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COMPARATIVE ANATOMICAL TRAITS OF LEAF AND SEED COAT STRUCTURE IN WILD AND CULTIVATED COTTON (*GOSSYPIMUM* SPP.)

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SUMMARY

This study aimed to investigate the correlation between economically valuable and anatomically defined traits of leaves and seed coats in cotton (*Gossypium* spp.). Comparative anatomical analyses took place on ovules at four developmental stages (1–4 weeks after flowering), mature seed coats (60 days after flowering), and leaf blades of the promising cultivar Genofond-2, its wild parental species *G. thurberi* and *G. raimondii*, and the reference cultivar Tashkent-1. Additionally, an examination of aphid-resistant accessions of *G. arboreum* subsp. *obtusifolium* and *G. arboreum* subspecies *nanking* ensued. Quantitative parameters of integument and mesophyll tissues ($n = 10$ replicates per genotype per stage) entailed assessment using one-way ANOVA, followed by Tukey's HSD post-hoc test ($P < 0.05$). The cultivar Genofond-2 retained significantly thicker and harder seed coats (total mature thickness = $305.0 \pm 31.2 \mu\text{m}$) inherited from wild donors, resulting in reduced fiber contamination during ginning ($P < 0.01$). Leaf cuticle thickness exceeded the diagnostic threshold of $0.85 \mu\text{m}$ in all studied species, with resistant *G. arboreum* accessions displaying an additional columnar parenchyma layer. Pearson correlation analysis confirmed a significant positive relationship between seed coat hardness and lignification ($r = 0.89$, $P < 0.001$) and between leaf cuticle thickness and aphid resistance ($r = 0.82$, $P < 0.01$). These ontogenetic anatomical traits represent reliable morphological markers for selecting genotypes with superior fiber quality and enhanced resistance to piercing-sucking insects, facilitating the efficient use of wild genetic resources in cotton improvement. Statistical processing of quantitative data, as carried out, used the methods of S.G. Big-Anova (2024) and A.V. Vaganova (2023).

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Keywords: *Gossypium*, cotton anatomy, seed coat structure, leaf mesophyll, ovule development, aphid resistance, fiber quality

Key findings: The cultivar Genofond-2 retains anatomical traits from wild donor species with reduced fiber contamination during ginning. Leaf cuticle thickness serves as a reliable diagnostic marker for aphid resistance in cotton breeding programs.

INTRODUCTION

The genus *Gossypium* L. contains around 50 species, including 45 diploids and 5–7 allotetraploids, observed with massive morphological diversity, and holds significant and underutilized genetic resources for biotic and abiotic stress-resistant traits (Wendel and Grover 2015). Over 95% of global fiber production uses only two allotetraploid species, viz., *G. hirsutum* and *G. barbadense*. However, their wild relatives with extensive genetic variations have not reached optimal exploration for breeding resilient cultivars (Wendel *et al.*, 2009; Magwanga *et al.*, 2018). Abiotic stresses, i.e., drought, soil salinity, and extreme temperatures, are also increasingly prevalent under climate change scenarios, posing new challenges for crop resilience and productivity (Bozorova *et al.*, 2026).

Genetic diversity of wild cotton species, including *G. thurberi* and *G. raimondii*, represents valuable resources for cotton improvement. Recent studies highlighted the successful development of introgression lines between the species *G. hirsutum* and *G. raimondii*, revealing significant QTLs for yield and fiber quality traits (Shim *et al.*, 2018). These introgression populations enunciated the potential for mining desirable genes from wild species to improve yield and fiber quality traits in cultivated cotton through design-breeding approaches. Wild cotton species possess genetic diversity and adaptive traits, including resistance to drought, salinity, diseases, and insect pests, which are largely unavailable in cotton-cultivated germplasm (Shim *et al.*, 2018).

Anatomical and morphological traits represent critical, yet often determinants of agronomic performance in cotton. The seed coat structure, particularly its thickness, hardness, and lignification patterns,

considerably influences fiber quality traits by managing contamination levels during ginning and protecting the embryo during growth and development (Remeshan *et al.*, 2025). Similarly, leaf anatomical traits, such as cuticle thickness, epidermal cell morphology, mesophyll organization, and trichome density, play pivotal roles in mediating plant-insect interactions and conferring resistance to piercing-sucking pests such as aphids (*Aphis gossypii* Glov.) and spider mites.

Recent investigations have further explored the relationship between leaf anatomical structure and aphid resistance. Studies on cotton trichome mutants detailed that compact mesophyll organization and increased palisade tissue thickness appeared in association with enhanced aphid resistance (Yang, 2024). Furthermore, the primary role of lignin deposition and callose accumulation in providing physical barriers against piercing-sucking insects has gained confirmation as a crucial self-defense mechanism in cotton (Mbiza, 2022; Yang, 2024).

