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BREEDING AND GENETICS

Visual Ratings and Relationships of Trichomes on Bracts, Leaves, and Stems of Upland Cotton

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ABSTRACT

Reduction of trichome density on various organs of cotton (*Gossypium hirsutum* L.) plants may enhance the ability to clean seedcotton and lint. Visual ratings of trichomes could facilitate characterization of genotypes and selection for specific types of pubescence. The objectives of this study were to determine if trichomes on leaves, stems, and bracts can be effectively characterized by visual ratings and to determine the relationships of trichome density on abaxial leaf surfaces, leaf margins, stems, and bract margins. Random plants from segregating populations were selected from plantings at Keiser, AR, in 2001 and 2002. Visual ratings from 1 (glabrous) to 7 (very hairy) were made in the field on a mid-canopy, first-position leaf and bract, and a stem about five nodes from plant apex. Trichome density associated with each organ was then determined and regressed against the visual ratings. Significant correlations between visual ratings and trichome densities indicated that trichome density on abaxial leaves, stems, and bract margins can be visually rated. With established standards and a wide range of variation, ratings of abaxial leaf trichomes were more effective than ratings of trichomes on stems or bract margins. Densities of trichomes on abaxial surfaces and margins of leaves were not correlated. All other correlations were significant, but the low coefficients suggest that the traits have some degree of independence. It may be possible to genetically reduce the degree of marginal bract pubescence of cultivars while still retaining higher degrees of pubescence on leaves and stems.

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Hair-like protrusions (i.e. trichomes) occur on various surfaces of cotton (*Gossypium hirsutum* L.) plants (Fig. 1). The primary advantage of reduced pubescence levels (or lower trichome density) is increased cleaning efficiency of seedcotton resulting in improved grades of the ginned cotton (Novick et al., 1991). As summarized by Jenkins and Wilson (1996), trichomes on leaves and stems are related to either increased resistance or susceptibility of cotton to different insect pests. The glabrous trait was generally associated with neutral to positive effects on yield, while the pilose trait often had negative effects.

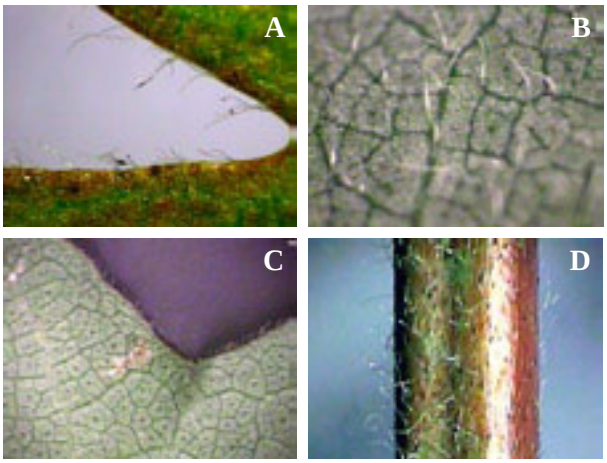

Figure 1. Examples of trichomes typically associated with bract margin (A),abaxial leaf (B), leaf margin (C), and stem (D) of Upland cotton.

Visual ratings of pubescence of different plant organs are easier and faster than counting trichomes, so they can be important tools for developing, screening, and describing genotypes for specific pubescent characteristics. Rayburn (1986) proposed a hairiness index system for cotton leaf pubescence classification as follows: 1 = less than 300 trichomes cm^{-2}; 2 = 300 to 600 trichomes cm^{-2}; and 3 = more than 600 trichomes cm^{-2}. Norman and Sparks (1997) observed that over a 3-yr period, some cultivars had stable leaf hair counts, while others exhibited large variations from year to year, so a system based on trichome numbers may be difficult to use. Bourland et al. (2003) developed a leaf

pubescence rating system that included ratings from 1 through 9 with five major grades and four intermediate grades. The system included representative cultivars as standards and a brief description of the pubescence characteristics for each major grade. Leaf pubescence rating and abaxial leaf trichome density were highly correlated. Visual rating systems for pubescence on stems and bracts have not been reported.

