*Pak. J. Bot.*, 56(1): 85-95, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-1(31)

# LEAF MICROMORPHOLOGICAL TRAITS OF LEGUMES FROM TAKKAR WILDLIFE SANCTUARY

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**Abstract**

This study elucidates the micro-morphology of leaf among wildlife sanctuary inhabited leguminous species adapted to arid environment. The foliar structures that enable its survival in arid conditions are little recognized. The comparative anatomical attributes of 10 leguminous species were analyzed using bio imaging microscopic techniques. Both surfaces of the leaves exhibited differences in their leaf anatomical traits including type of stomata, epidermis, anticlinal wall, and diversity of trichomes. The epidermal cells shape was reported as polygonal, tetragonal to pentagonal and wavy. The largest epidermal cells were examined in *Dalbergia sisso* (47.5 µm) on adaxial side and *Prosopis cineraria* (38.5 µm) on abaxial surface. Largest stomatal complex was recorded for *Dalbergia sisso* (24.9 µm) on abaxial, while the smallest (8.28 µm) for *Prosopis juliflora* on adaxial side. Unicellular trichomes were observed on both surfaces of *Crotalaria burhia* while glandular trichomes were located in the coastal zone of *Prosopis juliflora*. The easy and quick identification of micromorphological markers of leguminous species reflect their adaptations to aridity in a wildlife sanctuary habitats. The current findings of the foliar micromorphological traits are of special interest for plant taxonomists for the correct identification of leguminous species.

**Key words:** Foliar traits; Paracytic stomata; Microscopic peculiarities; Wildlife Sanctuary.

## Introduction

Fabaceae comprises 730 genera and 19,325 species (Soares *et al*., 2021). Family Fabaceae is also referred to as the bean, pea, or legume family. The Fabaceae is the third largest terrestrial plant family after the Asteraceae and Orchidaceae. The family is widely scattered in tropical regions and is thought to be worldwide due to the large number of species diversity (Barrett *et al*., 2021; Anjum *et al*., 2022). The Fabaceae plants are very diverse; it primarily consists of annual and perennial herbaceous plants, shrubs, and trees that are easily identified by their fruit or legume (Group *et al*., 2016). Plants in the family are also distinguished by the complex stipulate leaves (Shaheen *et al*., 2020). They are abundant in Pakistan's temperate, sub-temperate, grassland and timber grassland habitats, as well as subtropical regions. Plants of the Fabaceae are widely distributed in dry grassland areas. Despite systematic differences, the Fabaceae origin appear to be monophyletic (Uzma *et al*., 2012).

The introduction of microscopy has introduced a new dimension to the morphological features that are available for systematic implications (Chen *et al*., 2020; Majeed *et al.,* 2023). The diversity of trichomes was analyzed using imaging techniques, and their taxonomic relevance was discussed (Melo *et al*., 2010). Using the SEM technique, several trichome types were found to cover the leaf surface, with stomatal complex features having a substantial taxonomic value (Jabeen *et al.,* 2022; Manzoor *et al.,* 2023). The foliar epidermal structure provides significant relevant details to distinguish between the angiosperm groups. Various epidermal aspects, like stomata, hairs, and the length and shape of epidermal cells, have become key identification features for categorizing species within the dicot angiosperm families (Hong *et al*., 2011).

Two methods of identifying plants are employed in the field of plant taxonomy. Macromorphological traits such as inflorescence types, phyllotaxy, stems, aroma, and fruit observation are used to first observe plants. Second, plants are studied on a micromorphological scale to better understand their surface characteristics (Esfandani-Bozchaloyi and Zaman, 2018). Anatomical attributes are very helpful in identifying relationships between different orders and taxa, and their aspects have gained significance in evolutionary relationships. In a variety of plant groups, comparative foliar anatomy has proven to be taxonomically and diagnostically significant. Anatomical microstructure were examined using a variety of botanical techniques for their histological description (Majeed *et al*., 2022).

