International Journal of Tropical Insect Science
https://doi.org/10.1007/s42690-020-00416-0

ORIGINAL RESEARCH ARTICLE

Evaluation of resistance in cotton genotypes against leafhoppers *Amrasca biguttula biguttula* (Ishida), (Homoptera: Cicadellidae)

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Received: 4 July 2020 / Accepted: 22 December 2020

Abstract

Cotton production is affected by various insect-pests attack. Among the insect pests, sucking pests impose serious crop damage. In sucking pests, the leafhopper *Amrasca biguttula biguttula* (Ishida), considered as a severe pest. Cultivation of susceptible hybrids and reduction in conventional insecticide applications wreak havoc of sucking pest problem. Up to 50% yield losses been reported for the pest leafhopper alone. Host plant resistance is an important component of integrated pest management strategy for leafhopper resistance. Identification of sources of resistance is way to minimize yield losses. In this study, 54 genotypes along resistant check (NDLH1938) and susceptible check (DCH32) screened for resistance in field. Based on initial resistance evaluation studies at field level, 21 genotypes were selected for further studies such as host preference studies and nymphal emergence studies in greenhouse and host plant resistance by pest infestation evaluation in field. In field screening, nine genotypes namely AKH1355, GISV 216, AKH 2012–8, GSHV 173, GISV 267, AKH 1301, GSHV 171, NDLH 2010 and AKH 2006–2 constantly showed resistance on par with resistant check (NDLH1938). Both host preference studies and nymphal emergence tests identified seven genotypes RS 2711, GISV 267, LHDP 1, AKH 1355, RS 2765, F 2164, and GISV 216, which performed on par with resistant check. Leaf anatomical parameters like distance from the lower epidermis to phloem, thickness of phloem bundle are major obstacles for stylet penetration and affect the feeding of leafhoppers. However, leaf thickness, which facilitates the insect to feed easily, succulent nature of such thick leaves preferred by the insects. Biochemical profiles revealed that higher level of chlorophyll, nitrogen, protein and amino acids favors the leafhopper infestation. In contrast, phenol compound act as feeding deterrent, as most of the resistant genotypes showed higher level of phenol.

Keywords Cotton leafhopper · Host plant resistance · Field screening · Nonpreference · Biochemical profiling

Introduction

Cotton is considered as a major commercial crop for its multiple uses as fibre, cattle feed and oil. In India, *Bt* cotton impacted tremendous change in cotton cultivation. Successful adoption of *Bt* results in the area under *Bt* cotton has raised to 98% of cotton area. Area of cotton cultivation in India is 12.42 mha, out of which 11.6 mha is covered under *Bt* (ISAAA 2018). *Bt* cotton has effectively controlled the lepidopteron pests which includes the boll worm complex (Wu and Guo 2005). Among the sucking pests, the leaf hopper *Amrasca biguttula biguttula* (Ishida), considered to be a severe pest. Cultivation of susceptible hybrids and reduction in conventional insecticide applications wreck havoc. Adults and nymphs of leafhopper attack the crop from vegetative to boll bursting and cause significant yield losses. Cultivation of susceptible hybrids, low usage of conventional pesticide in *Bt* cotton and emergence of insecticide resistance particularly in leafhoppers were the reasons for higher occurrence of leafhopper population and damage to cotton (Kranthi 2017; Prabhakar et al. 2011). Up to 50% yield reduction been reported for the pest leafhopper alone (Kalkal et al. 2009; Murugesan et al. 2009). Integrated pest management is an ideal strategy to control leafhopper pest in cotton. Among the IPM strategies, cultivation of resistant variety is a vital component (Khan and Saxena 1998). Screening of germplasm to identify the stable source of resistance is a predominant step in any resistance breeding programme. Study of insect plant

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interaction helps in understanding the role of plant architecture in insect resistance. The most destructive leafhoppers are those whose feeding causes burning symptom referred as hopper-burn of foliage (Uthamasamy 1985; Backus et al. 2005). Leafhoppers employ a lacerate-and-flush-feeding strategy for food intake. Adults and nymphs of leafhopper penetrate their stylets either continuously or intermittently, they secret saliva inside the leaf tissues, this saliva after solidification forms a sheath like structure called salivary sheath; it protects the stylet from external damage while feeding. Once the stylet enters into the phloem sap, it sucks the sap and causes injury to the tissues, which results in turning of the tissues into yellow, after wards, the leaf starts to curling downward and finally withered and detached. Painter (1968) has postulated the concept of non-preference is one the mechanism of resistance; it is the plants character, which affects the insects feeding, oviposition and shelter preference. Many morphological traits contribute to the resistance of plants to pests. Plant defense through morphological modifications like trichomes, thickening of cuticles, parching of leaf and thickening of cell walls and unique plant parts are important nonpreference factors for insects (Yallappa Harijan et al. 2017). Characters like leaf thickness, thickness of phloem, distance of phloem from lower epidermis, palisade cell length and trichome length are found to be important parameter against leafhopper for confereing resistance (Manivannan et al. 2017; Murugesan and Kavitha 2010). The current study was envisaged to understand the leaf anatomical differences for leafhopper resistance in cotton genotypes.

Biochemical compound present in the plants imparts resistance. Some of the chemical triggers insect feeding by act as feeding stimulants and some interfere with the metabolism of the insects and deter the insect growth, development and reproduction. These chemical constituents are major hues in host plant selection of the insects for feeding and oviposition (Maxwell and Jennings 1980). Profiling of a variety for biochemical components indirectly helps in identification of resistance lines.

The objectives of this study were therefore to (i) screening cotton germplasm for resistance to leafhoppers, and (ii) to study the role of leaf anatomical structure in deterrence of leafhopper feeding (iii) to understand the biochemical basis of resistance.

