THE HAIRY ANTHER PHENOTYPE IS CONDITIONED BY TWO GENETIC SYSTEMS IN COTTON

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Abstract

A densely pubescent mutant, 61038, with hairy anthers was isolated as a pilose upland cotton which conferred resistance to spider mites and showed more trichomes on the plant body than the T_1 pilose phenotype. Genetic studies indicated that the mutant is conditioned by one dominant gene that is allelic to the pilose gene T_1, hence it is designated T_1^a. The trichomes are of two types: stellate and simple. This mutant had many more stellate trichome branches (10-50 times) than normal pubescent cottons, while its simple trichome density was only 2-5 times higher. Another hairy anther mutant was isolated from interspecific crosses between upland cotton with T_1 and Pima cotton. No hairy anther plants were observed in more than 1500 F_2 plants from 17 interspecific crosses between long-staple and upland cotton without T_1. We propose a model where a dominant inhibitor gene, I_t, from upland cotton inhibits T_1 expression on anthers. Double recessive status at both loci in the Pima cottons, when combined with T_1 from upland cotton, results in expression of the hairy anther phenotype ($T_{1\_}$ I_t I_t) in the F_2.

Introduction

Plant hairs, trichomes, develop from single epidermal cells on the aerial surfaces of plants. Two main types of trichomes, glandular and non-glandular, can be found playing various functions including protecting plants from insect damage. The development of plant trichomes provides an excellent system for studies of cell developmental biology. Because they are easily observable, simple and easily dissected genetically, qualitative and quantitative genetics of trichomes have been investigated in many plant species including cotton (*Gossypium* spp.) and *Arabidopsis*. The molecular genetic analysis of trichome development in *Arabidopsis* has been extensively studied in recent years (Marks, 1997).

In the cultivated cotton species, the non-glandular trichomes are normally observed on leaves and stems, petals, bracts, and calyx, but not on carpels. Also, the presence of trichomes on sepals has not been reported. The morphology of trichomes varies from unbranched (i.e. simple) to multi-branched or stellate. Genetic variation in cotton trichomes exists in density, distribution, and length. Classic genetic studies have identified five genetic loci (t_1 to t_5) and a total of at least 19 alleles (Lee, 1985). The pilose gene, T_1, on chromosome 6 of the A subgenome, confers heavy pubescence on leaves, stems and fruits. The second major gene, T_2, on chromosome 25 of the D subgenome, only expresses dense hairs on leaves and stems. The interaction between the two loci was found to affect trichome density (Kloth, 1995). The two loci were confirmed by the molecular mapping studies of Wright et al. (1999). Three other quantitative loci (QTL) on chromosome 1, 23 and LGA05 were additionally detected to affect pubescence in cotton.

The objectives of the present studies are to (1) report the discovery of the hairy anther mutant, and (2) conduct genetic studies concerning the inheritance of the hairy anther.

Materials and Methods

Test 1

61038, a natural mutant isolated from *G. hirsutum* cv. Liaoyang Domao, is an early-maturing, densely pubescent line that was found to be resistant to spider mites, while these lines with T_1 or T_2 and other local Chinese pubescent lines were susceptible (Zhang et al., 1993). To understand the genetic mechanism in this mutant, we made crosses involving it and TM-1, T582, T586, Deltapine (DPL) 65, and five Chinese cotton lines (e.g. Emian 18, Zhong 12, Sumian 3, Zhong 99, and Hua 102). On an early morning before flowers from other lines had shed pollen, the flowers from 61038 at first appeared to have pollen. However, following pollination attempts no pollen grains could be seen on the stigmas. When the anthers were re-checked, what were originally thought to be pollen grains were actually trichomes on the anther surfaces.

F_1, F_2, and testcrosses involving F_1 and one of the parents were grown in the field and tested for trichome density. The trichome density was scored by two methods: (1) A qualitative rating system based on scores from 0 to 4 where 0= glabrous; 1= normal pubescent; 2-3= moderately pubescent; 4= pilose or heavily pubescent. (2) A quantitative method where simple trichomes or branches of stellate trichomes on the abaxial surface of the 3rd leaf from the top of a plant or anther surface were observed or counted using a dissecting microscope. The counts were made in a 10 x 10 mm sector adjacent to the mid-vein near the confluence of one of the major lateral veins. Since a stellate trichome branch contributes to plant hairiness equally as a simple trichome, each of the branches from a stellate trichome was considered as a different hair for calculation of hair density.