Micromorphological studies provide an important role in identifying and categorizing plant species within certain Angiosperm land plants (Abbas *et al*., 2022). The characteristics of leaf epidermal anatomy contribute significantly to the resolution of taxonomic issues. Many authors have described the use of epidermal morphology in their taxonomic research and botanical reviews. Furthermore (Ayodele & Olowokudejo, 2006) are the scientists who have successfully used aspects of leaf epidermal structure to address taxonomic issues. (Ahmed *et al*., 2016) the leaf is the non-reproductive organ that is most frequently utilized in plant taxonomy, and according to (Alege & Shaibu, 2015), the leaf epidermis is the second-most significant characteristic after cytology for resolving taxonomic and evolutionary issues. The epidermis of the leaves acts as a dynamic barrier between the internal environment of the plant and the outside

environment (Alege & Shaibu, 2015; Gonzalez & Marazzi, 2018). Stomata, epidermis, cuticle, trichomes, and subsidiary cells are examples of traits that have been shown to be effective (Hong *et al*., 2011).

Due to their limited utility in identifying species or taxa of intraspecific rank, foliar anatomical features have received little attention in taxonomic studies. Statistical examination of a large number of samples is required since the differences between species are typically quantifiable rather than qualitative (Zoric *et al*., 2012). The largest genera include *Acacia*, *Astragalus* and *Mimosa* (Dzoyem *et al*., 2014). (Martínez Quesada, 1997) investigated the trichomes and leaf epidermis of seven taxa of *Indigofera* (Fabaceae). Anisocytic stomata were reported in most cases while leafs were amphistomatic. Trichomes on leaflets were found to be multicellular multiseriate and uniseriate. (Retallack & Willison, 1988; Zoric *et al*., 2012) studied foliar epidermal characters of some species of *Trifolium.* Characters that were analysed in the research were stem and leaf anatomy as well as absence or presence of glandular, non-glandular trichomes on flowers and leaves. Shah *et al*. (Shah *et al*., 1972) studied stomata and trichomes on leaves of twenty-one Mimosaceae species. Mostly paracytic stomata were reported while in *Mimosa pudica* non-glandular trichomes were recorded.

Light and scanning electron microscopy are essential for determining the minute features of plant materials. This microscopy has numerous applications in a variety of biological disciplines. Using SEM techniques, it was helpful to find out surface information and their role in identification (Majeed *et al*., 2023). SEM combines high magnification with precise ultrastructure features, allowing the detection of qualitative differences not visible with optical microscopy (Oak *et al*., 2021). No such detailed taxonomic studies regarding leaf epidermal anatomy via microscopic visualization from Takkar Wildlife sanctuary exist. This micromorphological examination provides micro-characters of foliar ultrastructure which are very helpful for delimitation and identification of leguminous species from Takkar Wildlife sanctuary rangeland.

The current research aims to examine the micromorphological visualization of foliar epidermis patterns among leguminous taxa utilizing microscopic imaging protocol. The description of the leaf microstructure of leguminous species not only broadens the taxonomic character of the species, but also provides a better understanding of the Fabaceous diversity found in Wildlife sanctuary and their conservation.

## Material and Methods

**Plant sampling:** Fresh plant samples of leguminous species were collected from various regions of Takkar Wildlife Sanctuary. Takkar Wildlife Sanctuary is very diverse topographically. Eastern part of the sanctuary is deserted; with rocky plain area in some parts of this sanctuary. The sand dunes have covered Rocky Mountains. Takkar wildlife sanctuary is spread over 107,520 acres of land in two districts: Khairpur mir's and Sukkur of Sindh province, Pakistan (Khaskheli *et al*., 2022). The sampling collection localities of Takkar area were mentioned in (Table 1) and also illustrated in (Fig. 1). Field trips were organized from March to April (spring) and July to August (monsoon) 2021. Specimens were dried, pressed and mounted according to standard protocol of (De Vogel, 1987) and deposited in the herbarium of Pakistan (ISL). Taxonomic identification was carried out by using herbarium specimens, literature, catalogues, different Floras and previous work on legumes in Pakistan. The correct taxonomic names and synonyms were verified by using authentic database the plant naming index; (www.ipni.org).

