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RESEARCH ARTICLE

Comparative Morphological and Anatomical Studies on Young Leaves of *Ficus* Species

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ABSTRACT

Background: The genus *Ficus* comprises a diverse group of plant species known for their ecological, ornamental, and medicinal value. Its species exhibit significant variation in leaf morphology, anatomical structures, and physiological traits, making them ideal candidates for comparative botanical studies.
Objective: A comparative study was conducted to investigate the morphological and anatomical characteristics of selected *Ficus* species collected from the New Botanical Garden, University of Agriculture, Faisalabad, between November and February.
Methodology: The second fully emerged leaf from each species was sampled for analysis. Key parameters included leaf area, petiole length, and features of the lower epidermis. Epidermal layers were peeled and preserved in FAA solution for microscopic evaluation of stomatal density, number of subsidiary cells, pore size, and guard cell dimensions.
Results: *Ficus elastica* exhibited the highest stomatal density, while *F. natalensis* showed the lowest. The petiole length of *F. elastica* increased gradually over time, whereas *F. natalensis* maintained a consistently short petiole without a clear pattern of change. The leaf area of *F. elastica* decreased from November to December and increased again in January. *F. natalensis* consistently showed the smallest leaf area throughout the study period. The guard cell length of *F. elastica* decreased gradually over time, whereas in *F. hawai,* it showed an increasing trend. Guard cell width increased in *F. macrophylla* but decreased in *F. elastica* over the study period.
Conclusion: Significant anatomical and morphological variations between Ficus species were found in this study. *F. natalensis* had the smallest and most stable leaf area with the least amount of variation in petiole length, whereas *F. elastica* displayed the highest stomatal density and increasing petiole length over time. Species-specific responses to seasonal circumstances were recorded in pore diameters, guard cell size, and stomatal features across *Ficus* species and observation months.

INTRODUCTION

The genus Ficus is among the premier genera of shrubs and trees, which are known for its medicinal, socioeconomic and religious importance. As regards its medicinal importance, the *Ficus* spp. are known to biosynthesize bioactive compounds including phenolics, flavonoids, terpenes etc., which are used to treat the ailments such as diabetes, toothache, gum infection, piles etc. (Salehi et al. 2021; Devi *et al.* 2022). Interestingly all parts of plant including bark, roots, leaves and fruits are used in the treatment of various ailments (Murugesu *et al.* 2021).

Morphological features of plants have close association with the biochemical pathways operative in leaves and other parts (Klem *et al.* 2019). Photosynthesis, which depends upon the stomatal functions in the exchange of water and gases, is one of the fundamental processes involved in the production of primary and secondary metabolites. Hence, the stomatal density and function in the leaves may directly influence the synthesis of primary metabolites and indirectly the secondary metabolites (Ördög et al. 2013).

Plants have historically been categorised according to the appearance of their fruits and blooms. In order to accurately identify and classify closely related taxa, plant

scientists have been using leaf epidermal morphology (Abdulrahaman and Oladele 2010; Qiu *et al*. 2023; Hussain *et al*. 2025). Moreover, to differentiate between various taxonomic ranks, such as families, tribes, genera, and species, morphological traits are essential (Alaida and Aldhebiani 2022). However, several species of flowering plants have similar morphological attributes, so it might be difficult to differentiate them based only on morphological traits (Nazir *et al*. 2013). Determining the taxonomic placements of these species thus requires an awareness of both the similarities and differences within a sub-genus. Numerous crucial micromorphological characteristics were identified through the examination of *Ficus* epidermal surfaces; some of these features exhibit noteworthy interspecific variations that are significant from a taxonomical standpoint (Mubo *et al*. 2004).

Essential diagnostic features of the epidermis that offer helpful suggestions for identification include size, stomata orientation, stomata form, guard cell shape, and structural features of epidermal cells (Munir *et al*. 2011; Trofimov and Rohwer 2018). In a related study, it was shown that trichome size and shape, stomata size and form, and the presence or absence of stomatal clusters were useful diagnostic traits for differentiating distinct fern species (Rahman *et al*. 2017).

Although it is now thought that epidermal traits are important for taxonomy, there is little information on the epidermal morphology of *Ficus* (Hussain *et al*. 2025). Notable characteristics such as epidermal cell structure, shape, orientation, stomatal complex size, and trichome types have major taxonomic implications (Rahman *et al*. 2017).

