Many insect pests cause economic damage in cotton growing areas; among them sucking pests particularly leafhoppers are more threatening in present scenario (Makwana and Dulera 2018). Management through chemicals leads to many hazardous like environmental pollution, negative effects on existing flora and fauna and also leads to imbalance of tri-trophic interaction. Control of any pest to below its economic threshold level by single management practice is difficult. Integrated pest management is the best way to manage any pest population. Hence, identification of source of resistance in host plant is needful. In majority of the plants secondary metabolites and mineral constituents are involved in offering resistance against pest species. With this background, the study was formulated with an objective to study biochemical factors associated for resistance in cotton genotypes against leafhoppers'.

## MATERIAL AND METHODS

**Field screening of cotton genotypes:** Total of 60 cotton genotypes were field screened against leafhopper during *Kharif* 2020. At All India Coordinated Research Project on cotton, Haradanahalli, Chamarajanagara. Each genotype was sown in replicated trial in 3 rows of 90 plants with spacing

of 90 × 60 cm, between rows and plants, respectively. The plants of different genotypes were raised as per package of practice, except the plant protection measures (Anonymous 2016). In each genotype, the observations on nymphs and adults of leafhopper were recorded on 45 and 60 DAS on ten randomly selected plants. In each plant three leaves - one each from top, middle and bottom strata- were observed and mean population per three leaves was worked out. The hopper-burn assessment was rated by adopting 1-4 Grade Scale (Indian Central Cotton Committee). Based on LHRI (Leaf Hopper Resistance Index) the genotypes were categorized as highly resistant, resistant, intermediate, susceptible and highly susceptible with 1.0-1.5, 1.51-2.0, 2.01-2.5, 2.51-3.0 and 3.01-4.0 (Rao 1973). The genotypes of cotton representing resistant category were selected for sampling. The un-infested leaves of selected cotton genotypes were sampled at 60 days after sowing. The sampled genotypes were collected separately in a butter paper for the estimation of the important biochemicals viz., the total and reducing sugars, total phenols, total free amino acids, tannins and crude proteins and major nutrients viz., N, P, K were estimated. The leaf samples of selected genotypes were dried under shade for two weeks. The dried samples were ground using grinder. The powdered samples were stored in plastic covers until analysis.

**Estimation of biochemical components:** The leaf samples of selected cotton genotypes were collected, washed with

distilled water and dried under shade. 10 g of leaf sample was taken in separate conical flask and 150 ml of 80 percent ethanol was added and refluxed for 30 minutes on hot water bath. After boiling, the extract was cooled and tissues were ground thoroughly in a mortar with pestle in slight ethanol. The supernatant was decanted into another flask and residue was again re-extracted with small quantity of hot ethanol and decanted. This extract was filtered through Whatman's No.1 filter paper and made up to a known volume with 80 percent ethanol. The ethanol part of extract was stored in refrigerator at 4 °C and used for the estimation of biochemical components present in plant sample. The total and reducing sugars in each test genotypes were estimated by the method suggested by Somogyi. Estimation of total phenols and tannins in stem samples of test genotypes was done by following Folin-Ciocalteu method suggested by Bray and Thorpe (1954). The amount of total free amino acid present in the samples was estimated by Ninhydrin method. Nitrogen and crude proteins were estimated by micro-Kjeldahl method, phosphorous by spectrophotometric method and potassium by flame photometric method.

**Statistical analysis:** The mean data was processed after suitable transformation, using SPSS Software.

## RESULTS AND DISCUSSION

**Total phenols:** The higher amount of phenol was recorded in cotton genotypes Br-24b 2671 and Br-24b 2675 and were on par with each other and lower amount of phenol content was in highly susceptible genotypes viz., Br-2b 376, Br-2b 356 and Br-2b 373 (Table 1). The correlation study concluded that phenol content had significant negative correlation with leafhopper incidence ( $r=-0.91^{**}$ ) (Table 2). Higher leaf phenol content will lead to a lower leaf hopper injury index (Rohini et al 2011), which is generally determined by the smaller number of leafhopper population (Halder et al 2016, Raju et al 2020) owing to the phenols antibiosis nature in resistant cultivars.

**Tannins:** The amount of tannins increased as resistance increases. The highly resistant genotype has higher tannins in Br-24b 2671 and Br-24b 2675 and genotypes. Br-2b 376, Br-2b 356 and Br-2b 373 recorded lower amount of tannins as they belong to highly susceptible genotypes (Table 1). The tannins had significant negative association with leafhopper incidence ( $r=-0.90^{**}$ ) (Table 2). More tannins decrease leafhoppers (Sandhi et al 2017), due to tannin's significant contribution in conferring cotton's defence system against sucking insects (Nikhath et al 2019, Raju et al 2020).