Materials and methods

In 2016, a field trial was conducted to screen the cotton germplasm of 54 genotypes including one resistant check (NDLH 1938) and susceptible check (DCH32) (Table 1). Genotypes were graded with level of resistance with respect to infection injury grade. Based on field screening, sum of 21 resistant genotypes along with two contrasting checks were evaluated in 2017 in randomized block design with two replications under unprotected condition.

Leafhopper infestation under field conditions

Leafhopper population counting, ten plants from each genotype were selected randomly and tagged properly. For hopper counting, two leaves from upper portion of first plant, two leaves from middle portion of second plant and three leaves from bottom portion of third plant of each genotype observed periodically in the same serious manner. In such a way, two top leaves, two middle leaves and two bottom leaves were taken into consideration. Nymphs and adults were counted on each tagged leaves in the early morning hours. Average population was calculated by arithmetic mean. Observations were started from 30 days after sowing and recorded up to 105 days with an interval of 7 ± 1 days. For our analysis we have taken the crucial infestation stage of 45, 70 and 90 days and it was observed the population was above ETL in all the stage.

Culturing of leafhopper insects

Susceptible cotton genotype DCH32 was sown at weekly intervals in 10×10 m^2 area inside a greenhouse and grown without any chemical insecticide spray. Seedlings were covered with nylon cages of mesh size 0.15×0.15 cm to prevent the escape of leafhoppers. The caged cotton plants, about 20–25 days old, were inoculated with the field collected leafhopper adults and the leafhoppers were cultured continuously.

Host preference studies

To identify host preference the survival of leafhopper nymphs in different genotypes was studied in pot culture under green house. All the 23 genotypes were raised in pots. The completely randomized design was followed with two replications per genotypes. Twenty numbers of second instar nymphs were released in each pot and the pot was covered with screen cage to avoid the escape of nymphs. The survival of nymphs was recorded up to 7 days on daily basis. Number of nymphs survived was recorded as percentage of survival.

Oviposition preference studies

From field, shoots of the plants were cut and brought into the laboratory to study the nymphal emergence as a measure of ovipositional preference. All the 23 genotypes raised in two replications were taken into account of study. For each genotype two plants were selected and leaves were tagged for counting the nymphal emergence. The shoots were kept in screen cage and nymphs were counted on daily basis up to 10 days.

Table 1 Leafhopper infestation on cotton genotypes in field

S. No	Genotypes	45th DAS	70th DAS	90th DAS
1	PUSA 5760	1.69	2.37	2.03
2	H 1464	1.48	2.48	1.98
3	H 1454	1.33	2.45	1.89
4	RS 2711	1.27	2.42	1.85
5	RS 2765	1.45	2.08	1.77
6	LHDP 1	1.15	2.38	1.77
7	F 2164	1.48	2.18	1.83
8	GSHV 173	1.24	2.13	1.69
9	GSHV 171	1.18	2.19	1.69
10	JK 35	1.31	2.16	1.74
11	GISV 267	1.18	2.15	1.67
12	GISV 216	1.14	2.03	1.59
13	SCS 1062	1.40	2.45	1.93
14	NH 630	1.19	2.14	1.67
15	AKH 09–5	1.28	2.41	1.85
16	AKH 2006–2	1.26	2.3	1.78
17	AKH 2012–8	1.19	2.05	1.62
18	AKH 1301	1.22	2.17	1.70
19	AKH 1351	1.22	2.36	1.79
20	AKH 1355	1.13	1.92	1.53
21	NDLH 2010	1.28	2.19	1.74
22	NSDL1938 (Resistant Check)	1.42	1.93	1.68
23	DCH 32 (Susceptible Check)	3.61	4.80	3.05
	LSD (P=0.05)	1.01	1.18	1.15
	P values of ANOVA	P<0.001	P<0.001	P<0.001

DAS days after sowing

Leaf anatomical studies

Trichome density

Trichome density on the upper and lower surface of leaves of the five selected individual plants of each entry was estimated following the procedure of Maite et al. (1980). The third fully expanded leaf was used for the estimation. Leaf samples were cut into 1 cm leaf bit and boiled in 20 ml of water in small glass vials for 15 min then removed, 20 ml of 96% ethyl alcohol was added, and the leaf bits were boiled approximately for 20 min at 80 °C. After boiling, alcohol was drained off and same boiling process with fresh alcohol was repeated until the chlorophyll content of the leaf bits were removed completely. Alcohol was removed from the boiling tube and 90% lactic acid was added, the vials were stoppered and heated at 85 °C until leaf segments cleared (approximately for 35–45 min). After heating processes over, the vials were kept to cool, leaf bits were taken and mounted on glass slide using pinch of lactic acid to count trichomes. Trichomes present in one centimeter area were counted under Leica stereo zoom binocular microscope.

Leaf thickness measurements

Each genotype same aged leaves were taken for leaf thickness measurement. Each leaf to avoid dominant secondary veins, top, bottom and leaf margins were left only the leaf area located between midrib and leaf margin of central portion was taken for consideration. Leaves were thin sectioned using sharp surgical blades. These leaf sections were placed on slide and examined through Leica stereo zoom binocular microscope. For each genotype, ten sections were taken in two replicates; the mean data was used for analysis.

Leaf anatomical measurements

Leaf anatomical parameters namely distance of phloem from lower epidermis, palisade cell height, thickness of phloem were measured for conferring the anatomical basis of resistance for leafhopper feeding through leaf tissues. Leaf samples were collected from all the genotypes at 45 days after sowing. For anatomical investigation, microtome sectioning method was followed.

