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Cross-Dependent Inheritance of Stem and Leaf Pubescence in Cultivated Tetraploid Cotton: Morphometric Assessment, F_2 Segregation, and Backcross Response

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Abstract Stem and leaf pubescence is a useful breeding trait in cotton, but its expression depends on the parental combination and the organ examined. Contrasting *Gossypium hirsutum* L. and *G. barbadense* L. genotypes and their F_1, F_2, and BC_1 progenies were evaluated during 2023–2025. A modified 0–5 visual scale was assessed against digital morphometry using ten genotype-organ means from five representative parents. Visual scores were positively associated with trichome density ($r = 0.905$; $R^2 = 0.819$). Across 17 F_2 populations ($n = 189–270$), standard deviations ranged from 0.45 to 1.29 for stems and from 0.41 to 1.28 for leaves. Reciprocal F_1 comparisons differed by cross: L-10 × A-4384 and A-4384 × L-10 showed no significant difference in either organ ($P = 0.086$ and 0.301), whereas Afsona × Marvarid and its reciprocal differed by 0.68 stem-score units and 0.67 leaf-score units (both $P < 0.001$). The L-10/A-4384 F_1 progenies showed incomplete dominance toward the more pubescent parent ($hp = 0.66–0.74$). Stem and leaf scores were strongly correlated in the two focal F_2 populations ($r = 0.95$ and 0.98). Relative to F_2, backcross means shifted toward the recurrent parent, although substantial variation remained. These findings support the morphometric basis of visual genotype ranking and show cross-dependent phenotypic segregation. Neither the reciprocal differences nor the organ-level correlations establish a specific inheritance mechanism.

Keywords *Gossypium hirsutum*, *Gossypium barbadense*, Trichomes, Pubescence, Reciprocal crosses, F_2 segregation, Backcrossing

1. Introduction

Visual assessment of cotton pubescence offers a practical way to characterize variation on stems and leaves [1,2]. Trichomes differ in density, length, branching, and distribution, so a visually similar surface need not have the same underlying architecture [3,4,5]. These differences are relevant to breeding because cotton pubescence has been associated with insect preference and with agronomic or fiber traits [6,7,8,9]. The value of a pubescence phenotype therefore depends on its genetic background and the breeding objective, rather than on hairiness alone.

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Molecular studies also indicate that cotton pubescence cannot be represented by one universal genetic model. Genotyping-by-sequencing has identified quantitative trait loci associated with trichome-based defense [10], and the genetic basis of stem trichome development differs between cultivated tetraploid species [11]. Other studies have linked a hairless-stem phenotype in *G. barbadense* to HD1 [12], identified GoSTR as a regulator of stem trichome formation [13], and demonstrated multilocus control of leaf-hair development in upland cotton [14]. Transcriptomic work further illustrates the regulatory complexity of trichome initiation [15].

These advances do not remove the need for efficient field phenotyping. Visual systems remain useful for screening large segregating populations [1,2], but image-based analyses reveal quantitative variation within visual classes [16]. Here, we assessed the morphometric basis of a modified field scale, compared pubescence variation across F_2 populations,

examined selected reciprocal F_1 combinations, and characterized the backcross response of the strongly contrasting L-10/A-4384 pair.

2. Materials and Methods

2.1. Plant Material and Field Evaluation

The study was conducted during 2023–2025 at the Do'rmon experimental station and the Plant Immunity Laboratory of the Institute of Genetics and Plant Experimental Biology, Academy of Sciences of the Republic of Uzbekistan, in Qibray District, Tashkent Region. Plants were grown in 5-m plots. Field trials comprised two replications in 2023, two in 2024, and three in 2025.

The parental material included four *G. hirsutum* genotypes: L-10 (pilose), Afsona and A-3638 (moderately pubescent), and A-6140 (sparsely pubescent). The two *G. barbadense* parents were Marvarid (densely pubescent) and A-4384 (very sparsely pubescent or nearly smooth). In 2023, 50 plants of each parent were phenotyped. Parents and F_1 combinations were evaluated using 50 plants per genotype in 2024. Parents, F_1, F_2, and BC_1 materials were reassessed within the same field setting in 2025. Approximately 9,000 images were collected over the three years; these were photographic records, not 9,000 independent experimental units.

