

Correspondence of Trichome Mutations in Diploid and Tetraploid Cottons

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Quantitative variation for leaf trichome number is observed within and among *Gossypium* species, varying from glabrous to densely pubescent phenotypes. Moreover, economically important cotton lint fibers are modified trichomes. Earlier studies have mapped quantitative trait loci (QTLs) affecting leaf pubescence in *Gossypium* using allotetraploids. In this study, we mapped genes responsible for leaf trichome density in a diploid A genome cross. We were able to map 3 QTLs affecting leaf pubescence based on trichome counts obtained from young leaves (YL) and mature leaves (ML). When the F₂ progeny were classified as pubescent versus glabrous, their ratio did not deviate significantly from a 3:1 model, suggesting that glabrousness is inherited in a simple Mendelian fashion. The glabrous mutation mapped to linkage group A3 at the position of major QTL YL1 and ML1 and appeared orthologous to the *tl* locus of the allotetraploids. Interestingly, a fiber mutation, *sma-4(ha)*, observed in the same F₂ population cosegregated with the *glabrous* marker, which indicates either close linkage or common genetic control of lint fiber and leaf trichomes. Studies of A genome diploids may help to clarify the genetic control of trichomes and fiber in both diploid and tetraploid cottons.

Trichomes are unicellular or multicellular epidermal structures found on leaves and stems of many plants. They serve various functions including protection against herbivores, insect pests, and drought (Kärkkäinen and Ågren 2002). Unicellular trichomes are extensively studied in *Arabidopsis* as models of cell fate and development (Marks 1997; Hulskamp 2004). In cotton, leaf trichomes are associated with increased resistance to leaf hoppers (Jenkins and Wilson 1996; Bourland et al. 2003). As in *Arabidopsis*, cotton leaf trichomes are unicellular and branched. However, the epidermal seed trichomes termed “lint fibers,” which are the cotton of commerce, are unbranched and extremely elongated (Hulskamp et al. 1994; Kim and Triplett 2001). Cotton lint fibers and trichomes, though morphologically different, may still share a similar pathway in the earlier stages of development (Arpat et al. 2004; Rong et al. 2005).

Wang et al. (2004) reported that a cotton myeloblastosis (MYB) gene specifically expressed in *Gossypium arboreum* fiber initials was able to restore trichome production in an *Arabidopsis gl1* mutant (*GL1* or *Glabra1* is an MYB transcription factor essential for the initiation of trichomes in *Arabidopsis* [Oppenheimer et al. 1991]) and also induce (albeit limited) seed trichomes.

Although considerable work has been done on mapping trichome genes in allotetraploid species of *Gossypium* (Endrizzzi et al. 1984; Wright et al. 1999; Lacape and Nguyen 2005), we are not aware of literature related to leaf trichomes in diploids. Individual loci in allotetraploids have a suspected homoeology with the diploid species, but specific genetic relationships have not been tested. It is of value to better understand whether genes that account for phenotypic variation at the tetraploid level are the same as those that account for variation at the diploid level. The genetic complexity of allotetraploids resulting from genome duplication and associated intergenic and intergenomic interactions might be better resolved by studying the corresponding genes in diploids. As evolution of fiber occurred in A genome progenitors of cotton, studies on the A genome may also help to dissect trichome and fiber development in cotton. Accordingly, the objective of this research was to investigate the genetic control of variation in leaf trichome density in diploid (A genome) cotton and begins to explore its relationship to trichome and fiber-related genes in tetraploids.

Materials and Methods

A population of 176 F₂ progeny from an interspecific cross between *G. arboreum* (acc. SMA4) and *Gossypium herbaceum* (accession A1-97) grown under field conditions at the Coastal Plain Experiment Station, University of Georgia-Tifton Campus, GA, was used in this study (for more details, see Desai et al. 2006). Leaf pubescence was assessed by counting the number of trichomes inside a 6-mm ring (28.27 mm²) using a stereomicroscope as described in

Wright et al. (1999). Three trichome counts were made on the underside of 1 young leaf (YL, fully expanded but still glossy) and 1 mature leaf (ML, fully expanded, no longer glossy) from each of the parents and F₂ progeny; 1 on each side of the midrib just above the convergence of the 2 large lateral veins and on the midrib (to obtain midrib pubescence count). The averages of the 3 counts were used in subsequent analyses; variation of the counts is presented as standard error (SE). The Pearson correlation coefficient between YL and ML trichome counts was estimated, and the normality of phenotypic distribution was tested using Statistical Analysis Software (SAS, Cary, NC). Chi-square contingency testing for Mendelian inheritance was tested using SAS ($P < 0.05$).