Microtomy

Fresh leaves were collected and fixed in FAA (formalin, acetic acid and 70% alcohol (1:1:18)) solution for 48 h. Then the fixed leaves were washed in 70% alcohol initially and further washed by series of 80, 90 and 100% alcohol (absolute alcohol). Washed leaves were further dehydrated in a combination of n-butanol to ethanol in a combination of mixing ratio of 1:3; 1:1 and 3:1 solutions and finally washed through absolute n-butanol. In each combination, leaves were processed for 3 h dehydration. Dehydrated leaves were transferred into a test tube, which contains small portion of melted paraffin (melted at 58 to 60 °C) and samples were allowed for cold infiltration of paraffin into leaves samples and kept in room temperature for 24 h. Then the test tube was kept in hot air oven and 60 °C temperature was maintained. Fresh molten paraffin was added with an interval of 4 h until the trace of butanol removed from the leaves samples. Dehydrated material, which was free of butanol, was embedded in paraffin as per the procedure of paper boat technique (Jensen 1962). Required size and shape were taken and inner side of the paper boat was smeared with glycerin. Dehydrated leaves with molten wax were poured inside the paper boat immediately. After that, already boiled molten wax was added. Paraffin block were cut into appropriate size usually 6–7 μm using rotary microtome and specimens were kept for hardening under cold water bath.

Dehydration

For fixing of sections to slides, gelatin is used as an adhesive and 1% of gelatin was prepared using warm water. In this gelatin solution, small quantities of potassium dichromate crystals were added and finally gelatin was filtered and used for fixing. A smear of gelatin spread over the slide and ribbon of sections were carefully laid over and it was allowed further stretching by keeping the slides in hotplate at 45 °C. These sections were dried in room temperature for 72 h and stored for further process. Processed sections paraffin were removed by washing in xlyene, xylene with absolute alcohol, these were later hydrated by washing in series of alcohols (100, 90, 70 and 50% dilutions) in a 5 min interval and finally washed in water.

Staining

Sections were stained using safranin (1%) as staining and fast green as counter staining agent. Deparaffinzied and hydrated sections finally stained in 1% safranin for 24 h. Excess of stains were removed by running water and again sections were hydrated in a series of alcohol wash of 50, 70, 90 and 100%. After that, sections were counter stained in 0.5% fast green for very quickly in 15 s and washed through passing in 90% and 100% alcohol. Excess of stains were removed by washing in a clove oil mixture, which was prepared by mixing 50% clove oil with 25% xlyene. Then section was washed through xlyene and mounted for examination. Different leaf tissues and cells were measured by calibrating oculometer using stage micrometer.

Leaf anatomical measurements of distance of phloem from lower epidermis, palisade cell height, thickness of phloem, observations were recorded (10X) and group means were analyzed using "t" test.

Biochemical analysis

Chlorophyll *a*, total chlorophyll, total nitrogen content, free amino acid content, soluble protein content and total phenol content were estimated in cotton leaves of the accessions. The plants were grown in a controlled environment following the completely randomized design. Two separate plants were used at 10 weeks after sowing. Six leaves from plants (two each from the top, middle and bottom of the plant) were collected and dried at −56 °C for 7 days in a freeze dryer. The dry samples were ground in an electric blender. The samples were replicated five times for each accession, with two plants per replication.

Leaf sample of 0.2 g was extracted with 80% acetone and the filtrate was used to read their absorbance in spectrophotometer at 645 nm (chlorophyll *a*), 665 nm (chlorophyll *b*) and 652 nm (total chlorophyll) (Sadasivam and Manickam 1992). Total free amino acid content was estimated using the ninhydrin method (Moore and Stein 1948). 0.5 g leaf sample was extracted with 10 ml of 80% ethanol and 1.0 ml extract was mixed with 1 ml ninhydrin (oxidizing agent). After boiling (20 min), 5 ml diluents were added and kept for 15 min. Then the intensely formed bluish purple colour was measured at 570 nm. Phenol content was estimated by using 0.5 g of leaf and sample was extracted with 10 ml of 80% methanol. After obtaining the particle free extract, the filtrate was dried at 40 °C under vacuum in rotary evaporator. Then the solid residue dissolved in 5 ml methanol and an aliquot (0.2 ml) was added with Folin ciocalteau phenol reagent and 7% Sodium carbonate. After 24 h, the blue coloured complex read at 765 nm. Micro-Kjeldahl's distillation method was used for estimation of total nitrogen. The dried leaf sample (1 g) was digested with diacid mixture and the sample was made up to 100 ml for estimating the nitrogen content. Lowry method was used for the estimation of soluble protein content. One gram of leaf sample was ground with 10 ml of Tris-HCl buffer. Then 0.1 and 0.2 ml of filtrate was mixed with reagent mixture (1 ml of 1: 1 ratio mixture of 2% Sodium tartarate and 1% CuSO4.5H2O in 50 ml of 1% Na2CO3 in 0.5 N NaOH). After 10 mins, 0.5 ml of 0.2 N Folin Ciocalteau reagents was added and kept for 30 min before taking reading at 660 nm. Mean data of the biochemical parameters were used for the statistical analysis.

Results

Leafhopper screening under field conditions

Across the growth period, there was increasing trend of leafhopper population observed at 45th and 70th days after sowing; there was a decreasing trend at 90th days after sowing in all the genotypes (Table 1). There was high level of leafhopper infestation recorded for susceptible genotypes compared with all other genotypes. In case of 45th days after sowing there was less range of infestation observed; however AKH 1355 and GISV 216 showed lesser infestation. At 70th days after sowing genotypes AKH 1355 and NSDL1938 showed low level of infestation. Among the 23 genotypes screened at 45 days after sowing the following 18 genotypes were on par with leaf hopper resistant check NDLH 1938 at 45 days after sowing were AKH 1355. GISV 216, LHDP 1, CSH 3129,GISV 267, GSHV 171,NH 630,AKH 2012–8,AKH 1351, AKH 1301, GSHV 173, AKH 2006–2, RS 2711, AKH 09–5, NDLH 2010, JK 35 and H 1454. At 60 DAS, 13 genotypes AKH1355, GISV 216, AKH 2012–8, RS 2765, GSHV 173, NH 630, GISV 267, JK 35, AKH 1301, F 2164, GSHV 171, NDLH 2010 and AKH 2006–2 were on par with leaf hopper resistant check NDLH 1938.