The cross-population comparison was restricted to 17 F_2 populations for which sample counts and descriptive summaries were internally consistent. Sample sizes ranged from 189 to 270 plants. The focal L-10 × A-4384 and A-4384 × L-10 F_2 populations contained 244 and 224 plants, respectively; their BC_1 populations contained 18–30 plants. Each F_1 group comprised 50 plants. Preliminary F_1 observations were reported previously [17]. The present analysis extends that work through parental morphometry, F_2 segregation, and BC_1 phenotypes.

2.2. Visual Scoring of Stem and Leaf Pubescence

Stem and leaf pubescence were scored separately using a modified scale informed by the approaches of Bourland et al. [1] and Hornbeck and Bourland [2]. The categories were 0, glabrous; 1, very sparsely pubescent or nearly smooth; 2, sparsely pubescent; 3, moderately pubescent; 4, densely pubescent; and 5, pilose. Half-point intermediate classes were used. Repeated observations of the same plant at different developmental stages were averaged, so final plant-level values could include increments smaller than 0.5.

Observations extended from budding and flowering to the end of the growing season. To reduce variation attributable to developmental position, the stem was divided into vertical tiers defined by fruiting-branch position. The abaxial leaf surface and its corresponding stem region were examined at comparable positions.

2.3. Digital Morphometry

Trichomes were documented using a Levenhuk DTX500 digital microscope, mobile macro-imaging equipment, and Apexel optical accessories. Calibrated images were analyzed in Fiji (ImageJ). Regions of interest (ROIs) were delineated, individual trichomes were marked with the Point Tool, and density was expressed as trichomes cm^{-2}. Trichome length, width, and branching were also examined in the parents. The photographic panel was used only to illustrate morphology; all quantitative density measurements came from separate calibrated ROI images.

Agreement between visual scores and trichome density was explored using Pearson correlation and ordinary least-squares regression across ten genotype-organ means: five representative parental genotypes, each contributing a stem and a leaf mean. The fit was descriptive; the paired organ means do not constitute ten independent genotypes, and the analysis was not an individual-plant validation or a calibration model. Differences in density among vertical tiers were evaluated by one-way analysis of variance followed by Tukey's honestly significant difference test.

2.4. Genetic and Statistical Analyses

Population summaries are presented as mean ± standard deviation (SD). The coefficient of variation was calculated as CV = 100 × SD/mean and used only as a supplementary descriptor. Because scores were bounded at 0 and 5 and CV depends mathematically on the mean, biological comparisons were based primarily on means, SDs, and the reported frequency classes.

The F_1 dominance coefficient was calculated as $hp = (F_1 - MP)/(HP - MP)$, where MP is the midparent mean and HP is the mean of the more pubescent parent. Values between 0 and 1 indicate incomplete dominance toward that parent; negative values indicate displacement toward the less pubescent parent. The coefficient describes the position of the F_1 mean on the scoring scale and is not a locus-specific genetic estimate.

Pearson correlations summarized the relationship between stem and leaf scores, treating plant-level averages of repeated ratings as quasi-continuous variables. Selected reciprocal F_1 means were compared using Welch's independent-samples t-test, with 50 plants per direction and 95% confidence intervals (CIs) for the mean difference. $P < 0.05$ was the significance threshold. Values above this threshold are reported as non-significant, including $P = 0.086$. These comparisons assess phenotypic differences between cross directions; they do not distinguish maternal, cytoplasmic, or parent-of-origin mechanisms. Backcross shifts were interpreted descriptively.

3. Results

3.1. Parental Contrast and the Morphometric Basis of Visual Scores

The six parents spanned a broad range of pubescence (Table 1). L-10 was close to the maximum visual score in both organs, whereas A-4384 had the lowest scores. Afsona

and A-3638 occupied the intermediate class, A-6140 was sparsely pubescent, and Marvarid was densely pubescent. The representative micrographs illustrate these contrasts and the diversity of trichome architecture (Figure 1).

