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CHARACTERIZATION AND INHERITANCE OF COTTON LEAF PUBESCENCE

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Abstract

Upland cotton (*Gossypium hirsutum* L.) genotypes have varying densities of trichomes on the leaves. Absence of trichomes increases the attractiveness of the cotton plant to some major insect pests, thus increasing the reliance on pesticides. Leaf trichome density was quantified by two methods. Qualitative grading system is based on the visual examination of the relative density of the abaxial trichomes. Quantitative measure system counts the total trichomes in a specific unit area. Number of trichomes defined a total number of trichomes and trichome branches. Analysis of variance revealed significant variation for number of leaf trichomes in all the 6 generations of the 3 crosses. The segregation pattern for trichome counts in F_2 generations indicated the discontinuous variation, which confirmed the qualitative nature for this trait. The leaves tended to become less hairy as they approach towards maturity and moreover, cotton leaf trichome counts decreased from the apex to the bottom of the same plant canopy not only in the parental populations as proposed earlier but also progressed in the similar fashion in different genetic backgrounds, which meant that the transference of the gene for hairiness in different genetic backgrounds may not effect the pattern of hairiness. There existed a strong relationship between leaf pubescence ratings and trichome counts, indicating its sound morphological basis which is a step forward in the direction of research on trichomes.

Introduction

Trichomes are unicellular outgrowths from the epidermis of leaves, shoots and roots. The trichome cover of a plant surface is collectively called pubescence. Hairiness/ trichomes act an important insect non-preference trait against the sucking insect pests of cotton. It is clear that trichomes play a role in plant defence, especially with regard to phytophagous insects. The degree of hair or trichome density on the leaves of *Gossypium* species and cultivars is related to varying degrees of resistance/susceptibility to sucking pests (Meagher *et al*., 1997). The degree of jassid resistance had definite correlation with the pilosity of the plant. The more tufted types were less prone to jassid attack (Sikka *et al*., 1966). The oviposition behaviour of *Creontiades signatus*, a relatively new plant bug pest of South Texas Cotton, was investigated by Armstrong *et al*. (2009) with respect to the trichome density on okra and normal leaves. Trichome density increased in the progressing nodes of okra leaf with less oviposition sites than the normal leaf with abundant oviposition sites.

There may be one state of hairiness and the other one of glabrousness on the basis of the densities of the trichomes. Pubescence phenotypes are described as smooth (no trichomes), hirsute (moderate pubescence) or pilose (dense pubescence). Profusely state of hairiness is termed as pilose or velvet hairiness. Most modern cultivars of cotton are smooth (glabrous). Pubescence can be measured by two methods. There is a qualitative grading system (Kloth, 1995) based upon the distribution of trichomes on the leaves and leaf veins. The classification system uses five grades: 1 is the absence of trichomes at all stages and 5 is the presence of trichomes on the petiole and at all stages of vein branches

(Wright *et al*., 1999; Bourland *et al*., 2003; Stiller *et al*., 2004; Lacape & Nguyen, 2005) whereas, there is also a quantitative means of measurement of leaf trichome density on leaf surfaces. The density of stem trichomes was scored in the upper 5 cm of each plant, on a scale 1 (glabrous) to 5 (highly pubescent) (Wright *et al*., 1999; Lacape & Nguyen, 2005). In a study by Bourland *et al*. (2003) the trichome counts were made with help of an index card of 0.65 cm diameter hole (0.33 cm^{-2}). All the trichome counts were made within the specific unit area with the aid of a stereo-microscope. Smith (1964) observed the average number of trichomes on leaf blades from 2 to 205 trichomes cm^{-2}. On the basis of this he defined a cultivar Deltapine as smooth leaf with 5 trichomes cm^{-2}. Bourland *et al*. (2003) found that the leaves in the top of the plant always exhibited the highest leaf pubescence ratings, and leaves from the bottom of the canopy tended to have lowest pubescence ratings.

In view of the resistance rendered by trichomes against the insect-pests, this study of characterizing leaf trichomes and pubescence rating system based on visual examination of the relative distribution and density of the trichomes on the abaxial leaf surface is helpful in breeding cotton varieties with specific reference to the reduced insect/pest population.