Host preference studies

Leafhopper survival was studied under pot culture (Table 2). Except two genotypes (SCS 1062 and PUSA 5760) almost all others showed lesser survival when compared with susceptible check DCH32. Genotypes superior to NDLH 1938 (Resistant Check)in terms of minimum percentage of survival are RS 2711, RS 2765, LHDP 1, F 2164, GISV 267, GISV 216, AKH 09–5, SCS 1062, JK 35, AKH 1301, AKH 2012–8, AKH 1351, PUSA 5760, AKH 1355, CSH 3129.

Oviposition preference studies

Nymphal emergence from the plants was recorded (Table 2). There was a high amount of nymphal emergence was recorded in susceptible check (DCH 32) compared with other genotypes except NH630. A set of 12 genotypes were superior to the resistant check (NDLH1938) viz., H 1464, RS 2711, PUSA 5760, AKH 1351, GISV 267, LHDP 1, SCS 1062, AKH 1355, RS 2765, F 2164, GISV 216 and H 1454.

Leaf anatomical parameters

Leaf anatomical traits revealed wide difference among the resistant and susceptible genotypes (Table 3). Except leaf thickness, all other traits were negatively correlated with leafhopper infestation. Trichome length varied significantly amongst the tested genotypes and ranged from 0.313 to 0.434 mm. Genotype F 2164 possessed significantly shorter trichome length of 0.313 mm and the longer trichome was observed in PUSA5760 (0.434 mm). Four genotypes namely PUSA 5760, RS 2711, NDLH 2010 and AKH 2012–8 had lengthier trichome over the resistant check (NDLH 1938). Trichome density on leaf lamina varied significantly amongst all the entries and ranged from 70 to 320.6 trichomes per mm2 leaf area. Genotype DCH 32 possessed significantly lower trichome density (70 trichomes/mm^2) and the higher was found on AKH 1301 (320.6 trichomes/mm^2). Seven genotypes namely AKH 1301, AKH 1355, AKH 1351, NH 630, GISV 216, H 1454 and H1464 showed superior trichome density over resistant check (NDLH 1938). The results revealed significant difference among cotton entries regarding trichome density on leaf midrib. Leaf thickness in the present experiment varied from a range of 0.301 to 0.420 μm. The genotype DCH 32 possessed significantly lower leaf thickness (0.301 μm), while higher leaf thickness was found in AKH 2012–8 (0.420 μm). Seven genotypes namely AKH 2012–8, NDLH 2010, H 1454, H 1464, F 2164, PUSA 5760 and AKH 1351 showed higher leaf thickness over resistant check (NDLH 1938). Distance of phloem from lower epidermis was maximum in RS 2765 (400 μm) and showed significant difference from other genotypes. The mean distance of phloem from lower epidermis varied significantly amongst all the genotypes and ranged from 250 to 400 μm. The genotype DCH32 possessed significantly the shortest distance (250 μm) and the longest distance was observed in RS 2765 (400 μm). A set of 10 genotypes namely RS 2765, NDLH 2010, NH 630, AKH 2006–2, H 1464, H 1454, NDLH 1938, JK 35, GISV 216 and AKH 1301 possessed significantly longer phloem distance (306.55 μm). In this study, the mean phloem thickness varied significantly amongst all the genotypes and ranged from 231 to 362.5 μm. The genotype DCH32 possessed significantly thinner phloem (231 μm), whereas thicker phloem was observed in AKA 2012–8 (362.5 μm). Three genotypes namely AKH 2012–8, H 1454 and NDLH 2010 showed thicker phloem over resistant check (NDLH 1938). Palisade cell height varied significantly amongst all the genotypes and ranged from 101 to 225 μm. The lowest height (101 μm) was observed in DCH32, whereas the highest was recorded in AKH 1351 (362.5 μm). Palisade cell length in ten genotypes namely AKH 1351, JK 35, H 1454, PUSA 5760, GISV 267, RS 2765, NDLH 2010, GISV 216, AKH 1301 and RS 2711 were superior over the resistant check (NDLH 1938).

Biochemical bases of resistance

Biochemical parameters revealed that there was a difference between susceptible and resistant genotypes. In case of chlorophyll *a* content (Fig. 1a), susceptible check DCH 32 showed higher chlorophyll *a* (1.61 mg/g), however all other genotypes were less. Total chlorophyll content (Fig. 1b) was higher in susceptible check (2.30 mg/g) compared with other

genotypes. As like chlorophyll *a* and total chlorophyll content; total nitrogen content (Fig. 1c), was also higher in susceptible genotypes (DCH 32) (2.35%) than others were. Free amino acid (Fig. 1d) was found to be higher in susceptible check (DCH 32) (3.85 mg/g) when compared to resistant genotypes. In case of soluble protein (Fig. 1e), again susceptible check was higher (15.1 mg/g) than other genotypes. The resistant check NLDH 1938 showed a significantly lesser amount of soluble protein (5.0 mg/g). In contrast to the above trend, total phenol content (Fig. 1f) was lower in susceptible check (DCH 32) (4.8 mg/g) than other genotypes. Genotype RS2711 was observed with higher tannin (9.1 mg/g). A clear trend of general higher amount of chlorophyll a, total chlorophyll, total nitrogen, free amino acid and soluble protein was found in susceptible genotypes. However, total phenol was lower in susceptible genotypes than others were.