Table 1. Visual stem and leaf pubescence in the parental genotypes (2023; n = 50 plants per genotype)

Genotype	Species	Class	Stem mean ± SD	CV (%)	Leaf mean ± SD	CV (%)
L-10	*G. hirsutum*	Pilose	4.98 ± 0.06	1.28	4.99 ± 0.06	1.14
Afsona	*G. hirsutum*	Moderate	2.97 ± 0.12	3.93	2.96 ± 0.13	4.37
A-3638	*G. hirsutum*	Moderate	2.92 ± 0.19	6.58	2.94 ± 0.19	6.43
A-6140	*G. hirsutum*	Sparse	2.06 ± 0.22	10.84	2.09 ± 0.26	12.49
Marvarid	*G. barbadense*	Dense	3.78 ± 0.37	9.80	3.88 ± 0.31	7.98
A-4384	*G. barbadense*	Very sparse	1.06 ± 0.14	13.28	1.07 ± 0.15	14.09

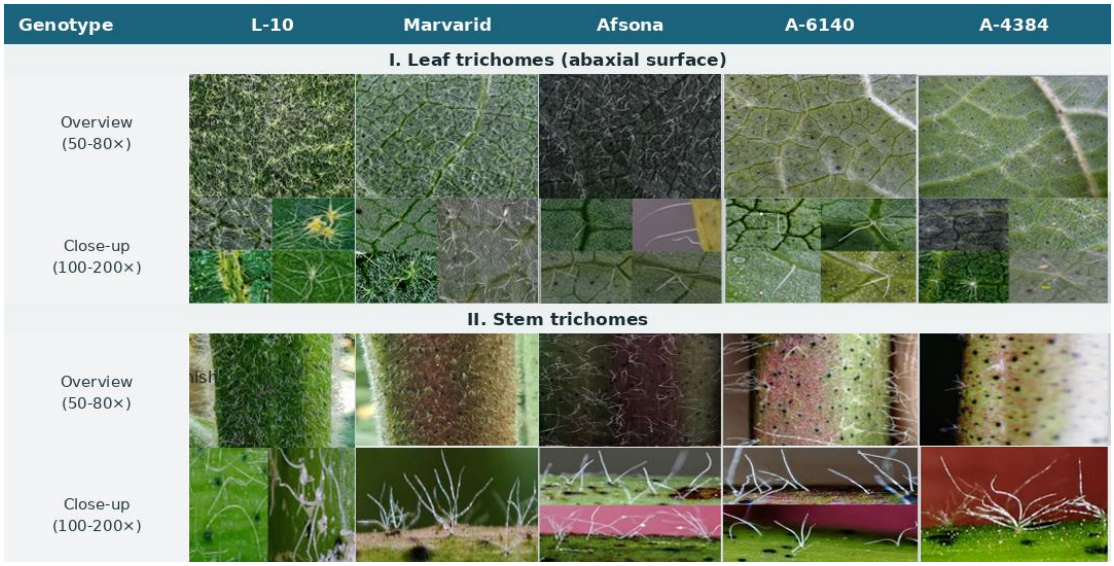


Figure 1. Representative micrographs of the abaxial leaf surface and stem trichomes in five parental genotypes. Overview and close-up labels give the acquisition magnification ranges reported for the images, not calibrated dimensions in this resized panel. The images illustrate qualitative morphology only. Quantitative morphometry used separate calibrated ROI images in Fiji (ImageJ)

