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Comparative analyses of morphological, anatomical and nutritional traits of cotton cultivars in relation to *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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ABSTRACT

Leaf morphology, anatomy and nutritional traits of ten cotton cultivars were correlated with the whitefly, *Bemisia tabaci* (Gennadius) incidence. The cultivars namely, LD 327, P 59, HS 1300 and P 1752 had less incidence of *B. tabaci* ranging from 1.9-2.8 adults per leaf with higher density of glandular and non-glandular trichomes; in terms of length of trichomes and distance to phloem tissue the cultivar F 2036 had the longest while it was the shortest in LD 327. The latter was also unique in having the thinnest leaf lamina (216 µm) and the least protein content in the leaf (0.40 mg/ml). The cultivars with higher *B. tabaci* incidence were found to have maximum calcium, chlorine and sodium contents in their leaves, while the cultivars with less incidence had higher magnesium, sulphur and potassium contents. The cultivar LD 327 which showed least incidence of *B. tabaci* revealed a positive correlation as regards density and length of trichomes, distance to phloem tissue, and sulphur and potassium contents with whitefly incidence.

Key words: *Bemisia tabaci*, EDX, *Gossypium* sp, Leaf anatomy, Protein content, Trichomes

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) has been an economic pest of cotton throughout the world (Stansly and Naranjo 2010). The development of insect resistant cultivars will lead to insect population suppression. This is true in case of a polyphagous pest like *B. tabaci*. As combining resistance with yield and quality traits is a difficult proposition, plant traits should be explored to minimize the damages (Norman *et al*. 1996). The plant characters like length and density of trichomes and high gossypol content had been found significant in reducing *B. tabaci* infestation in cotton (Butter and Vir 1989). The glandular trichomes on the leaf result in their mortality and thereby reduced susceptibility (Channarayappa *et al*. 1992). The thickness of leaf tissue had been documented to serve as a mechanical obstacle to insect feeding and the distance between abaxial epidermal tissue and phloem tissue was found to be a contributing factor for resistance in cotton (Puri *et al*. 1993). The concentration of phenol, tannin, P, Mg, N and Fe were lower in resistant cotton genotypes than the susceptible ones (Rao *et al*. 1990). Some secondary metabolites (Baldwin 2001, Benderoth *et al*. 2006) and induced proteins and enzymes (Chen *et al*. 2005, Harfouche *et al*. 2006) often play a direct defensive role. Taking these into account the present study evaluated the interactions between the cultivars and *B. tabaci* in terms of morphological, anatomical, and nutritional plant traits to identify those providing resistance to *B. tabaci* in cotton.

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MATERIALS AND METHODS

The study was conducted at the IARI farm (213m, 28°38'28.1"N, 077°10'12.2"E) during *kharif* (June-October) season of 2009-2011. The cultivars namely, P 1752, P 59, P 86, LRA 5166, LRK 516, RS 810, F 2036, Abadita, HS 1300 of *Gossypium hirsutum* and LD 327 of *G. arboreum* were evaluated in 12 m×14 m plots with six replications in a randomized complete block design. Row to row and plant to plant distance was 100 cm and 10 cm, respectively, and the plots were drip or sprinkler irrigated according to the optimal regime.

The *B. tabaci* incidence was evaluated through fortnightly observations. Fishpool *et al*. (1995) were followed for the sampling of adults. Forty five leaves of three randomly chosen plants were observed in such a sequence of five leaves each from upper, middle and bottom in each plant. The incidence of *B. tabaci* was recorded in terms of total number of adults per leaf.

A fully expanded 90-120 days old leaf from the terminal of the ten randomly selected plants from each cultivar was observed for trichome density. The collected leaves washed in distilled water and fixing in FAA (formalin: acetic acid: ethyl alcohol) for 24 hr and then in 70% ethyl alcohol for 24 hr at room temperature. These leaves were then washed

in distilled water and cut into cm^2 squares and macerated in Jeffrey's solution (mixture of 1% aqueous CrO_3 and 10% aqueous HNO_3, 1:1) for 48 hr at room temperature changing the solution every 24 hr. Upper cuticular layer of abaxial epidermis was separated and rinsed well in distilled water before staining with 2% safranin for 20 min. Thereafter dehydrated in TBA (tertiary butyl alcohol) and mounted in canada balsam for examining the density of glandular trichomes in an area of 790 µm under Leica DM 1000 phase contrast microscope at 200x. Non-glandular trichome density was evaluated through the examination of abaxial surface of leaves per cm^2 under Leica EZ4 stereozoom microscope at 100x. The observations were taken from three regions of the same leaf, two of these away and one near the petiole.