Discussion

This study, we identified cotton genotypes resistant to leafhoppers using field level screening, green house experiments, leaf anatomical studies and biochemical profiling. Among the 54 genotypes which were screened in the year 2016, 21 lines showed resistance, these lines were again screened with two contrasting checks namely resistant check (NDLH1938) and susceptible check (DCH32) in 2017. Two years screening certainly helped in selecting the stable resistant lines. In field level screening, nine genotypes namely AKH1355, GISV 216, AKH 2012–8, GSHV 173, GISV 267, AKH 1301, GSHV 171, NDLH 2010 and AKH 2006–2 showed resistance on par with check (NDLH1938). Host plant resistance is an important component of integrated pest management strategy for leafhopper resistance. These lines can be used as donor source for breeding leafhopper resistant cotton varieties.

Both host preference and oviposition preference studies revealed that seven genotypes RS 2711, GISV 267, LHDP 1, AKH 1355, RS 2765, F 2164, and GISV 216 performed on par with resistant check. In combining field screening and lab screening, three genotypes AKH1355, GISV 216 and GISV 267 consistently showed resistance on par with resistant check. Host preference studies by Mohankumar (1996) and Manish (1998) also found that higher number of

Table 2 Leafhopper survival percentage and nymphal emergence

S. No	Genotypes	Host preference (Survival percentage)	Oviposition preference (Nymphal emergence)
1	PUSA 5760	3.79	1.37
2	H 1464	2.58	1.21
3	H 1454	2.43	2.12
4	RS 2711	2.37	1.21
5	RS 2765	2.55	1.73
6	LHDP 1	2.25	1.50
7	F 2164	2.58	1.83
8	GSHV 173	2.34	2.53
9	GSHV 171	2.28	2.45
10	JK 35	2.41	2.58
11	GISV 267	2.28	1.41
12	GISV 216	2.24	1.91
13	SCS 1062	3.15	1.57
14	NH 630	2.29	4.39
15	AKH 09–5	2.38	3.62
16	AKH 2006–2	2.36	3.42
17	AKH 2012–8	2.29	2.80
18	AKH 1301	2.32	2.67
19	AKH 1351	2.32	1.37
20	AKH 1355	2.23	1.62
21	NDLH 2010	2.38	3.91
22	NSDL1938 (Resistant Check)	2.52	2.44
23	DCH 32 (Susceptible Check)	3.96	4.47
	LSD (P=0.05)	1.96	1.81
	P values of ANOVA	P<0.001	*P*<0.001

survival of leafhopper nymphs and adults observed on susceptible lines than resistant lines.

Plants have the innate potential to evade the insect pest attacks through various adoptive mechanisms. In this defense role, plant morphological traits ensure the infestation and attack from the herbivore. Such as leaf and stem modifications contribute in the form of non preference or antixenosis. These morphological modifications deter the insects' locomotion, feeding and oviposition. In this present study, we have measured these anatomical parameters to ascertain their relationship to impart leafhopper feeding deterrence.

Trichomes are epidermal extension of the cell, which protrudes as hair growth on aerial parts of the plants. Which confer resistance to majority of the insect pests especially sucking pests in cotton i.e. leaf hopper (Parnell et al. 1949; Butler et al. 1991; Singh and Lal 1993), aphids (Nibouche et al. 2008) and whiteflies (Jindal et al. 2008; Rustamani et al. 2014). Lot of studies has shown that leaf hairiness is negatively associated with leafhopper incidences so far (Parnell et al. 1949; Khan and Agarwal 1984; Butler et al. 1991). However, few studies were in support of positive association with leaf hairiness and leafhopper incidences (Nibouche et al. 2008). Some studies were clearly mentioned about the efficiency of hairiness for resistance depends on not only the intensity of leaf hairiness but also the hair length (trichome length) (Parnell et al. 1949; Nibouche et al. 2008). Leaf trichomes are of two types namely glandular and non glandular. It was proven that glandular type has the nature of secreting plant defense chemicals, which deters or induces antibiosis in insect body (Shaheen et al. 2009; Osawaru et al. 2011). In these way; types of trichomes plays a major role in host plant resistance. Since we did not analyze the