Materials and Methods

The present investigation was prompted by the pronounced variation in the densities of trichomes among the six generations. Four genotypes were selected and were selfed for four generations by growing twice a year, in a glasshouse and field during 2003 to 2004. The selected parents with contrasting traits (Table 1) were planted during November, 2004 in a greenhouse. Crosses were attempted in three groups to obtain F_0 seed during February through March, 2005. The detail of crossing scheme is presented in Table 2. The experiment in the field was laid out in a Randomized Complete Block Design with three replications of each of the six generations of the three crosses. Trichomes represent the presence of small hairs on the cotton plant (Fig. 1). The data on number of trichomes/unit area were recorded in each replication during 2006-07. The trichome density on leaves was estimated following two criteria proposed by Bourland *et al*. (2003).

Qualitative grading system for trichomes: Three leaves at random each from upper, middle and lower portion of the selected plants was used to assess for the trichome density rating. A rating system of trichomes on the abaxial surface of leaf, using a scale of 1 for sparsely (non) hairiness, 2 for moderate number of trichomes, 3 for (pilose) hairiness was carried out.

Quantitative measure of leaf trichomes: The same leaves mentioned above for the study of qualitative grading were used to assess for the quantitative measure of trichomes on the abaxial leaf surface. Observations pertaining to the number of trichomes were recorded with the help of an index card within an area of 0.1 cm^2 (Fig. 2) laid over the abaxial side of each leaf from three different positions and averaged. Trichomes in the 0.1 cm^2 area were counted with the aid of high magnifying power microscope (Olympus Z61). Each bunch of stellate trichomes was counted as a separate trichome. The mean and standard deviation for trichomes cm^{-2} were determined separately for each trichome count on three different positions of the plant canopy.

Table 1. Distinctive morphological features of the upland cotton accessions.

S. No.	Variety/ accession	Parentage	Hairiness stage	Origin
1.	Acala 63-74	-	Glabrous	Exotic
2.	CIM 446	CP 15/2 × S 12	Lightly hairy	CCRI, Multan Pakistan
3.	FH 1000	S 12 × CIM 448	Lightly hairy	CRI, Faisalabad Pakistan
4.	HRVO-1	B-557/2/Gambo Okra/ Rajhans/3/Rajhans	Pilose hairiness	CRI, Faisalabad Pakistan

CRI = Cotton Research Institute, Faisalabad, Pakistan
CCRI = Central Cotton Research Institute, Multan, Pakistan

Table 2. Scheme of crossing.

S. No.	Cross	Trait considered
1.	HRVO-1 × FH-1000	Pilose hairiness × Lightly hairy
2.	HRVO-1 × CIM-446	Pilose hairiness × Lightly hairy
3.	HRVO-1 × Acala 63-74	Pilose hairiness × Glabrous

(a) Pilose hairiness

(b) Normal hairiness

(c) Intermediate class of hairiness

Fig. 1. Variable classes in leaf trichomes

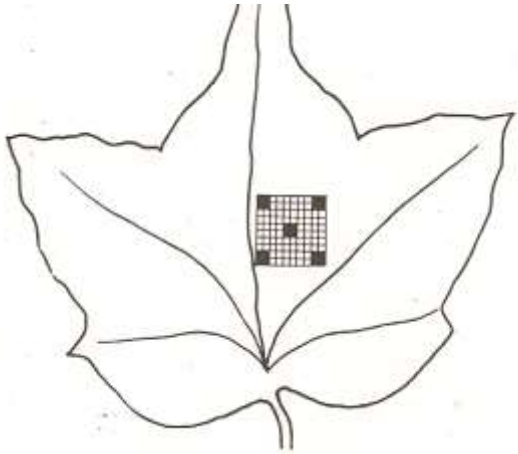

Fig. 2. Trichome counts on the abaxial side of cotton leaf with an area of 0.1 cm^2 of an index card.

Phenotypic and genotypic correlation coefficients between pubescence ratings and trichome counts were also determined using the F_2 data. Phenotypic correlation coefficients were calculated following Dewey & Lu (1959) using Minitab computer programme. The genetic correlations (r_g) between two characters X and Y were calculated following Falconer (1981).

