Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 591-594

Received: 13-11-2019
Accepted: 15-12-2019

KPM Dhamayanthi
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

K Rameash
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

A Manivannan
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Annie Sheeba
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

S Abirami
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Corresponding Author:
KPM Dhamayanthi
ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore, Tamil Nadu, India

Studies on leaf hairiness and sucking pest resistance in Egyptian cotton (*Gossypium barbadense* L.)

KPM Dhamayanthi, K Rameash, A Manivannan, Annie Sheeba and S Abirami

Abstract
Trichomes play a key role in imparting host plant resistance against important insect pests in cotton. They act as significant insect non-preference trait against the sucking pests of cotton thus decreasing the reliance on pesticides. In the present study, qualitative and quantitative analysis was carried out to find out the density of trichomes in *Gossypium barbadense* cotton. Qualitative grading was done based on the visual examination of the relative density of the abaxial trichomes and the total trichomes in a specific unit area were measured in each generation for the quantitative analysis. The presence of the trichomes in abaxial surface of the leaves, on the stem and branches in *G. barbadense* cotton were being taken for quantification. Significant variation was observed in number of leaf trichomes in four generations of three inter-mating crosses of *G. barbadense.* The segregating population studies reveals that variation for the hairiness character was not uniform in F_2 generation indicating the presence of the qualitative nature of the trait. Hairiness was very prominent in early days of the different generation of the crop and declined slowly as they approach towards maturity. A notable finding from the study is irrespective of the parents and generations, trichome population appears more in the apex region of the plants and when it goes down to the plant canopy it get gradually decreased. It is an evident that the genes responsible for hairiness is not get transferred and does not have any effect in the fashion of producing trichomes in different genetic backgrounds. The finding has broadened our knowledge in genetics of trichomes in cotton. In view of the resistance rendered by trichomes against the insect-pests, this study of genetics of leaf trichomes based on the relative distribution and density of the trichomes on the abaxial leaf surface is helpful in breeding cotton genotypes with specific reference to the reduced insect/pest population.

Keywords: Gossypium barbadense, trichomes, genetics, breeding, cotton genotype

Introduction
Cotton fibers are differentiated epidermal cells developed from the outer integument of ovules. As a single cell, cotton fiber otherwise known as trichomes has become a model system for studies of cell initiation, elongation, differentiation and cell wall biosynthesis (Bourland and Hornbeck 2007) [1]. There are extensive studies pertaining to role of trichomes in contributing host plant resistance against insect pests by several research workers (Levin (1973) [7], Steffen Hagenbucher (2013) [11] and Sushma Deb *et al*. (2015) [13]. Leaf trichomes can be of different shapes (straight, spiral, hooked, branched, or unbranched), sizes and it can be glandular or non-glandular (Hanley *et al.* 2007) [4]. Non-glandular trichomes have been reported to restrict the insect pests mechanically and the degree of resistance depends on their shape, length, and density. Dense trichomes found to affect the plant-insect interactions by interfering with the movement of insects and other arthropods on the plant surface and limiting their access to leaf epidermis. Levin (1973) [7] proposed that trichome density is negatively related to the feeding, nutrition, and ovipositional behaviour of insects. Cotton plants have trichomes over the plant parts and it act as an important insect non-preference trait against the sucking insect pests of cotton (Steffen Hagenbucher *et al.* 2013) [11]. In general, the plants covered by trichomes is collectively called pubescence and the levels of pubescence in cotton ranging from glabrous or smooth to very hairy or pilose. The degree of pubescence or trichome density on the leaves of *Gossypium* species or cultivars is related to varying degrees of resistance/susceptibility to sucking pests and the more hairiness was less prone to jassid attack (Nawab *et al*. 2011) [8]. In recent years, Bt. cotton era has brought a massive change in the pest scenario of cotton and the sucking pests become the major pests resulting a heavy yield loss in India.