Trichomes occur in various types and forms on the cotton plant. Webber (1938) described three types of epidermal outgrowths that occur on the leaves and stems of the cotton plant: multi-cellular capitate hairs, stellate hairs, and simple hairs. Simple hairs are described as being derivatives of stellate hairs, appearing similar to a single ray of a stellate hair. Stellate hairs occur on both adaxial and abaxial leaf surfaces, typically being more numerous on the abaxial than the adaxial surface. Simple hairs are usually scattered among stellate hairs on mature leaves of most *Gossypium* species. Additionally, the epidermal hairs of cotton were described as usually being well developed near the plant apex, with numbers decreasing as epidermal tissues age. Bourland and Hornbeck (2007) confirmed this distribution of trichomes for leaves and bracts over the plant.

Trichomes on cotton leaves (both adaxial and abaxial surfaces), leaf margins, and stems are genetically controlled by multiple alleles at five loci with some alleles affecting trichomes on different plant organs (Percy and Kohel, 1999). Therefore, trichome density on different organs should be related. In a study of 31 cotton cultivars and strains, Smith (1964) found that trichome density on different leaf parts (blade, petiole, mid-vein, and margin) to be strongly and positively correlated. Smith suggested that trichome counts on abaxial surfaces of leaves from the middle to top portions of plants should be used to characterize trichome numbers on cotton genotypes. Variation within several of the cultivars and strains suggested that selection could be used to reduce trichomes on leaves.

Marginal trichomes on bracts have received little attention. Bourland and Hornbeck (2007) found significant variation on marginal bract trichomes over plant nodes, positions, canopy sites, sample dates, and cultivars. They proposed that bracts should be sampled from mid-canopy, first-position bolls near the time of cessation of flowering and that bract trichomes could be characterized by evaluating plants at one location if highly stressed environments were avoided.

The objectives of this study were to determine if trichomes on leaves, stems, and bract can be effectively characterized by visual ratings and to determine the relationship of trichome density on abaxial leaf surface, leaf margin, stem, and bract margin.

MATERIALS AND METHODS

In 2001 and 2002, random cotton (*Gossypium hirsutum* L.) plants were sampled from segregating F_2 populations in Keiser, AR. Each year, the populations were grown on a Sharkey silty clay soil (very-fine, smectitic, thermic, chromic Epiaquerts) using standard practices for irrigated cotton in the region. Plots were 12 rows, each 12.5 m long, on about 1.0-m centers and were not replicated. After stand establishment, plants were thinned to about six plants per row meter. Seven populations in 2001 and six populations in 2002 were derived without selection from crosses among commercial cultivars and advanced breeding lines developed by University of Arkansas Cotton Breeding Program (Bourland, 2004). Plants from crosses of glabrous (or slightly hairy) by hairy (or very hairy) leaf parents comprised 75 and 100 of the plants in 2001 and 2002, respectively. The remaining plants (45 in 2001 and 20 in 2002) were from crosses between glabrous (or slightly hairy) parents.

In late August of each year, a full-sized leaf on each randomly selected plant was rated for abaxial leaf pubescence using the rating system described by Bourland et al. (2003). Primary grades of this rating system are as follows: 1 (glabrous), 3 (lightly hairy), 5 (moderately hairy), 7 (very hairy), and 9 (pilose). Intermediate grades, i.e. 2, 4, 6, and 8, may be assigned if a plant does not appear to fit one of the primary grades. Using the same rating scale, a portion of the main stem (about five nodes from the plant apex) was visually rated for stem pubescence and a bract from a full-size, first-position boll near the center of the plant (using sampling procedures suggested by Bourland and Hornbeck, 2007) was visually rated for marginal pubescence. A hand lens was used in conjunction with a dark background (black construction paper) to facilitate the rating of marginal trichomes on bracts.

After visual ratings were completed, the bract, leaf, and stem samples were labeled and taken to the lab, where trichome counts were made. To facilitate counting of trichomes, a hole-punch (6 mm diameter) was made in an index card and placed over the plant tissue. Using a viewing microscope,

the number of trichomes exposed through the hole was then counted. Each branch of a stellate trichome was counted as an individual trichome. Abaxial leaf trichome density was determined by counting trichomes for two abaxial, mid-vein areas of each leaf and converted to trichomes per square centimeter. Trichome density for margins of bracts and leaves was determined by counting trichomes on two representative areas of the center tooth of each bract and center lobe of each leaf, then converting to trichomes per centimeter. Similarly, stem trichome density was estimated by counting the trichomes extending from one edge of the stem and converting the number to trichomes per centimeter.