**Total free amino acids (TFA):** Differences in TFA among genotypes were significant and showed an increasing trend with susceptibility and exhibited significant positive

( $r=0.76^{**}$ ) relationship with incidence of leafhopper (Table 2). Among the selected genotypes, highly resistant genotypes Br-24b 2671 and Br-24b 2675 recorded least amount of TFA. Lower amount of TFA was observed in CET H × B 20605 this followed by CET H × B 20609 and Br-13a 2668 (Table 1).

**Crude proteins:** Crude proteins were found lower in highly resistant genotypes i.e., Br-24b 2671 and Br-24b 2675. However, the highest per cent crude proteins was observed in highly susceptible genotypes Br-2b 376, Br-2b 356 and Br-2b 353 and were found differed significantly (Table 1). The increasing trend of crude proteins was observed in cotton genotypes with increase susceptibility which was positively correlated with incidence ( $r= 0.85^{**}$ ) (Table 2). Elevated levels of proteins in the plant also favour the incidence of leafhoppers in cotton (Amin et al 2016, Manivannan et al 2021) which are built from amino acids and these amino acids are essential for the growth and development of insects (Manivannan et al 2021). Contrarily, a non-significant correlation between protein content and leafhopper infection in cotton genotypes was also found (Murugesan and Kavitha 2010).

**Total soluble sugars (TSS):** The total soluble sugars varied significantly and a lower amount of TSS was observed in highly resistant genotypes viz., Br-24b 2671 and Br-24b 2675. However, in highly susceptible genotypes the amount of TSS was found significantly highest in Br-2b 376, Br-2b 356 and Br-2b 353 were on par with each other. The increasing trend of TSS in different genotypes showed a significant positive impact on leafhopper incidence ( $r=0.90^{**}$ ). However leafhoppers had negative non-significant association of total sugars on okra (Sandhi et al 2017) and on Bt cotton genotypes (Nikhath et al 2019).

**Total reducing sugars (TRS):** In different genotypes, total reducing sugar varied between 1.24 to 4.16 mg g<sup>-1</sup>. The significant and lower amount of TRS was recorded in highly resistant categories viz., Br-24b 2671 and Br-24b 2675. However, in highly susceptible genotypes total reducing sugar varied between 4.00 and 4.16 mg g<sup>-1</sup> (Table 2). TRS had positive significant influence ( $r= 0.88^{**}$ ) on leafhopper population (Table 2). Reducing sugars had positive and highly significant correlation between pest populations in Bt cotton genotypes (Nikhath et al 2019).

**Nitrogen (N):** The nitrogen content was low in highly resistant genotypes Br-24b 2675 and Br-24b 2671 were on par with each other. Higher amount of nitrogen content was quantified in highly susceptible genotypes. Correlation study revealed that percent nitrogen in leaf sample exerted significant positive influence on incidence of leafhopper ( $r=0.87^{**}$ ) (Table 2). Higher N content makes leaves more succulent, which supports more sucking pests (Sonalkar,

2019). N is also a key component of amino acids, which promotes higher population of leafhoppers.

**Phosphorus (P):** Highly resistant genotypes Br-24b 2671

and Br-24b 2675 had higher amount (0.36%) of phosphorus and were on par with each other. In highly susceptible genotypes Br- 2b 376, Br-2b 356 and Br-2b 353 were on par

