INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2025, Vol. 4, NO. 1, 49-59.
DOI: https://doi.org/10.56612/ijaaeb.v1i1.110
ARK: https://n2t.net/ark:/71142/IJAaEB.v1i1.110
https://eminentscientists.com/

Taxonomic significance of leaf epidermal features for identification of some *Ficus* species

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Abstract
Morphological similarities among plant genera and species pose challenges in accurate identification, but leaf epidermis, with its diverse anatomical characteristics, serves as a valuable tool for differentiation. The study focused on the taxonomic significance of leaf epidermal features for the identification of various *Ficus* species. Leaves from different taxa were collected and preserved in 70% ethanol, and their epidermis carefully peeled off for microscopic examination. The staining process with safranin enhanced the visibility of various features. The observed anatomical characteristics included epidermal cell area, guard cell shape, stomatal size, stomatal shape, stomatal types, stomatal density, subsidiary cell shape, and types of trichomes. Significant variations were found in epidermal cell area, stomatal size, and stomatal density, etc. Clustering analysis categorized the species into four distinct groups, revealing associations and commonalities. Stomatal characteristics, subsidiary cell shapes, and trichomes presence are documented, which provide a detailed characterization. Positive and negative parameter-to-parameter correlations offered insights into trait interactions. The findings of this study contribute valuable information for the taxonomic identification of *Ficus* species, emphasizing the importance of leaf epidermal features in distinguishing among closely related taxa. This research enhances our understanding of plant diversity and provides a practical approach for accurate plant identification based on microscopic observations of leaf epidermal characteristics.

ARTICLE TYPE
Research Paper (RP)

SECTION
Plant Biology (PB)

HANDLING EDITOR
İbadullayeva, S.J. (PB)

ARTICLE HISTORY
Received: 05 Jan, 2024
Accepted: 19 Jun, 2024
First Online: 28 Jun, 2024
Published: 06 Jan, 2025

KEYWORDS
Anatomical characteristics;
Clustering;
Diversity;
Leaf epidermis;
Taxonomy

Introduction

Traditionally, plants have been classified based on the morphology of their fruits and flowers. Plant scientists have employed leaf epidermal anatomy to facilitate accurate identification and classification of closely related taxa (Abdulrahaman and Oladele, 2010; Qiu et al., 2023). Morphological characteristics play a crucial role in distinguishing between different taxonomic ranks, including families, tribes, genera, and species (Alaida and Aldhebiani, 2022). However, certain flowering plant taxa pose challenges in differentiation based solely on morphological traits (Nazir et al., 2013), as some species share similar physical characteristics. Therefore, understanding both the similarities and variations within a sub-genus is imperative for determining the taxonomic positions of these species.

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CITATION (APA): Hussain, A., Ahmad, M.S.A., Riffat, A., Rani, W. (2025). Taxonomic significance of leaf epidermal features for identification of some *Ficus* species. *International Journal of Applied and Experimental Biology* 4(1): 49-59.

Microscopic analysis plays a significant role in anatomical assessments of plant subgenera within a family. Leaf epidermal features, such as trichomes and stomata, have proven to be essential in resolving taxonomic issues (Abdulrahaman et al., 2011; Patel et al., 2021). Many plant species can be identified using the physical characteristics of leaf epidermis, such as trichomes and stomata, in conjunction with taxonomic aids (Xiang et al., 2010; Patel et al., 2021; Qiu et al., 2023).

The value of leaf epidermal anatomy in plant identification has also been applied in forensic botany. For example, leaf epidermal anatomy was used to identify the source of plant material found at a crime scene; the study found that the leaf epidermal characteristics of the plant material matched those of a known plant species, which was used as an evidence in court (Daeid et al., 2021). Thus, the identification of plant species relies heavily on foliar anatomical features. Many scholars have already researched the leaf anatomical properties of plants (Kadiri et al., 2005; Xiang et al., 2010; Abdulrahaman et al., 2011; Sheikh and Kumar, 2017; Patel et al., 2021), and foliar epidermal anatomy of various families has been published (Shah et al., 2018).

The investigation of *Ficus* epidermal surfaces revealed a variety of essential micromorphological features, some of which show remarkable interspecific differences that are taxonomically important (Mubo et al., 2004). Size, orientation of stomata, form of stomata, shape of guard cells, and structural characteristics of epidermal cells are all essential diagnostic traits of the epidermis that provide useful indications for identification (Munir et al., 2011; Trofimov and Rohwer, 2018). In another analogous study, it was reported that the size and shape of stomata, presence or absence of stomatal clusters, and size and shape of trichomes had been useful diagnostic characteristics for distinguishing between different fern species (Rahman et al., 2017).

Although it is now believed that epidermal features are crucial for taxonomic purposes, the information on the epidermal morphology of *Ficus* is limited. Notable features such as epidermal cell structure, shape, orientation, stomatal complex size, and trichome types carry significant taxonomic significance (Rahman et al., 2017). Thus, the current research aimed to provide a comprehensive understanding of the role of leaf epidermal anatomy in plant identification. This research will contribute to the existing literature on the use of leaf epidermal anatomy in plant identification and provide insights for researchers and practitioners in the field of plant taxonomy and classification.

