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Grewia tembensis Fresen and *Grewia trichocarpa* Hochst. ex A.Rich. (Grewioideae Hochr; Malvaceae Juss.) Micromorphological Study and Comparison via Electron Microscopy

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Abstract: *Grewia tembensis* and *Grewia trichocarpa* inhabit dry tropical zones and arid environments, adapting to extreme climatic conditions and limited moisture supplies. Overall, *Grewia* L. possesses a significant variety of bioactive chemical constituents of great therapeutic importance. Indeed, for these species, precise morphological analyses are poor, and their detailed characterization is almost non-existent. This research attempts to investigate and compare the micromorphological traits of *G. tembensis* and *G. trichocarpa* species through scanning electron microscopy (SEM). Micromorphological characteristics of the leaf and fructiferous structures turned out to be highly effective in separating the two species, *G. tembensis* and *G. trichocarpa*, especially regarding the type, density, and distribution of trichomes on the lower and upper surfaces of the leaves, along with the stomatal and trichome types on the surfaces of the fruits. Statistical analyses using principal component analysis, *t*-tests, and hierarchical clustering conducted on micromorphological data of the leaves, flowers, and fruits showed considerable variation within samples of *G. tembensis* and samples *of G. trichocarpa*. On the basis of their morphological assessment characteristics, the samples of both species were distinct and clustered into separate groups. This study emphasizes the necessity of performing detailed morphological studies of species by means of an electron microscope and proves that the leaf features are important for separating species. Such morphological traits of trichomes would offer an efficient tool to distinguish the species. Within the findings, this suggests that such diagnostics are likely to be highly useful for species identification in *Grewia*, especially in cases where there are no fruits available.

Keywords: comparison; description; *Grewia*; morphology; SEM; systematic; taxonym

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1. Introduction

Order Malvales Juss. ex Bercht. and J. Presl contains four families: Tiliaceae (Juss.), Sterculiaceae (Vent.), Bombacaceae (Kunth.), and Malvaceae (Juss.) [1]. It is worth mentioning that the classification in this order is facing several challenges, as numerous genera have been reassigned among families. Initially, the genus *Grewia* L. was classified under the family Tiliaceae; however, it has been moved to the family Malvaceae according to the results of phylogenetic analyses, and the Tiliaceae has become a subfamily of Malvaceae (Tilioideae) [2,3]. Within Malvaceae, there are currently nine subfamilies: Bombacoideae (Burnett), Brownlowioideae (Burret), Byttnerioideae (Burnett), Dombeyoideae

(Beilschm.), Grewioideae (Hochr.), Helicteroideae (Meisn.), Malvoideae (Burnett), Sterculioideae (Beilschm.), and Tilioideae (Arnott) [2]. The genus *Grewia* L. belongs to the subfamily Grewioideae [4–6]. The *Grewia* genus comprises 280–300 species, as mentioned by Al-Hawshabi [7]. Currently, 274 species have been accepted within the *Grewia* genus, as recorded in data from the Royal Botanic Gardens, (Kew) & Plants of the World Online [8]. *Grewia* species are distributed primarily in tropical Africa, Madagascar, the Arabian Peninsula, the Himalayas, Pakistan, India, China, Myanmar, Thailand, Indo-China, Malesia, the Pacific Islands (e.g., Tonga and Samoa), and northern Australia [5,9].

In the genus *Grewia*, several taxonomic studies have highlighted the value of morphological and anatomical characters. Hashmi and Qaiser [10] looked at two species of *Grewia* (*G. erythraea* and *G. tenxa*) from Pakistan and indicated that these species can be distinguished from each other based on these morphological features. The investigation by Chung [9] explored several species of *Grewia* in Malaysia; *G. multiflora, G. leavigata, G. polygama,* and *G. huluperakensis.* It mentioned that these species can be distinguished from one another through a range of morphological characteristics. In addition, Nuha and Fatima [11] studied *Grewia* spp. in Sudan and noted that the species *G. tenax* is identifiable by its flower's colors. More recently, Hawshabi [7] examined the vegetative and reproductive phenotypic traits of seven *Grewia* spp. in Yemen and reported that the identified characteristics have significant taxonomic relevance.

Regarding molecular and phylogenetic studies, there are some works on *Grewia* species that published the complete chloroplast genome, such as *Grewia biloba* Xu [12] and *Grewia biloba* var. *parviflora* reported by Hou [13]. Dorr and Wurdack [14] reconstructed the *Grewia* phylogenetic tree involving several species from genera found in the subfamily Grewioideae, using *ITS* sequences. The interrelationships within *Grewia* suggest a biogeographical scenario of complexity.

The medicinal significance of *Grewia* species has been reported. These constituents include quercetin, β-sitosterol, lupeol, stigmasterol, triterpenoids, lipid compounds, flavonoids, steroids, saponins, and tannins [15,16]. It is used in traditional medicine to treat ailments in Africa, such as coughs. It is used to treat microbial infections, typhoid fever, gastroesophageal reflux, hypochondriasis, decreased appetite, and a distended diaphragm [17].

The flora of Saudi Arabia is one of the most diverse ecological regions in the Arabian Peninsula and contains important genetic resources of crops and medicinal plants. The components of this flora are an illustration of a mixture of effects coming from Asia, together with African assets, as well as the Mediterranean region [18]. Chaudhary, Khalik, and Al-Ruzayza [19,20] recorded 13 genera and 54 species of Malvaceae in Saudi Arabia, including cultivated varieties. Currently, the Flora of Saudi Arabia list (latest updated on 1 September 2024 [21]) includes about 16 genera and 64 species distributed in three subfamilies, namely: Dombeyoideae, Grewioideae, and Malvoideae. A total of nine species of *Grewia* have been reported in the Kingdom of Saudi Arabia, namely, *Grewia arborea* (Forssk.) Lam., *Grewia erythraea* Schweinf., *Grewia gillettii* Sebsebe and B. Mathew, *Grewia mollis* Juss., *Grewia tenax* (Forssk.) Fiori, *Grewia tembensis* Fresen., *Grewia trichocarpa* Hochst. ex A. Rich., *Grewia velutina* (Forssk.) Lam., and *Grewia villosa* Willd. [21]. However, these species lack full taxonomic studies, and detailed descriptions are needed to determine their taxonomic positions.

It grows as a shrub in a desert biome and dry shrubland biome. Morphological characters of *G. tembensis* and *G. trichocarpa* are similar, and divergence is often based on flowering and fruiting. Microscopic examinations of micromorphological features play an essential role in the processes of distinction and identification of samples to species and sex at the taxonomic level. However, few studies have evaluated *Grewia* with scanning electron

microscopy (SEM), such as in *Grewia pindanica* R.L. Barrett [22]. This work aims to study and compare the morphological features of "*G. tembensis* and *G. trichocarpa*" by scanning electron microscopy.