The costs of chemical control and growing ineffectiveness of these pesticides against insects have necessitated finding out an effective alternative method. Host plant resistance merits profound attention in the search for alternative pest management strategies and plays a keys role in formulating eco-friendly pest management programmes. In built resistance against insect pests like plant pubescence provides a dependable umbrella against several insect pests of cotton. Hairiness to confer jassid resistance appears typically to be controlled by a major hairiness gene associated with a complex of minor genes (Knight 1952) [6]. In view of the resistance rendered by trichomes against the insect-pests, this study of characterizing leaf trichomes and its distribution over the plant parts is helpful in breeding cotton varieties with specific reference to the reduced insect/pest population. Genetic variation in cotton trichomes exists in density, distribution and length. Classic genetic studies have identified five genetic loci (t1 to t5) and at least 19 alleles in total (Rahman and Khan 1998) [10]. The study of plant hairiness is of significant importance in cotton especially in India where insect pests are growing in to a very serious menace. Keeping in view of that the four hairy accessions of *G. barbadense* germplasm have been undergone field evaluation with the glabrescent variety Suvin as control.

Materials and Methods

The experiments and field trials were conducted during 2012-16 at the research farm of ICAR - Central Institute for Cotton Research, Regional Station, Coimbatore. Five germplasm accessions *viz*., ICB-124, ICB-264, ICB-284, HAG-02 and EC-18 have been selected for the study to find out the variation in the trichomes density along the six generations. Crosses were attempted in three combinations to obtain seeds rising F_1 generation. The parents of the identified hairy and non-hairy plant types were planted during 2012-13 winter crop season and the F_1 seeds were sown in summer season. The F_2, BC_1 and BC_2 generation were raised in 2013-14 and 2015-16 winter and summer crop seasons. The field experiment was laid out in a Randomized Block Design with three replications of each of the six generations of the three crosses. The data on number of trichomes/unit area were recorded in each replication. The trichome density on leaves was estimated following two criteria proposed by Bourland and Hornbeck (2007) [1]. The details of the accessions/variety have been given in Table-1 and the crosses made for the trait were given in table-2.

Table 1: Genotypes used in the study

Variety/acc	Parentage	Hairiness stage	Wax content	Variety/Origin
HAG-02	Exotic Collection	Pilose	305 µg/cm-2	Exotic
EC-18	Exotic Collection	Hirsute	343 µg/cm-2	Exotic
ICB-124	IC germplasm	Slightly hairy	132 µg/cm -2g	CICR - Nagpur
Suvin	Sujatha x St. Vincent	Glabrescent	-	CICR-Coimbatore

Table 2: Crossing details

Crosses	Traits
HAG-01 x EC-18	Pilose (Highly hairy) x Moderately hairy
HAG-01 x ICB-124	Pilose (Highly hairy) x Moderately hairy
HAG-01 x Suvin	Pilose (Highly hairy) x glabrous (Non-hairy)

Qualitative grading system for trichomes

Three leaves at random each from upper, middle and lower portion of the selected plants was used to assess the trichome density. 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 used for the study of qualitative grading were utilized 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 0.1cm^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 compound microscope. Each bunch of stellate trichomes was counted as separate trichome. The mean and standard deviation for trichome cm^2 were determined separately for each trichome count on three different positions of the plant canopy. Phenotypic and genotypic correlation coefficients between pubescence ratings and trichome counts were also determined using the F_2 data. Phenotypic correlation coefficients and the genetic correlations (rg) between two characters X and Y were calculated following Falconer and Mackay (1996) [3].

Results

In general, *Gossypium barbadense* species is highly affected by the sucking pest especially the jassids and white fly and need almost 4-5 insecticidal spray for a season. Some of the hairy germplasm lines as HAG-02 EC-18 and ICB-124 are exhibiting the tolerance for the sucking pests (Fig.1a and 1b). Only those lines have been utilized for grading the trichomes and crossing purpose. The analysis of variance exhibits a significant differences (P<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 HAG-02, EC18, ICB-124 and Suvin (Table 3). 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.

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 all accessions/variety studied. 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 Fig.1. Maximum trichomes counts were recorded for the common parent P_1 (HAG-02) in all the three cross combinations. The mean number of trichomes for P_1 (HAG-02) at the top, middle

and bottom of the plant canopy were recorded as 326.10, 322.03 and 326.10 per 0.1 cm² respectively.