Results

The significant differences ($P \leq 0.05$) among the P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of all the three crosses presented in Table 3. Significant differences of the means for number of trichomes were found between the parents HRVO-1, FH 1000, CIM 446 and Acala 63-74 (Table 4).

Table 3. Mean squares for number of trichomes in six generations of three crosses.

Source	d.f	HRVO-1 × FH 1000	HRVO-1 × CIM 446	HRVO-1 × Acala 63-74
Replication	2	8.220	2.762	149.867
Genotypes	5	14557.706	19792.663	16497.202
Error	10	7.740	1.327	52.094

A higher magnitude of variances in F_2 and backcrosses of all the three crosses was observed as compared to parental and F_1 generations. The variances in F_2 for almost all the three crosses were higher than their respective backcrosses. In order to elaborate the extent of variation for the number of trichomes the frequency distributions for number of trichomes in F_2 generations of three crosses are presented in Fig. 3. The trichome counts of the leaves vary from top to the bottom of the plant canopy in cotton plant. The mean values for the number of leaf trichomes at the top, middle and bottom of the plant canopy along with their respective standard deviation values on the same plant in each of the six generations of the three crosses are presented in Table 5.

Table 4. Generation means and variances for number of trichomes in three single crosses.

Generation	HRVO-1 × FH-1000		Generation	HRVO-1 × CIM-446		Generation	HRVO-1 × Acala 63-74	
	Mean	Variance		Mean	Variance		Mean	Variance
P_1(HRVO-1)	240.20	12.84	P_1(HRVO-1)	240.20	4.84	P_1(HRVO-1)	239.80	4.16
P_2(FH-1000)	40.76	10.09	P_2(CIM-446)	31.73	4.43	P_2(Acala 63-74)	43.76	4.51
F_1	103.70	14.58	F_1	60.73	4.67	F_1	198.40	4.23
F_2	122.73	5218.67	F_2	102.52	7108.62	F_2	173.20	633.71
BC_1	158.79	4259.46	BC_1	169.57	2907.23	BC_1	217.93	384.37
BC_2	77.00	4140.61	BC_2	47.64	2617.77	BC_2	108.16	413.62
LSD (0.05)	**5.06**		**LSD (0.05)**	**2.09**		**LSD (0.05)**	**13.13**	

Table 5. Trichome density count on the abaxial leaf surface at three different positions on the plant in six generations in different crosses.

Crosses	Generations	Plant position No. of trichomes 0.1 cm^{-2}		
		Top	Middle	Bottom
		Mean ± SD	Mean ± SD	Mean ± SD
HRVO-1 × FH 1000	P_1	253.58 ± 5.65	240.04 ± 4.96	226.91 ± 3.60
HRVO-1 × CIM 446	P_1	253.58 ± 5.65	240.04 ± 4.96	226.91 ± 3.60
HRVO-1 × Acala 63-74	P_1	253.58 ± 5.65	239.93 ± 4.92	225.87 ± 4.11
HRVO-1 × FH 1000	P_2	53.96 ± 3.82	40.80 ± 1.62	27.47 ± 2.59
HRVO-1 × CIM 446	P_2	39.64 ± 2.33	31.44 ± 2.34	24.13 ± 2.34
HRVO-1 × Acala 63-74	P_2	53.96 ± 3.82	43.33 ± 2.59	34.11 ± 3.09
HRVO-1 × FH 1000	F_1	134.10 ± 7.26	97.62 ± 5.22	79.42 ± 3.70
HRVO-1 × CIM 446	F_1	78.79 ± 3.16	61.36 ± 2.27	42.07 ± 1.94
HRVO-1 × Acala 63-74	F_1	217.27 ± 1.92	197.42 ± 3.77	180.51 ± 2.72
HRVO-1 × FH 1000	F_2	142.65 ± 71.35	119.04 ± 73.29	106.50 ± 74.04
HRVO-1 × CIM 446	F_2	115.28 ± 85.53	102.35 ± 84.39	89.91 ± 84.85
HRVO-1 × Acala 63-74	F_2	191.10 ± 70.03	188.96 ± 70.99	186.13 ± 69.59
HRVO-1 × FH 1000	BC_1	180.75 ± 59.90	154.53 ± 71.19	141.08 ± 72.92
HRVO-1 × CIM 446	BC_1	183.54 ± 86.43	169.00 ± 87.46	156.15 ± 90.30
HRVO-1 × Acala 63-74	BC_1	234.50 ± 17.78	216.65 ± 20.46	202.65 ± 21.56
HRVO-1 × FH 1000	BC_2	96.93 ± 39.24	73.17 ± 27.29	60.90 ± 27.46
HRVO-1 × CIM 446	BC_2	58.73 ± 18.36	47.95 ± 15.71	36.24 ± 11.11
HRVO-1 × Acala 63-74	BC_2	122.92 ± 83.90	108.22 ± 78.03	93.32 ± 77.52