Materials and Methods

The study aimed to investigate the leaf epidermal anatomy of eight *Ficus* species (*Ficus racemosa, Ficus religiosa, Ficus retusa, Ficus benjamina, Ficus benghalensis, Ficus lyrata, Ficus carica,* and *Ficus elastica*) sourced from the historic botanical garden at the University of Agriculture Faisalabad. Three samples per plant were meticulously selected for the analysis. To preserve the leaves, a solution comprising 70% ethanol and 30% distilled water served as their sanctuary for three days. Subsequently, the samples were transferred to a permanent preservation medium of acetic ethanol solution (25% acetic acid and 75% alcohol), following a protocol with slight modifications based as described elsewhere (Cotton 1974). For the sectioning and staining process, suitable leaf portions were delicately chosen and separated from their epidermis using a sharp blade. The thinnest slices were then selected for examination, with the process guided by the technique outlined by Adedeji and Jewoola (2008). These sections underwent a series of ethanol treatments, beginning with 30% ethanol treatment for 15 minutes, followed by a 50% ethanol bath for another 15 minutes, and finally, immersion in 70% ethanol for 15 minutes to eliminate excess moisture. Following this, a safranin stain was applied, and the sections were immersed in 90% alcohol for drying. Subsequently, the samples were exposed to a xylene solution for further staining. After staining, the samples underwent careful washing thrice with distilled water to remove any residual staining agents. The stained sections were then meticulously mounted on glass slides using needles and forceps. Excess water and dye were removed with a tissue paper before covering each slide with a coverslip. The prepared slides were then examined under a light microscope, and photographs captured using a digital camera. The investigation of the leaf epidermis adhered to the methodology outlined by Adedeji and Jewoola (2008).

Measurement of anatomical parameters

Anatomical parameters were measured using microscopic images captured with a digital camera. These images were cropped using the MS PowerPoint crop tool to focus on distinct regions of both the upper and lower leaf surfaces, and each examined individually. Three replicates were analyzed for each parameter. The investigated anatomical parameters included three measurable parameters and five qualitative aspects.

For the measurable parameters, epidermal cell area was determined by measuring the dimensions (length and width) of epidermal cells in micrometers across three replications using a scale, with MS

Excel used to calculate cell areas. Stomatal number was assessed by counting stomata on both upper and lower leaf sides, recording the counts in Microsoft Excel, and applying appropriate formulas to determine stomatal density. Stomatal size was determined by measuring length and width in cm using a scale, converting the measurements to micrometers, and calculating stomatal size using a proper formula.

For the qualitative parameters, observations were made regarding stomatal types, stomatal shapes, subsidiary cell shapes, guard cell shapes, and trichome types. These observations were conducted using a microscope, and images were captured with a digital camera, following the methodology outlined by Ahmad et al. (2009).

Statistical analysis

Data was represented graphically using Microsoft Excel. Clustered heatmaps and correlograms were generated using R Studio (Version 1.1.463, RStudio, Inc.). Microphotographs were captured using a CCD digital camera (Model: DK 5000) attached to a Leica light microscope (Model: DM 1000). These micrographs proved invaluable for identifying and distinguishing epidermal cells based on their microscopic features.

Results

Epidermal cell area

The study highlights significant variations in adaxial and abaxial epidermal cell areas among the studied *Ficus* species. The recorded results for adaxial epidermal cell area were highly significant. *Ficus racemosa* and *F. lyrata* exhibited substantial epidermal cell areas, while *F. carica* and *F. religiosa* displayed moderate-sized epidermal cells. *Ficus benjamina* and *F. elastica* had smaller epidermal cell areas. In the case of abaxial epidermal cell area, the results were also highly significant. Large epidermal cell areas were observed in *F. carica* and *F. racemosa*, while *F. lyrata* and *F. religiosa* had moderately sized epidermal cells. *Ficus benjamina* and *F. elastica* showed smaller epidermal cell areas. These findings contribute valuable insights into the leaf anatomy of each species, aiding in a comprehensive understanding of their morphological diversity (**Figure 1a**).

Stomatal density

The current study underscores the significant variations in both adaxial and abaxial stomatal densities across the examined *Ficus* species, shedding light on their distinct physiological adaptations. Moving on to adaxial stomatal density, the recorded results were highly significant. Large stomatal densities were observed in *F. elastica* and *F. benghalensis*, while *F. religiosa* and *F. lyrata* displayed moderate stomatal densities. *Ficus benjamina* and *F. retusa* exhibited lower stomatal densities. Finally, for abaxial stomatal density, the results were highly significant. Large stomatal densities were found in *F. racemosa* and *F. elastica*, while