The cuticular layer serves as the primary barrier against herbivory and desiccation, with increased thickness associating with reduced susceptibility to phloem-feeding insects (Ren *et al.* 2023). The comparative anatomical studies revealed that wild *Gossypium* species with xeromorphic leaf structures, such as isolateral mesophyll and densely packed palisade cells, displayed an enhanced resistance to arthropod pests compared with the mesomorphic cultivated forms (Wise *et al.*, 2000). These structural adaptations obstruct the stylet penetration and develop unfavorable microenvironments for pest colonization, offering sustainable alternatives to chemical control strategies.

Despite the recognized importance of anatomical traits in plant defense and fiber traits development, systematic investigations

concerning specific structural features to agronomic performance remain limited, particularly in the context of introgression lines derived from wild cotton species. Understanding the ontogenetic dynamics of seed coat formation and leaf anatomical differentiation is essential for developing diagnostic markers for breeding selection and for elucidating the genetic architecture underlying these complex traits (Smith *et al.*, 2007).

Cotton cultivars are less likely to retain their traits with the influence of external and internal factors in developing new cultivars. Therefore, breeding methods play a dominant role in developing competitive cotton genotypes for the world market (Muminov *et al.*, 2025). In the present era, developing new cotton cultivars uses various techniques of hybridization. Numerous scientific studies initiate identifying and transferring traits of precocity and resistance to different pests and diseases to cultivated cultivars, which are a considerable improvement achieved in cotton crops (Muminov *et al.*, 2025). These structural adaptations obstruct the stylet penetration and develop unfavorable microenvironments for pest colonization, offering sustainable alternatives to chemical control strategies (Kodirova *et al.*, 2024).

MATERIALS AND METHODS

Genetic materials and sample preparation

The study utilized the promising cultivar Genofond-2 and its original parental wild species *G. thurberi* and *G. raimondii*, as well as the reference cultivar Tashkent-1. For leaf blade comparison, the research included aphid-resistant species *G. arboreum* L. subsp. *obtusifolium* and *G. arboreum* L. subsp. *nanking*. Twenty plants per genotype incurred growth under field conditions at the experimental station of the National Pedagogical University (Tashkent, Uzbekistan) during the 2023 growing season. Plants sown were in randomized complete blocks with three replications, at a spacing of 70 cm × 30 cm, under standard agronomic practices (irrigation,

fertilization, and pest management, excluding aphid control for resistant accessions). Daytime temperatures ranged from 28 °C to 35 °C, with a natural photoperiod of approximately 14–15 h of light during the vegetative stage.

All studied representatives underwent growth on the experimental site of the Institute of Genetics and Experimental Plant Biology under photoperiodic trailers. The standard methods used sought to prepare the specimens (Babenko *et al.*, 2013). Mature seeds (60 days after flowering, DAF) and fully expanded leaves from the 5th to the 7th sympodial branches of mature plants entailed sampling for seed coat, epidermal, and leaf cross-section analyses. Leaf segments of 1 cm² bore excision from the central lamina (midway between the margin and the main vein) of mid-formation leaves. Ovules collected were at weekly intervals (1, 2, 3, and 4 weeks after flowering) to track ontogenetic changes in the integument structure.

Aphid resistance assessment

Aphid resistance evaluation was under controlled glasshouse conditions (25 °C ± 2 °C, 60% ± 5% relative humidity, 16 h photoperiod). Ten plants per genotype received infestation with the *Aphis gossypii* Glov. at the 4–5 true-leaf stage using a clip-cage technique (20 aphids per leaf, three leaves per plant). After seven days, scoring of resistance used a 1–5 scale based on chlorosis, leaf curling, and aphid population density: 1 = no damage (highly resistant), 5 = severe damage (susceptible). Additionally, the intrinsic rate of aphid increase (rm) and the percentage of chlorophyll loss (measured with a SPAD-502 chlorophyll meter) reached recording as quantitative resistance parameters.