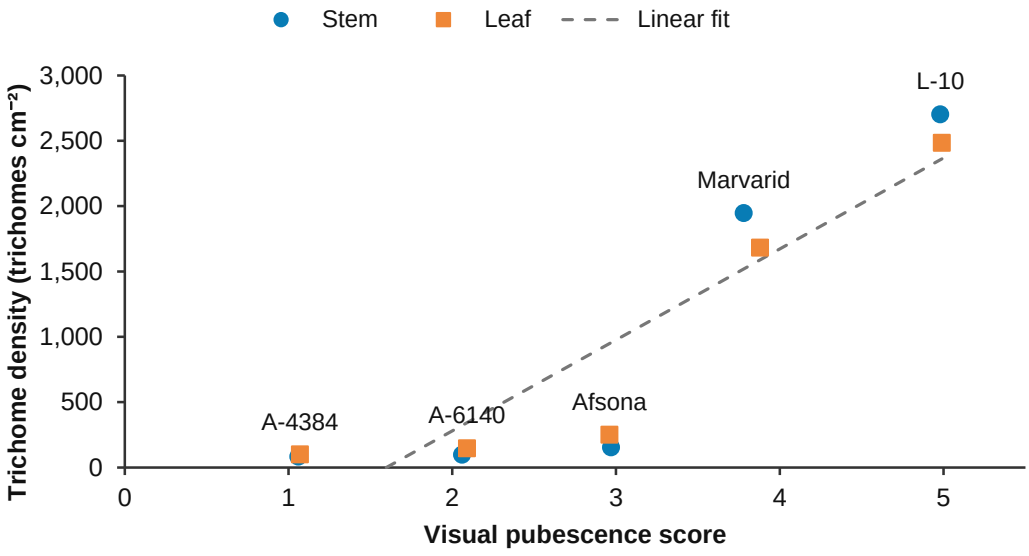


Figure 2. Visual pubescence scores and trichome density in five parental genotypes. Circles denote stems and squares denote leaves; each point is a genotype-organ mean. The dashed line is the descriptive least-squares fit to all ten means ($r = 0.905$; $R^2 = 0.819$), displayed within the non-negative density range. Paired organ means share a genotype and are not ten independent biological replicates. This comparison is not an individual-plant validation of the scale

Table 2. Stem and leaf pubescence in the 17 F_2 populations included in the cross-population comparison

Cross	n	Stem mean ± SD	CV (%)	Leaf mean ± SD	CV (%)
L-10 × Afsona	234	4.15 ± 0.74	17.95	4.21 ± 0.76	17.97
Afsona × L-10	217	4.15 ± 0.72	17.26	4.25 ± 0.70	16.50
L-10 × A-6140	226	3.81 ± 1.29	33.84	3.97 ± 1.28	32.29
A-6140 × L-10	208	3.91 ± 1.21	30.95	3.99 ± 1.18	29.70
Afsona × A-3638	204	2.67 ± 0.45	16.74	2.73 ± 0.41	14.99
Marvarid × A-4384	190	2.58 ± 0.95	36.66	2.70 ± 0.87	32.31
A-4384 × Marvarid	189	2.35 ± 1.08	46.05	2.41 ± 1.08	44.85
Afsona × Marvarid	201	3.25 ± 0.63	19.37	3.29 ± 0.61	18.46
Marvarid × Afsona	203	3.12 ± 0.52	16.65	3.17 ± 0.51	16.20
L-10 × Marvarid	270	3.57 ± 1.00	27.98	3.64 ± 0.99	27.23
Marvarid × L-10	215	3.80 ± 0.75	19.69	3.81 ± 0.73	19.25
L-10 × A-4384	244	3.27 ± 1.11	33.83	3.41 ± 1.09	31.85
A-4384 × L-10	224	3.26 ± 1.16	35.48	3.29 ± 1.17	35.59
A-6140 × A-4384	224	2.07 ± 0.94	45.36	2.09 ± 0.91	43.69
A-4384 × A-6140	215	1.87 ± 0.78	41.44	1.87 ± 0.78	41.44
Afsona × A-4384	202	1.97 ± 0.80	40.55	1.97 ± 0.80	40.50
A-4384 × Afsona	254	2.23 ± 0.79	35.62	2.26 ± 0.78	34.57

M and SD are displayed to two decimals; CV values are retained from the source summaries.
SD describes phenotypic dispersion, not uncertainty in the estimated mean.