2. Materials and Methods

2.1. Plant Specimen Collection and Study Area

For the purpose of morphological evaluations, specimens of foliage, flowers, and fruits from the species *Grewia tembensis* and *Grewia trichocarpa* were collected from the Faifa Mountains in the Jazan region of southwestern Saudi Arabia (17°14′45.1″ N 43°05′27.2″ E), which is situated in the southwestern segment of the Arabian Peninsula as well. This area holds significant importance from both a floristic and phytogeographical standpoint, serving as a biogeographical connection between the continents of Asia and Africa [23,24]. The Faifa Mountains constitute a pivotal hotspot for botanical diversity in Saudi Arabia. This region is characterized by a complex array of environments and varied habitats, which collectively foster a rich and diverse floral assemblage [25]. The heightened levels of plant species diversity in the southwestern region of Saudi Arabia can be attributed to substantial annual precipitation exceeding 300 mm and an altitudinal gradient that ascends from sea level to 3100 m. The climatic conditions in the Faifa Mountains remain temperate throughout the year; however, temperatures are prone to decline during winter at higher elevations and increase during summer at lower altitudes. Specifically, summer temperatures in these mountains range from approximately 16 to 28 degrees Celsius, whereas winter temperatures vary between 3 to 25 degrees Celsius.

This collection occurred from September to November 2024. Five individuals (trees) of the species were studied. All study samples were collected from Jabal Fifa and lived in the same environmental conditions. The identification of the samples was validated by specialists, who utilized both herbarium specimens and morphological characteristics documented in relevant literature. Voucher specimens were meticulously prepared, and thereafter, these specimens were archived within the Herbaria of the Biology Department at the Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia (Table S1), showing information about the vouchers.

2.2. Examination of Micromorphological Characteristics by Scanning Electron Microscopy

The samples were dried, and the dry flowers and fruit specimens, as well as the abaxial (AB) and adaxial (AD) surfaces of the leaves of the species studied in this investigation, were fixed to stubs by double-sided adhesive tape. The samples were then platinum-coated for 2 minutes prior in a Cressington 108 coating unit (Micro to Nano/Haarlem, The Netherlands). Observation and photography were conducted using an LTD JSM7610F, with a magnification power of 1,000,000, contrast power of 1 NM, and 15 kV (JEOL/Peabody, MA, USA) at the Central Research Laboratory of King Saud University. The qualitative features that were visible on either side of the leaves (AB and AD), as well as the general surface patterns related to flowers and fruits fruits were systematically recorded. The complex relief of the leaf surface and trichomes was described using the previously specified terminology in Chung [26]. The outer stomatal ledge and outer stomatal aperture were described using terminology in Pautov et al. [27]. The photomicrographs were taken in the same position for all samples when measuring. A total of 31 qualitative characters (for leaves, flowers, and fruits) were defined to be recorded, along with 21 quantitative characters measured (Tables S2 and S4). Measurements of length, width, and area for leaves, fruits, petals, and ovaries are shown in the schematic drawing provided in the Supplementary File (Figure S1). The measurements were conducted using ImageJ v. 1.53t analysis software [28]. The processes of measurement using ImageJ software involved uploading sample photos

into ImageJ, setting a scale, preparing the image for measurement, and using Adjust Jope in the menu. Then, image analysis for estimating the area was performed by choosing the submenu Tools-ROI Manager, followed by exporting the ImageJ data file for further analysis [29].

2.3. Statistical Analysis

The quantitative micromorphological features (Table S4) were prepared for immediate application, and the morphometric dataset was used in the PCA analysis. This PCA was conducted with XLSTAT version 2024.2.1 [30]. The PCA helps us examine how well the selected traits (variables) can cluster samples based on their resemblances. In addition, the collection of summary statistics concerning morphological traits and graphical representations and assay the association of the phenotypic traits to observe the correlation between them and the association according to the species.

The correlation coefficients between the different morphological characters were determined in XLSTAT version 2024.2.1 [30], with Bootstrap observations chart: Number of samples: 50, Seed (random numbers).

It was applied box plot and *t*-test analysis (GraphPad Software version 10.4.1 for Windows, [31]) to compare the means in this respect for two species, *Grewia tembensis* and *Grewia trichocarpa*. Statistical significance was tested using 5 replicates per sample and 95 permutations; a *t*-test *p*-value < 0.05 was considered significant, while *p*-value > 0.05 indicated an insignificant result.

Where R-Squared (R^2 or the coefficient of determination) was calculated; R-squared (R^2 or the coefficient of determination) is a statistical measure of how close the data are to the fitted regression line. So, R-squared is essentially what percentage of how well the data fits the regression itself. Using the R-squared, which ranges between 0 and 1, describes the degree to which the model fits the data: 1 = perfect fit of the model to the data; 0 = the model explains none of the variance in your dependent variable. As a rule of thumb, in academic research, one can expect R-squared to be statistically significant somewhere between 0.50 and 0.99.

It was used the Heatmapper web server [32] to generate a heatmap of variation and hierarchical clustering based on the *G. tembensis* and *G. trichocarpa* morphological data. Additionally, a morphological dendrogram grouping was built through the 'factoextra' package Version 1.0.7 following [33] and RStudio software Version 2.0 [34]. Consequently, it enhances the potential of exploring the relations between two species, *Grewia tembensis* and *Grewia trichocarpa*, of morphological traits. Hierarchical representation and clustering: Samples were clustered (branch and subbranch formation) based on dissimilarity.

3. Results

3.1. Morphological and Micromorphological Characteristics of the Leaf Epidermis, Stomata, Ovaries, and Fruits

Characteristics of the Grewia species, *G. tembensis* and *G. trichocarpa*, via SEM are presented in Table S2 and Figures 1–6. The *G. tembensis* species is distinguished by long cuticular striations on the (AB and AD) leaf surfaces, whereas they are short and less frequent on the AD leaf surface of *G. trichocarpa*.

In this study, we observed the presence of several types of glandular and nonglandular trichomes on the leaf surfaces in both species (Table S2). a. Nonglandular trichomes are as follows: (1) simple long trichomes that are tapered at the tip (Figure 1b,g; (2) 2, 3, or 4-armed tufted trichomes on the (AB) and (AD) leaf surfaces, which are observed only in *G. tembensis* (Figure 1c–e,h); (3) stellate trichomes without a central cushion that are present on both the (AB) and (AD) leaf surfaces; these trichomes had more arms (3–10 arms)

and were more highly dense on the AB leaf surface in *G. trichocarpa* (Figure 2a–d) than in *G. tembensis* (5–8 arms/Figure 1f), and they had a twisted appearance and were swollen at the tips of *G. trichocarpa* on both the AB and AD leaf surfaces (Figure 3a–c); and (4) stellate trichomes with a central cushion present only on the leaf surfaces of *G. trichocarpa*, which were 6–8-armed, long, twisted, and swollen at the tips of both the (AB) and (AD) leaf surfaces (Figure 3a,d). b. Glandular trichomes were observed on the AB and AD leaf surfaces of both *G. tembensis* and *G. trichocarpa*, with short stalks and elliptical, broadly elliptical, or rounded heads (Figure 4c,f).