A fully expanded 90-120 days old leaf from the terminal of the ten randomly selected plants from each cultivar was observed for the measurements on trichome length. Leaves were thoroughly washed with distilled water to remove the contaminants and cm^2 sections were made from three regions, two from the marginal region and one from the area next to mid vein. These were evaluated under scanning electron microscope (ZEISS EVO MA10) at low vaccum mode at 20 KV/EHT and 100-150 Pa using cold stage at 168x to 780x.

A fully expanded 90-120 days old leaf from the terminal of the ten randomly selected plants from each cultivar was observed for evaluating the lamina thickness. The leaves were fixed in FAA for 24 hr and then incubated in 70% ethyl alcohol for 24 hr at room temperature. Transverse sections were made from the area next to mid vein and stained with 2% safranin for 20 min. These stained sections were subjected to dehydration in a series of 50%, 70%, 90% ethyl alcohol for 10 min each, followed by 5 min in 100% ethyl alcohol. After dehydration, it was kept in a mixture of 100% ethyl alcohol: TBA (3:1) for 20 minutes followed by 10 min incubation in mixture of 100% ethyl alcohol: TBA (1:1). It was then kept in 100% TBA for 10 min. These sections were then mounted in canada balsam, and lamina thickness and the distance to phloem tissue from abaxial epidermis were measured at 400x under Leica DM 1000 phase contrast microscope.

A fully expanded 90-120 days old leaf from the terminal of the ten randomly selected plants from each cultivar was evaluated for the protein content. 0.5 g of leaf was ground in 5 ml of phosphate buffer (0.1 M, pH 7.0) in ice followed by centrifugation for 10 min, at 10000 rpm at 4ºC. The supernatant was removed carefully into a fresh microcentrifuge tube without any disturbance to the pellet. The volume of supernatant was measured and added equal amount of 10% TCA (trichloro acetic acid) followed by centrifugation at 10000 rpm for 10 min at 4ºC. The pellet was separated by removing the supernatant completely and dissolved in 1.0 ml of 1N NaOH and mixed well. Total protein content was determined using BSA (Bovine Serum Albumin) as the standard. The absorbance was read at 595 nm using BioTeck Power wave ^{Tm}XS2 ELISA absorbance spectrophotometer.

A fully expanded 90-120 days old leaf from the terminal of the ten randomly selected plants from each cultivar was evaluated for their chemical contents in a Oxford model Energy Dispersive X ray detector system (EDX). The leaves were washed with distilled water and dried in oven at 40^{o}C for 1 hr. The leaf sections of cm^2 size were mounted on aluminium stubs and coated with palladium (18 nm thickness) in a sputtering device (SC7620-Quorum Tech.) and were subjected to EDX for estimating the concentration of nutrients and images from three regions of each leaf recorded under scanning electron microscope (ZEISS EVO MA10) at an accelerating voltage of 20 KV/EHT and 10 Pa, at high vacuum mode.

The data on the mean population of *B. tabaci* per leaf, morphological, anatomical and nutritional characters of the leaves of ten cultivars were subjected to single factor ANOVA and correlation analyses using SAS version 9.1 (SAS Institute Inc.) and scattergraph plotted between incidence and leaf traits. The incidence index ratios for the determination of resistance in the ten cultivars were obtained with the following formula.

$$\text{The index ratio (resistance)} = \frac{\text{Mean of the least incidence cultivars}}{\text{Mean of the tested cultivar}} \times 100$$

RESULTS AND DISCUSSION

B. tabaci incidence

The data on the *B. tabaci* incidence given in Table 1 indicate that the variations are significant when subjected to single factor ANOVA ($P \leq 0.001$); the highest incidence was in LRA 5166, LRK 516 and P 86 (5.4/leaf), followed by F2036, RS810 and Abadita; and the least was on LD 327 (1.9/leaf), which is 3x less than highest incidence followed by P 59, HS 1300, and P 1752 (Table 1). The incidence index ratio of LD 327, P 59, P 1752 and HS 1300 was > 60%, with Abadita 57% while <50% was observed with LRA 5166, LRK 516, P 86, F 2036 and RS 810.