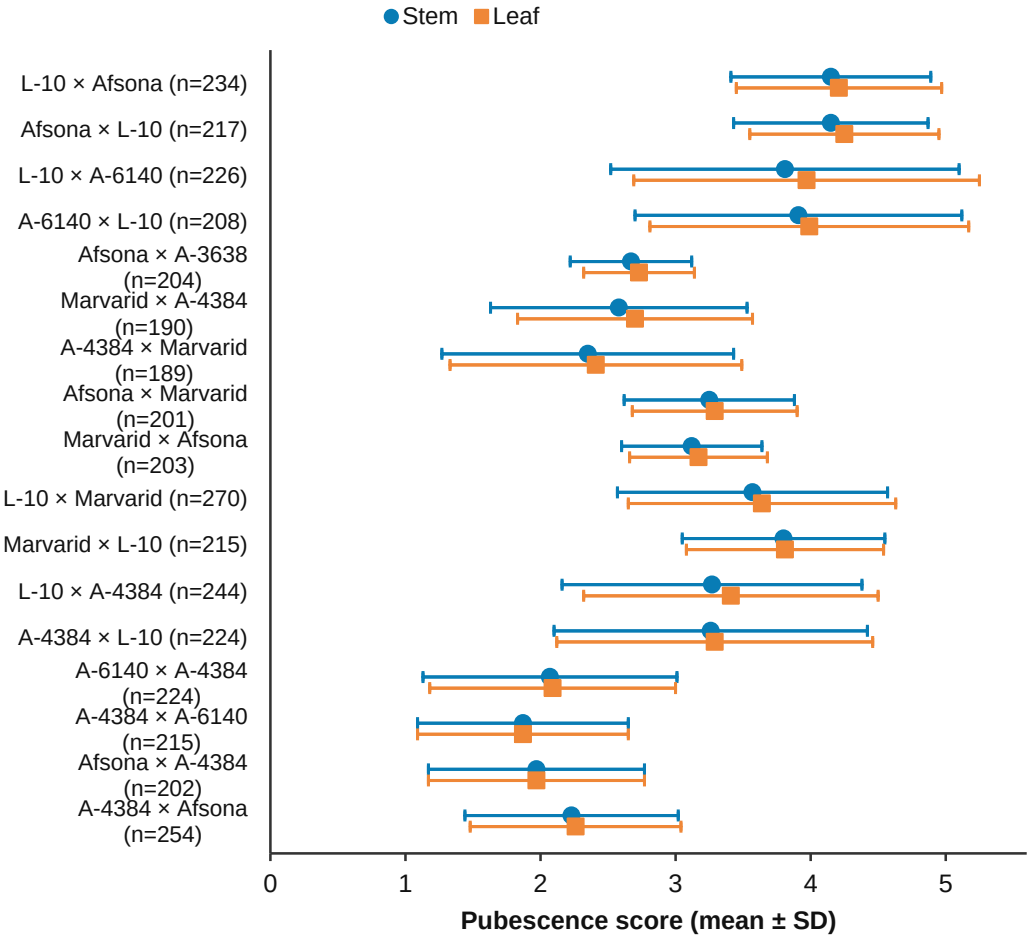


Figure 3. Mean stem and leaf pubescence in 17 F_2 populations. Every error bar represents the corresponding population's SD, and labels give the number of plants. The scoring scale is 0–5; a mean ± SD interval is not the observed range and may extend beyond the scale limits

Trichome density increased toward the upper tiers in all examined parents (reported $P < 0.001$). In L-10, leaf density increased from 1,350 ± 80 trichomes cm^{-2} at tier 1 to 4,050 ± 157 at tier 5; stem density increased from 1,465 ± 103 to 4,247 ± 157. The corresponding A-4384 values were 25.00 ± 6.24 to 220.00 ± 15.10 for leaves and 22.00 ± 2.65 to 178.00 ± 17.69 for stems.