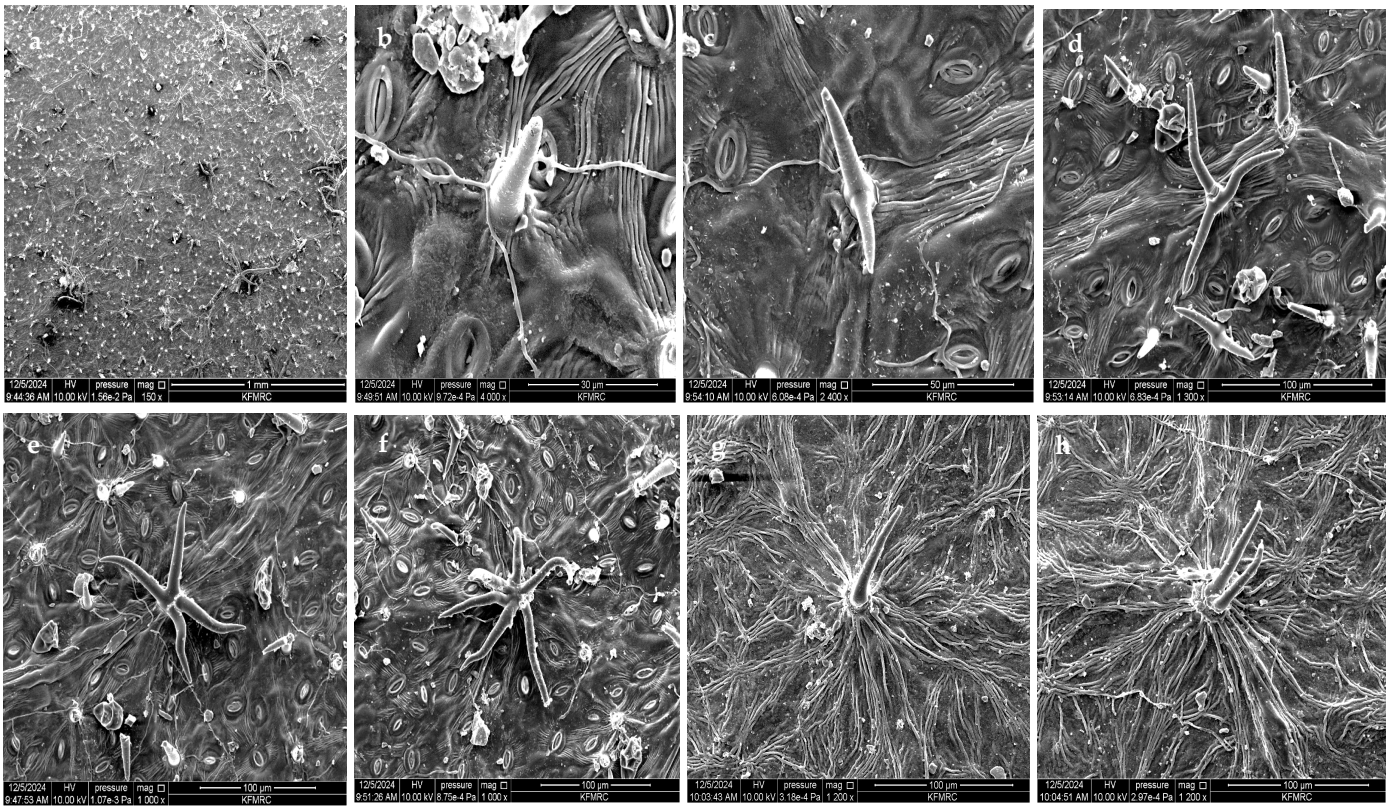


Figure 1. (**a**). Abaxial leaf (AB) surface in *G. tembensis*; (**b**). simple long trichomes tapered at the tip; (**c**–**e**). 2, 3 and 4-armed tufted nonglandular trichomes; (**f**). stellate trichomes with 5 arms without a central cushion. (**g**). Adaxial leaf surface (AD) of *G. tembensis*, simple trichomes; (**h**). tufted 2-armed nonglandular trichomes and long cuticular striation on the AD leaf surface of *G. tembensis*.

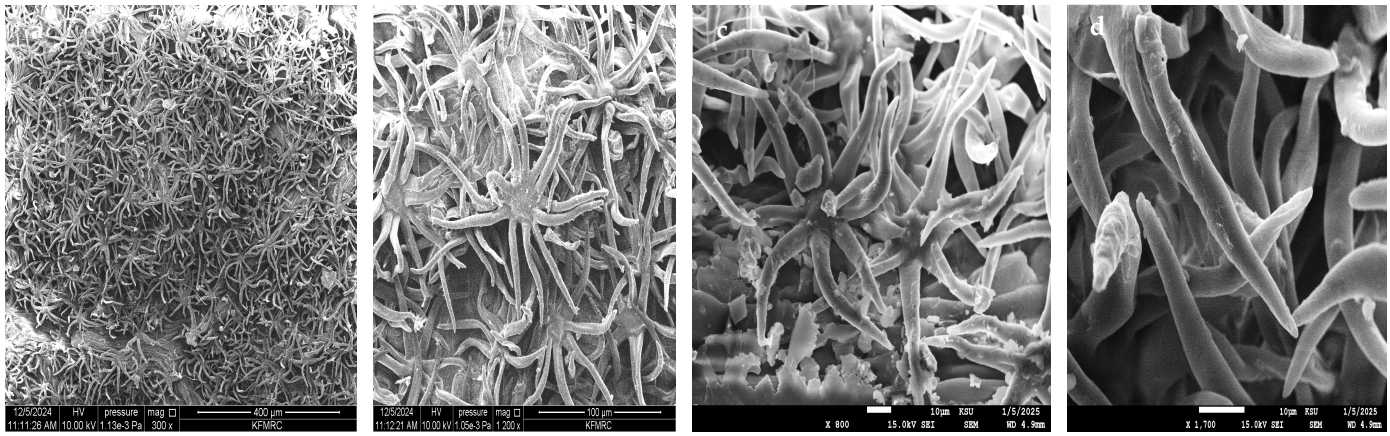


Figure 2. (**a**–**d**). Dense stellate trichomes on the abaxial leaf surface of *G. trichocarpa*.

Stomata were present only on the AB leaf surfaces of *G. tembensis* and *G. trichocarpa*; they were elliptical (*G. tembensis*) to broadly elliptical (*G. trichocarpa*) and were elevated slightly above the surrounding epidermal cells. The stomatal shape was anomocytic in *G. tembensis* (Figure 4a,b), and it was paracytic in *G. trichocarpa;* however, the high density of stellate trichomes on the AB surface of *G. trichocarpa* obscures the clear visibility of the stomata (Figure 4d,e).