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The trichomes from 61038 and several other lines were observed under a scanning electron microscope for morphological characterization.

Test 2

Hairy anther plants were also found in interspecific F_2 populations between T586 (*G. hirsutum*) having the pilose gene (T_1) and Pima 57-4 or Sev7 (*G. barbadense*). Segregation for hairy anther and glabrous anther was recorded. Plants in F_2 of T582 and 57-4 and 16 other F_2 crosses between four normal upland cotton lines (Hua 101, Emian 18, AC 239 and PD 6520) and four *G. barbadense* lines (designated B1 to B4) were also evaluated for hairy anther in the field.

Results and Discussion

A Hairy Anther Mutant in Upland Cotton

Characterization of trichomes was made using light and scanning electronic microscopes (Figure 1). Under the light microscope, 61038 was seen to have more branches in stellate trichomes with 6 to 12 branches per trichome, while other cotton lines including Zhong 99 had only 2 to 4 branches per trichome.

The number of branches per mm^2 from the stellate trichomes in normal pubescent lines were 0.59 to 2.8, while the densely pubescent Chinese cotton line, Zhong 99 was 11.9 (20- 4 times), and 61038 was 31.6 (50- 11 times). However, the difference in the density of simple hairs between normal lines and pubescent lines was not great. Normal lines ranged from 0.14 to 0.55, while Zhong 99 was 1.04, and 61038 was 0.83. Therefore, all cotton lines had more stellate branches (ST) than simple hairs (SI) with a ratio ranging from 2.1 to 38.0 (Table 1). The ST/SI ratio for normal lines was 2.1 to 9.6, while Zhong 99 was 11.4, and 61038 was 38.0. The total hair density (including stellate trichome branches) was 0.86 to 3.33 for normal cotton, 12.93 for Zhong 99, and 32.43 for 61038. Statistical analysis showed that the density of stellate branches and total hair density in 61038 was significantly higher than these in Zhong 99, but no significant difference in simple trichome density was detected between the two genotypes.

However, the difference in the density of stellate trichomes between the two pubescent genotypes and the normal cottons was reduced. The stellate trichome density for 61038 and Zhong 99 was 3.51 and 3.96, respectively, while the normal cottons tested ranged from 0.20 for T582 (smooth leaf) to 0.92 for Zhong 12 (normal pubescent). Apparently, the hairiness in Zhong 99 is only due to its significantly higher density in stellate trichomes than the normal genotypes, but the hairiness in 61038 is attributed to both its higher density of stellate trichomes and higher number of branches per stellate trichome. The normal cotton genotypes had a total trichome density (the sum of stellate trichome density and simple trichome density) from 0.43 for T582 to 1.47 for Zhong 12, while the trichome density for 61038 and Zhong 99 was 4.34 and 5.00, respectively. Both 61038 and Zhong 99 had similar stellate trichome density and total trichome density. Our results were comparable with that reported by Kloth (1995) who observed 4.3 to 5.6 trichomes/mm^2 for the pilose genotype. The spider mite resistance in 61038 might be due to greater number of branches per stellate trichome.

In all the F_1 populations, no glabrous or normal pubescent plants were observed, and all the plants showed the pilose phenotype, indicating that the new pilose genotype is dominant. Similarly, the hair density in three F_1 crosses was very close to that for 61038, showing a dominant status of 61038 phenotype, whereas the intermediate hair density data from two other F_1 crosses suggested that the heavily pubescent phenotype was incompletely dominant (Table 2).

The trichome density was qualitatively graded and quantitatively counted on abaxial leaves, petioles and young stems in the F_2 and testcrosses. The two measuring methods were in good agreement (Table 3). In six F_2 or testcrosses with 23 to 57 plants each, the correlation between the rating system and trichome count was highly significant, indicating that the grading system would be sufficient for the genetic analysis. Furthermore, the grading and trichome counting on the three organs gave similar results. Here, only the segregation data based on grading from the abaxial leaf surface are presented (Table 4). Ratios of three pilose to one normal pubescence in all the F_2 populations, and 1:1 ratios in all the testcrosses involving F_1 and a normal line, were obtained. The testcrosses of F_1 with 61038 also showed the pilose phenotype. The segregation data indicated that the densely pubescent mutant is conditioned by one dominant gene.