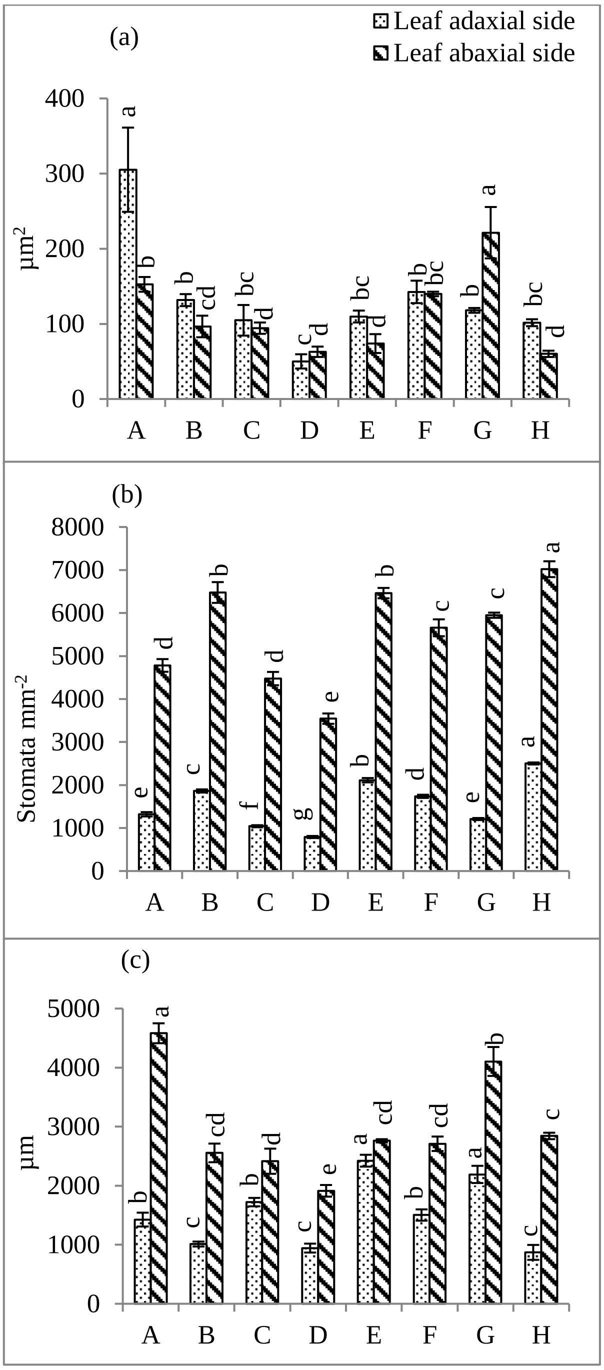

Figure 1: Epidermal cell area of leaf adaxial and abaxial side (a), Stomatal density of leaf adaxial and abaxial side (b), Stomatal size of leaf adaxial and abaxial side (c) of the different species of *Ficus. Ficus racemosa* (A), *Ficus religiosa* (B), *Ficus retusa* (C), *Ficus benjamina* (D), *Ficus benghalensis* (E), *Ficus lyrata* (F), *Ficus carica* (G), and *Ficus elastica* (H) from the botanical garden, University of Agriculture Faisalabad

F. lyrata and *F. benjamina* exhibited moderate stomatal densities. *Ficus benghalensis* and *F. retusa* had relatively lower stomatal densities. These findings contribute to our understanding of the intricate stomatal characteristics within the diverse *Ficus* genus (**Figure 1b**).

Stomatal size

The study reveals significant variations in both adaxial and abaxial stomatal sizes among the examined *Ficus* species. Regarding adaxial stomatal size, the findings were highly significant as well. Large stomatal sizes were recorded in *F. carica* and *F. benghalensis*, while *F. retusa* and *F. lyrata* had moderate stomatal sizes. *Ficus benjamina* and *F. elastica* exhibited smaller stomatal sizes. In the case of abaxial stomatal size, highly significant results were obtained. Large stomatal sizes were observed in *F. racemosa* and *F. carica*, while *F. elastica* and *F. benghalensis* had moderate stomatal sizes. *Ficus benjamina* and *F. retusa* showed smaller stomatal sizes. The observed differences contribute valuable insights into the stomatal characteristics of each species, enhancing our understanding of their unique physiological adaptations (**Figure 1c**).

Stomatal characteristics

All examined species, including *F. elastica, F. benjamina, F. racemosa,* and *F. retusa*, consistently featured oval-shaped stomata on their adaxial leaf surfaces, although there were variations in the number of these stomata (**Figure 2**). Additionally, on the adaxial leaf surface, they all possess a specific type of stomatal structure known as "paracytic," but precise number of these stomata differs among the