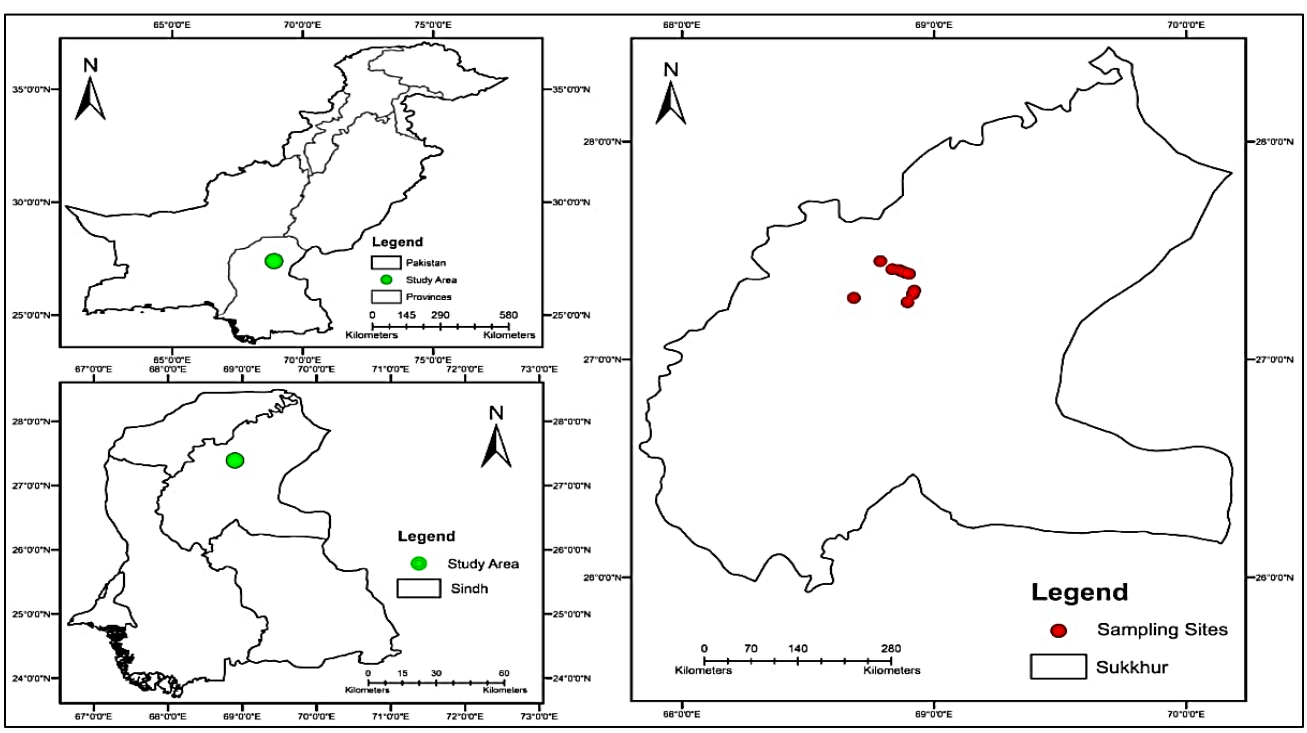


Fig. 1. Map of the study area localities (Takkar Wildlife Sanctuary).