Table 3 Leaf morphological and anatomical parameters

S. No	Genotype	Trichome length (mm)	Trichome density (per mm^2)	Leaf thickness (μm)	Distance of phloem from lower epidermis (μm)	Phloem thickness (μm)	Palisade cell length (μm)
1	PUSA 5760	0.434^{a}	119.6^{c}	375.1^{b}	300.3^{e}	300.0^{b}	175.2^{b}
2	H 1464	0.357^{d}	168.6^{b}	390.0^{b}	337.5^{d}	300.2^{b}	100.0^{e}
3	H 1454	0.350^{d}	175.6^{b}	395.0^{b}	337.5^{d}	325.0^{b}	175.0^{b}
4	RS 2711	0.419^{a}	165.2^{b}	257.5^{e}	237.5^{h}	200.0^{f}	162.5^{bc}
5	RS 2765	0.331^{de}	130.2^{c}	270.0^{e}	400.0^{a}	237.7^{d}	162.5^{bc}
6	LHDP 1	0.360^{bc}	110.2^{c}	365.0^{b}	287.5^{f}	312.5^{b}	150.0^{bc}
7	F 2164	0.313^{e}	114.6^{c}	385.0^{b}	287.5^{f}	325.0^{b}	112.5^{e}
8	GSHV 173	0.367^{bc}	102.8^{c}	313.6^{c}	275.0^{f}	275.0^{c}	125.0^{d}
9	GSHV 171	0.331^{de}	92.4^{d}	317.5^{c}	275.0^{f}	237.5^{d}	100.0^{e}
10	JK 35	0.322^{de}	72.0^{d}	295.0^{cd}	325.1^{ed}	225.0^{d}	187.5^{b}
11	GISV 267	0.351^{d}	142.6^{c}	295.0^{cd}	275.0^{f}	225.0^{d}	175.0^{b}
12	GISV 216	0.325^{de}	176.2^{b}	342.5^{c}	325.0^{ed}	250.0^{cd}	162.5^{bc}
13	SCS 1062	0.391^{a}	157.6^{bc}	307.5^{c}	250.0^{g}	237.5^{d}	137.5^{d}
14	NH 630	0.381^{a}	180.8^{b}	312.5^{c}	350.0^{c}	287.5^{c}	100.0^{e}
15	AKH 09–5	0.367^{bc}	150.6^{bc}	262.5^{e}	300.0^{e}	212.5^{d}	150.0^{bc}
16	AKH 2006–2	0.365^{bc}	130.6^{c}	335.0^{c}	350.3^{c}	287.5^{c}	100.0^{e}
17	AKH 2012–8	0.399^{a}	84.2^{d}	420.0^{a}	312.5^{ed}	362.5^{a}	112.5^{e}
18	AKH 1301	0.381^{a}	320.6^{a}	320.0^{c}	325.0^{ed}	250.0^{cd}	162.5^{bc}
19	AKH 1351	0.369^{bc}	197.0^{b}	370.0^{b}	250.0^{g}	300.0^{b}	225.0^{a}
20	AKH 1355	0.368^{bc}	199.4^{b}	357.5^{b}	275.0^{f}	275.0^{c}	125.0^{d}
21	NDLH 2010	0.411^{a}	117.6^{b}	405.0^{b}	362.5^{b}	325.0^{b}	162.5^{bc}
22	NDLH 1938 (Resistant Check)	0.391^{a}	166.2^{b}	320.0^{c}	330.1^{d}	325.0^{b}	140.0^{d}
23	DCH 32 (Susceptible Check)	0.211^{e}	70.0^{d}	411.2^{a}	250.2^{g}	231.2^{d}	101.2^{e}
	LSD (P=0.05)	0.096	63.2	129.0	126.1	105.2	38.4
	P values of ANOVA	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
	Correlation with leafhopper (r)	−0.48*	−0.32*	0.27	−0.36*	−0.31*	−0.22*

Means with the same letter in a column are not significantly different at $P < 0.05$ by Tukey's HSD