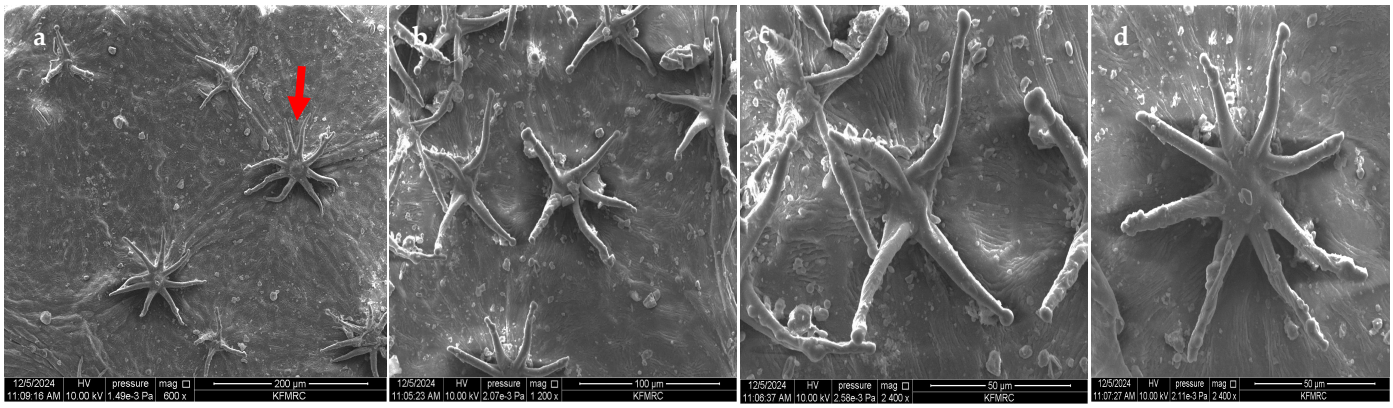


Figure 3. The Adaxial leaf surface of *G. trichocarpa*. (**a**). stellate trichomes, the arrow indicates trichome with a central cushion and multiple arms. (**b**,**c**). twisted appearance and swollen stellate trichomes without a central cushion. (**d**). trichome with a twisted appearance, swollen at the tips, and a central cushion.

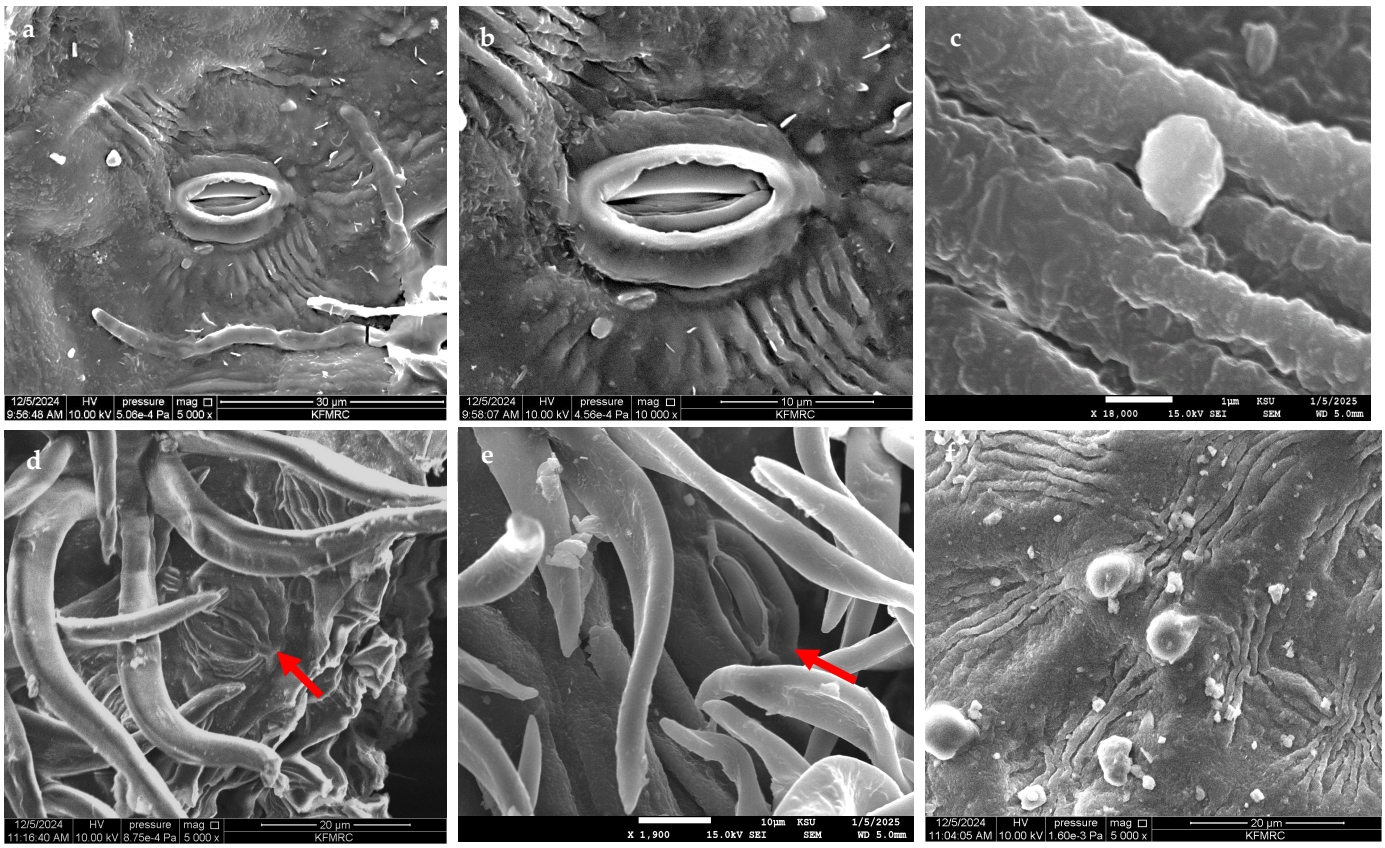


Figure 4. (**a**,**b**). Stomata on the abaxial leaf (AB) surface of *G. tembensis*; (**c**). Glandular trichomes in *G. tembensis*. (**d**,**e**). Stomata on the abaxial leaf (AB) surface of *G. trichocarpa*. The arrow indicates that the stomata of *G. trichocarpa* are covered by dense trichomes. (**f**). Glandular trichomes in *G. trichocarpa*.