Trichome density

As far as the glandular trichomes are concerned significant variations among cultivars were evident ($P \leq 0.001$) and were significantly greater on LD 327 (15.4/790 µm) followed by P 1752 (15/790 µm), P59 (14.5/790 µm), HS 1300 (14/790 µm); the lowest density was observed on LRA 5166, LRK 516 and F 2036 (5.3/790 µm) followed by RS 810 (6.7/790 µm), P 86 (7.8/790 µm), and Abadita (9.6/790 µm) (Table 1). Correlation coefficients of this data with whitefly incidence revealed that the majority of the cultivars namely LD 327, P 59, HS 1300, Abadita, RS 810, P 86 exhibited positive relationships while few like P 1752, LRA 5166, LRK 516 and F 2036 exhibited negative relationships (Table 2). Two groups were formed from the ten populations in the scattergraphs plotted between *B. tabaci* incidence and density of glandular trichomes; the one with populations from P 1752, P 59, HS 1300 and LD 327 which

Table 1 *Bemisia tabaci* incidence and leaf characters of cotton cultivars (mean ± SE)

Cultivar	Incidence / leaf (n=24)	Non glandular trichomes/ cm^2) n=12	Glandular trichomes/ 3 mm (n=12)	Length of non-glandular trichomes (mm) (n=18)	Lamina thickness (µm) (n=10)	Distance to phloem tissue (n=10)	Protein concentration mg/µl (n=10)
HS1300	2.7+0.4	135.5+3.4	14 + 0.2	489.1	216	142	0.53
LD327	1.9+0.2	132.5+9.3	15.4 +0.3	195.5	299	265	0.40
P59	2.6+0.3	135.5+3.4	14.5+0.2	528.2	333	213	0.58
P1752	2.9+ 0.5	130.1+ 10.2	15+0.1	385.6	259	212	0.77
ABADITA	3.3+0.3	120.7+7.6	9.6+0.4	447.6	332	284	0.76
RS810	4.3+0.7	95.4+2.9	6.7+0.2	480.6	314	239	1.46
F2036	4.8+0.6	95.1+3.35	5.3+0.1	614.7	345	314	1.24
P86	5.4+0.6	89.1+2.9	7.8+0.3	518.8	238	151	1.33
LRA5166	5.4+0.8	83.3+4.7	5.3+0.1	597.7	323	307	1.30
LRK516	5.4+1	83.3+1.9	5.3+0.1	527.4	366	282	1.09

Table 2 Correlation coefficients of *Bemisia tabaci* incidence vs leaf characters

Parameters	LD 327	HS 1300	P 59	P 1752	Abadita	F 2036	R S810	P 86	LRK 516	LRA 5166
Non-glandular	0.1	–0.2	–0.1	0.14	–0.2	–0.1	0.1	–0.0	0.1	0.2
Glandular	0.6	0.0	0.7	–0.7	0.3	–0.1	0.5	0.2	–0.1	–0.1
Length	0.09	–0.20	0.1	0.1	–0.2	–0.1	0.1	–0.04	0.06	0.16
Lamina thickness	–0.70	0.72	–0.15	0.16	–0.07	0.01	–0.14	0.70	0.50	0.50
Distance to phloem tissue	0.20	–0.13	0.04	0.38	–0.04	–0.70	–0.50	0.23	0.40	–0.40

showed more density, and the other with populations from the cultivars LRK 516, LRA 5166, P 86, F 2036, RS 810 and Abadita which showed less density of glandular trichomes.

The non-glandular trichome density showed significant variations among the cultivars at P≤0.001; HS 1300 and P 59 showed highest density (135.5/cm^2) followed by LD 327, P 1752; least density was on LRK 516 and LRA 5166 (83.3/cm^2) followed by P 86, F 2036, RS 810 and Abadita (Table 1). Correlation coefficients of these with *B. tabaci* incidence revealed that the cultivars LRK 516, LRA 5166, RS 810, P 1752, and LD 327 were positively correlated with density of non glandular tichomes (Table 2). From the populations of ten cultivars, two groups were formed in the scattergraphs plotted between *B. tabaci* incidence and density of non glandular trichomes; the one with populations from P 1752, P 59, HS 1300 and LD 327 which showed more density, and the other with populations from the cultivars LRK 516, LRA 5166, P 86, F 2036, RS 810 and Abadita with less density of nonglandular trichomes.