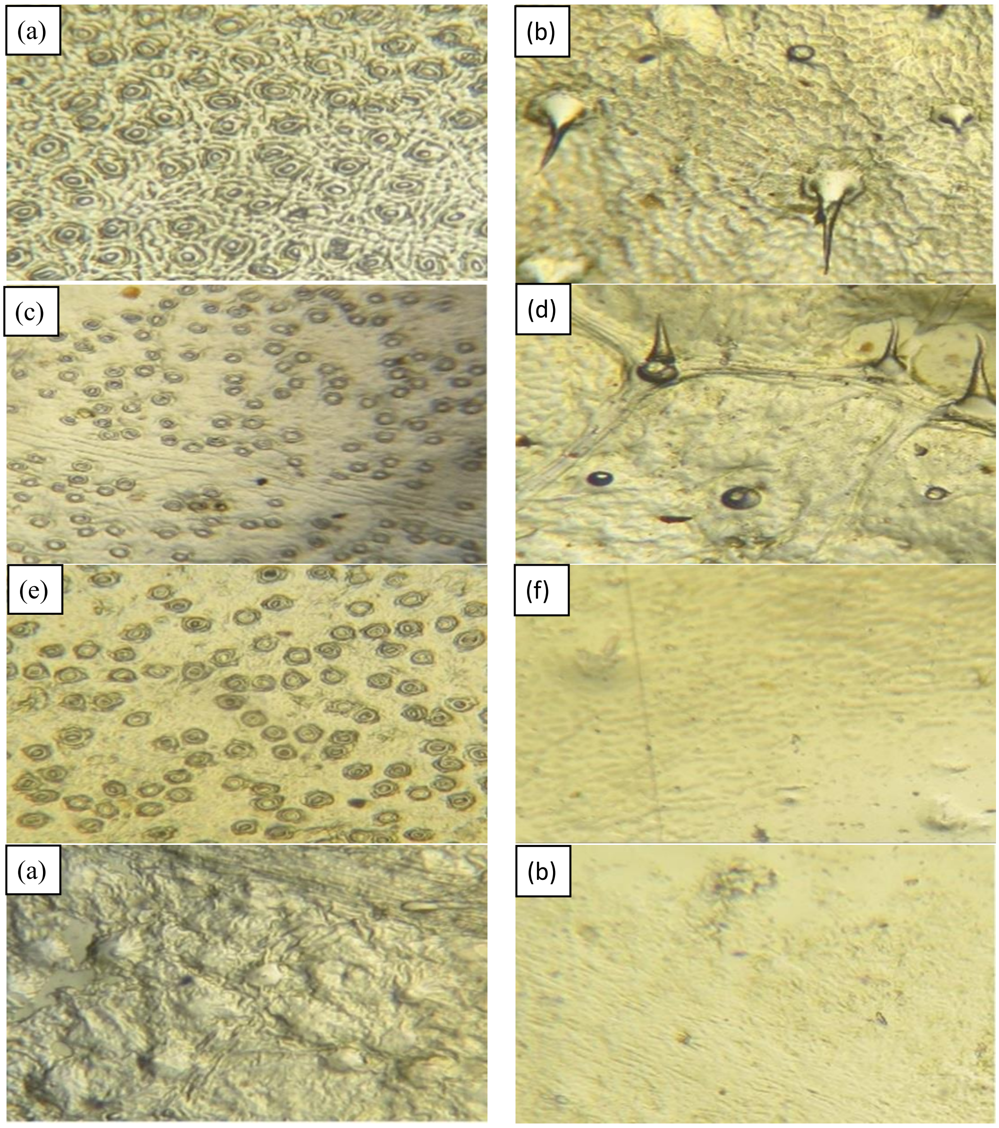

Figure 2. Photographs depicting the abaxial leaf epidermal anatomy of *Ficus elastica* (a), adaxial leaf epidermal anatomy of *Ficus elastica* (b), abaxial leaf epidermal anatomy of *Ficus benjamina* (c), adaxial leaf epidermal anatomy of *Ficus benjamina* (d), abaxial leaf epidermal anatomy of *Ficus racemosa* (e), adaxial leaf epidermal anatomy of *Ficus racemosa* (f), abaxial leaf epidermal anatomy of *Ficus retusa* (g), and adaxial leaf epidermal anatomy of *Ficus retusa* (h)

species. On the abaxial leaf surface, these plant species also exhibit the "paracytic" stomatal type, with variations in number, while their guard cells, responsible for regulating stomatal openings, maintained a consistent kidney-like shape on both adaxial and abaxial leaf surfaces. When the subsidiary cell characteristics on the adaxial leaf surfaces of various *Ficus* species, including *F. elastica, F. racemosa, F. benjamina,* and *F. retusa*, were examined, it was noted that *F. elastica* exhibited polygonal to isodiametric subsidiary cells, while the others displayed either polygonal shapes (*F. racemosa and F. benjamina*) or an irregular pattern (*F. retusa*). On the abaxial leaf surfaces, the subsidiary cells of *F. elastica and F. retusa* appeared irregular, while *F. racemosa and F. benjamina* exhibited polygonal-shaped subsidiary cells. In terms of trichomes, all species were devoid of such subsidiary cells on the adaxial leaf surfaces. However, on the abaxial leaf surfaces, *F. elastica* lacked trichomes, but *F. racemosa* had unicellular, thin, and non-glandular trichomes. However, *F. benjamina* lacked trichomes, and *F. retusa* featured covering and glandular trichomes that were larger than those on the adaxial side. In short, the comprehensive analysis of stomatal characteristics across the examined *Ficus* species, including *F. elastica, F. benjamina, F. racemosa,* and *F. retusa,* reveal consistent features such as oval-shaped adaxial stomata with varying quantities and a 'paracytic' stomatal structure. Notably, guard cells maintained a uniform kidney-like shape on both leaf surfaces. Subsidiary cell variations were observed, with *F. elastica* displaying unique polygonal to isodiametric cells, while others exhibited polygonal shapes or irregular patterns. The absence of adaxial trichomes was consistent across all species, whereas on the abaxial surfaces, trichome presence and characteristics varied, providing valuable insights into the leaf anatomy of these *Ficus* species (**Figure 2**).