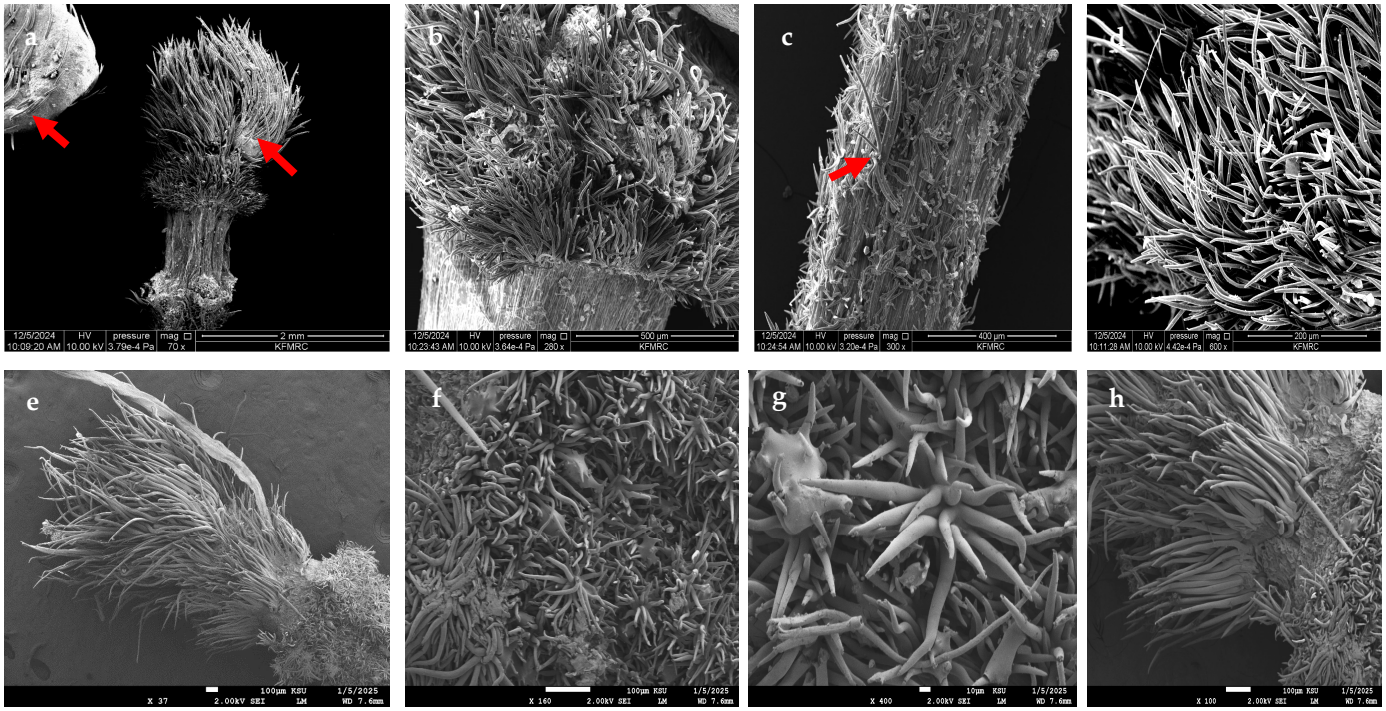


Figure 5. (**a**,**b**) Ovary surface and floral stalk of *G. tembensis*, simple trichomes, and the arrow indicates 2-armed trichomes; (**c**). stellate trichomes on the floral stalks, the arrow indicates long trichomes *in G. tembensis*. (**d**). high magnifications for simple trichomes on the ovary surface of *G. tembensis*. (**e**). ovary surface and floral stalk of *G. trichocarpa* with simple trichomes; (**f**,**g**). Multiple-armed nonglandular trichomes on the ovary of *G. trichocarpa.* (**h**). High magnifications for simple trichomes on the ovary surface of *G. trichocarpa*.

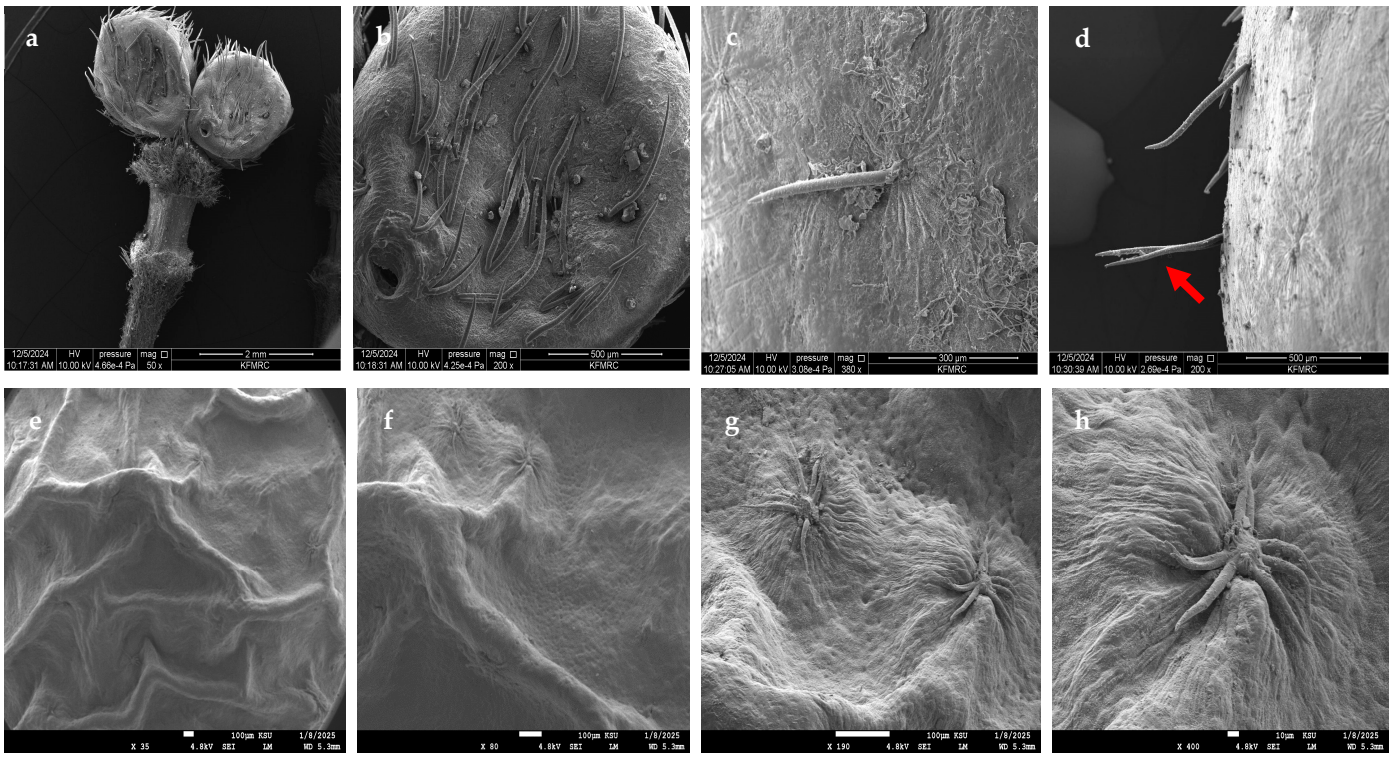


Figure 6. (**a**,**b**). Fruit surface of *G. tembensis*; (**c**). simple, long nonglandular trichomes that are tapered at the tips; (**d**). tufted 2-armed nonglandular trichomes; (**e**,**f**). fruit surface of *G. trichocarpa*; (**g**,**h**). stellate nonglandular trichomes.