* Significant correlation with leafhopper @5%

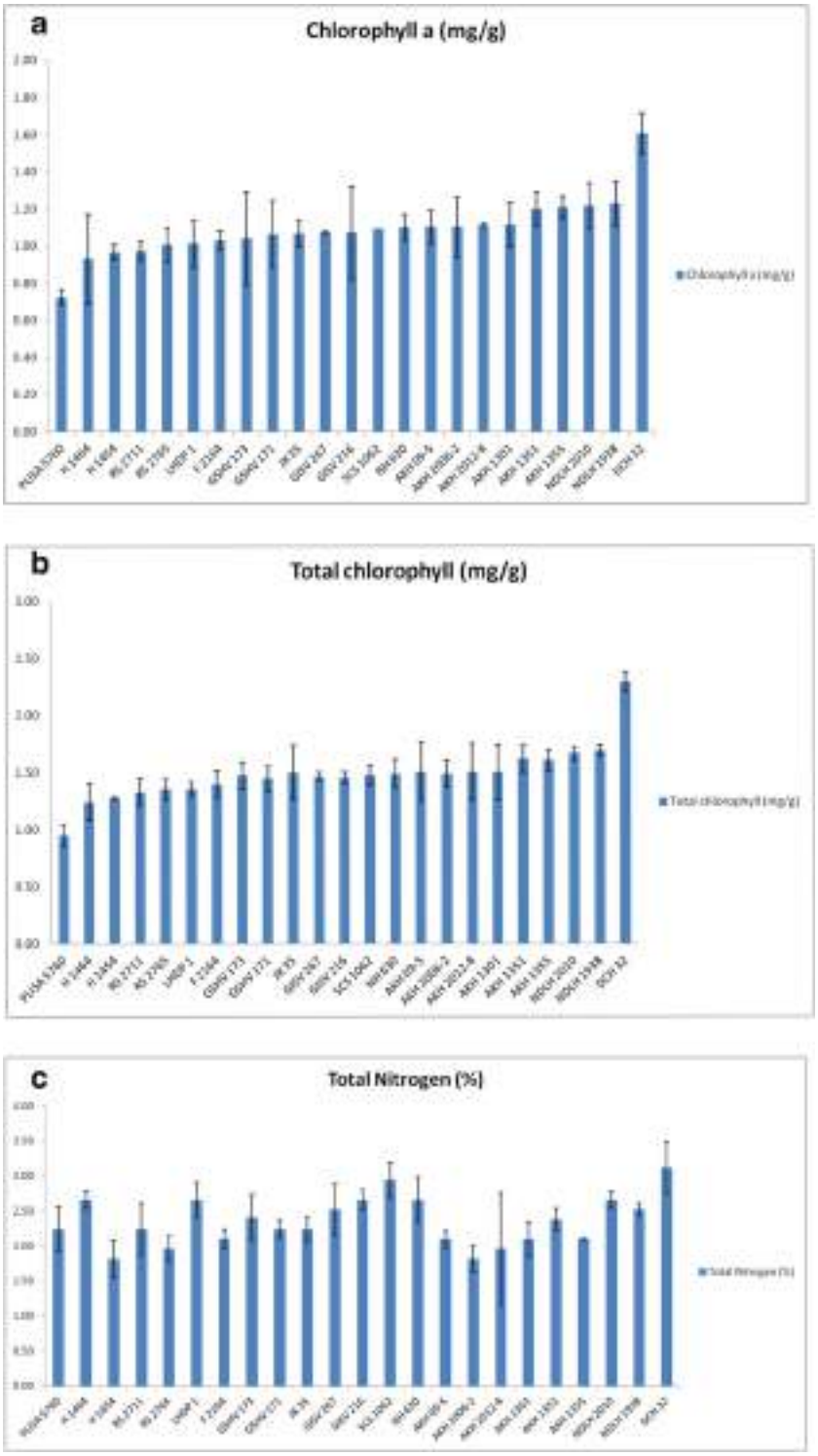

Fig. 1 **a** Chlorophyll *a* content among the genotypes, **b** Total Chlorophyll content among the genotypes, **c** Nitrogen content among the genotypes, **d** Free amino acid content among the genotypes, **e** Soluble protein content among the genotypes, **f** Phenol content among the genotypes

nature of trichome in screening the genotypes, we could do the detailed study in future to unravel the role of secretary glandular trichomes in imparting resistance to leaf hopper. Trichome length deters the free movement of leafhopper, which affects their locomotion, feeding and reproduction. We found in our screening these lines PUSA 5760, RS 2711, NDLH 2010, AKH 2012–8 and SCS 1062 are clearly showing prominent trichome length when compare with

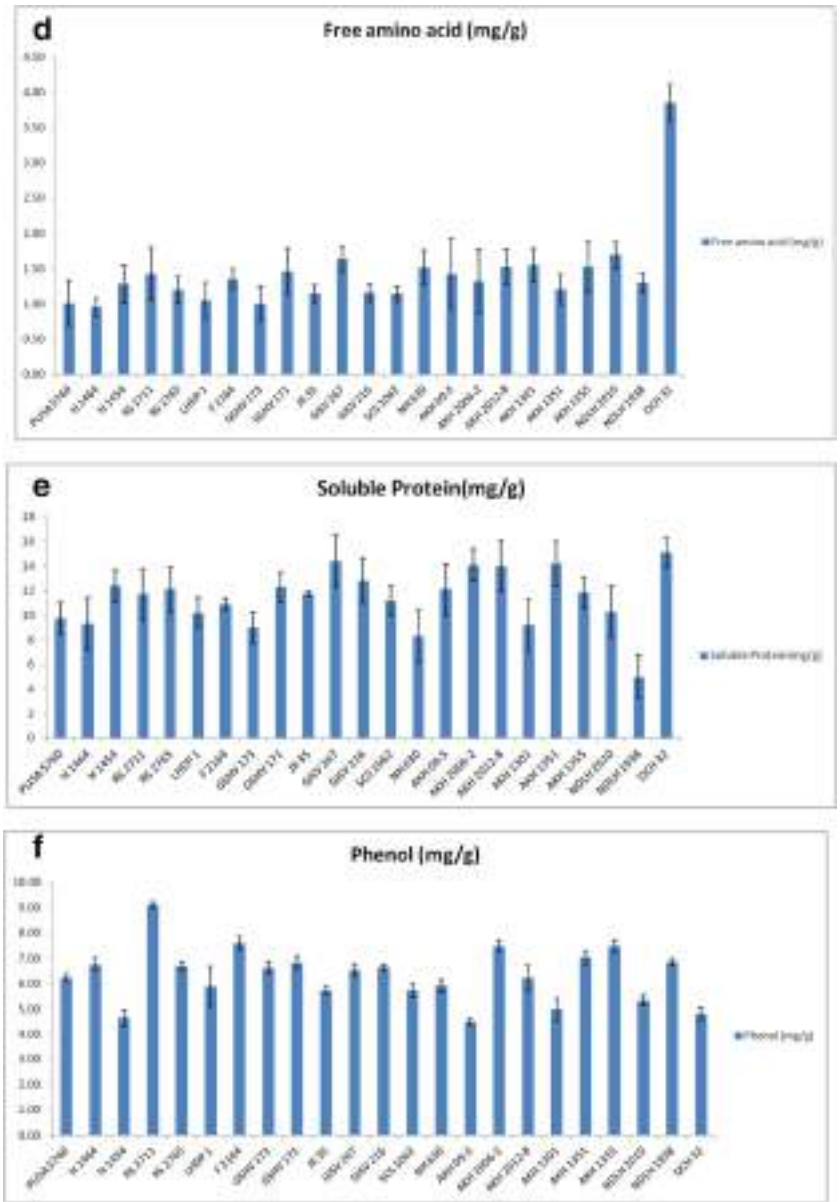

Fig. 1 (continued)

resistant check NDLH1938. Hence, leaf trichome may be considered as important criteria for leafhopper resistance. Pubescence is one of the main traits of nonpreference for insect pests in cotton (Manivannan et al. 2017). Degree density of trichomes on leaves in cotton decides contrasting resistance for leafhoppers (Meagher et al. 1997). Dense mat of trichomes on leaf surface or thick outer layers of cells deter the minute early instar nymphs from reaching the feeding sites or the adults to deposit eggs (Traw and Dawson 2002).

Leaf lamina, leaf thickness and leaf toughness provide the scope for pest preference. Most of the succulent leaves preferred by sucking pest for easy feeding of saps (Agarwal et al. 1978; Eittipibool et al. 2001). AKH 2012–8 and NDLH 2010 had very thick and rough leaves, eventually these genotypes showed higher level of resistance in field as well as lab screening. Compact leaf comprises of higher density of mesophyll, upper and lower epidermal cells. Thinner the leaf lamina serve as a bigger resistance for sucking pests. Leaf toughness could limit population buildup of certain pests (Kadapa et al. 1988; Muhammad et al. 2011). Most of the earlier studies revealed that leaf thickness positively coincide with the leafhopper incidences (Mahal et al. 1993; Taylo and Bernardo 1995).