Ficus religiosa, F. carica, F. lyrata, and *F. benghalensis* consistently displayed oval-shaped stomata on their adaxial leaf surfaces, although there were variations in the number of these stomata (**Figure 3**).

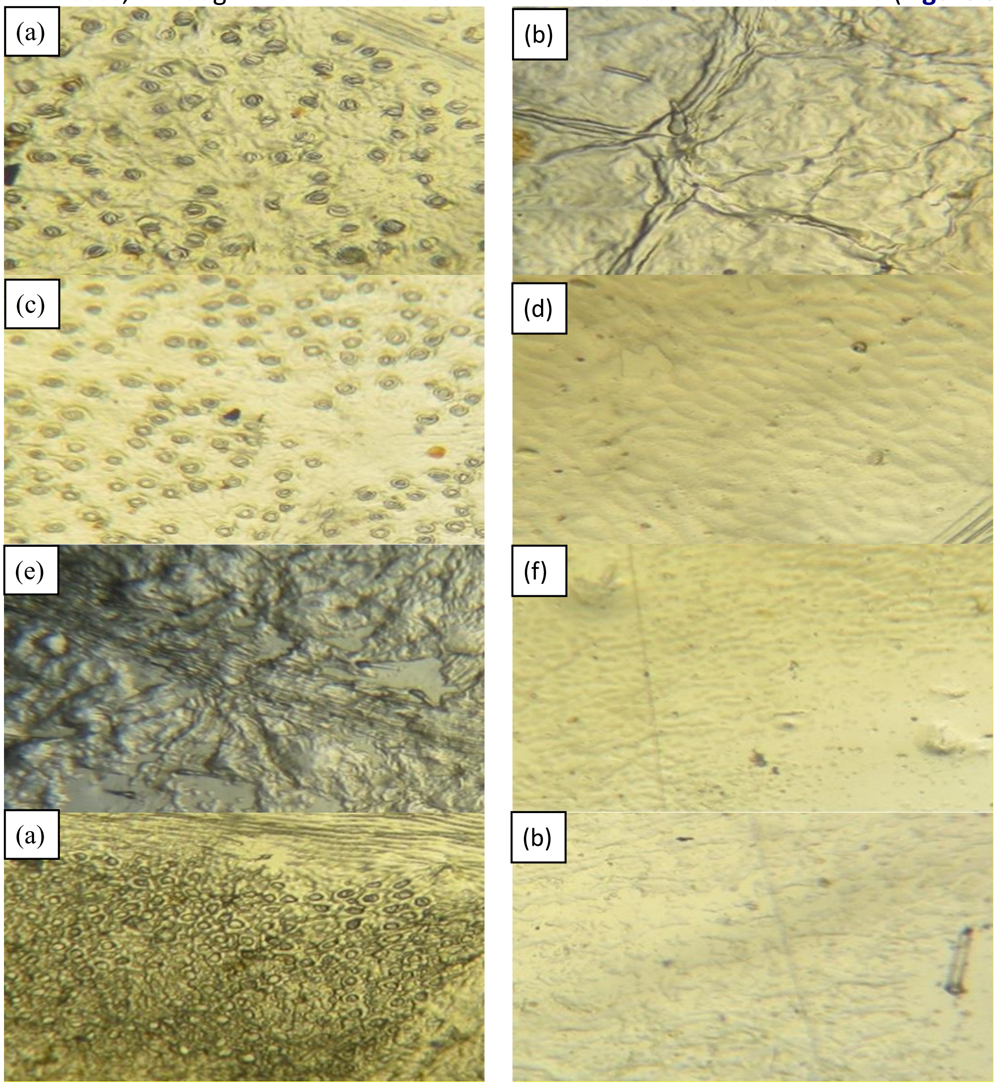

Figure 3. Photographs of abaxial leaf epidermal anatomy of *Ficus religiosa* (a), adaxial leaf epidermal anatomy of *Ficus religiosa* (b), abaxial leaf epidermal anatomy of *Ficus carica* (c), adaxial leaf epidermal anatomy of *Ficus carica* (d), abaxial leaf epidermal anatomy of *Ficus lyrata* (e), adaxial leaf epidermal anatomy of *Ficus lyrata* (f). abaxial leaf epidermal anatomy of *Ficus benghalensis* (g), and adaxial leaf epidermal anatomy of *Ficus benghalensis* (h)

Moreover, on the adaxial leaf surface, they all share a specific type of stomatal pattern, known as "paracytic," but the exact count of these stomata differs. On the abaxial leaf surface, these species also exhibited the same "paracytic" type of stomata, again with variations in number, while their guard cells, responsible for regulating these stomatal openings, maintained a consistent kidney-like shape on both the adaxial and abaxial leaf surfaces. When the subsidiary cell shapes on the adaxial leaf surfaces of various species, including *F. religiosa, F. carica, F. lyrata*, and *F. benghalensis*, were examined, it was observed that they all exhibited a common polygonal shape. However, a subtle distinction arose on the abaxial leaf surfaces, where *F. lyrata* departed from the polygonal pattern and exhibited an irregular shape, while the others maintained the polygonal configuration. Shifting the focus to trichomes, on the adaxial leaf surfaces, both *F. religiosa* and *F. lyrata* lacked trichomes, whereas *F. carica* boasted unicellular prickle-type trichomes, and *F. benghalensis* shared the absence of trichomes with the former two. On the abaxial leaf surfaces, *F. religiosa* and *F. lyrata* remained trichome-free, while *F. carica* possessed thin and dense trichomes, and *F. benghalensis* bore unicellular and non-glandular trichomes.