The petals and stamen of *G. tembensis* were yellow in color. The petal color of *G. trichocarpa* was light orange, whereas the stamen color was orange. Different types of trichomes were observed as well on the ovary surface (Table S2) as follows: (1) simple, long trichomes tapered at the tip, present on the ovary and highly dense at the base of the ovaries of *G. tembensis* and *G. trichocarpa* (Figure 5b,d,h); (2) tufted trichomes with 2 arms, present only on the surface of the ovary and base of the ovary in *G. tembensis* (Figure 5a); (3) stellate trichomes without a central cushion, which are 8- to 12-armed and present on the floral stalks of *G. trichocarpa* (Figure 5g), but long and 6- to 8-armed in *G. tembensis* (Figure 5c); and (4) 5- to 8-armed stellate trichomes with a central cushion, which have only been found on the floral stalks of *G. tembensis* (Figure 5c). Fruit colors are orange in both species, with 4 fruit lobes in *G. tembensis* and 2 in *G. trichocarpa*. The fruit surface of *G. tembensis* is distinct due to nonglandular trichomes that are simple, long, and tapered at the tip, and 2-armed tufted trichomes, which are present only in *G. tembensis* (Figure 6b–d). Stellate trichomes without a central cushion are observed only on the fruit surface of *G. trichocarpa* (Figure 6g,h).

3.2. Outcomes of Data Analysis

The PCA outcomes demonstrated significant variability in the morpho- and micromorphological attributes of the leaves, flowers, and fruits of *G. tembensis* and *G. trichocarpa*. This phenomenon was evident in the allocation of the specimens within the two main groups on the basis of the PCA results.

Table S3 and Figure S2 in the Supplementary Materials show the eigenvalues and variability of the principal component analysis (PCA) axes, where Axes 1, 2, and 3 represent the highest values (38.383, 14.550, and 13.114, respectively). The characteristics that exhibited the most substantial positive and negative loadings on Axis 1, 2, and 3 are shown in Table S4. The blade width, fruit lobe length, fruit lobe width, and fruit area characteristics exhibited the most significant positive contributions on Axis 1 of the PCA (0.849, 0.727, 0.866, and 0.822, respectively). In contrast, the blade length, area of the outer stomatal aperture, outer ledge on the abaxial (AB) surface of the leaf, and ovary area exhibited the most significant negative contributions on Axis 1 of the PCA (−0.749, −0.808, −0.864, and −0.817, respectively). The outer stomatal ledges length on the abaxial (AB) leaf surface and the ovary width characteristics presented the most significant positive and negative loadings on Axis 2 of the PCA (0.869 and −0.581, respectively). The petal length and petal area characteristics had the highest positive loadings (0.710 and 0.619, respectively) on Axis 3 of the PCA, whereas the blade area had the highest negative loadings (−0.502).

Figure 7 and Figure S3 in the Supplementary Materials show the loading plots and cluster loading distributions of samples on Axes 1 and 2 of the principal component analysis. The samples of *G. tembensis* and *G. trichocarpa* are clearly distributed within two groups according to the species. The PCA results show that the species under study differ along axis 1, and that axis 2 variables contribute to intraspecific variability. A similar effect of micromorphological characteristics on sample distribution was also observed for (Figures S4 and S6 in the Supplementary Materials) Axes 1 and 3 of the PCA, as well as Axes 2 and 3 of the PCA (Figures S5 and S7 in the Supplementary Materials). They obtained similar results from grouping the study samples into two separate groups according to species in heatmaps of the variation and hierarchical clustering analysis and cluster dendrogram, as shown in Figures S8 and S9 in the Supplementary Materials.

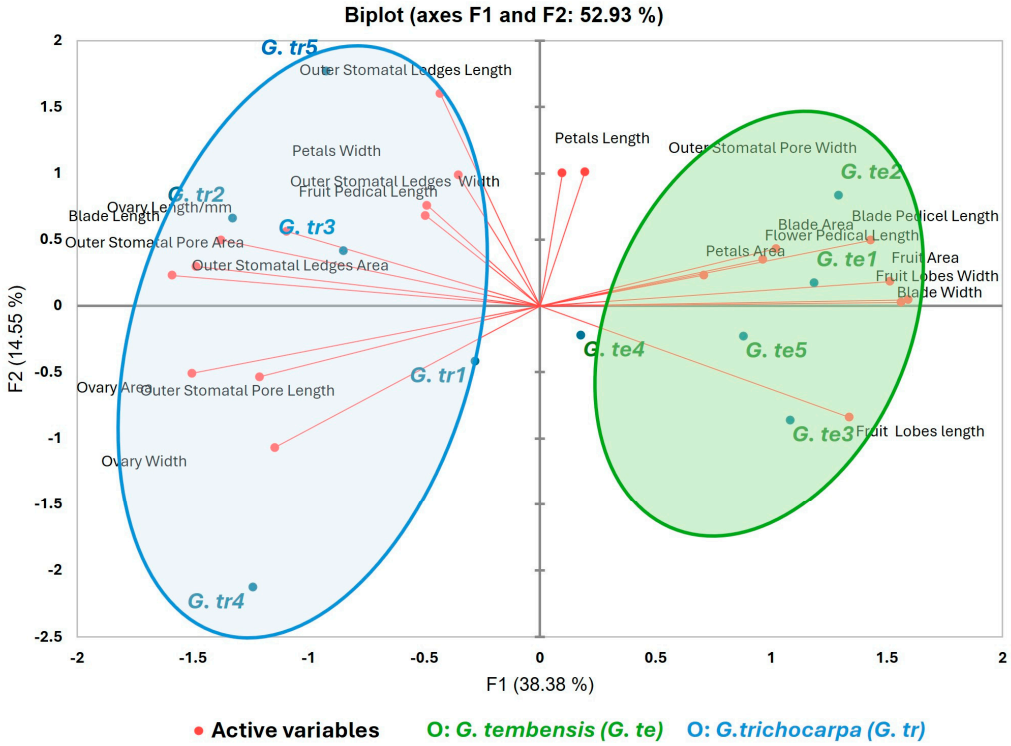


Figure 7. Visualization of the loadings and cluster loadings of the micromorphological traits in specimens of *G. tembensis* and *G. trichocarpa*, displayed on PCA Axes 1 and 2.

Table S5 represents the correlation matrix among the micro-morphological features of the leaves, flowers, and fruits of *G. tembensis* and *G. trichocarpa*. A robust positive correlation was observed between the length of the flower pedicel and blade pedicel length characteristics (0.888). However, the most negative correlation was between the fruit lobe width and outer stomatal ledges area (−0.806).