Among the parents the maximum trichome count was observed in top portion of the plant of pilose parent HAG-02 followed by P_2 (EC-18) which is lightly hairy type and the trichome level is 49.32, 41.55, 30.37 as top, middle and bottom respectively while the check variety Suvin - a glaberacent type does not have hairs either on the top or bottom of the plants (Fig.2a, b & c). In F_1 generation, of the cross HAG-02 × EC-18, the mean number of leaf trichomes recorded from the top, middle and bottom positions were 152.2, 102.40 and 81.03 per 0.1cm² respectively. The F_1 generation of the crosses, HAG-02 × ICB-124 and HAG-02 x Suvin the mean number of leaf trichomes were recorded per 0.1 cm² as 97.30, 93.47, 58.41 and 289.9, 194.31 and 191.07 at the top, middle and bottom of the plant canopy respectively. In the backcross generations (BC_1) of (P_1) the crosses, HAG-02 x EC-18, HAG-02 x ICB-124, HAG-02 x Suvin, the mean number of leaf trichomes at three different positions of the plant canopy were recorded as 195.71, 165.73, 145.19 per 0.1 cm², 190.52, 99.04, 83.07 per 0.1 cm² and 224.34, 216.38, 205.13 per 0.1 cm² respectively whereas, the mean number of leaf trichomes in the backcross with parent 2 (BC_2) of these two crosses resulted in 102.33, 99.20, 79.22 per 0.1 cm², 61.49, 54.19, 52.91 per 0.1 cm² and 134.71,117.71,and 121.37per 0.1 cm² respectively.

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) [12] 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 the segregation pattern for trichomes 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) [2].

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) [6]. It was however, noticeable from that a very small proportion of plants fell in another intermediately resembling hairiness category.

The phenotypic expression of the intermediate hairy state in heterozygous condition was probably affected by the genetic background of the parents indicating, modifying gene effects (Rahman & Khan, 1998 and Huma Khalil *et al*. 2017**)** [10, 5]. 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 as total number of trichome branches, with each branch of stellate trichomes counted as a trichome (Bourland and Hornbeck, 2007) [1]. 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. A Scnning Electron Microscopic study have been conducted to grade the trichome level of the three types *viz*., Pilose, hirsute and glabrescent (Fig 3. a, b, c, d, e, f, g, h, i). 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 graphically shown in Fig.1.

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

Source	d.f	HAG-01	EC-18	ICB-124	ICB-264	ICB-284
Replication	2	6.31	3.67	149.86	72.33	8.52
Genotypes	4	12467.15	17775.33	16497.20	11153.09	10215.46
Error	12	5.63	0.13	52.09	13.29	1.42

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

Generation	HAG-01 x EC-18		Generation	HAG-01 x ICB-124		Generation	HAG-01 x Suvin	
	Mean	Variance		Mean	Variance		Mean	Variance
P_1(HAG-01)	312.05	9.14	P_1 (HAG01)	308.19	7.35	P_1 (HAG01)	311.01	5.24
P_2 (EC-18)	36.11	11.21	P_2 (ICB124)	33.87	4.33	P_2 (Suvin)	41.23	4.33
F_1	117.04	8	F_1	58.44	5.02	F_1	200.25	5.10
F_2	128.37	6027.24	F_2	135.36	6708.56	F_2	161.71	719.04
BC_1	139.52	5016.53	BC_1	156.01	2261.06	BC_1	177.12	522.31
BC_2	803.33	3821.06	BC_2	55.32	3104.77	BC_2	99.30	1002.33