A total of 167 F₂ plants were used to construct the genetic map. Extraction of genomic DNA and restriction fragment length polymorphism (RFLP) mapping was carried out as described (Rong et al. 2004; Desai et al. 2006). The linkage map was constructed using MapMaker/EXP 3.0 (Lander et al. 1987) and included 275 RFLP loci distributed on 12 linkage groups. Quantitative trait loci (QTLs) analysis was performed using MapMaker/QTL (Lander and Botstein 1989) with a logarithm of odds (LOD) threshold of 2.5 (genome-wide likelihood of one or more false positives is <5%). To test for the presence of additional QTLs masked by QTLs with large effects, the position of a QTL with the highest LOD score was fixed at its maximum likelihood position and the genetic map was searched for the presence of new peaks (QTLs) using the “SCAN” command. If the maximum LOD value for a QTL fell in a marker interval of more than 15 cM, the LOD score at the nearest marker locus was considered for that QTL. QTL likelihood maps and biometric parameters for individual QTLs were determined using MapMaker/QTL. The mode of gene action (additive, dominant, or recessive) was tested using the “TRY” command (MapMaker/QTL), as described by Paterson et al. (1991). If more than one mode of gene action could not be deemed unlikely, the candidate modes were declared in order of decreasing likelihood. A dominance over additive ratio ≥ 3 was considered to indicate heterosis (Paterson et al. 2003).

Sequences of cotton probes (Rong et al. 2004) mapped to linkage group A3 (A genome) and Chr.06 (AD genome) were compared with *Arabidopsis* nucleotide and translated protein sequences using BlastN and BlastX with a threshold of $E < 10^{-10}$.

Results and Discussion

The frequency distribution of trichome counts for YL and ML of 176 F₂ progeny is shown in Figure 1. The *G. arboreum* parent (SMA-4) was glabrous with 0 trichomes, whereas the pubescent *G. herbaceum* parent (A1-97) had a trichome count of 100.2 (SE = 8.08) and 106.6 (SE = 7.27) for YL and ML, respectively. The means of YL and ML for the F₂ population were 19.2 (SE = 1.67) and 21.4 (SE = 1.93). These findings in diploid cotton differed somewhat from

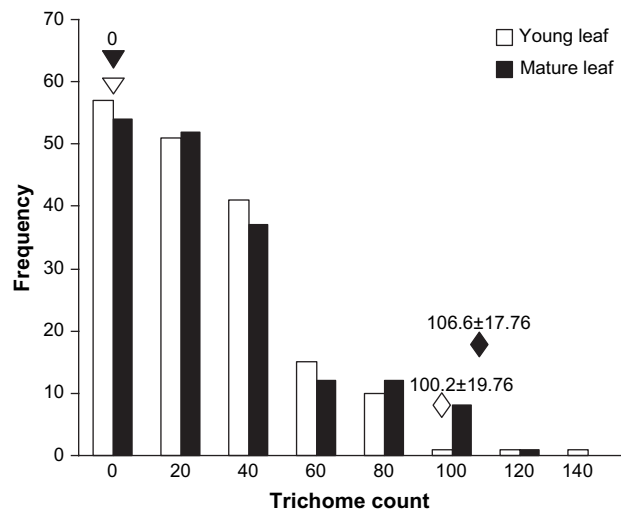


Figure 1. Frequency distribution of leaf pubescence counts for YL and ML of 176 F₂ progeny. Parental means are represented by symbols Δ and \diamond for *Gossypium arboreum* and *Gossypium herbaceum*, respectively.

our prior studies of tetraploid cotton, in which we observed slightly higher trichome densities on YL (Wright et al. 1999). The distribution of the trichome number deviated significantly from a normal distribution based on the Shapiro–Wilk test. Normality was not restored even after the glabrous individuals were excluded from the analysis. The 2 traits, YL and ML, were highly correlated ($r = 0.90$). As 55 among 176 F₂ progeny were glabrous, we scored the leaf pubescence as a dominant marker with *G. arboreum* carrying the homozygous recessive allele. A chi-square test indicated that the segregation of the F₂ phenotype did not deviate from the ratio of 3 (pubescent):1 (glabrous) expected for a single gene model ($P = 0.055$) while showing a significant deviation from ratios of 9:7 and 13:3 for epistatic interactions ($P < 0.001$).