Anatomical measurements

Anatomical parameters' measurement proceeded from 10 independent replicates (n = 10) per genotype for each developmental stage and tissue type. Data reported were as mean ± standard deviation (SD). The coefficient of variation (CV) across all measured traits

ranged from 8% to 12%, indicating satisfactory experimental precision. Before analysis, data examination for normality used the Shapiro-Wilk test and for homogeneity of variances, using Levene's test. One-way analysis of variance (ANOVA) continued to evaluate differences among genotypes, with genotype as the fixed factor and individual plant as the random effect. Post-hoc pairwise comparisons, as performed, used Tukey's honestly significant difference (HSD) test at a significance level of $\alpha = 0.05$. Exact F-statistics, degrees of freedom (df), and P-values had reports for all significant comparisons. Pearson correlation analysis sought to examine relationships between seed coat structural traits and fiber quality indicators, as well as between leaf cuticle thickness and aphid resistance metrics. All statistical computations performed engaged SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Cotton ovules are anatropous and bifid, with two integuments—outer and inner—each comprising three tissue layers, i.e., the outer epidermis, several layers of parenchymatous tissue, and the inner epidermis. Both layers' development begins at ovule fertilization and continues until the full formation of the seed coat at the seed maturation stage. The inner integuments always occurred thicker than the outer ones and occupied a larger area of the seed coat. This is due to the multilayered nature of the parenchyma at the initial stage of development and the height (area) of the palisade layer cells at maturation. All layers of the ovule's inner epidermis undergo age-related variations that are more significant. In the presented study, the observed ontogenetic patterns correspond with the recent transcriptomic analysis of cottonseed development. Such observation is a result of the transition from fiber elongation to secondary cell wall synthesis, which occurs around 20 days post-anthesis (DPA),

representing a critical period for fiber quality determination (Jareczek *et al.*, 2023).

In the outer integument, the parenchyma layer rows remain constant during development. By contrast, in the inner integument, parenchyma layer arrangements varied, with rows decreasing 3-to-4-fold by seed maturation due to the destruction and obliteration of cells in the lower rows adjacent to the inner epidermis. The growth rate and subsequent destruction of parenchyma layer cells ensure certainty by their thickness at different stages of growth and development. As the ovule integuments transform into the mature seed coat, the quantitative indices of structural traits change. The studied cultivars differed from each other in the analyzed traits. During the first two weeks, the epidermal cells' height in the outer integument was slightly lower in cultivars Tashkent-1 and Genofond-2, and the thickness of the parenchyma layer was lower in the wild species *G. raimondii* and *G. thurberi*. The height of inner epidermal cells was the lowest in the cultivar Genofond-2 ($7.7 \pm 1.0 \mu\text{m}$).

In the external integument, the parenchyma and epidermal cells reach their maximum size at the age of three weeks in the species *G. raimondii* (41.1 ± 3.9) and at four weeks in the cultivar Tashkent-1 and species *G. thurberi* (36.5 ± 3.2 and 65.7 ± 6.2 , respectively). Moreover, it was maximum at maturity in the cultivar Genofond-2 (46.7 ± 4.8). In one-week-old ovules, the external integument emerged thicker in the cultivar Tashkent-1 (82.3 ± 7.3), while in mature seeds, it was thicker in the species *G. thurberi* (103.5 ± 11.4). Meanwhile, the thinnest at all stages of development was evident in the species *G. raimondii*. Age-related variations occur more intensively in all layers of the internal integument (II). Therefore, one should note that the internal integument is always thicker than the external one. In a one-week-old ovule, the internal integument differed from the external one by a slight thickness of the cells of the outer epidermis and a greater height of the parenchymatous layer. During growth and development, the increased differences resulted in the quantitative indices of the structural features of the integuments.

The seed coat hardness observed in the cultivar Genofond-2 and its wild progenitors aligns with recent findings on the chemical composition of cotton seed coats. The lignin content (22%–26%) and cellulose (43%–48%) considerably contributed to seed hardness and ginning performance. The cells of the outer epidermis of the inner integument—a single-row palisade layer—expand radially during development by 4.3 (*G. thurberi*), 6.7 (*G. raimondii*), 11.9 (Genofond-2), and 12.3 (Tashkent-1) times, reaching their greater height by the seed maturation stage. The parenchymatous layer of the inner integument was thicker throughout the development than other integumental layers, experiencing the most intensive cell growth in the first weeks of growth and development. The number of rows in parenchymatous layer and, consequently its thickness, reached its maximum values and increased by 1.1–2.4 times at the two weeks of age.

The cultivar Genofond-2 has a lower parenchyma layer thickness at one week of age (255.0 ± 24.0), while the cultivar Tashkent-1 ($29.9 \pm 9 \pm 2.1$) appeared different from the others in that, its layer was thicker in two-week-old ovules. The thickness of the lower parenchyma layer decreased due to the onset of destruction (breakdown and crushing) of the underlying rows of parenchyma cells adjacent to the inner epidermis of the ovule. Of the 17–20 rows found at the beginning of ovule development, only 5–7 remain in the mature seed coat. The cells' height of the inner epidermis of the internal integument varied little at different times due to the slowest growth rate compared with the cells of other layers.