**Table 1. Sampling and geography of of leguminous species from Takkar Wilife Sanctuary.**

| Leguminous species | Locality/District | Accession No. | Latitude (N) | Longitude (E) | Collector |
|---|---|---|---|---|---|
| *Acacia jacquemontii* Benth. | Choon Diko/ Khairpur | 133395 | 27°21'14.3"N | 68°47'20.6"E | Salman, Jamil |
| *Acacia nilotica* (L.) Delile | Lal Juryo Khan Shambani/Shukkar | 133396 | 27°23′36.4″ | 68°53′36.5″ | Jamil, Shoukat |
| *Acacia modesta* Wall. | Dargah Sultan Badshah/ Khairpur | 133375 | 27°24′ 52.3″ | 68°50′ 02.3″ | Jamil, Salman |
| *Astragalus hamosus* L. | Lal Juryo Khan Shambani/Shukkar | 133398 | 27°23′38.1″ | 68°53′33.6″ | Salman, Ghulam Yaseen |
| *Crotalaria burhia* Benth. | Zawar Mehar Ali Khaskheli/ Khairpur | 133371 | 27°16′ 57.8″ | 68°40′ 47.9″ | Jamil, Salman |
| *Dalbergia sissoo* DC. | Lal Juryo Khan Shambani | 133390 | 27°23′38.1″ | 68°53′35.8″ | Jamil |
| *Prosopis cineraria* (L.) Druce | Lal Juryo Khan Shambani/Shukkar | 133367 | 27°23′37.9″ | 68°53′35.2″ | Jamil, Shoukat |
| *Prosopis juliflora* (Sw.) DC. | Dargah Sultan Badshah/ Khairpur | 133397 | 27°24′36.0″ | 68°51′35.7″ | Jamil |
| *Parkinsonia aculeata* L. | Sultan Bhambhiro/Shukkar | 133378 | 27°18′ 02.6″ | 68°54′ 54.8″ | Salman, Jamil |
| *Tephrosia purpurea* (L.) Pers. | Wariwaro/ Khairpur | 133399 | 27°23′ 49.5″ | 68°53′ 17.3″ | Jamil |

**Foliar micromorphology:** Plants specimen were collected from different localities of Takkar Wildlife Sanctuary and then researched for epidermis sectioning under optical microscope. In case of small leaves whole leaf, while large leaves were cut into small pieces and put in the test tube in the solution of Lactic Acid (90.08%) and Nitric Acid (65%), heated for sometimes to decolorize and then washed sensibly. Debris on the surface of specimens on the slide were prepared by adding Lactic Acid or bleach and then washed. Small section was placed on standard glass slide and covered with coverslips (Ashfaq *et al*., 2019). For each species, 6 to 10 slides were made for the abaxial and adaxial surfaces, and all epidermal features were measured using a microscope. All anatomical features were examined under light microscope Nikon & Meiji (Japan) and photographed were made by using light microscope (LECA-DM-1000).

**Stomatal index**

**Stomatal index:** The stomatal index was determined using the formula:

$$SI = S/S + E \times 100$$

where SI = Stomatal Index, S = No. of Stomata per unit area and E = No. of epidermal cells per unit area.

**Statistics quantification:** Data was analyzed statistically to find out the mean and standard error (SE) using SPSS statistical software. For statistical analysis of mean ± SE; almost 15 to 20 readings were taken for each parameter (Raza *et al*., 2020).

**Results**

Ten leguminous species belonging to seven genera were selected for anatomical examination with the help of light and scanning electron microscopy. The genera that were analyzed include *Acacia, Astragalus, Crotalaria, Dalbergia, Prosopis, Parkinsonia,* and *Tephrosia*. Significant variations in micromorphological foliar epidermal characters were examined (Tables 2, 3 & 4). The field pictorial view and light micrographs of Fabaceous taxa were illustrate in Figs. 2, 3 and 4.

**Table 2. Foliar epidermal morphology based qualitative characters of leguminous species.**

| Leguminous species | Ad/Ab | Shape of epidermal cells | Anticlinal walls | Stomata type | Trichomes |
|---|---|---|---|---|---|
| *Acacia jacquemontii* Benth. | Ad | Polygonal | Entire | Paracytic | Absent |
| | Ab | Polygonal | Entire | Paracytic | Absent |
| *Acacia nilotica* (L.) Delile | Ad | Polygonal | Entire | Paracytic | Absent |
| | Ab | Polygonal | Entire | Paracytic | Absent |
| *Acacia modesta* Wall | Ad | Tetragonal to Pentagonal | Entire | Absent | Absent |
| | Ab | Tetragonal to Pentagonal | Entire | Paracytic | Absent |
| *Astragalus hamosus* L. | Ad | Polygonal | Sinuate and deeply sinuate | Paracytic | Absent |
| | Ab | Polygonal | Sinuate and deeply sinuate | Paracytic | Absent |
| *Crotalaria burhia* Benth. | Ad | Polygonal | Entire | Absent | Unicellular |
| | Ab | Polygonal | Entire | Paracytic | Unicellular |
| *Dalbergia sissoo* DC. | Ad | Polygonal | Entire and slightly wavy | Paracytic | Absent |
| | Ab | Polygonal | Entire and slightly wavy | Paracytic | Absent |
| *Prosopis cineraria* (L.) Druce | Ad | Tetragonal | Entire | Paracytic | Unicellular |
| | Ab | Tetragonal | Entire | Paracytic | Unicellular |
| *Prosopis juliflora* (Sw.) DC. | Ad | Polygonal | Entire | Anisocytic | Glandular |
| | Ab | Polygonal | Entire | Anisocytic | Glandular |
| *Parkinsonia aculeata* L. | Ad | Wavy | Sinuate | Absent | Absent |
| | Ab | Wavy | Sinuate | Paracytic | Absent |
| *Tephrosia purpurea* (L.) Pers. | Ad | Polygonal | Entire | Absent | Unicellular |
| | Ab | Polygonal | Entire | Paracytic | Unicellular |