Length of trichomes

Length of trichomes evaluated in the ten cotton cultivars given in Table 1 reveal that there are significant differences among these at P≤0.001. The maximum length of trichome was observed on F 2036 (614.7µm) while the smallest was on LD 327 (195.5 mm). The trichome length of cultivars namely LRK 516, LRA 5166, RS 810, P 1752, P 59 and LD 327 were found positively correlated with whitefly incidence (Table 2). Scattergraphs plotted between *B. tabaci* incidence and trichome length revealed the overlapping of the populations from the ten cultivars.

Lamina thickness and distance to phloem tissue

Thickness of the leaf lamina when compared in the ten cotton cultivars indicate that the variations are significant at P≤0.001 (Table 1) with the maximum thickness being in LRK 516 (366 µm) and the least being in LD 327 (216 µm). The populations from the ten cultivars were found overlapping with each other in the scattergraph plotted between *B. tabaci* incidence and leaf lamina thickness.

Distance to phloem tissue from abaxial epidermis in the ten cotton cultivars is given in Table 1 was found to significantly vary among the cultivars (P≤0.001), the mean distance was maximum in F 2036 (314 µm), and minimum in LD 327 (142 µm). As far as the relationships were considered the cultivar LD 327 was negatively correlated with *B. tabaci* incidence in case of lamina thickness while it was positively correlated with distance to phloem tissue (Table 2). *B. tabaci* populations from the ten cultivars were found overlapping with each other in scattergraph plotted between *B. tabaci* incidence and distance to phloem tissue.

Protein content

Protein contents of the leaves in the ten cultivars revealed significant difference at P≤0.001 (Table 1), with the highest protein content in RS810 (1.46 mg/ml) and the least content was observed on LD 327 (0.40 mg/ml). The correlation studies showed positive relationship with *B.*

tabaci incidence in cultivars LRK 516 and RS 810. The populations from the cultivars P 1752, P 59, HS 1300 and LD 327 were found overlapping with each other in the scattergraph plotted between *B. tabaci* incidence and protein content.

Nutritional analyses

Statistically significant variations were observed in the EDX analyses of potassium (K), calcium (Ca), sulphur (S), magnesium (Mg) and chlorine (Cl) at Pd"0.001; differences in the elements like silicon (Si), sodium (Na), aluminium (Al) were found to be significant. Higher Mg, S, and K contents were detected in the cultivar LD 327 and Ca and Cl content in P 86. Correlation coefficients with *B. tabaci* incidence revealed that these elements are insignificant in their relationships.

The evaluation of plant-insect relationships in cotton and relating these with *B. tabaci* incidence led to the conclusion that the ten cultivars studied fall under two groups, of which one is considered susceptible and the other moderately resistant. LD 327, P 59, P 1752 and HS 1300 fall under the latter and these had *B. tabaci* incidence ranging from 1.9 to 2.8 per leaf. LRA 5166, LRK 516, F 2036, Abadita and P 86 were found to be comparatively susceptible with *B. tabaci* incidence ranging between 3.3 and 5.4 per leaf. Maximum incidence was found on LRA 5166 and P 86, and minimum on LD 327. Similarly, Raghuraman *et al*. (2004) concluded that LRA 5166 and LRK 516 are more susceptible to *B. tabaci*. Differences in incidence among the *G. hirsutum* cultivars were observed by Chu *et al*. (2000) and these reveal that plant-insect relationships play a major role in *B. tabaci* incidence. The scattergrams of trichome density in relation to *B. tabaci* incidence were found to corroborate the categorization of the cultivars on the basis of incidence. Number, length, type and spatial arrangement of leaf trichomes were found to influence the population density of *B. tabaci* on different crops (Kishaba *et al*. 1992, Heinz and Zalom 1995).