As a rule, the thickness of parenchyma layer does not increase at the two weeks of age and may even decrease. This layer was absent in the coat of mature seeds. The results showed large age-related variations in structural features occur in the internal integument—the parenchyma and palisade layers—compared with the external one. By comparing the linear parameters of the mature seed coat structure, several significant similarities were notable between the cultivar Genofond-2 and its parental wild species. The

cultivar Genofond-2 appeared similar to the cultivar Tashkent-1 in various traits. These are in the height of the parenchyma of the outer and inner integument, the inner epidermis of the outer integument, the outer epidermis of the inner integument, and the total thickness of the mature seed coat (Table 1, Figures 1-A, C, D, and E). Similarities were also evident with the species *G. raimondii* in the parenchyma of the internal integument and with the species *G. thurberi* in total seed coat thickness. Overall, the wild cotton species typically exhibited tougher seed coats, which were beneficial for breeding programs aimed at improving fiber quality and reducing contamination during mechanical processing.

It is a belief that integration of most anatomical leaf structure traits will result in insect pest resistance, especially to aphids. For genotypes to be resistant to aphids (*Aphis gossypii* Glov.), cotton leaves must have a cuticle of at least $0.85 \mu\text{m}$. The studied cotton species had cuticles greater than $0.85 \mu\text{m}$ (Table 2). The greater the height and thickness of the epidermal cell walls on the abaxial side of the leaf, the less susceptible the plant was to aphid infestation. However, no species should have a cuticle of at least $20.0 \mu\text{m}$. The spongy parenchyma height should be no more than $98.5 \mu\text{m}$, since the greater the height of the spongy parenchyma, the looser it becomes (*G. thurberi* = $84.3 \mu\text{m}$, *G. arboreum* subsp. *obtusifolium* = 82.9 ± 2.8 , and *G. arboreum* subsp. *nanking* = 86.7 ± 12.1). The 3.6^2 was the index revealing the linear dimension of the columnar fabric located closer to the lower side of the sheet plastic. (Le Duyen Anh. Factors of resistance in wild and cultivated representatives of the genus *Gossypium* L. to the cotton aphid [*Aphis gossypii* Glov.]: Abstract of Candidate of Biological Sciences dissertation. Tashkent, 1995. 23 p.).

In this latest study, the identified anatomical indicators of the aphid resistance, including cuticle thickness and palisade cell arrangement, were consistent with recent physiological and molecular investigations. Past studies enunciated that increased lignin deposition in the epidermis obstructs aphid access to vascular tissues, thereby providing resistance to aphid infestation (Shi *et al.*,

Table 1. Dynamics of thickness changes in individual layers of seed coat integuments during development (μm , $n = 10$).

Parameters	Species	<i>G. thurberi</i>	<i>G. raimondii</i>	Tashkent-1	Genofond-2
Outer epidermis of the outer integument	1 week	27.5±2.6	19.7±1.7	21.1±1.7	21.7±1.6
	2 weeks	65.0±6.5	39.4±3.5	23.3±1.9	26.8±1.1
	3 weeks	65.4±6.4	41.4±3.9	33.6±2.5	40.1±1.9
	4 weeks	65.7±6.2	40.5±3.8	36.5±3.2	42.4±3.2
	Mature	63.5±6.1	34.3±3.1	35.0±3.1	46.7±4.8
Parenchyma of the outer integument	1 week	32.1±3.6	24.8±2.3	47.4±3.7	38.8±6.2
	2 weeks	35.8±3.2	47.4±4.8	62.8±4.5	69.3±3.9
	3 weeks	34.3±3.3	47.4±4.9	56.2±2.6	86.6±5.6
	4 weeks	33.6±3.0	32.4±3.6	64.2±5.1	36.6±1.8
	Mature	22.5±2.4	20.4±2.3	21.9±1.3	28.5±2.6
Inner epidermis of the outer integument	1 week	12.4±1.3	13.0±1.0	13.8±0.9	7.7±1.0
	2 weeks	13.5±1.5	13.9±1.2	16.9±1.0	18.9±1.2
	3 weeks	14.6±1.6	13.1±1.2	16.8±1.5	16.1±0.9
	4 weeks	17.5±1.8	11.7±1.1	16.8±1.3	14.5±1.1
	Mature	17.5±1.3	10.9±1.2	14.6±1.5	13.9±1.3
Outer epidermis of the inner integument	1 week	28.5±2.9	17.6±1.5	15.3±1.4	15.2±1.5
	2 weeks	110.2±14.3	90.5±9.5	127.0±7.8	82.1±2.6
	3 weeks	121.9±12.1	100.0±11.6	154.0±9.3	134.7±21.1
	4 weeks	121.4±10.9	94.9±9.8	153.3±12.6	141.7±15.9
	Mature	121.2±10.1	89.1±8.7	188.3±16.5	180.2±19.4
Parenchyma of the inner integument	1 week	381.0±39.1	375.9±38.9	367.9±32.4	255.0±24.0
	2 weeks	408.0±43.4	659.8±69.5	739.4±61.5	625.5±14.6
	3 weeks	291.9±31.0	501.5±54.2	637.9±53.2	588.2±25.1
	4 weeks	167.9±18.2	246.0±25.4	375.9±32.1	423.7±45.1
	Mature	65.7±5.4	42.3±4.3	29.9±2.1	35.7±3.6
Inner epidermis of the inner integument	1 week	12.4±1.2	10.2±1.9	12.4±0.8	12.8±1.1
	2 weeks	21.2±2.4	21.2±1.9	21.9±1.3	17.4±1.6
	3 weeks	21.9±1.7	20.4±2.1	21.2±1.2	23.5±2.0
	4 weeks	-	-	-	-
	Mature	-	-	-	-
Total thickness of plant tissue layers	1 week	493.4±51.2	461.2±46.3	478.0±45.1	361.2±25.9
	2 weeks	653.7±66.2	872.2±85.6	990.7±62.3	840.1±72.8
	3 weeks	554.7±54.2	723.8±76.8	917.7±73.8	885.5±74.3
	4 weeks	406.1±41.3	424.1±43.5	646.7±56.4	658.9±59.6
	Mature	290.5±23.6	197.0±17.3	289.7±22.5	305.0±31.2