To test whether this gene is a new locus or linked to certain other genes, 61038 was crossed with T582 and T586. The resulting F_2 and testcross populations were evaluated in the field for the segregation of trichome density and other morphological traits. In the F_2 population of 61038 x T582 consisting of 100 plants, four phenotypes with a 9:3:3:1 ratio were observed as the densely pubescent gene and one of the five loci (v_1, gl_1, cu, fg, and cl) were considered. 1:1:1:1 ratios were obtained from 57 plants of the testcross between F_1 and T582. The data indicated that this new gene is independent to the five recessive markers. Segregation of the new gene was tested against nine dominant markers including the pilose gene T_1 in T586. No normal pubescent or glabrous plants were observed in the F_2 population between T586 and 61038 consisting of more than 150 plants. If T_1 were non-allelic to and independent of the new gene, there should be 1/16 normal pubescent plants segregated in the F_2 population. We concluded that the new gene is allelic to T_1, and assign

the gene symbol T_1^a for the hairy anther mutant isolated in upland cotton.

A Hairy Anther Mutant Isolated in an Interspecific Hybrid Between Upland Cotton and Pima Cotton

Interspecific cross-breeding between upland cotton and long staple (*G. barbadense*) cotton has been extensively practiced since the turn of the last century, resulting in the isolation of many new mutants and transgressive segregants. Pronounced genetic variation in trichome density on leaves and stems has been observed in an interspecific F_2 population (Wright et al., 1999). However, the phenomenon of trichomes on the anther surface has not been reported from interspecific crosses.

In our present studies, we made all 16 possible crosses between four upland cotton cultivars and four long staple cotton cultivars including Pima and Egyptian cottons. None of the eight cultivars carry the pilose gene T_1. Among more than 1500 F_2 plants, we found no hairy anther segregants. Surprisingly, when T586 having the T_1 gene was crossed to two Pima cottons (57-4 and Sev7), plants with hairy anthers were observed in the F_2 populations of the two crosses. In the F_2 populations, normal pubescent plants and most of the pilose plants did not show the hairy anther phenotype. The segregation ratio fit a 13 glabrous anther : 3 hairy anther ratio. When two of the hairy anther plants were testcrossed onto Sev7, a 1:1 ratio was obtained (Table 5). Obviously, for trichomes to be present on the anther surface in the interspecific crosses, the pilose gene T_1 should be present. The segregation ratios suggest that another dominant gene present in upland cotton inhibits T_1 expression on the anther. We denote this inhibiting gene as I_t. Therefore, T586 has genotype $T_1T_1I_tI_t$, while the Pima cottons are double recessive ($t_1t_1i_ti_t$). The heterozygous F_1 showed the T586 phenotype, as expected. However, among 4 phenotypes in the F_2, only the phenotype ($T_1\_i_ti_t$) will show hairy anther because the dominant inhibitor gene I_t is absent. The genetic model explaining the results is shown in Figure 3. According to this model, 1/3 of the hairy anther plants should be homozygous ($T_1T_1i_ti_t$) and 2/3 heterozygous at the locus T_1 ($T_1t_1i_ti_t$). Since a ratio of 1 glabrous anther :1 hairy anther was obtained in the testcross progeny, the two hairy anther F_2 plants that were chosen for testing were heterozygous at the T_1 locus. A plant homozygous for hairy anther that would produce all hairy anther progeny in a testcross with Pima cotton was not among the two selected for testing, due in part to the small size of the hairy anther sub-population.

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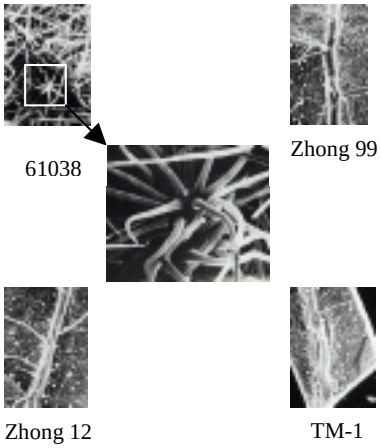

Figure 1. Trichomes on abaxial leaf surface in 61038 and three other upland cottons.

Table 1. Leaf hair density (no./mm^2) in 61038 and other upland cottons

Genotyoe	No. ST	No. SI	No. total	ST/SI
61038	31.60	0.83	32.42	38.0
Zhong 99	11.89	1.04	12.93	11.4
Zhong 12	2.77	0.55	3.33	5.0
Emian 18	1.32	0.14	1.46	9.6
TM-1	2.18	0.34	2.52	6.5
T582	0.59	0.23	0.86	2.1
37-9	2.08	0.17	2.25	12.5

ST-stellate hair; SI-simple hair.