Comparatively less density of glandular trichomes was observed on the cultivars categorized as susceptible (Table 1). These findings are in line with Ashfaq *et al*. (2010) and Zia *et al*. (2011) who reported less glandular trichomes whenever there is high *B. tabaci* population density. As well the entries of this group are completely overlapping in the scattergraphs depicting the close relationship between incidence and glandular trichomes, and thus are very difficult to differentiate. The number of glandular trichomes was higher in the moderately resistant cultivars when compared to the susceptible ones. These findings are in agreement with Channarayappa *et al*. (1992) who reported that glandular trichomes on the leaf reduced the susceptibility. Afzal *et al*. (1999) reported that six cotton cultivars out of the twenty studied showed comparative resistance against *B. tabaci* and these resistance traits were found mainly associated with glandular trichomes. The data obtained on glandular trichomes in relation to plant resistance derive support from the findings of Kaloshian and Walling (2005) that the plants display chemical deterrents on their cuticular surfaces or store toxic compounds in vacuoles or trichomes for release upon tissue damage. Among these glandular trichomes produce an array of volatile and nonvolatile secondary metabolites, including acyl sugars, which deter insect settling, phloem feeding and increase frequency of probing leading to host plant resistance.

Comparatively less density of nonglandular trichomes was observed on the cultivars categorized as susceptible and more density was observed in the other moderately resistant group. This derive support from the findings of Meagher *et al*. (1997) that genotypes within *G. arboreum* and *G. hirsutum* showed higher trichome density and lower *B. tabaci* numbers, or low trichome density and high *B. tabaci* numbers. Kishaba *et al*. (1992) suggested that high density and arrangement of leaf hairs were the likely cause of reduced *B. tabaci* oviposition. On the contrary, Chu *et al*. (2000) reported that those with more *B. tabaci* density on leaves had higher number of non glandular trichomes. Leaf pubescence had been reported as very important morphological trait for oviposition preference (Boica *et al*. 2007). More density of nonglandular trichomes was observed in the areas of the leaf nearer to the petiole than the areas away from the petiole (Thomas *et al.* 2014). Maximum density of non glandular trichomes was observed on HS 1300 which falls under moderately resistant group and minimum density was recorded on LRK 516 of the susceptible group. Hence, the present study revealed that cultivars with high density of non glandular trichomes are moderately resistant to *B. tabaci* and those with less density are susceptible.

Likewise, analysis of results on the trichome length in the present study derives support from Acharya and Bhargava (2008) who reported that shorter trichomes led to lesser incidence. The leaf lamina thickness was maximum in the susceptible group of cultivars such as LRK 516, F 2036 and Abadita, and minimum thickness was observed in the moderately resistant cultivars. These results are in agreement with Puri *et al*. (1993) who reported positive correlation of *B. tabaci* incidence with leaf lamina thickness. However, Zia *et al*. (2011) concluded that thickness of leaf lamina and *B. tabaci* incidence are negatively correlated in cotton cultivars. Afzal *et al*. (1999) reported that the resistant traits of cotton cultivars were found mainly associated with lamina thickness.

In contrast to the reports of Puri *et al*. (1993) and Chu *et al*. (2000), the distance to phloem tissue was varying among the susceptible and moderately resistant cultivars. Rao *et al*. (1990) reported that there was significant reduction in *B. tabaci* populations in cultivars with more distance to phloem tissue. In contrast to Puri *et al*. (1993), the protein content was maximum in the susceptible cultivars and the entries of this group were found overlapping with each other in the scattergraph plotted for relationship with *B. tabaci* incidence. EDX analyses in the present study revealed that Mg, S, and Cl contents are

maximum in LD 327 and K in HS 1300, and these are moderately resistant to *B. tabaci*. Rao *et al*. (1990) observed negative relationships between *B. tabaci* incidence and P, Mg and Mn contents. It was observed that the susceptible cultivar P 86 showed maximum Ca and Cl contents.

The results obtained on the host plant morphological, anatomical and biochemical aspects along with incidence of *B. tabaci* reveal that the cultivars with smaller and more density of glandular as well as nonglandular trichomes were enhancing the resistance of host plant to *B. tabaci*. It was also concluded that the cultivars susceptible to *B. tabaci* had longer trichomes, thicker leaf lamina, high protein content, and Mg, S and Cl contents. These indicate the relationship of plant traits with insect pest's host plant selection phenomena. In view of the problems due to insecticide resistance, resurgence, outbreaks as a secondary pest and environmental hazards, resistant cultivars could be an alternative for management of whitefly. This awareness on the role of plant-relationships in the incidence of *B. tabaci* will enable us to understand the underlying mechanisms and contribute to ecologically sustainable pest management.

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