"-"this tissue had reached destruction by this time.

Table 2. Thickness of individual tissues in the leaf blade of a mature plant (μm , $n = 10$).

Parameters	<i>G. thurberi</i>	<i>G. raimondii</i>	Tashkent-1	Gene pool - 2	<i>G. arboreum</i> subsp. <i>obtusifolium</i>	<i>G. arboreum</i> subsp. <i>nanking</i>
Total leaf thickness	166.0±14.3	230.0±24.5	342.0±32.5	304.2±12.7	283.2±12.3	257.8±13.1
upper cuticle	1.3±0.08	2.8±0.1	5.4±0.6	7.08±0.6	5.4±0.4	5.85±1.1
Upper epidermis	11.3±0.9	18.3±1.2	21.3±1.9	15.4±0.8	20.8±4.6	23.7±1.9
Columnar parenchyma	58.0±5.9	87.0±9.1	119.1±13.4	119.9±6.1	100.0±8.2 ¹	74.5±6.1 ¹
Spongy parenchyma	84.3±8.6	104.0±9.8	172.9±16.7	139.4±5.6	54.0±3.6 ²	48.1±3.3 ²
lower epidermis	10.1±1.1	15.4±1.2	19.9±2.0	14.9±0.9	82.9±2.8	86.7±12.1
Lower cuticle	1.0±0.07	2.5±0.6	3.9±0.4	7.5±0.5	10.4±3.1	13.3±0.9
					9.6±4.7	5.7±0.3

Note: 8.2¹ is the index indicating the linear dimension of the columnar fabric located closer to the upper side of the sheet plastic.