Leaf is the source tissue where the biosynthesis of majority of medicinally and economically important compounds takes place. *Ficus* is an important genus known for the synthesis of quite a few metabolites of medicinal value (Devi *et al.* 2022), which may be associated to the metabolite profiles. Thus, the current study was conducted to gain a comprehensive understanding of the importance of leaf epidermal structure in the identification of *F. elastica*, *F. elastica* and *F. natalensis*. This study will add to the existing literature on the use of leaf epidermal architecture in plant identification and provide insights for researchers and practitioners in plant taxonomy and categorisation.

MATERIALS AND METHODS

An experiment was conducted in order to explore stomatal modifications in lower leaf lamina, petiole length and leaf surface area of different *Ficus* species in November, December and January. The leaves were collected from the New Botanical Garden, University of Agriculture, Faisalabad. Samples were preserved in FAA (formaldehyde-acetone-alcohol) fixative for 24 hours and then shifted to 70% ethanol for preservation. The hand sectioning of the leaf lamina was performed. The leaf lamina was peeled off from the selected leaf samples and immediately placed in 30% ethanol for 10–15 minutes. These tissues were shifted to 50% ethanol, followed by 70% ethanol for 10–15 minutes, respectively. After 70% ethanol treatment, a few drops of safranin were added for 5 minutes. Safranin with 90% ethanol was used for 5 minutes. Then samples were washed 2–3 times with 100% ethanol. The samples were treated with 25%, 50% and 100% xylene for clearing them. Each section was permanently preserved in a drop of Canada balsam. The images of the samples were taken under an electron microscope.

RESULTS

Length of petiole

The comparison of different *Ficus* species petiole length recorded at different intervals (November, December and January) showed significant variations. Data showed a gradual increase in the petiole length of *F. elastica* over time. In contrast, *F. natalensis* consistently exhibited the minimum petiole length throughout all the experimental months, whereas other *Ficus* species showed no specific pattern in increase or decrease in length (Fig. 1A).

Leaf area

Results revealed that *F. elastica* var. varigata leaf area was decreased from November to December and again increased from December to January. The *F. natalensis* had the smallest leaf area but remained constant throughout all the experimental months (Fig. 1B).

Number of stomata

The comparison of *Ficus* species leaves epidermis collected at different time intervals (dawn, dusk, mid-day) in winter months revealed that there's a subsequent increase in the opening of stomata during mid-day. Among the species studied, *F. natalensis* had the maximum no. of stomata in November and December, but in January, *F. elastica* var. varigata had the maximum no. of stomata. Moreover, *F. elastica* 'Burgundy' had the least no. of stomata in almost all data taking months (Fig. 1C).

Number of subsidiary cells

Data showed that *F. natalensis* showed the maximum no. of stomata and *F. hawai* and *F. macrophylla* had the least no. of subsidiary cells (Fig. 1D).

Stomatal pore area

Results showed that the stomatal pore size of *F. macrophylla* and *F. elastica* 'Burgundy' gradually increased across the observation periods, while *F. natalensis* pore size increased from November to December and then decreased from December to January (Fig. 2A–4).