Keywords: Ad = Adaxial, Ab = Abaxial

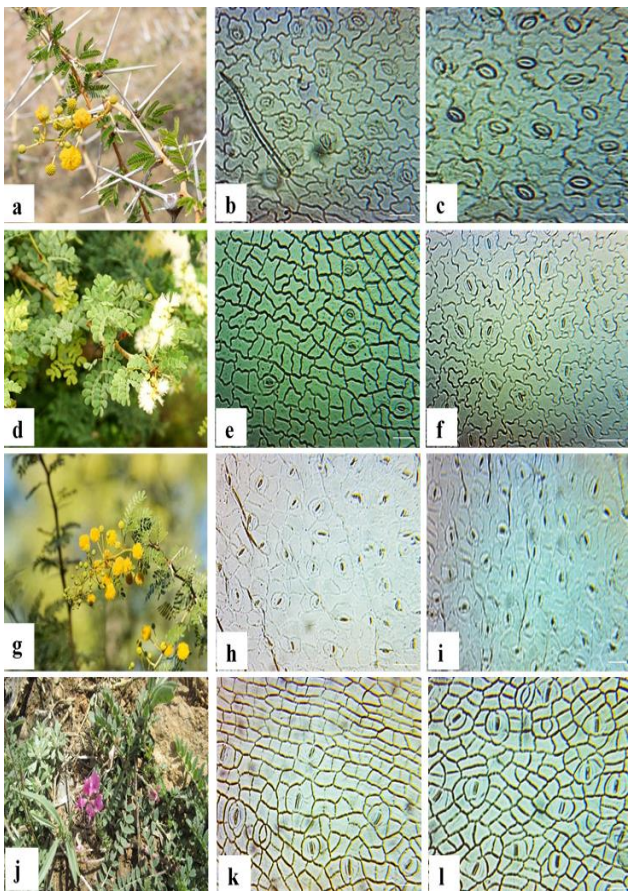


Fig. 2. Field Pictorial view and light microscopic anatomical photographs, scale bar 10 µm: *Acacia jacquemontii* Benth. (a) Field view, (b) Adaxial surface 40x, (c) Abaxial surface 40x; *Acacia nilotica* (L.) Delile (d) Field view, (e) Adaxial surface 40x, (f) Abaxial surface 40x; *Acacia modesta* Wall. (g) Field view, (h) Adaxial surface 40x, (i) Abaxial surface 40x,; *Astragalus hamosus* L. (j) Field view, (k) Adaxial surface 40x, (l) Abaxial surface 40x.

**Foliar epidermis:** The shape of epidermal cells is polygonal or wavy with entire, slightly wavy, or sinuate walls. The shape of epidermal cells was the same on both abaxial and adaxial surfaces except in *Acacia modesta* and *Parkinsonia aculeata.* Where it was tetra to pentagonal in *Acacia modesta* while wavy in *Parkinsonia aculeata* on both sides. The pattern of anticlinal wall was entire, slightly wavy, sinuate, and deeply sinuate. The pattern of the wall was similar on both abaxial and adaxial surfaces. The length of epidermal cell ranges from (38.5± 6.35µm) in *Prosopis cineraria* to (17.5± 1.0µm) in *Astragalus homosus* on the abaxial surface. On the adaxial surface it varies from (47.5± 2.5µm) in *Dalbergia sisso* to (17.5± 1.76µm) in *Tephrosia purpurea* (Fig. 5). Subsidiary cells have also been found in all species with considerable variations. Three types of subsidiary cell arrangement were noted; margin sinuous, enclosing guard cells, followed by lobed margins enclosing guard cells, and lobed wavy margins, partly enclosing guard cell shapes. The largest subsidiary cell length (42±6.49 µm) along the adaxial side and the maximum length (40±4.67 µm) on the abaxial side were noted in *Dalbergia sisso*. The width of the largest subsidiary cell was noted in *Acacia jacquemontii* (22±3.5 µm) on the adaxial surface, while in *Dalbergia sisso* on the abaxial surface (13±1.2 µm), as mentioned in (Fig. 6).