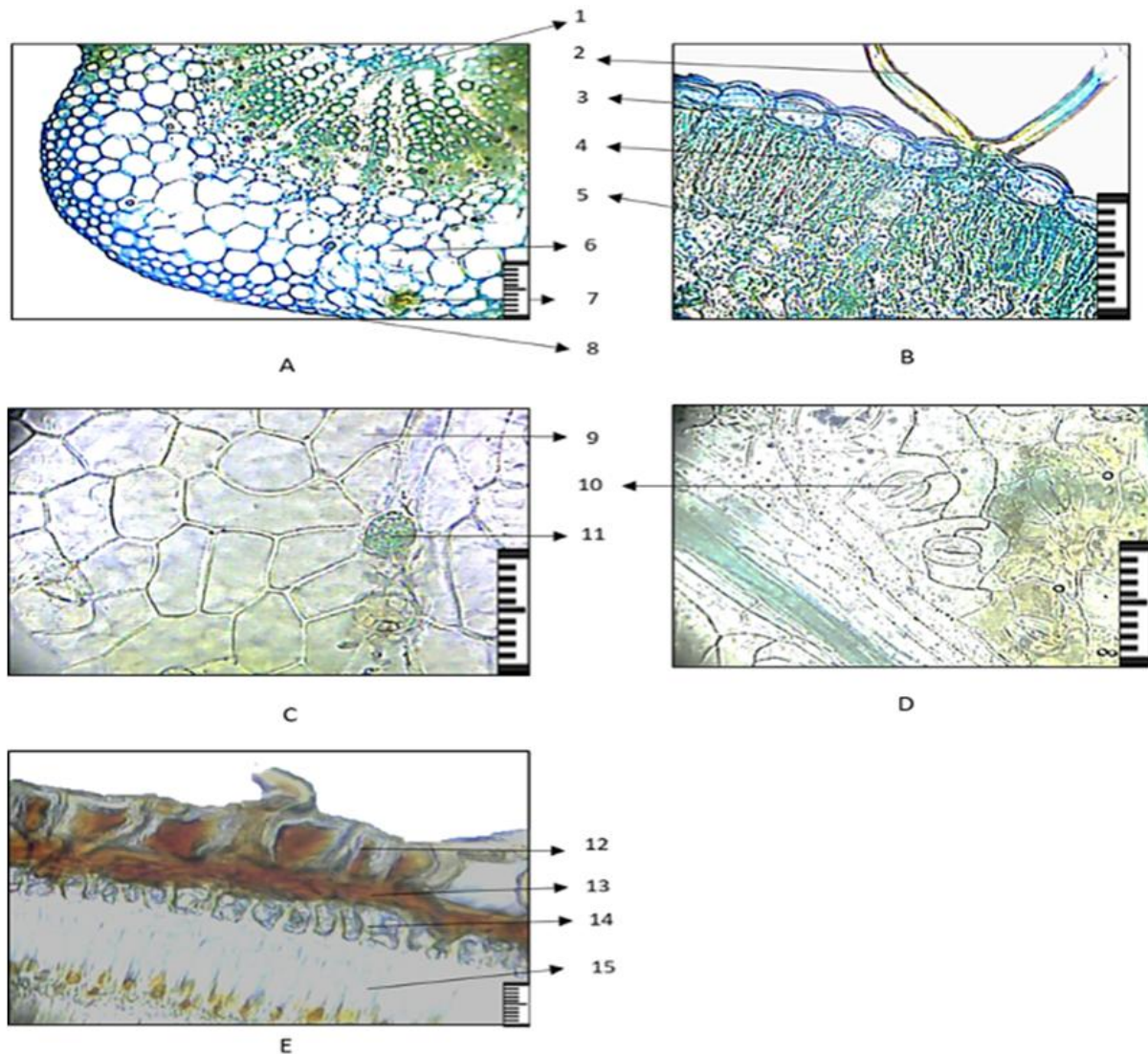


Figure 1. Fragments of a leaf and seed coat of the cotton variety Genofond-2: A–fragment of the central vein of a leaf; B–leaf mesophyll; C–adaxial epidermis of the leaf; D–abaxial epidermis of the leaf; E–the husk of a mature seed: 1–conducting bundle, 2–trichome, 3–adaxial epidermis, 4–palisade parenchyma, 5–spongy parenchyma, 6–parenchyma cells, 7–gossypol receptacles, 8–abaxial epidermis, 9–epidermal cell, 10–stoma, 11–glandular trichome, 12–outer epidermis of the outer integument, 13–parenchyma of the outer integument, 14–inner epidermis of the outer integument, and 15–outer epidermis of the inner integument.

2024). The additional columnar parenchyma layer observed in the *G. arboreum* subspecies provides enhanced structural barriers against aphid stylet penetration. Such results have the support from a recent comparative analysis of resistant and susceptible cotton genotypes (Iqbal *et al.*, 2019).

However, it was noteworthy to mention that the mesophyll structure of the species *G. arboreum* L. subsp. *obtusifolium* and *G. arboreum* L. subsp. *nanking* differed somewhat from the other species studied. Specifically, these two species have an additional, rather high, and lower columnar layer (Figure 1-B). This, in turn, provides better leaf protection

Table 3. Anatomical features of the leaf blade of a mature plant (n = 10).

Parameters in 1 mm ²		<i>G. thurberi</i>	<i>G. raimondii</i>	Tashkent-1	<i>Gene pool -2</i>	<i>G. arboreum</i> subsp. <i>obtusifolium</i>	<i>G. arboreum</i> subsp. <i>nanking</i>
Epidermal cell count	Upper	2528.0 ±261.8	1992.0 ±195.3	1230.0 ±135.6	2524.4 ±185.8	1170.0 ±127.4	1140.0 ±132.8
	Lower	2732.0 ±294.3	1800.0 ±159.4	1365.3 ±142.5	3315.6 ±281.9	1730.0 ±160.8	1250.0 ±142.7
Number of cells around stomata	Upper	3	3	3	3	3	3
	Lower	3	3	3	3	3	2-4
Number of stomata	Upper	96.0 ±8.6	80.0 ±5.6	56.3 ±8.2	180.0 ±14.4	160.0 ±15.4	160.0 ±12.6
	Lower	204.0 ±15.3	130.0 ±10.6	102.8 ±6.9	513.0 ±30.3	249.0 ±28.2	250.0 ±25.7
Number of hairs	Upper	3.7±0.1	3.1±0.1	-	48.6±5.1	12.0±2.1	10.0±1.9
	Lower	44.0±2.6	4.2±0.1	1.9±0.06	77.4±8.1	14.0±2.1	5.0±0.8