Averaging the tier-level values gave L-10 densities of 2,484.20 trichomes cm^{-2} for leaves and 2,702.60 for stems. Across the ten genotype-organ means, visual scores were positively associated with density ($r = 0.905$; $R^2 = 0.819$; Figure 2). This agreement supports the biological basis of genotype ranking, but the aggregate analysis does not establish the accuracy of scores for individual plants.

3.2. Cross-Dependent Variation in F_2 Populations

The 17 F_2 populations differed in both mean score and spread (Table 2; Figure 3). Stem SD ranged from 0.45 to 1.29 and leaf SD from 0.41 to 1.28. Afsona × A-3638 had the narrowest distributions. Wider variation occurred in contrasting combinations, including L-10 × A-6140, A-6140 × L-10, and the reciprocal L-10/A-4384 pair. CV ranged from 14.99% to 46.05% across organs and populations, but was not treated as an independent measure of genetic variation.

3.3. F_1 Dominance and Reciprocal Cross Direction

In the L-10/A-4384 pair, both reciprocal F_1 progenies were closer to the more pubescent L-10 parent than to the midparent value. Dominance coefficients were 0.70 for stems and 0.74 for leaves in L-10 × A-4384, and 0.66 and 0.72, respectively, in A-4384 × L-10. Thus, both directions showed incomplete dominance toward the more pubescent parent.

The reciprocal comparison depended on the parental combination (Table 3). For L-10/A-4384, differences of 0.08 stem-score units and 0.04 leaf-score units were non-significant, and both CIs included zero. In contrast, Afsona × Marvarid exceeded Marvarid × Afsona by 0.68 stem-score units and 0.67 leaf-score units (both $P < 0.001$). These results show a cross-direction association in the latter pair, not a general directional effect across all crosses.

3.4. F_2 Segregation and Backcross Response in the L-10/A-4384 Pair

L-10 and A-4384 differed by 3.92 visual-score units in both organs. Stem pubescence in the two reciprocal F_2 populations averaged 3.27 ± 1.11 and 3.26 ± 1.16 (Table 4). The reported modal stem class was 4.00 in both populations, containing 108 plants in L-10 × A-4384 and 102 in its reciprocal. Lower and intermediate classes between 1.00 and 3.50 were also present, showing why the mean alone cannot describe the segregating population.

Relative to the F_2 means, backcross means moved toward the recurrent parent in both reciprocal backgrounds (Figure 4). In the L-10 × A-4384 background, stem scores averaged 3.50 ± 0.92 after backcrossing to L-10 and 2.35 ± 1.26 after backcrossing to A-4384. The corresponding reciprocal-background means were 3.37 ± 1.38 and 2.31 ± 1.15. Variation remained substantial in several BC_1 populations; these results indicate directional shifts rather than full restoration of the recurrent-parent phenotype.

3.5. Joint Expression of Stem and Leaf Pubescence

Stem and leaf scores were strongly correlated in L-10 × A-4384 F_2 ($r = 0.95$; $n = 244$) and A-4384 × L-10 F_2 ($r = 0.98$; $n = 224$). BC_1 correlations ranged from 0.82 to 1.00. The organs therefore varied together, but these associations do not establish pleiotropic control by a single gene or an identical developmental mechanism.

Table 3. Welch comparisons of selected reciprocal F_1 means (n = 50 plants in each direction)

Reciprocal comparison	Organ	Difference	95% CI	t (df)	P
L-10 × A-4384 vs. reciprocal	Stem	0.08	–0.012 to 0.172	1.73 (95.2)	0.086
L-10 × A-4384 vs. reciprocal	Leaf	0.04	–0.036 to 0.116	1.04 (89.5)	0.301
Afsona × Marvarid vs. reciprocal	Stem	0.68	0.595 to 0.765	15.90 (86.3)	<0.001
Afsona × Marvarid vs. reciprocal	Leaf	0.67	0.585 to 0.755	15.67 (86.3)	<0.001

Difference = first-listed direction minus its reciprocal. CI, confidence interval; df, degrees of freedom.
The test does not identify the biological mechanism behind a reciprocal difference.