Cluster analysis

The comprehensive cluster analysis unveiled distinctive groupings among the examined *Ficus* plants, shedding light on the relationships and shared characteristics within the botanical dataset. Through this analytical lens, a valuable insight has been obtained into the connections that categorize these plant species into four distinct groups, enriching the understanding of their intricate associations and individual traits. The analysis of the provided connections and relationships revealed that the plants under consideration can be distinctly categorized into four groups. These groupings are the result of a comprehensive cluster analysis, which helped us better understand the associations between these plant species.

Group 1: In the first group, we find *F. carica* and *F. lyrata*. What stands out is the strong connection between these two species, indicating a notably close relationship between them. This connection suggests that *F. carica* and *F. lyrata* share certain characteristics or traits that set them apart from the other species in the study.

Group 2: Moving on to the second group, it comprises *F. retusa* and *F. benjamina*. These two species are closely linked to each other, which imply that they may share specific features. This shared characteristic distinguishes them from the species in the other groups.

Group 3: The third group is an interesting cluster, including *F. elastica*, *F. religiosa*, and *F. benghalensis*. These three species are interconnected, indicating a degree of similarity among them. This suggests that *F. elastica, F. religiosa, and F. benghalensis* may have common traits that align them within this group.

Group 4: It consists of *F. racemosa*. While *F. racemosa* is not directly connected to any other species in the study, it exhibits some similarities to all the other plants. This suggests that *F. racemosa* possesses certain shared features with the broader set of plants, even if it doesn't have a direct connection to any single species within the dataset.

In essence, this clustering analysis not only categorizes these plants but also provides valuable insights into their relationships and commonalities. It highlights the distinct characteristics that define each group and, by extension, each plant species, contributing to our understanding of these *Ficus* taxa (**Figure 4**).

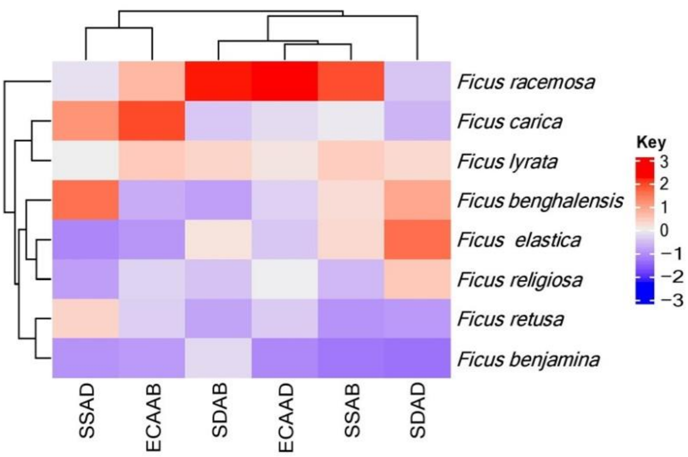

Figure 4. Cluster analysis of different leaf anatomical features of different taxa from old botanical garden, University of Agriculture Faisalabad.

ECAAD (Epidermal cell area adaxial), ECAAB (Epidermal cell area abaxial), SDAD (Stomatal density adaxial), SDAB (Stomatal density abaxial), SSAD (Stomatal size adaxial), and SSAB (Stomatal size abaxial)

In summary, the cluster analysis effectively categorizes the examined *Ficus* species into four distinct groups, revealing meaningful associations and relationships. These groupings, such as the close connection between *F. carica* and *F. lyrata* in Group 1 or the shared characteristics among *F. elastica, F. religiosa*, and *F. benghalensis* in Group 3, provide valuable insights into the underlying similarities and traits that define each plant group. The clustering approach enhances our understanding of the relationships and commonalities within the studied *Ficus* taxa, offering a comprehensive perspective on their botanical connections (**Figure 4**).

Correlation analysis

In the context of identification of different *Ficus* taxa, these relationships provide essential insights. Positive correlations often suggest similarities in anatomical features, while negative correlations indicate differences. The relationships can help taxonomists distinguish between species based on leaf anatomy, contributing to a deeper understanding of *Ficus* taxonomy. The parameter-to-parameter correlations further illustrate how these traits interact, offering valuable information for plant classification. To provide a comprehensive understanding of the relationships between plants, parameters, and parameter-to-parameter correlations, we have broken down the data step by step.