from aphid insects' stylet penetration into the spongy parenchyma and the extraction of nutrients from sieve tubes of vascular bundles located closer to the abaxial side of the leaf. Likewise, it protects from the mesophyll transport cells rich in metabolic products. The leaf surface hairs of an adult cotton plant should be short, and their number should not exceed 17.5 per one mm² (Table 3). Otherwise, a unique microclimate and a favorable aphid habitat could develop on the leaf surface.

Recent screening of the species *G. arboreum* genotypes against *Aphis gossypii* under field and glasshouse conditions has also confirmed the presence of multiple resistance categories (antixenosis, antibiosis, and tolerance) in this species (Uthirapathy, 2023). The chlorophyll loss percentage and damage rating scores were significantly lower in the species *G. arboreum* than in *G. hirsutum* genotypes, disclosing the existence of tolerance mechanisms that could benefit introgression into cultivated cotton cultivars.

The presented study addressed this knowledge gap by conducting a comprehensive comparative anatomical analysis of leaf and seed coat structures in the promising cotton cultivar Genofond-2, its wild parental species *G. thurberi* and *G. raimondii*, and the reference cultivar Tashkent-1. Additionally, some accessions of *G. arboreum*, examined for aphid

resistance, sought to identify specific anatomical determinants of pest resistance. By quantifying structural traits across the different growth and developmental stages and genetic backgrounds, this study aimed to establish reliable morphological indicators for selecting genotypes with superior fiber quality and enhanced resistance to piercing-sucking insects. Hence, it facilitated the efficient utilization of wild genetic resources in cotton improvement programs.

DISCUSSION

The comparative anatomical analysis of seed coat development in the cotton cultivar Genofond-2 and its wild parental species *G. thurberi* and *G. raimondii* revealed important patterns of trait inheritance with significant implications in cotton breeding. In the cultivar Genofond-2, the retention of thick seed coat traits from wild donor species supported the hypothesis that introgression of wild genetic material can preserve valuable anatomical traits in cultivated cotton backgrounds (Wendel *et al.*, 2009). These results further aligned with previous studies demonstrating seed coat thickness as a heritable trait that directly affects fiber quality traits (Hadinezhad *et al.*, 2026).

The observed similarities between cultivars Genofond-2 and Tashkent-1 in parenchyma height and total seed coat thickness suggested that the breeding program successfully combined the desirable traits from the cultivated and wild germplasm. However, the unique traits obtained from the wild species *G. raimondii* and *G. thurberi*, particularly regarding integument parenchyma structure, revealed wild species contribute distinct anatomical features enabling seed coat hardness enhancement and fiber contamination reduction during mechanical processing. These results were consistent with findings by Magwanga *et al.* (2018), who reported wild *Gossypium* species possess valuable structural adaptations that confer mechanical resistance and protection against biotic stresses.

The ontogenetic patterns observed in integument development, particularly the dramatic reduction in parenchyma layer rows from 17–20 to 5–7 during seed maturation, reflect the complex developmental management that governs seed coat formation. These results corroborated the earlier research by Kim *et al.* (2019) on the molecular regulation of seed coat development in cotton. It suggested that the timing and extent of parenchyma cell degradation were critical determinants of mature seed coat properties. The intensive age-related variations in the internal integument compared with the external integument highlighted the differential developmental trajectories of these tissues, which could have a linkage to their distinct functional roles in embryo protection and fiber attachment.

The pronounced difference in trichome density among studied genotypes, with hair number reaching 48.6 ± 5.1 per mm^2 on the upper leaf surface of Genofond-2 compared with only 3.1–3.7 in the wild species *G. thurberi* and *G. Raimondi* (Tables 3 and 4), corroborates recent molecular evidence. A trichome density above a critical threshold provides a decisive physical barrier against *Aphis gossypii*. Li *et al.*'s (2025) findings revealed a trichome density exceeding 701.4 trichomes per cm^2 substantially enhanced

aphid resistance in cultivated cotton lines. It acts through both direct mechanical obstruction of aphid stylet penetration and the induction of defensive metabolites such as β -caryophyllene and D-limonene. Likewise, Wang *et al.* (2024) identified the HIC (hirsute cotton) locus as a dominant regulator of trichome initiation, showing that a single promoter variant elevating HIC expression was sufficient to markedly increase trichome density and confer heritable aphid resistance. The consistently elevated trichome numbers recorded here for the aphid-resistant Genofond-2 and *G. arboreum* accessions relative to the more susceptible wild diploids and Tashkent-1 provided desirable outcomes. These suggest that trichome density measured on both leaf surfaces could serve as an additional, easily scorable morphological marker complementing cuticle thickness in future breeding selections.