Table 4. Pubescence summaries for the reciprocal L-10/A-4384 F_1, F_2, and BC_1 materials

Material	n	Stem mean ± SD	Leaf mean ± SD	CV (%) stem / leaf	hp stem / leaf	Stem-leaf r
F_1 L-10 × A-4384	50	4.39 ± 0.21	4.48 ± 0.22	4.83 / 4.87	0.70 / 0.74	—
F_2 L-10 × A-4384	244	3.27 ± 1.11	3.41 ± 1.09	33.83 / 31.85	—	0.95
BC_1 (L-10 × A-4384) × L-10	28	3.50 ± 0.92	4.21 ± 1.23	26.37 / 29.14	—	0.82
BC_1 (L-10 × A-4384) × A-4384	20	2.35 ± 1.26	2.35 ± 1.26	53.52 / 53.52	—	1.00
F_1 A-4384 × L-10	50	4.31 ± 0.25	4.44 ± 0.16	5.70 / 3.60	0.66 / 0.72	—
F_2 A-4384 × L-10	224	3.26 ± 1.16	3.29 ± 1.17	35.48 / 35.59	—	0.98
BC_1 (A-4384 × L-10) × A-4384	18	2.31 ± 1.15	2.36 ± 1.26	49.98 / 53.28	—	0.98
BC_1 (A-4384 × L-10) × L-10	30	3.37 ± 1.38	3.43 ± 1.45	41.08 / 42.20	—	0.99

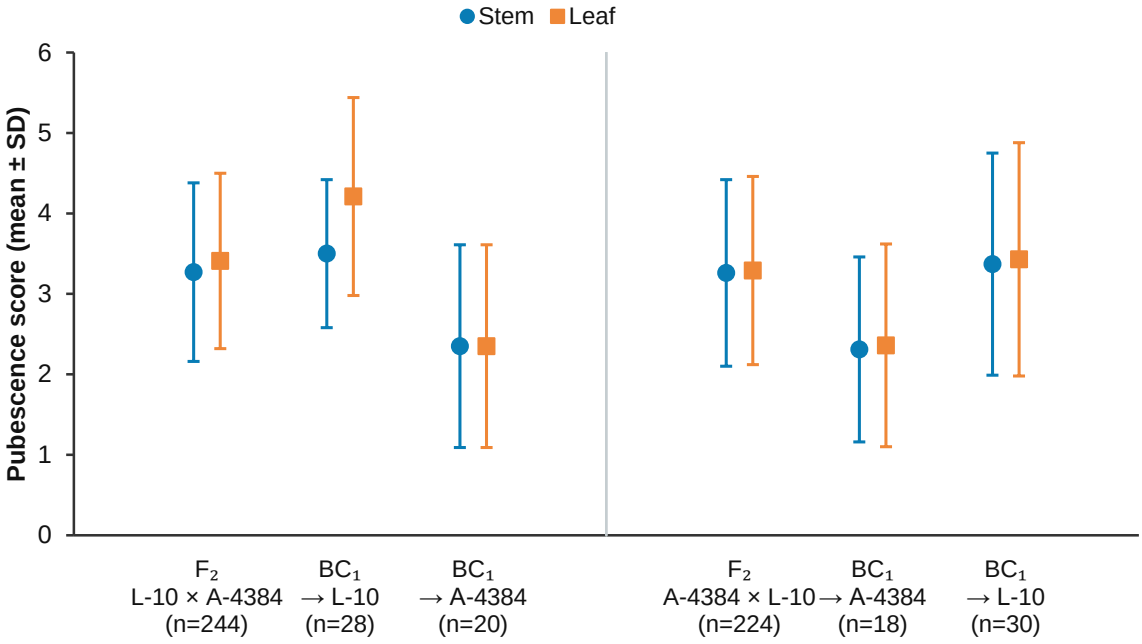


Figure 4. Stem and leaf pubescence in the two reciprocal F_2 backgrounds and their BC_1 progenies. Points show means and error bars show SD. Arrows indicate the recurrent parent; n is the number of plants. The vertical separator distinguishes the reciprocal backgrounds. Mean ± SD intervals are not confidence intervals or observed ranges

4. Discussion

This study brings together field scoring, image-based morphometry, and comparisons among hybrid generations. The positive score-density relationship is consistent with the morphological basis of visual cotton pubescence ratings described by Bourland et al. [1] and Hornbeck and Bourland [2]. Its scope is nevertheless specific: ten genotype-organ means support the ranking of contrasting parents, not the precision or repeatability of individual plant scores. The two organ means from each genotype also share a genetic background and should not be treated as ten independent genotypes.