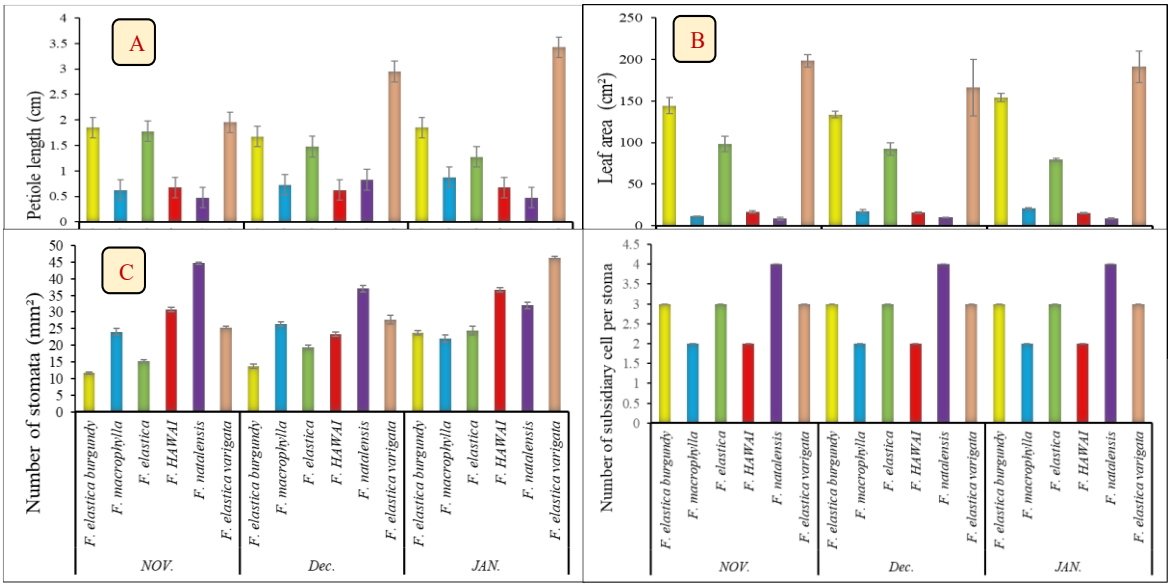

Fig.1: Petiole length (A), Leaf area (B), number of stomata (C) and number of subsidiary cells of different *Ficus* species during different winter months.

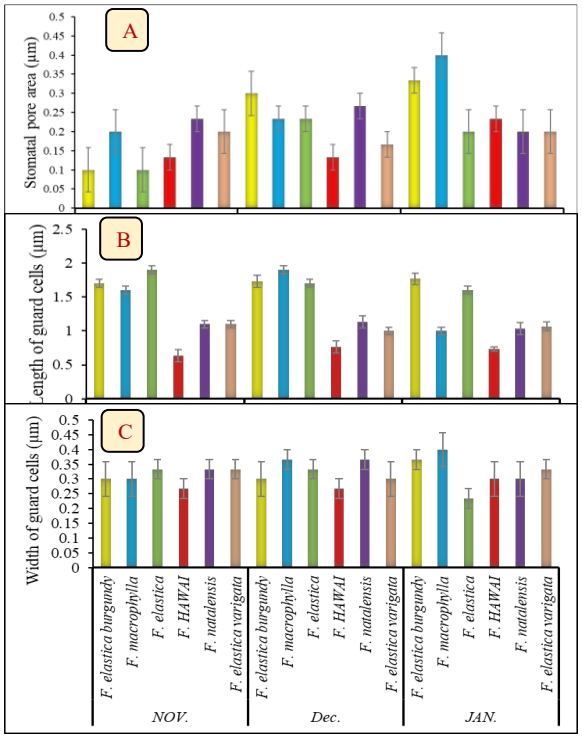

Fig. 2: Stomatal pore size (A), length of guard cells (B), and width of guard cells (C) of subsidiary cells of different Ficus species during different winter months.

Length of guard cells

The comparison of different *Ficus* species based on the length of guard cells recorded at different intervals in winter months (November, December and January) showed subsequent variations (Fig. 3–4). Results showed that the length of the guard cells of *F. elastica* gradually decreased over time, while the length of the guard cells of *F. hawai* increased across the observation periods (Fig. 2B).

Width of guard cell

The results for guard cell width showed a gradual increase over time in *F. macrophylla*, whereas in *F. elastica*, guard cell width decreased with the passage of time (Fig. 7).

DISCUSSION

The genus *Ficus*, commonly referred to as fig, belongs to the family Musaceae. *Ficus* is one of the largest plant genera, with more than 750 described species distributed worldwide, mainly in tropical countries. Fig trees are often ecologically important 'Keystone' components of tropical forests, because of the large number of vertebrates that feed on their figs, more than any other group of plants (Hussain *et al*. 2025).

A study was conducted to compare different morphological and anatomical characteristics of *Ficus* species. The result revealed that *F. elastica* had the maximum number of stomata, and the least were found in *F. natalensis*. The petiole length of *F. elastica* var. *varigata* increased over time. Over time, plants evolved different mechanisms to optimise their stomatal density in response to environmental cues to enhance their survival and fitness (Hou *et al*. 2023) (Fig. 1–2). Moreover, *F. natalensis* had shorter petiole length and showed no specific pattern in the increase and decrease in length during the observation period. The leaf area of *F. elastica* var. *varigata* decreased from November to