a. Relationships between species

Ficus lyrata is positively correlated with *F. benjamina* in stomatal density abaxial (SDAB), suggesting that these two species have a similar pattern of stomatal density. *Ficus lyrata* is positively correlated with *F. elastica* in stomatal size abaxial (SSAB), indicating similarities in stomatal size between these two plants. *Ficus benjamina* shows positive correlations with *F. elastica* in stomatal density abaxial (SDAB) and stomatal size abaxial (SSAB), implying similarities in these anatomical features. *Ficus benjamina* is also positively correlated with *F. carica* in epidermal cell area adaxial (ECAAD). *Ficus racemosa* has strong positive correlations with *F. elastica* in both stomatal density abaxial (SDAB) and stomatal size abaxial (SSAB), indicating shared anatomical characteristics in these parameters. It also shows positive correlations with *F. benjamina* and *F. carica* in epidermal cell area adaxial (ECAAD), suggesting commonalities in epidermal cell size. *Ficus elastica*, in addition to its correlations with *F. racemosa*, is positively correlated with *F. religiosa* in stomatal density abaxial (SDAB) and epidermal cell area adaxial (ECAAD). It also shows a negative correlation with *F. benghalensis* in stomatal size abaxial (SSAB), indicating differences between these species in stomatal size. *Ficus religiosa* has a positive correlation with *F. carica* in epidermal cell area adaxial (ECAAD), suggesting similarities in this parameter. *Ficus carica*, besides its correlations with *F. benjamina* and *F. racemosa*, has a positive correlation with *F. retusa* in stomatal density abaxial (SDAB). *Ficus retusa* shows positive correlations with *F. benjamina* in stomatal density abaxial (SDAB) and stomatal size abaxial (SSAB). *Ficus benghalensis*, as the top species on the y-axis, does not exhibit direct correlations with the other species in the x-axis. This suggests distinct differences in leaf anatomical features (**Figure 5a**).

b. Relationships between parameters

Stomatal density abaxial (SDAB) and epidermal cell area adaxial (ECAAD) show a negative correlation, indicating that, in general, as stomatal density increases, epidermal cell area tends to decrease. Stomatal density abaxial (SDAB) and stomatal size abaxial (SSAB) show a negative correlation, suggesting that an increase in stomatal density is often accompanied by a decrease in stomatal size. Epidermal cell area adaxial (ECAAD) and stomatal size abaxial (SSAB) have a positive correlation, meaning that larger epidermal cell areas are associated with larger stomatal sizes (**Figure 5b**).

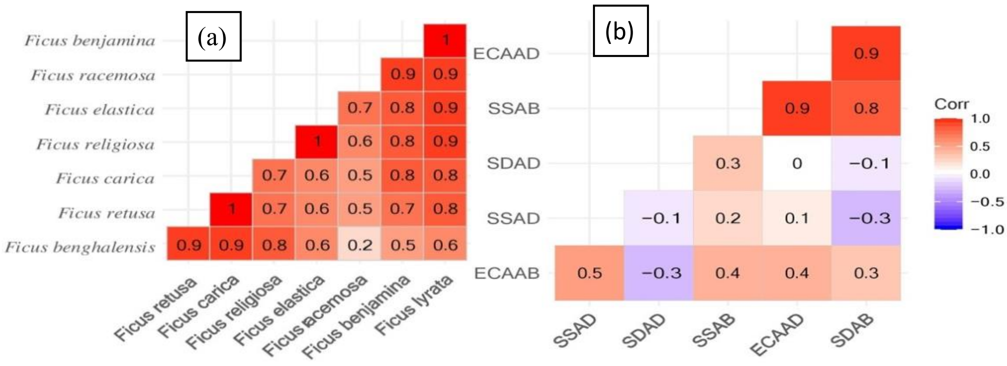

Figure 5. Correlation plot between species (A) and correlation plot between variables (B) of different leaf anatomical features of different *Ficus* species

ECAAD (Epidermal cell area adaxial), ECAAB (Epidermal cell area abaxial), SDAD (Stomatal density adaxial), SDAB (Stomatal density abaxial), SSAD (Stomatal size adaxial), and SSAB (Stomatal size abaxial)

Discussion

The taxonomic value of leaf epidermal characteristics is well documented and is useful in taxonomy (Adedeji and Jewoold, 2008; Qiu et al., 2023). The morphological and anatomical features of leaves are an important part of the plant that are used in plant identification. In the current study, leaf epidermal anatomical features were found to be the most important diagnostic characteristics of the *Ficus* species to differentiate. Leaf epidermal characteristics like epidermal cell area and shape of guard cells, stomatal size, shape, types and density of subsidiary cells, and types of trichomes were observed and used as identification tools.

The variations in adaxial and abaxial epidermal cell areas, stomatal size, and stomatal density provide valuable insights for taxonomists for classifying and differentiating these species. For instance, the substantial adaxial epidermal cell areas observed in *F. racemosa* and *F. lyrata*, in contrast to the smaller areas in *F. benjamina* and *F. carica*, could potentially be used as diagnostic features in taxonomic keys. Similarly, the differences in stomatal size and density, with *F. elastica* and *F. benghalensis* consistently showing larger stomatal sizes and densities, may serve as taxonomic markers. These findings contribute to a better understanding of the morphological diversity among *Ficus* species, aiding taxonomists in refining species descriptions and classifications within this genus, which is essential for accurate plant identification and the broader field of plant taxonomy. It is believed that cell shapes, anticlinal wall types, and the stomatal characteristics reveal some correlations among different plant taxa (Agbolade et al., 2011).