The length of the linkage map was 1147 cM and included 12 linkage groups assembled using 275 RFLP loci spaced at an average distance of 4.2 cM. The map has been described in detail in Desai et al. (2006). One QTL for the YL and 2 QTLs for the ML pubescence were detected as shown in Figure 2. Major QTL for ML (ML1) and YL (YL1) explaining 68.1% and 70.2% of the phenotypic variance (Table 1), respectively, were mapped to linkage group A3 in a region orthologous to the t_1 locus mapped on Chr.06 of the tetraploid genome (Wright et al. 1999; Lacape and Nguyen 2005). When scored as a simple Mendelian trait, the leaf pubescence mutant was also mapped on linkage group A3 at the same position as YL1 and ML1. The second QTL for mature leaf trichomes (ML2; explaining 9.2% of phenotypic variance) was mapped on linkage group A8 in a region orthologous to Chr.02/Chr.03 of the allotetraploid (Table 1). The presence of this second QTL may account for the slight divergence in the observed segregation ratio (121 pubescence vs. 55 glabrous or 2.2:1) from 3:1 although

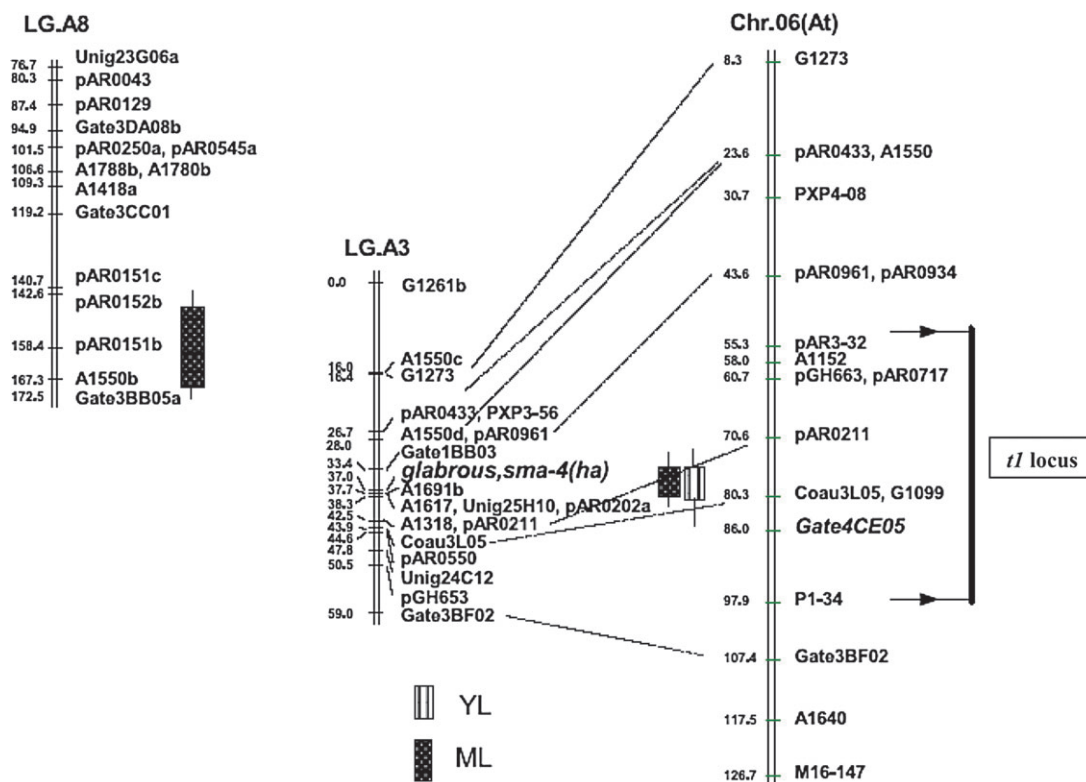


Figure 2. Location of genes/QTL affecting leaf pubescence on an A genome linkage map. Likelihood intervals for the positions of QTL are shown by bars (90%) and whiskers (99%). Chr.06 (previously published by Rong et al. [2004]) represents a linkage group in the allotetraploid A subgenome, orthologous to linkage group A3 (LG.A3). Orthologous marker loci between the diploid and tetraploid linkage groups are indicated by dashed lines. The region on Chr.06 indicated by 2 arrowed and 1 solid lines represents the location of QTL for leaf and stem pubescence in tetraploid populations of *Gossypium* fitting the description of the *tl* locus previously published by Wright et al. (1999) and Lacape et al. (2005). YL = young leaf pubescence count and ML = mature leaf pubescent count.

the ratio is not statistically different from the expected single-gene model. For all 3 QTLs, *G. herbaceum* alleles increased leaf pubescence.