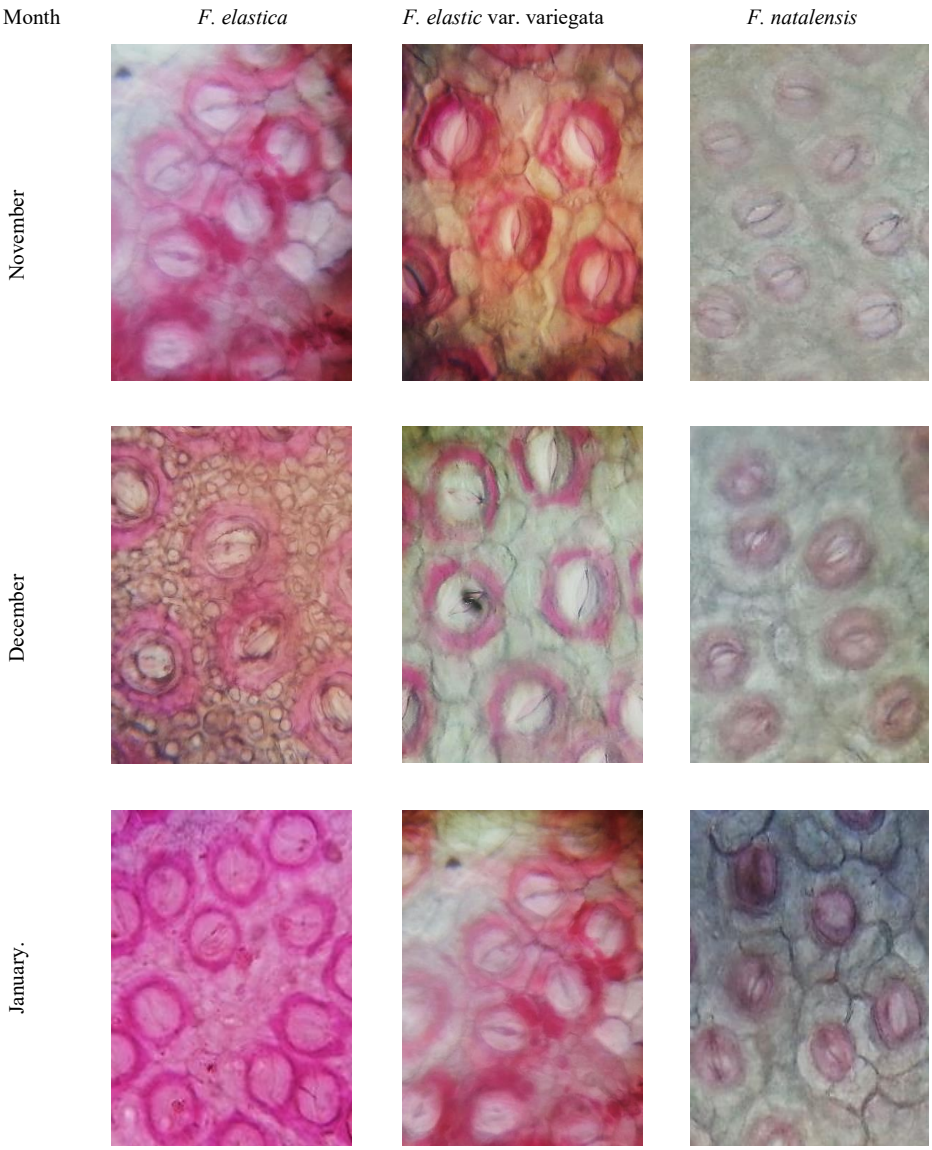

Fig. 3: Anatomical variations in stomatal complex of different *F. elastica*, *F. elastica* 'Burgundy' and *F. natalensis* during different winter months

December. and again, increased from December to January. In contrast, Shrestha *et al*. 2025) reported non-significant changes in the leaf area *of F. religiosa* during the winter season. In contrast, *F. natalensis* had the smallest leaf area, and no variation was marked from November to January. Furthermore, F. natalensis had the maximum number of stomata, and *F. hawai* and *F. macrophylla* had the least number of subsidiary cells. Stomatal pore area of *F. macrophylla* and *F. elastica* 'Burgundy' gradually increased with the passage of time, while *F. natalensis* pore area increased from November to December and then decreased from December to January (Fig. 3–4). Stomatal pore size or movement follows circadian rhythm, which is influenced by light exposure and the plants internal clock (Hou *et al*. 2023).