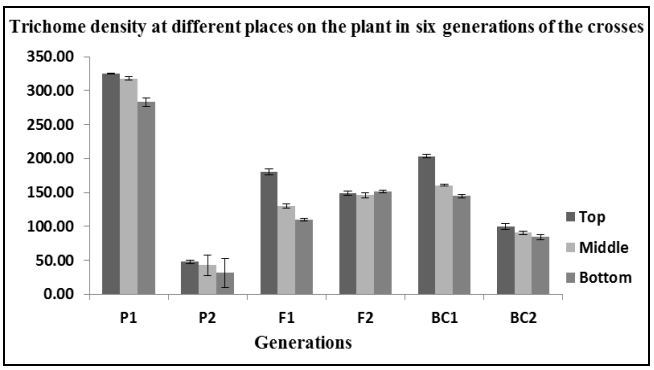

Fig 1: Trichome density at different places on the plant in six generations of the crosses

Discussion

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 trichomes 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- 5show a clear indication of the significant differences in trichomes 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 trichomes counts from the abaxial leaf

surfaces. The lower value of the standard deviation from that of the mean value of trichomes counts at three different plant positions on the same plant was an indicative that there existed small variations in trichomes counts within the particular plant position from where the leaves were used for trichomes density studies. The study by Bourland and Hornbeck (2007) [1] was based upon the trichomes 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 trichomes counts and trichomes ratings was also established from the present study. The positive and significant values of the correlation coefficients between trichomes ratings and trichomes 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.

The present research paved the way to learn the inheritance pattern involved in the trichomes studies. The rating system devised is easy, quickly understandable and effective in classifying the genotypes. The leaf pubescence ratings were strongly related to the trichomes counts, indicating its sound morphological basis. It is interesting to mention here that the leaves of all the cultures tended to become less hairy as they approach towards maturity and moreover, cotton leaf trichomes 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.

References

1. Bourland FM, Hornbeck JM. Variation in Marginal Bract Trichome Density in Upland Cotton. J Cotton Sci. 2007; 11:242-251.
2. Endrizzi JE, Turcotte EL, Kohel RJ. Qualitative genetics, cytology and cytogenetic, 1984.
3. Falconer DS, Mackay TFC. Introduction to quantitative genetics. 4th ed. Longman, Harlow, UK, 1996.
4. Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM. Plant structural traits and their role in anti-herbivore defense. Perspect. Plant Ecol. Evol. Syst. 2007; 8:157-178.
5. Huma Khalil, Abu Bakar Muhammad Raza, Muhammad Afzal, Muhammad Anjum Aqueel, Muhammad Sajjad Khalil, Muhammad Mudassir Mansoor. Effects of plant morphology on the incidence of sucking insect pests complex in few genotypes of cotton. Journal of the Saudi Society of Agricultural Sciences. 2017; 4:344-349.
6. Knight RL. The genetics of jassid resistance in cotton. I. The genes H1 and H2. J Genet. 1952; 51:46-66.
7. Levin DA. The role of trichomes in plant defense. Q. Rev. Biol. 1973; 48:3-15.
8. Nawab NN, Khan IA, Khan AA, Amjad M. Characterization and inheritance of cotton leaf pubescence. Pak. J Bot. 2011; 43(1):649-658.
9. Niles GA. Breeding cotton for resistance to insects. In: Cotton Breeding, II[nd] Edition, 2004 eds. Phundun Singh., Kalyani Publishers, New Delhi, 1980, 136-146.
10. Rahman H, Khan WS. Expressivity of H2 gene of hairiness and L0 gene of leaf shape of cotton under different genetic backgrounds. Pak. J Bot. 1998; 30(1):95-100.
11. Steffen Hagenbucher, Dawn Olson, John R. Ruberson, Jorg Romeis. Resistance Mechanisms against Arthropod Herbivores in Cotton and Their Interactions with Natural Enemies. Critical Reviews in Plant Sciences. 2013; 32(6):458-482.
12. Steel RGD, Torrie JH. Principles and Procedures of Statistics, A Biometrical Approach. McGraw Hill Book Co., New York, USA, 1980.
13. Sushma Deb, Bharpoda ATM, Suthar MD. Physico-Chemical basis of resistance in cotton with special reference to sucking insect pests. Department of entomology B. A. College of Agriculture Anand Agricultural University). ARRES an international e-journal. 2015; 4:87-96.