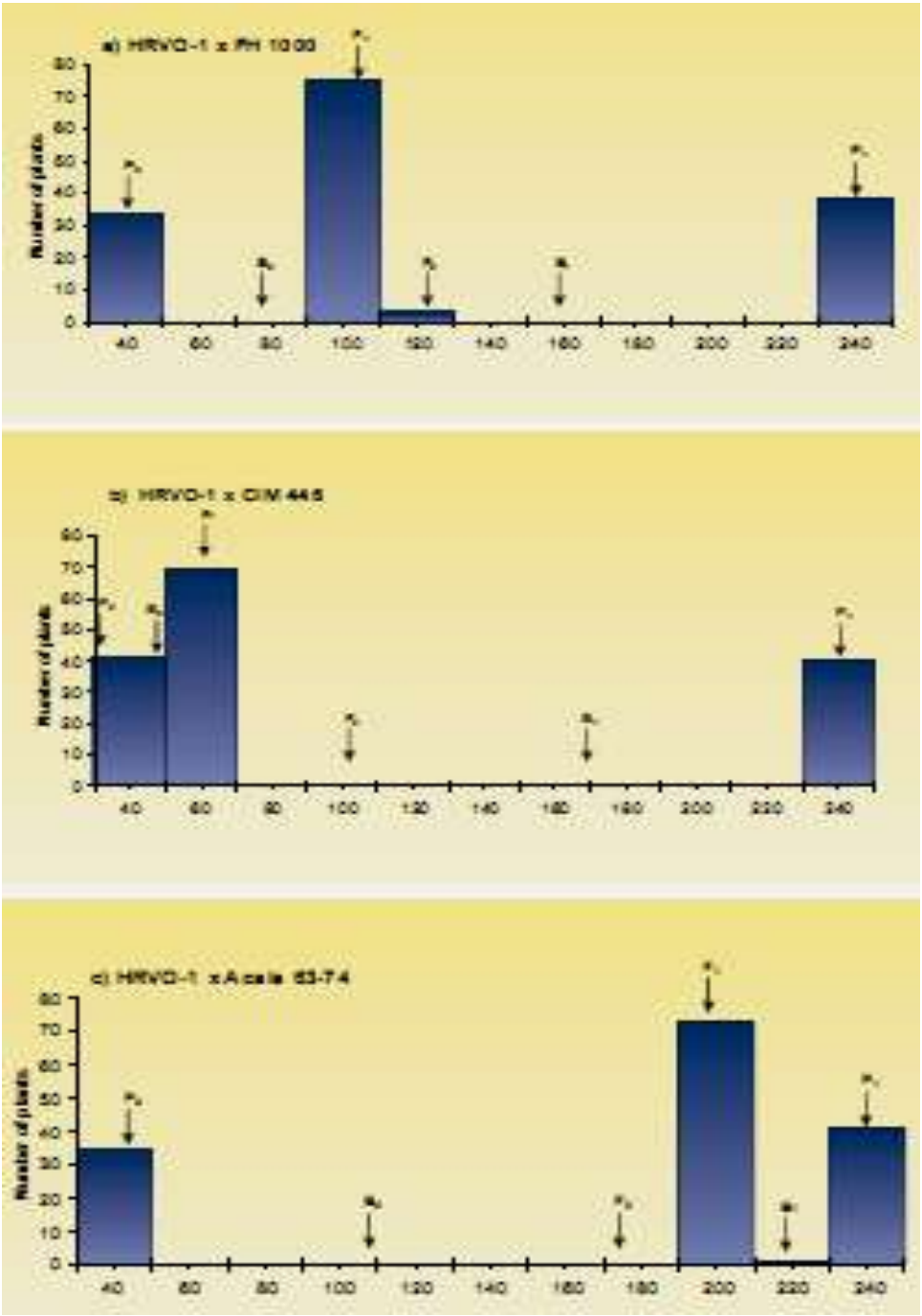

Fig. 3. Frequency distributions for number of leaf trichomes in F_2 generations of three crosses.

Maximum trichome counts were recorded for the common parent P_1 (HRVO-1) in all the three cross combinations. The mean number of trichomes for P_1(HRVO-1) at the top, middle and bottom of the plant canopy were recorded as 253.57, 239.93 and 225.86 per 0.1 cm^2 respectively. The mean number of leaf trichomes at the top, middle and bottom for P_2 (FH-1000) of the cross HRVO-1 × FH-1000, P_2 (CIM-446) of HRVO-1 × CIM-446 and P_2 (Acala 63-74) of HRVO-1 × Acala 63-74) were 53.95, 40.8, 27.67 per 0.1 cm^2, 39.64, 31.44, 24.13 per 0.1 cm^2 and 53.95, 43.33, 34.11 per 0.1 cm^2 respectively. In the F_1 generation of the cross HRVO-1 × FH-1000, the mean number of leaf trichomes recorded from the top, middle and bottom positions were 134.08, 97.62 and 79.42 per 0.1 cm^2 respectively. For the F_1 of the crosses, HRVO-1 × CIM-446 and HRVO-1 × Acala 63-74 the mean number of leaf trichomes were recorded per 0.1 cm^2 as 78.78, 61.35, 42.07 and 217.26, 197.42, 180.51 at the top, middle and bottom of the plant canopy respectively. In the backcross generations with parent 1 (BC_1) of the crosses, HRVO-1 × FH-1000, HRVO-1 × CIM-446 and HRVO-1 × Acala 63-74, the mean number of leaf trichomes at three different positions of the plant canopy were recorded as 180.75, 154.53, 141.08 per 0.1 cm^2, 183.54, 169.87, 156.15 per 0.1 cm^2 and 234.5, 216.65, 202.65 per 0.1 cm^2 respectively, whereas, the mean number of leaf trichomes in the backcross with parent 2 (BC_2) of these three crosses resulted in 96.93, 73.17, 60.9 per 0.1 cm^2, 58.73, 47.95, 36.24 per 0.1 cm^2 and 122.92, 108.22 and 93.32 per 0.1 cm^2 respectively. Mean comparison for the number of trichomes in the F_2 generation of three cotton crosses viz., HRVO-1 × FH-1000, HRVO-1 × CIM-446 and HRVO-1 × Acala 63-74 were recorded as 142.65, 119.04, 106.50 per 0.1 cm^2, 115.28, 102.35, 89.91 per 0.1 cm^2 and 191.07, 188.96 and 186.13 per 0.1 cm^2 at the top, middle and bottom of the plant canopy respectively.

In order to confirm the reliability of both the system of classification i.e., qualitative or quantitative, a positive and significant correlation was observed between leaf trichome counts and leaf trichomes ratings in the three crosses (Table 6).

Table 6. Genotypic (upper value) and phenotypic (lower value) correlation between leaf trichome counts and leaf trichome ratings in three crosses.