Table 2. Leaf hair density (no./mm^2) in F_1 and parents

Cross	Rating	No. total	Female parent	Male parent
T582 x 61038	2-4	29.92	0.86	32.43
T586 x 61038	3-4	29.28	nt	32.43
Emian18 x 61038	2-4	22.35	1.46	32.43
TM-1 x 61038	2-4	18.71	2.52	32.43
Zhong 12 x 61038	2-4	33.26	3.33	32.43

Table 3. Relationship between qualitative rating and hair density

Cross	Sample size	Coefficient correlation
(Emian 18 x 61038)F_2	57	0.8692**
(T582 x 61038)F_2	56	0.7990**
(TM-1 x 61038)F_2	46	0.8200**
(T582 x 61038)F_1 x T582	23	0.9100**
(TM-1 x 61038)F_1 x TM-1	38	0.8840**
(Emian 18 x 61038)F_1 x Emian 18	39	0.9011**

** significant at P=0.01 level.

Table 4. Number of plants segregating for plant hair density

Cross	No. plants 0-1	No. plants 2-4	χ^2
(T582 x 61038)F_2	63	199	0.13
(TM-1 x 61038)F_2	42	161	2.01
(DPL 65 x 61038)F_2	15	62	1.25
(Emian 18 x 61038)F_2	35	98	0.12
(Hua 102 x 61038)F_2	17	76	2.77
(Hua102 x 61038)F_1 x 61038	0	40	
(DPL 65 x 61038)F_1 x 61038	0	89	
(Emian 18 x 61038)F_1 x Emian 18	65	61	0.13
(T582 x 61038)F_1 x T582	24	37	2.78
(TM-1 x 61038)F_1 x TM-1	168	155	0.52

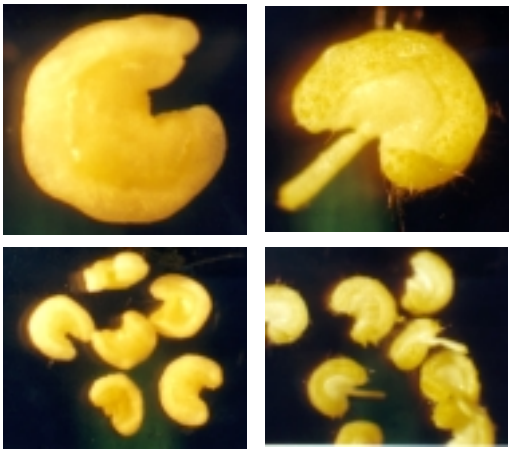

Figure 2. Hairy anther mutant isolated from an interspecific hybrid F_2 population between upland cotton and Pima cotton. Left: normal glabrous anther; Right: hairy anther.

Table 5. Number of plants segregating for hairy anther: interspecific crosses

Cross	No. S	No. H	Ratio (S: H)	χ^2
(T586 x 57-4)F_2	25	4	13:3	0.35
(T586 x Sev7)F_2	76	15	13:3	0.23
Hairy anther F_2-1 x Sev7	17	13	1:1	0.53
Hairy anther F_2-2 x Sev7	9	5	1:1	1.14
(T582 x 57-4)F_2	15	0		
(4 GH x 4 GB)F_2	1500	0		

S-glabrous anther; H-hairy anther.

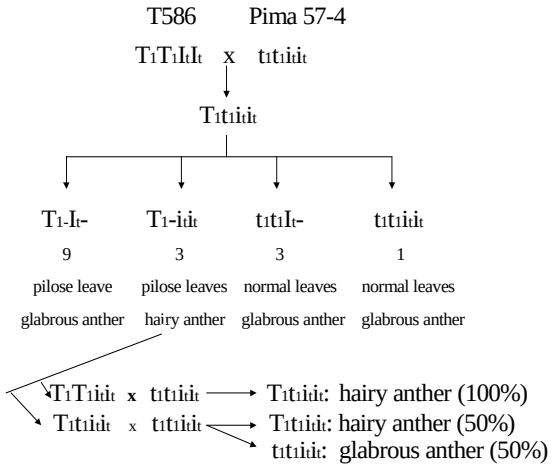

Figure 3. A genetic model explaining the segregation of hairy anther in interspecific F_2 populations.