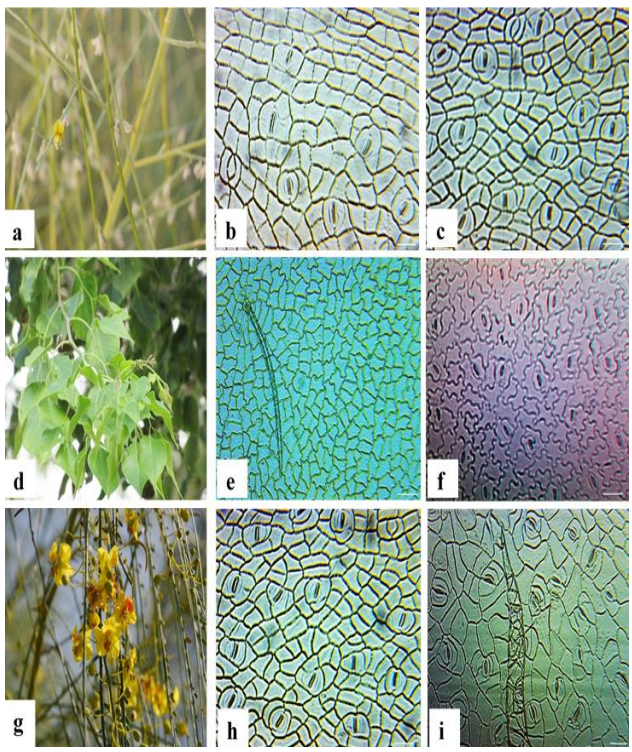


Fig. 3. Field Pictorial view and light microscopic anatomical photographs scale bar 10 µm: *Crotalaria burhia* Benth. (a) Field view, (b) Adaxial surface 40x, (c) Abaxial surface 40x; *Dalbergia sissoo* DC. (d) Field view, (e) Adaxial surface 40x, (f) Abaxial surface 40x; *Parkinsonia aculeata* L. (g) Field view, (h) Adaxial surface 40x, (i) Abaxial surface 40x.