Linear regression analysis (PROC REG; SAS Institute; Cary, NC) using visual ratings as dependent variable and trichome counts as an independent variable was performed for each respective plant part. Correlation coefficients among trichome density on the different plant organs within each year were determined using PROC CORR.

RESULTS AND DISCUSSION

Plants sampled in this study were from segregating F_2 populations derived from crosses of parents that expressed varying degrees of leaf and stem pubescence. These populations were expected to provide plants displaying a wide range of leaf and stem pubescence. Bourland and Hornbeck (2007) observed that density of trichomes on bracts and leaves appeared to be related. Therefore, these populations were expected to also generate variability in bract trichome density. Visual ratings of trichomes on various plant organs can be useful for selecting and describing genotypes if they are closely related to trichome numbers.

Visual ratings of leaf pubescence. Abaxial leaf trichome density increased linearly with visual leaf pubescence ratings in both 2001 and 2002 (Fig. 2). In each year, the slopes of the regression equations revealed similar rates of increase in trichome density (approximately 62 to 65 trichomes cm $^{-2}$) with each visual rating and similar densities associated with the respective ratings. Distinct differences in abaxial leaf trichome density were found among the primary rating grades of 1, 3, 5, and 7, with densities ranging from zero to approximately 350 trichomes cm $^{-2}$ (Table 1). Visual ratings of abaxial leaf surface and number of abaxial trichomes were highly correlated each year. These results confirm findings of Bourland et al. (2003) that leaf pubescence can be visually rated using the rating system that they described. Most abaxial trichomes on very hairy leaves were simple hairs. As trichomes became less dense, the frequency of stellate hairs relative to simple hairs seemed to increase.

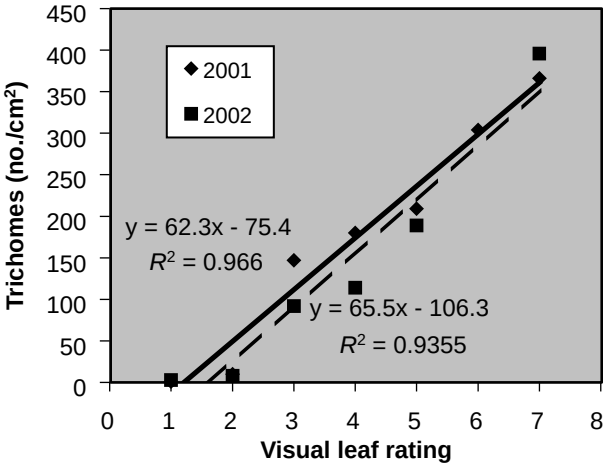

Figure 2. Regression analysis of abaxial leaf trichome density against visual pubescence ratings of abaxial leaf surfaces.

No attempt was made to visually rate trichomes on leaf margins. Trichome density on leaf margins varied little among the abaxial leaf surface ratings and was not significantly correlated to the ratings in either 2001 or 2002 (Fig. 3). Similar trichome density on leaf margins was found each year, ranging from approximately 35 to 45 cm $^{-1}$ over all plants (Table 1). As illustrated by Smith (1964), most trichomes on leaf margins were stellate hairs.

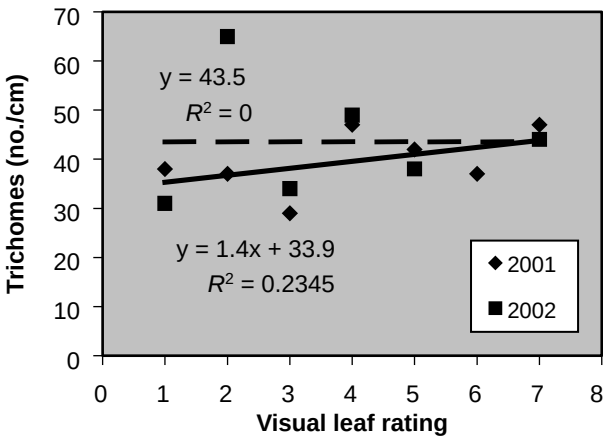

Figure 3. Regression analysis of marginal leaf trichome density against visual pubescence ratings of abaxial leaf surfaces.