**Table 1.** Biochemical constituents in cotton genotypes in relation to leafhopper incidence, *Kharif 2020*

Genotypes	LHRI	Category	Incidence (No./3 leaves)	Total phenols	Tannins	TFA	Crude proteins	TSS	TRS	N (%)	P (%)	K (%)
Br-24b 2671	1.00	HR	0.00 (0.71)	6.37 <sup>a</sup>	7.96 <sup>a</sup>	1.24 <sup>a</sup>	12.83 <sup>a</sup>	8.28 <sup>a</sup>	1.24 <sup>a</sup>	0.22 (2.66) <sup>a</sup>	0.36 (3.45) <sup>a</sup>	1.68 (7.44) <sup>a</sup>
Br-24b2675	1.00		0.00 (0.71)	6.35 <sup>a</sup>	7.86 <sup>a</sup>	1.25 <sup>a</sup>	12.75 <sup>a</sup>	8.32 <sup>ab</sup>	1.35 <sup>b</sup>	0.21 (2.65) <sup>a</sup>	0.36 (3.45) <sup>a</sup>	1.68 (7.44) <sup>a</sup>
Br-24b 2672	1.60	R	1.67 (1.47)	6.05 <sup>bc</sup>	7.38 <sup>b</sup>	1.36 <sup>a</sup>	13.98 <sup>ab</sup>	8.63 <sup>b</sup>	2.63 <sup>ef</sup>	0.24 (2.78) <sup>ab</sup>	0.35 (3.37) <sup>ab</sup>	1.63 (7.34) <sup>b</sup>
Br-24b 2673	1.80		2.57 (1.75)	5.95 <sup>bcd</sup>	7.28 <sup>b</sup>	1.37 <sup>a</sup>	15.09 <sup>bc</sup>	8.51 <sup>ab</sup>	1.52 <sup>c</sup>	0.25 (2.89) <sup>bc</sup>	0.35 (3.38) <sup>ab</sup>	1.62 (7.31) <sup>b</sup>
Br-24b 2676	2.00		2.23 (1.65)	6.08 <sup>b</sup>	7.47 <sup>b</sup>	1.36 <sup>a</sup>	13.82 <sup>ab</sup>	8.31 <sup>ab</sup>	1.31 <sup>ab</sup>	0.23 (2.76) <sup>b</sup>	0.34 (3.36) <sup>abc</sup>	1.58 (1.58) <sup>c</sup>
CET H × B 20601	2.20	I	4.33 (2.20)	5.89 <sup>bcd</sup>	6.63 <sup>c</sup>	1.36 <sup>a</sup>	15.63 <sup>bc</sup>	9.56 <sup>c</sup>	2.56 <sup>e</sup>	0.26 (2.94) <sup>bc</sup>	0.32 (3.27) <sup>bcd</sup>	1.48 (6.99) <sup>d</sup>
CET H × B 20605	2.20		5.53 (2.46)	5.84 <sup>cde</sup>	6.66 <sup>c</sup>	2.28 <sup>c</sup>	16.36 <sup>c</sup>	9.68 <sup>c</sup>	2.68 <sup>f</sup>	0.28 (3.01) <sup>cd</sup>	0.31 (3.21) <sup>d</sup>	1.46 (6.94) <sup>de</sup>
CET H × B 20606	2.40		5.20 (2.39)	5.64 <sup>e</sup>	6.61 <sup>c</sup>	1.35 <sup>c</sup>	15.59 <sup>bc</sup>	12.64 <sup>f</sup>	3.66 <sup>i</sup>	0.26 (2.93) <sup>bc</sup>	0.24 (2.82) <sup>f</sup>	1.47 (6.97) <sup>de</sup>
CET H × B 20609	2.40		5.57 (2.46)	5.78 <sup>de</sup>	6.58 <sup>c</sup>	2.29 <sup>c</sup>	16.54 <sup>cd</sup>	11.46 <sup>d</sup>	2.46 <sup>df</sup>	0.28 (3.02) <sup>cd</sup>	0.23 (2.78) <sup>f</sup>	1.47 (6.96) <sup>de</sup>
CET H × B 20610	2.40		6.47 (2.64)	5.76 <sup>e</sup>	6.54 <sup>c</sup>	2.37 <sup>c</sup>	16.96 <sup>cd</sup>	11.60 <sup>d</sup>	2.61 <sup>e</sup>	0.29 (3.06) <sup>cd</sup>	0.32 (3.26) <sup>cd</sup>	1.45 (6.92) <sup>e</sup>
Br-13a 2661	2.60	S	9.53 (3.17)	4.88 <sup>f</sup>	5.86 <sup>d</sup>	1.89 <sup>b</sup>	18.53 <sup>d</sup>	12.25 <sup>e</sup>	3.27 <sup>h</sup>	0.31 (3.20) <sup>d</sup>	0.27 (2.97) <sup>e</sup>	1.36 (6.69) <sup>f</sup>
Br-13a 2668	2.80		10.20 (3.27)	4.97 <sup>f</sup>	5.94 <sup>d</sup>	2.34 <sup>c</sup>	20.88 <sup>e</sup>	12.13 <sup>e</sup>	3.03 <sup>g</sup>	0.35 (3.40) <sup>e</sup>	0.29 (3.07) <sup>e</sup>	1.36 (6.69) <sup>f</sup>
Br-2b 376	3.40	HS	11.20 (3.42)	4.09 <sup>h</sup>	3.39 <sup>e</sup>	2.46 <sup>c</sup>	31.12 <sup>f</sup>	13.17 <sup>g</sup>	4.16 <sup>k</sup>	0.52 (4.15) <sup>f</sup>	0.31 (3.20) <sup>d</sup>	1.33 (6.62) <sup>f</sup>
Br-2b 356	3.80		14.37 (3.86)	4.21 <sup>gh</sup>	3.37 <sup>e</sup>	1.86 <sup>b</sup>	32.43 <sup>f</sup>	13.07 <sup>g</sup>	4.07 <sup>j</sup>	0.55 (4.23) <sup>f</sup>	0.22 (2.72) <sup>f</sup>	1.24 (6.39) <sup>g</sup>
Br-2b 373	3.60		15.30 (3.97)	4.40 <sup>g</sup>	3.33 <sup>e</sup>	2.46 <sup>c</sup>	32.96 <sup>f</sup>	13.01 <sup>g</sup>	4.00 <sup>j</sup>	0.55 (4.27) <sup>f</sup>	0.32 (3.24) <sup>d</sup>	1.22 (6.34) <sup>g</sup>
SE m ± CD@ p=0.05			0.021 0.061	0.048 0.138	0.037 0.107	0.035 0.101	0.403 1.168	0.067 0.195	0.018 0.053	0.036 0.106	0.023 0.067	0.013 0.038

Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1965); N- Nitrogen; P- Phosphorus; K- Potassium; \*Values in the parenthesis are square root transformed ( $\sqrt{x+0.5}$ ) in incidence and \*\*Arc sign transformed in minerals; LHRI- Leaf Hopper Resistance Index (Rao 1973)

**Table 2.** Correlation matrix between biochemical constituent, minerals and leafhopper incidence, *Kharif 2020*

Parameters	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>
Y-Leafhopper	-0.91**	-0.90**	0.76**	0.85**	0.90**	0.88**	0.87**	-0.62*	-0.86**
X <sub>1</sub> - Phenols	1.00	0.96	-0.64	-0.94	-0.86	-0.87	-0.95	0.51	0.83
X <sub>2</sub> -Tannin		1.00	-0.65	-0.98	-0.83	-0.87	-0.99	0.46	0.89
X <sub>3</sub> - TFA			1.00	-0.62	0.70	0.59	0.65	-0.35	-0.60
X <sub>4</sub> -Crude protein				1.00	0.77	0.81	0.99	-0.39	-0.84
X <sub>5</sub> -TSS					1.00	0.91	0.79	-0.73	-0.80
X <sub>6</sub> -TRS						1.00	0.82	-0.63	-0.83
X <sub>7</sub> - Nitrogen							1.00	0.56	0.78
X <sub>8</sub> -Phosphorus								1.00	0.79
X <sub>9</sub> - Potassium									1.00

N = 15; \*\* Significant at P ≤ 0.01; TSS- Total soluble sugars; TRS- Total reducing sugars; TFA- Total free amino acids

with each other except Br-2b 356 which records lowest (0.22%) amount of phosphorus percent. The phosphorus showed significant positive association ( $r=0.62^*$ ) with leafhopper incidence (Table 2).

**Potassium (K):** Decreased trend of potassium was observed as susceptibility increases. Among the selected genotypes higher amount of potassium was observed in highly resistant genotypes Br-24b 2671 and Br-24b 2675 and were on par with each other. The lowest amount of potassium was found in highly susceptible genotype Br-2b 353 followed by Br-2b 356 and Br-2b 376 (Table 1), potassium had significant negative relation with leafhopper incidence ( $r=-0.86^{**}$ ) (Table 2). Since potassium gives strong resistance to insect pests and high potassium levels increase secondary metabolite compounds, minimising carbohydrate deposition (Kiran et al 2018), potassium showed a negative non-significant link with leafhopper infestation on brinjal (Ali et al 2013). Increased K concentration in soil and rice plant lengthens the time it takes for *Nilaparvata lugens* to harm plant cells because K increases host plant tolerance (Rashid et al 2016).

### CONCLUSION

In the present investigation, tannins, total phenols, phosphorus and potassium contents were higher in highly resistant and resistant genotypes but, crude proteins, total free amino acids, nitrogen, total reducing and soluble sugars were found higher quantity in highly susceptible and susceptible genotypes so, these components may acting as a key factors in phytoimmunity against leafhoppers in cotton.

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