The length of the guard cell of *F. elastica* gradually decreased over time, while in *F. hawai* it increased from November till January. In addition, the width of the guard cells of *F. macrophylla* gradually increased across the observation period, and in *F. elastica* the guard cell width decreased, respectively (Fig 1–4). Plants are affected by the environmental conditions during all phases of growth and development. Especially, stomatal number reportedly changes when plants are grown in different seasons. Although the measurements were done on an area basis, this approach does not account for possible anatomical changes during different environmental conditions. It seems likely that the stomatal pattern may operate and respond to a range

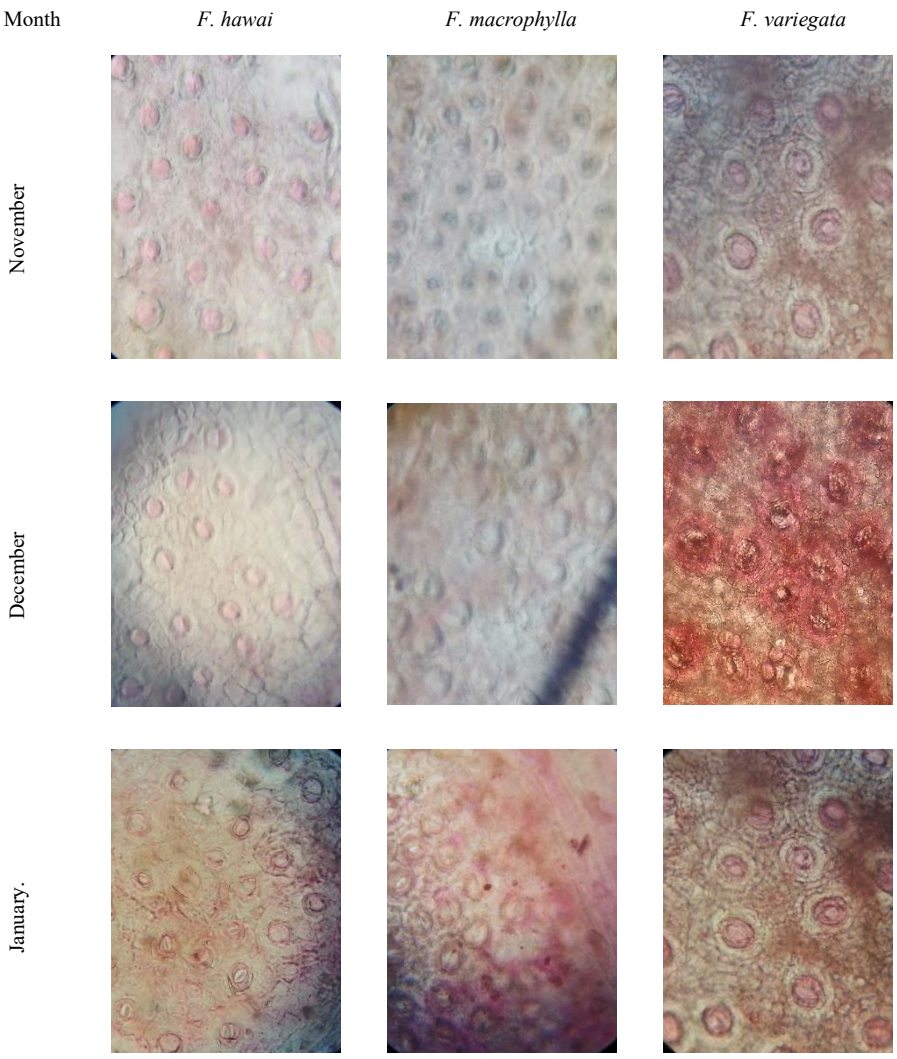

Fig. 4: Anatomical variations in stomatal complex of different *F. hawai*, *F. macrophylla* and *F. variegata* during different winter months.

of conditions that can be explored in further studies. Stomata frequency declined in response to increasing CO_2 and may have occurred over geological time (Hofmann *et al*. 2025).

CONCLUSIONS

changes in the number of epidermal cells, such as the number of stomata and subsidiary cells of *Ficus* species, were greatly affected by the environmental and climatic changes. These differences highlight the adaptive responses of each species to seasonal and diurnal changes. This study highlighted the taxonomic importance of leaf epidermal features, emphasising their effectiveness as diagnostic traits for distinguishing among *Ficus* species. Future research should focus on the molecular and physiological mechanisms underlying stomatal regulation and anatomical adaptations in Ficus species under varying environmental conditions. Long-term field studies and genetic analyses could provide deeper insights into their adaptive strategies.

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DATA AVAILABILITY

The data will be made available on a fair request.

ETHICS APPROVAL

Not applicable to this paper.

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