Table 1. Density of abaxial and marginal leaf trichomes associated with visual ratings of abaxial leaf pubescence on random plants from F_2 populations in 2001 and 2002

Visual leaf pubescence rating[x]	2001			2002		
	No. plants sampled	Abaxial trichomes (no. cm^{-2})[y]	Marginal trichomes (no. cm^{-2})[z]	No. plants sampled	Abaxial trichomes (no. cm^{-2})[y]	Marginal trichomes (no. cm^{-2})[z]
1	12	1 ± 5	38 ± 14	9	3 ± 4	31 ± 18
2	7	10 ± 10	37 ± 19	1	8 ± 0	65 ± 0
3	33	147 ± 66	29 ± 16	25	92 ± 55	34 ± 19
4	11	180 ± 81	47 ± 11	3	114 ± 53	49 ± 18
5	40	209 ± 76	42 ± 12	55	189 ± 88	38 ± 17
6	4	304 ± 107	37 ± 10	0	--	--
7	13	366 ± 122	47 ± 8	30	396 ± 127	44 ± 19
All	120	177 ± 123	38 ± 15	120	208 ± 151	39 ± 18

[x] Leaf pubescence was rated from 1 (glabrous) to 7 (very hairy) based on a scale developed by Bourland et al., 2003.

[y] Abaxial leaf pubescence rating and density of abaxial leaf trichomes were significantly correlated in 2001 (r = 0.78) and in 2002 (r = 0.77) at P = 0.05.

[z] Abaxial leaf pubescence rating and density of marginal leaf trichomes were not significantly correlated in 2001 (r = 0.27) or in 2002 (r = 0.17) at P = 0.05.

Visual ratings of stem pubescence. Trichome density on stems increased linearly with visual ratings of stem pubescence (Fig. 4). In each year, stem pubescence on plants varied from glabrous (rating = 1) to very hairy (rating = 7), but average stem trichome density was greater in 2002 than in 2001. The difference in years may be attributed to differences between the two sets of populations rather than the environment. Unlike the data for abaxial leaf surfaces, the rates of increase in stem trichomes varied considerably in 2001 and 2002, with a higher rate of increase for stem trichome density in 2002 than in 2001. Additionally, trichome density associated with each rating, except grade 1, was higher in 2002 than in 2001 (Table 2). Stem trichome density appeared to be lower than marginal leaf trichome density. Direct comparison of abaxial leaf trichome density (measured in number per area) to bract trichome density (measured in number per linear distance) could not be made.

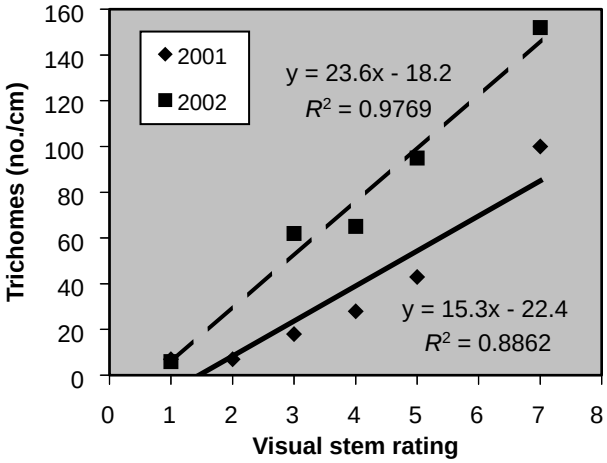

Figure 4. Regression analysis of stem trichome density against visual stem pubescence ratings.

Correlations between visual ratings and stem trichome density were positive and highly significant each year (Table 2). The rating scale may need additional standardization in view of the year-to-year variability in stem trichomes (slopes and trichome density by rating); however, the linear increase shown by the regression analysis and the significance of the correlations suggest that the visual rating system can be effectively used to distinguish variation in stem trichome density. Consistency of ratings of stem pubescence might be improved by establishing standards for each rating, as has been done for abaxial leaf pubescence. No standard types, other than the descriptive standard of no trichomes for rating 1, have been established for different grades of stem pubescence. Without standards, the ratings become more subjective, particularly as stem trichome density increases. The higher ratings will likely simply reflect the range of stem trichome density encountered.