The results of the T-test for the two species, namely, *G. tembensis* and *G. trichocarpa* (Table S6 and Figure 8), revealed that some micromorphological traits exhibited by the leaves, flowers, and fruit structures employed in this investigation significantly differed between the study samples. Specifically, the blade length, blade width, outer stomatal aperture area on the abaxial (AB) leaf surface, outer stomatal ledges area on the abaxial (AB) leaf surface, ovary width, ovary area, fruit lobe length, and fruit lobe width were significantly different between the two species, with p-values < 0.05 and R-squared values ranging between 0.537 and 0.804. The *G. trichocarpa* samples presented the largest values recorded for the blade length, blade width, outer stomatal aperture area AB, outer stomatal ledges area AB, ovary width, and ovary area in the box plot (Figure 8a,c–f), with means of 7.498, 16.163, 183.390, 4.650, and 8.031, respectively (Table S6 in the Supplementary Materials). The *G. tembensis* samples presented the greatest variation in the blade width, fruit lobe length, and fruit lobe width characteristics in the boxplot (Figure 8b,g,h), with means of 4.471, 0.699, and 0.519, respectively (Table S6).

Notably, these characteristics also reflect high loadings on the PCA axes, as shown in Table S6, and play a role in distinguishing the *G. tembensis* and *G. trichocarpa* samples (Figure 8).

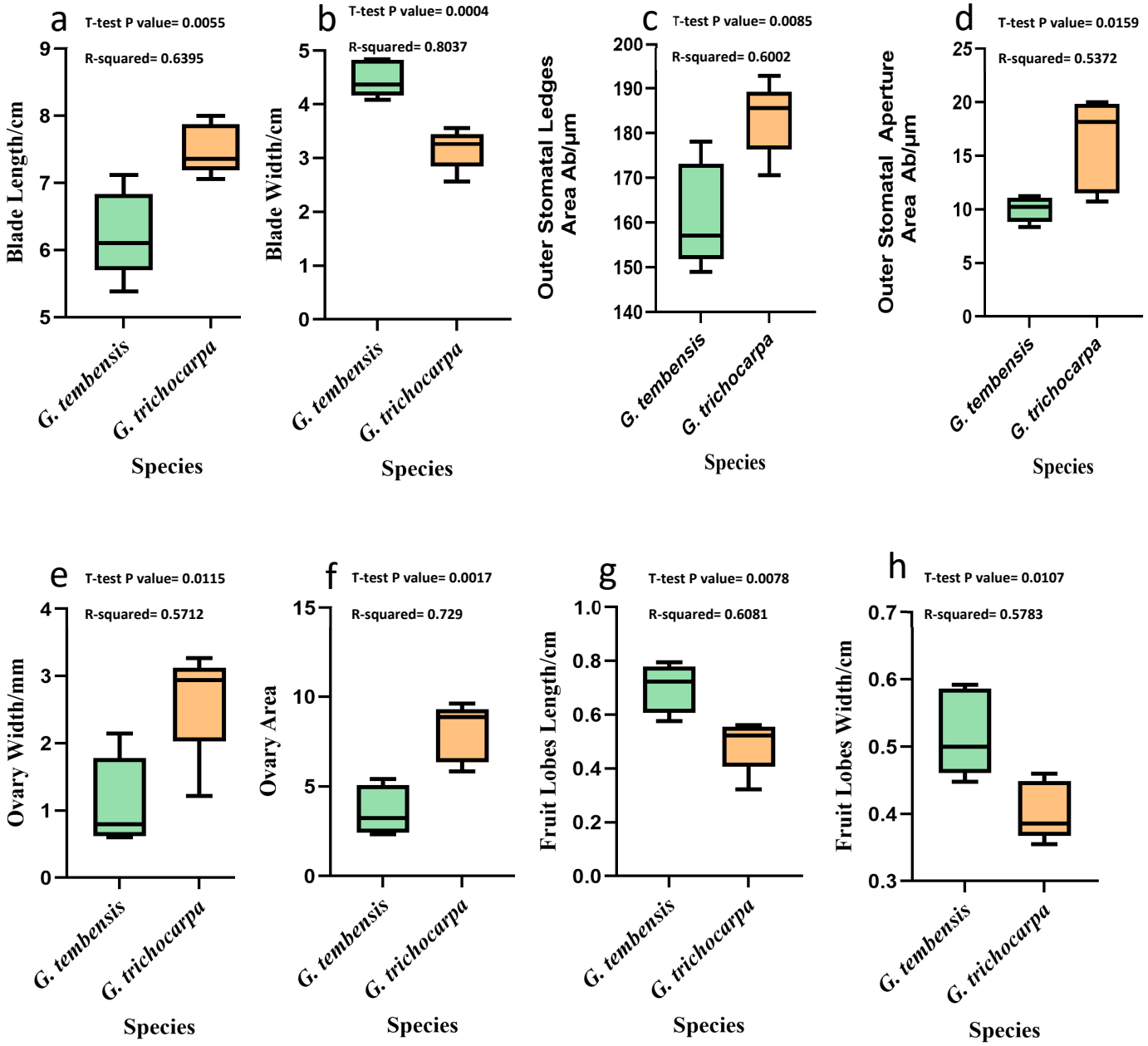


Figure 8. Boxplots and *t* test the results of the morphological traits within the species *G. tembensis* and *G. trichocarpa*. The samples of *G. trichocarpa* presented the most significant measurements for the blade length, outer stomatal aperture area, outer stomatal ledges area, ovary width, and ovary area, as illustrated in the boxplot (**a**,**c**,**d**,**e**,**f**). The *G. tembensis* samples presented the greatest variability for characteristics such as the blade width, fruit lobe length, and fruit lobe width, as depicted in the boxplot (**b**,**g**,**h**). Value of the spread represented with Confidence interval CI (95%).

4. Discussion

Micromorphological properties studied in this research strongly reflect that *G. tembensis* and *G. trichocarpa* share no close similarity. In fact, differences between these two species can be found in the abundance and types of trichomes on AB and AD leaf surfaces, ovary surfaces, and stalks, as well as fruit surfaces. The results of this study further suggest that the morphological characteristics of stomatal structures are important for the taxonomy of *Grewia* species. These findings are consistent with previous studies, which highlighted the importance of morphological characteristics in the classification of this genus. Other characters based on petiole length, leaf blade morphology, texture of blade surfaces, flower peduncle, ovary surface, and fruit morphology were also elucidated by

Hashmi and Qaiser [10] in an investigation of *Grewia* species. In addition, Chung [9] showed that *Grewia* species can be differentiated from each other along several morphological parameters, such as leaf margin types, inflorescence types and their number, flower sexuality, surface texture of petals, and number of fruit lobes.