Interestingly, the leaf pubescence mutation cosegregated with *sma-4(ha)*, a fiber mutation mapped in the same population (Rong et al. 2005). In addition, several QTLs for fiber fineness, length, elongation, color, and length uniformity have also been mapped to this region (Saranga et al. 2001; Paterson et al. 2003; Draye et al. 2005; Lacape et al. 2005; Chee et al. 2005a, 2005b) in tetraploid cotton populations. This suggests the possibility that both the glabrous and fiberless traits are under the influence of a single gene that affects both trichome and fiber

development or that the region is rich in genes governing trichome and fiber traits. In either case, the region is of much interest for further study of genes responsible for trichome and perhaps also fiber development.

As the trichome development pathway is well studied in *Arabidopsis* (Hulskamp 2004), we compared our cotton probe sequences (Rong et al. 2004) mapped on linkage group A3 and those in a 43-cM region encompassing the QTL for trichomes and other fiber quality traits on Chr.06 (orthologous to linkage group A3 in tetraploid cottons) to *Arabidopsis* nucleotide and protein sequences. The probe Gate4CE05 (obtained from a cDNA library of 7–10 days postanthesis *G. arboreum* fiber) mapping to Chr.06 of the AD

Table 1. Biometric parameters of individual QTLs affecting pubescence on YL and ML in an interspecific F₂ population (*Gossypium arboreum* × *Gossypium herbaceum*)

Trait	QTL	Linkage group	Nearest marker	LOD	a	d	d/a	PVE
YL	YL1	LG.A3	<i>Sma4-ha</i>	36.1	26.42	−8.15	−0.31	70.2
ML	ML1	LG.A3	<i>Sma4-ha</i>	29.6	29.99	−10.31	−0.34	68.1
	ML2	LG.A8	A1550	2.8	8.26	−6.65	−0.81	9.2

LG = linkage group, PVE = percentage of variance explained, a = additive effect, d = dominance deviation, and d/a = dominance over additive.

genome near the middle of the confidence interval for t1, showed highly significant homology ($E = 4 \times 10^{-21}$ and 5×10^{-36} for BlastN and BlastX, respectively) to the *Arabidopsis* trichome gene *Glabra1* (*GL1*). The *GL1* gene in *Arabidopsis* encodes a MYB-related transcription factor and a mutation in the regulatory or coding region of this gene is known to completely block the trichome production (Marks 1997; Hauser et al. 2001; Kärkkäinen and Ågren 2002). Also, various MYB transcription factors mainly expressed in developing cotton fibers were documented by Loguercio et al. (1999), Cedroni et al. (2003), and Suo et al. (2003). Restoration of trichomes in *Arabidopsis gl1* mutant was possible using GaMYB2, a cotton MYB transcription factor, which is predominantly expressed in developing fiber cells. Expression of GaMYB2 using a constitutive 35S promoter-induced trichomes on *Arabidopsis* seed (Wang et al. 2004), indicating possible similarity of genetic machinery for the regulation of *Arabidopsis* trichomes and cotton lint fiber. Gate4CE05 shows not only highest sequence homology ($E = 0$) to *GbMYB3* but also closely matches ($E = 2 \times 10^{-28}$) *GaMYB2* or *FIF*.

In summary, we confirmed the homoeology of the leaf trichome locus between diploid A genome and the allotetraploid cottons. *Gossypium herbaceum* is thought to be 1 of the 2 closest living relatives and the donor of the A subgenome of allotetraploid cottons (Desai et al. 2006) including *Gossypium hirsutum* and *Gossypium barbadense* that produce the bulk of world cotton fiber commerce. Because genetics in diploids are inherently much simpler than in allotetraploids, mapping of trichome mutants on the A genome may simplify the fine identification of these genes and, perhaps, may also contribute to characterization of genes involved in fiber development.

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