Visual ratings of bract trichomes. Trichome density on bract margins increased linearly with increasing visual bract rating (Fig. 5). Similar increases in bract trichome density were obtained each year, although the slope was much lower than those found for abaxial leaf surfaces or stems. The slopes of the regression equations revealed an

increase of 8 to 10 bract trichomes cm $^{-1}$ for each unit increase in visual rating, indicating much less variation in marginal bract trichome density than variation in abaxial leaf or stem trichome density. With this low variation, the small hand lens was useful in discriminating the visual ratings of marginal trichomes on bracts. Out of 120 plants sampled in each year, no plants were visually classed as having glabrous bracts (rating = 1) in 2001 and only two plants in 2002 (Table 3). Although not readily visible, marginal bract trichomes were present on the two glabrous-bract plants in 2002. Therefore, marginal bract trichomes were found on all plants in these segregating populations. Similarly, Bourland and Hornbeck (2007) did not find any glabrous bracts among the numerous Upland cultivars and breeding lines that they examined.

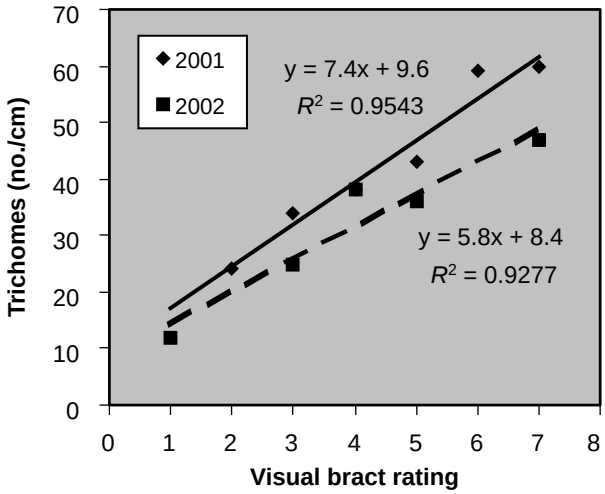

Figure 5. Regression analysis of marginal bract trichome density against visual bract pubescence ratings.

Table 2. Density of stem trichomes associated with visual ratings of stem pubescence on random plants from F_2 populations

Visual stem pubescence rating[y]	2001		2002	
	No. plants sampled	Stem trichomes (no.cm^{-1})[z]	No. plants sampled	Stem trichomes (no.cm^{-1})[z]
1	16	7 ± 8	26	6 ± 8
2	12	7 ± 8	0	--
3	13	18 ± 13	11	62 ± 27
4	21	28 ± 11	2	65 ± 0
5	21	43 ± 20	36	95 ± 34
6	0	--	0	--
7	55	100 ± 53	48	152 ± 59
All	119	58 ± 55	119	96 ± 70

[y] **Stem pubescence was rated from 1 (glabrous) to 7 (very hairy).**

[z] **Stem pubescence rating and density of stem trichomes were significantly correlated in 2001 (r = 0.72) and in 2002 (r = 0.84) at P = 0.05.**

Table 3. Density of marginal bract trichomes associated with visual ratings of bract pubescence on random plants from F_2 populations

Visual bract pubescence rating[y]	2001		2002	
	No. plants sampled	Marginal bract trichomes (no.cm^{-1})[z]	No. plants sampled	Marginal bract trichomes (no.cm^{-1})[z]
1	0	--	2	12 ± 4
2	2	7 ± 8	0	--
3	36	36 ± 8	29	25 ± 9
4	7	28 ± 13	1	38 ± 0
5	66	43 ± 10	64	36 ± 9
6	2	59 ± 5	0	--
7	5	60 ± 10	24	47 ± 12
All	120	58 ± 55	120	35 ± 12

[y] **Bract pubescence was rated from 1 (glabrous) to 7 (very hairy).**

[z] **Bract pubescence rating and density of marginal bract trichomes were significantly correlated in 2001 (r = 0.53) and in 2002 (r = 0.68) at P = 0.05.**