In leaf, epidermal cell, parenchyma cells, vascular bundle compactness and distance of vascular bundle from epidermal cells play important role in stylet penetration and sucking of phloem sap from the vascular bundle. These leaf anatomical structures impart resistance for phloem feeding. Very few works has been done on role of anatomical structure in

resistance to leaf hopper. Phloem feeding insects secret saliva which forms a salivary sheath to protect their stylet from mechanical damage of leaf tissues, with the stylet they suck the sap from the phloem and ingest their salivary slurry in turn results in injury of the leaf tissues. Distance between lower epidermis and phloem is considered as critical for imparting resistance to sucking pests. Since, the stylet of the leaf hopper has to deeply enter into phloem to feed the sap. More the distance, stylet penetration to reach the phloem is considered as difficult, this genotype generally shows resistance for leaf hopper feeding. A set of 10 genotypes were found to be having longer distance; these genotypes also exhibited resistance in field level screening. Ram Singh and Agarwal (1988) and Rana and Manzoor (2003) reported similar results for sucking pest resistance in cotton with respect to phloem distance. Recombinant Inbred Lines (RILs) for sucking pest tolerance exhibited the higher distance of lower epidermis and phloem when compare to susceptible parental line (Yellappa Harijan et al. 2017).

Leafhopper punctures the leaf surface, probes through stylet to reach the vascular bundle, and feeds the sap (Backus et al. 2005). Leafhoppers use lacerate and flush feeding strategy. Upon pulsing laceration by hoppers on vascular bundle is a permanent reduction in water translocation through the xylem, and a temporary blockage of photo assimilate translocation through phloem sieve elements. In process of feeding, however, this region undoubtedly becomes an energy sink, rather than a region through which photo assimilates are transported from source leaves to sink regions (Nielsen et al. 1990). When the phloem elements are compact, the chances of reaching the cells are less.

Our findings resulted in longer the distance from the lower epidermis to phloem, thicker phloem, lengthier palisade cells, longer and denser trichomes negatively correlated with leafhopper infestation. It gives the insight that these leaf anatomical features strongly associated with leafhopper resistance. These parameters have to be kept in mind to breed a variety for leafhopper resistance in future in addition with other morphological traits. Genotypes namely NDLH 2010 and H 1454 showed superior performance for all the anatomical traits. However, genotypes viz., RS 2765, H 1464, AKH 2012–8, AKH 1351, PUSA 5760 and GISV 216 performed well for the most of the traits. These genotypes were proven as tolerant for leaf hopper with anatomical evidences.

Plant biochemical constituents play important role in insect resistance. These components affect the insect's growth by their qualitative and quantitative nature. These chemicals act as feeding stimulants, physiological inhibitors, inducer of nutrient deficiencies or impairing insect growth metabolisms. These are known for their nonpreference and antibiosis mechanism of resistance.

Variety with high chlorophyll generally has more photosynthetic efficiency, such plants produces more biomass obviously. These genotypes are more preferred by sucking pest especially for their succulent nature. DCH 32 showed higher level of chlorophyll *a* and total chlorophyll content in comparison with other genotypes. Murugesan and Kavitha (2010) screened cotton germplasm using biochemical profiling for leafhopper resistance and observed that chlorophyll *a*, chlorophyll *b* and total chlorophyll found to be higher in susceptible accessions.

Secondary metabolite produced from plants provide effective defense against herbivores. Phenolic compounds hamper insect growth and reduce the survival rate (Schaller 2008). Phenol decreases the palatability of the plant sap to insects, which leads to feeding inhibition, metabolic changes and fertility reduction (War et al. 2012). High phenol content of a variety is directly linked with leafhopper resistance (Balakrishnan 2006; Hosagoudar and Chattannavar 2009; Rohini et al. 2011; Kanher et al. 2016). Our results are in accordance with Bhoge et al. (2019) who reported that less amount of phenol was found in susceptible check DCH32.

Plant nitrogen determines the quality of plant saps, which feed by insects. Nitrogen is the backbone for the formation of amino acids and protein. These protein and amino acids majorly act as feeding stimulant. Since amino acids are very much important in development of leafhoppers. Higher level of amino acids and protein contents were reported in most of the leafhopper susceptible varieties in cotton (Uthamasamy et al. 1971; Deguine and Hau 2001). Screening of germplasm especially for these biochemical profiles has to be given importance, since genotypes with lesser profiles may not preferred by the insects (Febvay et al. 1999).

Conclusion

Genotypes screened at field level was further conformed their level of resistance by host preference studies. These studies were in concordance with each other in identifying resistant genotypes for further breeding programme. Leaf morphology and anatomical structure play vital role in imparting resistant though nonpreference mechanism. In this study, we have shown the leaf structure modifications like trichome length and trichome density possess major hindrance for leafhopper locomotion and oviposition. These leaf appendages deter the insects settling behavior. Anatomical features like distance from the lower epidermis to phloem, thickness of phloem bundle are major obstacles for stylet penetration and affect the feeding of leafhoppers. However, leaf thickness, which facilitates the insect to feed easily, succulent nature of such thick leaves preferred by the insects. It was evident from the thickness of susceptible genotype. From biochemical profiling of these genotypes revealed that higher level of chlorophyll, nitrogen, protein and amino acids favors the leafhopper infestation. In contrast, phenol compound act as feeding deterrent,

as most of the resistant genotypes showed higher level of phenol.

Acknowledgments Authors wish to acknowledge ICAR-CICR for providing necessary support to carry out this research work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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