The consistent presence of oval-shaped stomata and "paracytic" stomatal structures across the examined *Ficus* species establishes a common feature, while variations in stomatal quantity and guard cell shape offer distinctions. Shape and size of stomatal complex can be used as an important character in taxonomic studies (Paul and Chowdhury, 2021). For example, *Plantago ovata* can be differentiated by amphianisocytic type of stomatal complex on both adaxial and abaxial surfaces as reported by Kanwal et al. (2012). Subsidiary cell shapes, particularly the polygonal to isodiametric cells in *F. elastica,* provide additional taxonomic cues. The presence or absence of trichomes, such as the larger glandular trichomes on the abaxial leaf surface of *F. retusa*, may further contribute to species differentiation. This research demonstrates the significance of leaf epidermal anatomy in accurate taxonomic identification, not only within the *Ficus* genus, but also in a broader botanical context, suggesting its potential for enhancing our understanding of plant diversity and aiding taxonomists in the classification of various taxa. Further research across a wider range of plant species can help validate and expand upon the taxonomic utility of epidermal characteristics as already highlighted elsewhere (Adedeji and Dloh, 2004; Patel et al., 2021; Qiu et al., 2023).

The studied *Ficus* species consistently exhibited oval-shaped, "paracytic" stomata on their adaxial and abaxial leaf surfaces, with variations in stomatal quantity, making these features valuable for taxonomic distinctions. The polygonal subsidiary cell shape on adaxial surfaces is a common trait, except for *F. lyrata*, which displays an irregular shape on the abaxial leaf surface, providing an additional taxonomic marker. Moreover, trichomes vary among species, with *F. carica* having unicellular prickle-type trichomes on the adaxial surface, and thin and dense trichomes on the abaxial surface, while *F. benghalensis* featured unicellular, non-glandular trichomes. For example, trichomes have been reported to have taxonomic significance and this can be used as an additional feature to differentiate the species (Uzunova et al., 2007; Chwil et al., 2015). These findings emphasize the role of leaf epidermal characteristics in taxonomic identification and highlight the morphological diversity within the *Ficus* genus, offering insights into broader plant taxonomy. Further research on a wider range of plant taxa can expand our understanding of the taxonomic utility of these traits.

Cluster analysis of the studied *Ficus* species reveals four distinct groups. Group 1 (*F. carica* and *F. lyrata*) shared close connections, indicating shared distinguishing characteristics. Group 2 (*F. retusa* and *F. benjamina*) exhibited common attributes that set them apart. Group 3 (*F. elastica*, *F. religiosa*, and *F. benghalensis*) showcased interconnectedness, hinting at shared traits. Group 4 (*F. racemosa*) displayed similarities to all others, despite no direct links. These clusters shed light on relationships, shared traits, and possible ecological or evolutionary adaptations among these *Ficus* species, enriching our understanding of their taxonomy and ecology. Further research into the specific characteristics within each group can provide deeper insights into their unique attributes. Over the last four decades micromorphological characters of the leaf epidermis have been scrutinized in several plant groups (Patel et al., 2021; Qiu et al., 2023; Zheng et al., 2023). These attributes have been informative at various taxonomic levels and valuable to differentiate among groups of extant taxa with putative relatives available in the fossil record (Reimer et al., 2007).

The research results provide valuable insights into the relationships and correlations between different *Ficus* species and various leaf anatomical parameters. Notable findings include positive correlations between species such as *F. lyrata* and *F. benjamina*, indicating similarities in stomatal density, and between *F. elastica* and *F. religiosa*, with shared characteristics in stomatal density and epidermal cell area. These relationships are critical for understanding the taxonomic distinctions between the species within *Ficus* genus. Conversely, *F. benghalensis* appears to be distinct, lacking direct correlations with other species, implying significant differences in leaf anatomical features. The parameter-to-parameter correlations, such as the negative correlation between stomatal density and epidermal cell area, highlight how these traits interact and can serve as valuable markers for taxonomic purposes. Overall, the data offers valuable insights into the relationships and anatomical features of different *Ficus* taxa, contributing to our understanding of their taxonomy and ecological adaptations.

Conclusion

This research underscores the significant taxonomic value of leaf epidermal characteristics, demonstrating their utility as diagnostic tools for differentiating *Ficus* species. The variations in adaxial and abaxial epidermal cell areas, stomatal size, shape, and density, subsidiary cell shapes, and presence or absence of trichomes provide valuable insights for accurate plant identification and classification. The consistency of oval-shaped stomata and "paracytic" stomatal structures across species establishes a common feature, while variations in stomatal number and guard cell shape offer distinctions. The cluster analysis highlights distinct groupings within the *Ficus* genus, shedding light on relationships and shared characteristics among the *Ficus* species. Positive and negative correlations between species and anatomical parameters contribute to our understanding of taxonomic relationships. These findings not only enhance our knowledge of *Ficus* taxonomy but also have broader implications for plant classification, emphasizing the significance of leaf epidermal anatomy in the field of plant taxonomy.

Author(s), Editor(s) and Publisher's declarations

Acknowledgement
This paper is derived from the M.Phil. study of Altaf Hussain submitted to Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Source of funding
None declared.

Contribution of authors
Conceptualized and designed the study: AH, MSAA, AR. Conducted research and wrote up the first draft of the manuscript: AH. Reviewed and edited the manuscript: AH, AR, WR, MSAA.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors have completely read the manuscript and agreed to publish it in IJAaEB.

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