Cross	Trait	Leaf trichomes ratings
HRVO-1 × FH 1000	Leaf Trichome counts	0.974*
		0.972**
HRVO-1 × CIM 446		0.926*
		0.925**
HRVO-1 × Acala 63-74		0.961*
		0.958**

*P<0.05, **P<0.01

Discussion

Keeping in view, the relative importance of trichomes as an umbrella against the sucking pests in cotton, it was important to study the inheritance and variation pattern of leaf trichomes in cotton. Analysis of variance (Steel & Torrie, 1980) revealed significant variation for number of leaf trichomes in all the six generations of three crosses (Table 3). The generation mean comparison (Table 4) based on LSD (0.05) values also indicated significant variation for this trait among the six generations of three crosses. This gives an understanding that the number of trichomes varies significantly in all of the six generations of the three crosses which is helpful in predicting the inheritance pattern for

leaf trichomes. The segregation pattern for trichome counts in F_2 generations of three crosses indicated the discontinuous variation, which confirmed the qualitative nature of inheritance for this trait (Endrizzi *et al*., 1984). Almost an equal number of plants showed pilose hairiness and normal/sparse hairiness, while a large number of plants exhibited intermediate hairiness in the segregating F_2 generations which indicated incomplete dominance for trichomes (Knight, 1952; Niles, 1980). It was however, noticeable from the Fig. 3(a) that a very small proportion of plants fell in another intermediately resembling hairiness category. This phenotypic expression of the intermediate hairy state in heterozygous condition was probably affected by the genetic background of the parents indicating, modifying gene effects (Falconer & Mackay, 1996; Rieseberg *et al*., 1996; Xu *et al*., 1997; Rahman & Khan, 1998; Schwarz-Sommer *et al*., 2003).

The qualitative leaf pubescence rating system proposed in this manuscript makes use the three classes. The intermediate class of pilosity allows one to distinguish between the normal and pilose hairiness. A quantitative grading system was developed to classify the number of trichomes. Number of trichomes on the leaves in the present study was counted (McLellan, 2005) as total number of trichome branches, with each branch of stellate trichomes counted as a trichome (Bourland *et al*., 2003; Bourland & Hornbeck, 2007). From most reports, it is not clear whether the trichome count was actual number of trichomes or a count of the total number of trichome branches. Trichome counts were concurrently made on the underside of a leaf from each plant. Abaxial leaf trichome counts showing variation on the same plant at three different positions i.e., top, middle and bottom of the plant in each of the six generations of the three crosses is shown in Table 5. The leaves in the top of the plant always exhibited highest trichome counts as compared to that of the leaves from the centre and bottom of the plant canopy. The decrease in the number of trichomes from the top to the middle of the plant may likely be due to thinning of the trichomes on the enlargement of the leaves. The decline in trichome counts from the middle to the bottom may be due to mechanical loss of trichomes associated with the movement and age of the leaves. The values of Table 5 show a clear indication of the significant differences in trichome counts found between the P_1 and P_2 values. The value of the standard deviation in each of the generations of the three crosses remained less than the mean recorded for trichome counts from the abaxial leaf surfaces. The lower value of the standard deviation from that of the mean value of trichome counts at three different plant positions on the same plant was an indicative that there existed small variations in trichome counts within the particular plant position from where the leaves were used for trichome density studies. The study by Bourland *et al*., (2003) was based upon the trichome counts on the leaves from top to the bottom of the plant canopy on the different cotton genotypes but it did not generate any information regarding the transference of the gene for hairiness in different genetic backgrounds. The present study has an edge as it further testifies the pattern of hairiness state at different plant positions on the same plant by transferring the gene for hairiness from one parent to another and in the subsequent generations. Moreover, the confirmation of the reliability of the proposed system of grading/categorizing leaf trichomes, a correlation between trichome counts and trichome ratings was also established from the present study. The positive and significant values of the correlation coefficients between trichome ratings and trichome counts help in understanding that the increased ratings would increase the pilosity and *vice-versa*. The high correlation coefficients indicate that the rating scale is an effective and reliable mean in characterizing the leaf pubescence of cotton cultivars.

Conclusions

From the present research it was easy to learn the inheritance pattern involved in the trichomes. The rating system devised is easy, quickly understandable and effective in classifying the genotypes. The leaf pubescence ratings were strongly related to the trichome counts, indicating its sound morphological basis. It is interesting to mention here that the leaves of all the cultivars tended to become less hairy as they approach towards maturity and moreover, cotton leaf trichome counts decreased from the apex to the bottom of the same plant canopy. Such type of investigations not only help in developing the natural resistance against insect pest by incorporating 'hairiness' trait but also helpful in predicting the insect pest activity at different plant positions with respect to the hairiness on the same plant.

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