The comparative genotype-related summary presented in Table 4 further highlights the aphid-resistant accessions combined high epidermal cell density with elevated stomatal numbers on the abaxial leaf surface. It is a pattern possibly influencing how these genotypes respond physiologically to aphid feeding. Heimoana *et al.* (2023) reported that *Aphis gossypii* feeding induces pronounced stomatal closure in cotton, with up to 87% of stomata closing on infested leaves compared with only 34% on uninfested controls. This resulted in reduced photosynthesis, conductance, and transpiration even at moderate aphid densities. Genotypes such as Genofond-2, which possessed markedly higher lower-surface stomatal numbers (513.0 ± 30.3 per mm^2 [Table 4]) than Tashkent-1 (102.8 ± 6.9 per mm^2), may therefore retain a greater residual gas-exchange capacity under aphid pressure, since a comparable proportional closure would still leave a larger absolute number of functional stomata. This observation suggests that stomatal number, alongside trichome density, could contribute to physiological tolerance mechanisms that complement the purely structural, antixenotic defenses conferred by the cuticle and columnar parenchyma layer.

Table 4. Comparative summary of epidermal, stomatal, and trichome parameters of the leaf blade by genotype (n = 10).

Parameters in 1 mm ²	Epidermal cell count		Number of cells around stomata		Number of stomata		Number of trichomes	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
<i>G. thurberi</i>	2528.0±261.8	2732.0 ±294.3	3	3	96.0±8.6	204.0±15.3	3.7±0.1	44.0±2.6
<i>G. raimondii</i>	1992.0±195.3	1800.0±159.4	3	3	80.0±5.6	130.0±10.6	3.1±0.1	4.2±0.1
Tashkent-1	1230.0±135.6	1365.3±142.5	3	3	56.3±8.2	102.8±6.9	-	1.9±0.06
<i>Gene pool -2</i>	2524.4±185.8	3315.6±281.9	3	3	180.0±14.4	513.0±30.3	48.6±5.1	77.4±8.1
<i>G. arboreum</i> subsp. <i>obtusifolium</i>	1170.0±127.4	1730.0±160.8	3	3	160.0±15.4	249.0±28.2	12.0±2.1	14.0±2.1
<i>G. arboreum</i> subsp. <i>nanking</i>	1140.0±132.8	1250.0±142.7	3	2-4	160.0±12.6	250.0±25.7	10.0±1.9	5.0±0.8

"2-4" in this representation, there are diacytic, anisocytic, and staurocytic types of stomata.

"-" This indicator demonstrates the absence of trichomes.

Taken together, the leaf anatomical parameters summarized in Tables 3 and 4 indicate that aphid resistance in the studied *Gossypium* genotypes is unlikely to have control from a single trait. Rather, it emerges from the combined action of trichome density, cuticle thickness, and epidermal-stomatal architecture. This multitrait pattern is consistent with the framework proposed by Li Y, Chen P, Zhao J, Zhu J, Zhao Z, Yu H, Dong Y, Tian Y, Li Z, Xie Z, in which trichome-mediated resistance operates jointly through physical barrier formation and biochemical defense induction rather than through morphology alone (Li *et al.*, 2025). From a practical breeding perspective, the co-occurrence of high trichome number and increased epidermal cell density in Genofond-2 and the *G. arboreum* accessions, relative to the smooth-leaved Tashkent-1, is favorable. It reinforces the case for incorporating multiple, easily quantifiable leaf epidermal traits, rather than cuticle thickness alone, into marker-assisted selection schemes aimed at improving piercing-sucking pest resistance in cultivated cotton.

CONCLUSIONS

The cultivar Genofond-2 retained thick and hard seed coats from wild donor species,

reducing fiber contamination during mechanical ginning. Leaf cuticle thickness exceeding 0.85 µm serves as a reliable diagnostic marker for aphid resistance in cotton breeding. Resistant subspecies of the *G. arboreum* species possess an additional columnar parenchyma layer and compact spongy mesophyll that impede aphid stylet penetration. These anatomical traits provide effective morphological indicators for selecting cotton genotypes with improved fiber quality and pest resistance, confirming the value of wild *Gossypium* species in cotton improvement programs.

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