Visual ratings of bract pubescence were positively correlated with trichome density on bract margins (Table 3), although not as highly as for abaxial leaf surfaces and stems. This study was the first time that the authors had attempted to visually rate marginal bract trichomes. The increase in the correlation coefficients from 2001 to 2002 suggests that visual rating ability may have improved with experience. Correlations between visual ratings and counts of bract trichomes were relatively lower than corresponding correlations of trichomes on abaxial leaves or stems. The lower correlations associated with bract trichomes may be because of the relatively low amount of variation found for this trait and the absence of true glabrous-bract plants. Both simple and stellate hairs were observed on the margins of bracts.

Relationships of trichome density on leaves, stems and bracts. Trichome densities on abaxial leaf surfaces, stems, and bract margins were significantly and positively correlated (Table 4). These correlations suggest some degree of common genetic control of trichome density on different plant parts. The values of the coefficients, however, were relatively low, ranging from 0.29 to 0.40, which suggests some independence of the traits.

Trichome density on leaf margins was not significantly correlated with abaxial leaf trichome density but was significantly correlated with trichome density on bract margins and stems (Table 4). These results are different from Smith (1964), who found a strong, positive correlation between trichome counts on abaxial leaf surfaces and leaf margins when he examined 31 cotton cultivars. Using plants from F_2 populations in this study, independent assortment of genes might have lowered the correlations among traits.

Since bracts are modified leaves, a strong association between trichome density of leaf and bract margins might be expected. Instead, correlations between leaf and bract margin trichomes were similar to the correlations between marginal leaf trichomes and stem trichomes (Table 4). Although not a focus of this research, the variation and importance of marginal leaf trichomes merits further investigation.

CONCLUSIONS

Positive and significant correlations of visual ratings with trichome counts were obtained for each respective plant organ (except for leaf margins, on which no attempt was made to visually rate trichome density), indicating the validity of the visual rating system for distinguishing variation in trichomes on abaxial leaves, stems, and bract margins. High correlations between leaf pubescence ratings and abaxial leaf trichome density confirmed the use of the leaf pubescence rating system established by Bourland et al. (2003).

Although the rating systems for leaves, stems, and bracts are subjective, the absence of standards (other than descriptive standard of no trichomes associated with glabrous types) increased the variation associated with stem and marginal bract pubescence. The differences in relationships of stem trichome density and stem pubescence ratings over years might be due to variation in stem pubescence of the different genetic pools. Stem pubescence can be visually rated, but specific ratings, other than glabrous, are not transferable among data sets.

The lower correlations between bract pubescence rating and marginal bract trichome density may be due to the relatively low amount of variation found for this trait and the absence of true glabrous-bract plants. Trichomes were observed on the bract margins of all plants in the segregating populations, regardless of leaf pubescence rating. Truly glabrous plant parts are easily rated because the objectivity

Table 4. Correlation coefficients among trichome density on leaves, stems, and bracts

Trichome location	Year	Trichome location[z]		
		Bract margin	Abaxial leaf	Leaf margin
Abaxial leaf	2001	0.35**		
	2002	0.33**		
Leaf margin	2001	0.41**	0.16	
	2002	0.30**	0.13	
Stem	2001	0.29**	0.40**	0.29**
	2001	0.31**	0.34**	0.35**

[z] Correlation coefficients designated with ** are significantly different from zero at $P \leq 0.01$.

associated with the absence of trichomes. Visually rating the degree of trichomes present becomes much more subjective. Because of low variability, it is doubtful that marginal bract trichome density can be effectively characterized using this unrefined visual rating system; however, visual discrimination of bract trichomes could be used to select for higher or lower density of marginal bract trichomes and to compare specific genotypes in one environment.

Pubescence on leaves, leaf margins, and stems are controlled by different alleles at five loci (Percy and Kohel, 1999). The genetics of marginal bract trichomes have not been established. It may be possible to genetically reduce the degree of marginal bract pubescence of cultivars while still retaining higher degrees of pubescence on leaves and stems. This would result in a cultivar with a reduced potential for lint contamination, while still possessing the advantages associated with higher levels of leaf and stem pubescence.

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