A visual class summarizes more than the number of trichomes. Differences in architecture are evident in the photographic panel, while previous image-based work has documented variation within visual hairiness classes [16]. The present images illustrate this distinction, but do not quantify how much length, width, or branching contributes to a visual score. Such contributions would require a separate analysis linking these measurements to plant-level ratings.

The F_2 results show that population mean and dispersion depend on the cross. The narrow spread of Afsona × A-3638 contrasts with the broader distributions of several combinations involving strongly divergent parents. This pattern is compatible with the cross- and species-dependent genetic architecture described in mapping studies [10,11,14]. It does not, by itself, reveal the number of segregating loci, distinguish genetic from environmental variance, or establish a particular Mendelian ratio. No heritability estimate is inferred here. CV is especially sensitive to lower means on a bounded scale, so comparisons should retain the accompanying mean and SD.

Reciprocal F_1 comparisons provide a second distinction between combinations. The L-10/A-4384 means were similar in both directions and showed the same dominance orientation. For Afsona/Marvarid, the larger reciprocal differences were significant under the reported Welch comparisons. A directional association is therefore present in that pair, but the statistical result alone cannot identify maternal provisioning, cytoplasmic inheritance, or parent-of-origin regulation. Additional reciprocal designs, replication across environments, and appropriate genotyping would be needed to separate those explanations.

Backcrossing produced a consistent directional pattern relative to F_2: means were higher with L-10 as recurrent parent and lower with A-4384. This is relevant to the development of breeding populations with different target pubescence levels. However, the BC_1 groups were smaller than the F_2 groups and often remained variable. The shifts were described rather than tested in a model accounting for field structure, and should not be interpreted as a demonstrated selection gain or complete genetic recovery. Strong stem-leaf correlations similarly suggest coordinated phenotypic expression, not necessarily one shared causal locus.

The earlier study described stem pubescence and other morphological characters in parental forms and F_1 hybrids [17]. The present analysis extends that work to F_2 variation,

backcross phenotypes, and a quantitative assessment of parental score-density correspondence. Its contribution therefore lies in the segregating and backcross generations and their connection to field phenotyping, rather than in presenting the earlier F_1 observations as an independent discovery.

4.1. Limitations

The principal limitations concern sampling and inference. The morphometric comparison uses aggregate means from only five genotypes, and the photographic panel is qualitative. Field observations span three years at one location; the population summaries do not separate year and block effects, and the Welch tests do not account for potential field-level clustering. The 17-population comparison is conditional on the stated data-consistency criterion. Smaller BC_1 samples also limit the precision of backcross comparisons. These constraints favor a descriptive, cross-specific interpretation rather than a universal model of cotton pubescence inheritance.

5. Conclusions

The modified 0–5 visual scale ranked contrasting cotton parents in agreement with aggregate trichome density ($r = 0.905$; $R^2 = 0.819$). Across 17 F_2 populations, stem and leaf pubescence showed cross-dependent variation. Reciprocal F_1 differences were small and non-significant in L-10/A-4384 but pronounced in Afsona/Marvarid. In the focal interspecific pair, both F_1 directions showed incomplete dominance toward the more pubescent parent, while backcross means shifted toward the recurrent parent and retained residual variation. These findings support the use of coordinated field and morphometric phenotyping to characterize breeding populations, without assigning the observed patterns to an untested molecular or cytoplasmic mechanism.

Data Availability

The underlying raw data are retained by the authors and may be made available upon reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest related to the content of this article.

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