In a micromorphological study on *Grewia* L. in Peninsular Malaysia and Borneo, [26] reported four general categories and nine subcategories of nonglandular trichomes, as follows: (1) Simple trichomes, characterized by their long, tapered apex. (2) Tufted trichomes: likely classified into 2- to 4-armed. (3) Stellate trichomes, in 4- to 6-armed formations without a central cushion. (4) Cushioned stellate trichomes in 4- to 8-armed and with a central cushion. In the current analysis of samples from *G. tembensis* and *G. trichocarpa*, these basic trichome types were observed. However, there are differences in the shape, number of arms, density, and distribution of the trichomes.

In our investigation, we observed a distinction in the morphology of cuticular striation on the epidermis between the two species, characterized by elongated and pronounced cuticular striation on the leaf surfaces of *G. tembensis*, contrasted with shorter and less frequently occurring cuticular striation on the leaf surfaces of *G. trichocarpa*. This observation aligns with the findings presented by Chung [26], who noted that cuticular striation in Grewia species can be categorized as either elongated, infrequent, or shortened in nature.

The micromorphological characteristics of the leaves, flowers, and fruits tested in this study showed significant differences between *G. tembensis* and *G. trichocarpa* based on differences identified using principal component analysis, *t*-tests, and hierarchical clustering.

Specifically, blade length, blade width, outer stomatal aperture area on the abaxial surface of leaves, outer stomatal ledges area on the abaxial surface of leaves, ovary width, ovary area, fruit lobe length, and fruit lobe width were determined to be effective in classifying the species samples into two classes.

The fine morphological features of the foliage, including the type, size, and abundance of trichomes on the abaxial and adaxial surfaces and types of stomata, were scrutinized in this study and were found to differ measurably between the two species (*G. tembensis* and *G. trichocarpa*); hence, these features provided the most complete separation of the two taxa. Identification of these species is also critical, owing to their importance in traditional medicines in many parts of Asia and Africa, as well as their value as a source of high-quality timber and as feed for herbivorous livestock. Given that most species identification methods rely heavily on the descriptive and morphological features of fruits and flowers, structures that are not always available throughout the year or are limited to certain seasons; it proposes that such leaf features may provide an important contribution to the taxonomy of *G. tembensis* and *G. trichocarpa* when only vegetative material is available and species identification must rely solely on foliar material.

Intensive studies [35,36] confirmed that the micromorphological features of the stem and leaf were valid features to differentiate genera and species in the order Malvales. In addition, ref. [26] emphasized the significance of the unique glandular trichomes in species under the Malvaceae family as an important prerequisite for species identification and reliable authentication of herbal medicine.

Karakish et al. [37] investigated the micromorphological traits of 17 species from within the Malvaceae family of Saudi Arabia. Notable characteristics of the stem, petiole, lamina, trichomes, crystals, ducts, and star-shaped idioblasts were significant; they used these characteristics for the development of an artificial key.

In general, many recently published studies from various families support the idea that the analysis of micromorphological traits of leaves, trichomes, or stomatal complexes could be useful taxonomic tools to separate specimens at the genus or even species level, as mentioned before in the Scrophulariaceae family [38]. The study of relations of *Silene*

leucophylla Boiss. and *Silene schimperiana* Boiss. demonstrated by [39]. Additionally, the study of micromorphological characteristics for species from the family Loranthaceae Juss [40] confirmed the importance of micromorphological characteristics.

5. Conclusions

The features of the qualitative and quantitative micromorphology analyzed throughout this study show the differences among the specimens of both species (*G. tembensis* and *G. trichocarpa*).

It observed fine variations between *G. tembensis* and *G. trichocarpa* samples in trichome features with respect to: the number of arms, trichome morphology, density, and distribution in leaves, ovaries, and fruits, in addition to variation in stomatal shape.

The results of the comparative analysis found differences between the microcharacters observed in: leaves, flowers, and fruits of both species. In particular, blade length, blade width, outer stomatal aperture area on the abaxial leaf, outer stomatal ledges area on the abaxial leaf, ovary width, ovary area, length of the fruit lobes, and fruit lobes width.

Also, encourage future studies to investigate the *Grewia* species through SEM methods, pursuing this powerful methodology to explore the highly diverse structural potential that exists within this genus.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050340/s1, Table S1: Information on herbarium vouchers. Table S2. Micromorphological descriptive features of the leaves, blossoms, and fruits of *G. tembensis* and *G. trichocarpa* within the Malvaceae family examined in this research. Table S3. Eigenvalues of the principal component analysis based on micromorphological traits. Table S4. Characteristic loadings in the principal component analysis (PCA) derived from the micromorphological gain characteristics. Table S5. Correlation matrix (Pearson (n)). Table S6. Results of the evaluation of the quantitative micromorphological characteristics of *G. tembensis* and *G. trichocarpa*. Figure S1. Schematic drawing Illustration of the measurement method used. Red arrows indicate length and width measurements for a. leaves, b. petals, c. ovaries, and d. fruits. Green indicates estimated area. The measurement procedures utilizing ImageJ software were executed by importing sample images into the program, establishing a scale, and employing the Adjust Jope function found in the menu. Subsequently, image analysis was performed to estimate the color area while excluding other regions by selecting the submenu Tools-ROI Manager, followed by the exportation of the ImageJ data file for subsequent analysis [29]. Figure S2. Principal component analysis of the eigenvalues of *G. tembensis* and *G. trichocarpa* on the basis of observations of the micromorphological characteristics of the leaves, fruits and flowers. Figure S3. Loading diagrams delineating the two principal component Axes 1 and 2, pertaining to *G. tembensis* and *G. trichocarpa*, derived from the examination of the micromorphological attributes of the leaves, fruit, and flowers. Figure S4. Loading diagrams delineating the two principal component Axes 1 and 3, pertaining to *G. tembensis* and *G. trichocarpa*, derived from the examination of the micromorphological attributes of the leaves, fruit, and flowers. Figure S5. Loading diagrams delineating the two principal components Axes 2 and 3, pertaining to *G. tembensis* and *G. trichocarpa*, derived from the examination of the micromorphological attributes of the leaves, fruit, and flowers. Figure S6. Loading plots and the corresponding clusters for the species *G. tembensis* and *G. trichocarpa*, depicted along the two principal component Axes 1 and 3, derived from the examination of the micromorphological traits of the leaves, fruit, and flowers. Figure S7. Loading plots and the corresponding clusters for the species *G. tembensis* and *G. trichocarpa*, depicted along the two principal component Axes 2 and 3, derived from the examination of micromorphological traits of the leaves, fruit, and flowers. Figure S8. Heatmap depicting the variation and hierarchical clustering of samples of *G. tembensis* and *G. trichocarpa* derived from the morphological characteristics of the leaves, flowers, and fruits evaluated in the present study. Figure S9. Dendrogram representation of the clusters of *G. tembensis* and *G. trichocarpa* specimens derived from analyses of the morphological traits of the leaves, flowers and fruits.

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