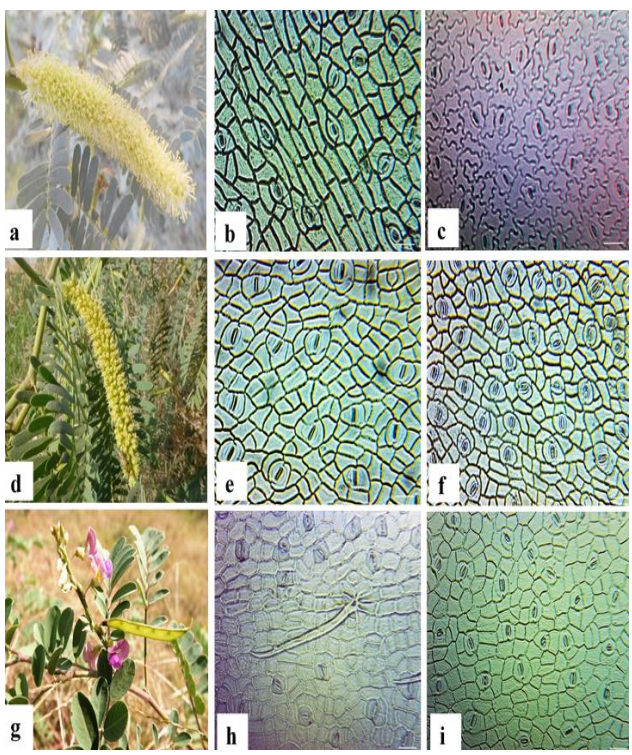


Fig. 4. Field Pictorial view and light microscopic anatomical photographs scale bar 10 µm: *Prosopis cineraria* (L.) Druce (a) Field view, (b) Adaxial surface 40x, (c) Abaxial surface 40x; *Prosopis juliflora* (Sw.) DC. (a) Field view, (b) Adaxial surface 40x, (c) Abaxial surface 40x; *Tephrosia purpurea* (L.) Pers. (a) Field view, (b) Adaxial surface 40x, (c) Abaxial surface 40x.

**Table 3. Quantitative analysis for stomatal index of selected leguminous species.**

| Leguminous species | Ad /Ab | Stomata P/A | Av. No. of Tri. per unit area | Av. No. of Ep. cells per unit area | Av. No. of St. per unit area | Stomatal Index (%) S/(S+E)× 100 |
|---|---|---|---|---|---|---|
| *Acacia jacquemontii* Benth. | Ad | P | - | 193 | 23 | 10.73 |
| | Ab | P | - | 247 | 52 | 17.5 |
| *Acacia nilotica* (L.) Delile | Ad | P | 3 | 92 | 8 | 8.18 |
| | Ab | P | - | 69 | 22 | 91.2 |
| *Acacia modesta* Wall | Ad | A | - | 45 | - | - |
| | Ab | P | - | 46 | 11 | 19.16 |
| *Astragalus hamosus* L. | Ad | P | - | 88 | 15 | 14.86 |
| | Ab | P | 2 | 30 | 26 | 21.9 |
| *Crotalaria burhia* Benth. | Ad | A | 3 | 200 | - | - |
| | Ab | P | 17 | 81 | 39 | 32.39 |
| *Dalbergia sissoo* DC. | Ad | P | 7.6 | 61 | 28 | 31.4 |
| | Ab | P | 5 | 80 | 3 | 4.28 |
| *Prosopis cineraria* (L.) Druce | Ad | P | 3 | 145 | 17 | 10.49 |
| | Ab | P | | 106 | 70 | 40.04 |
| *Prosopis juliflora* (Sw.) DC. | Ad | A | - | 191 | - | - |
| | Ab | P | - | 94 | 29 | 24.07 |
| *Parkinsonia aculeata* L. | Ad | P | - | 57 | 21 | 27.15 |
| | Ab | P | - | 94 | 59 | 38.75 |
| *Tephrosia purpurea* (L.) Pers. | Ad | A | 7 | 151 | - | - |
| | Ab | P | - | 92 | 30 | 24.55 |

Keywords: (Ad) = Adaxial, (Ab) = Abaxial, (P) = Present, (A) = Absent, (S) = Stomata, (E) = Epidermal cells, (%) = Percentage, (Av.) = Average, (No) = Number

**Stomatal complex:** Fabaceous taxa examined were all amphistomatic except *Acacia modesta, Crotalaria burhia, Parkinsonia aculeata* and *Tephrosia purpurea* which are hypostomatic. Stomata of paracytic type were observed in all species except *Prosopis juliflora* where stomata were anisocytic. The stomatal length varies from (24.9± 0.06µm) in *Dalbergia sisso* to (8.2± 0.5µm) in *Prosopis juliflora* on the abaxial surface. On the adaxial surface, it ranges from (24± 0.6µm) in *Dalbergia sisso* to (8.8± 0.61µm) in *Prosopis juliflora* (Fig. 7). The stomatal index ranges between 41.2-4.28% on the abaxial surface and 31.4-8.5% on the adaxial surface. On the abaxial surface, it is highest in *Acacia nilotica* (41.2%) and lowest in *Dalbergia sissoo* (4.28%). Whereas on the adaxial surface, it is highest in *Dalbergia sissoo* (31.4%) and lowest in *Acacia nilotica* (8.5%) as illustrated in (Fig. 8).

**Trichomes:** Unicellular, glandular trichomes are present on both surfaces in *Crotalaria burhia, Prosopis cineraria, Prosopis juliflora,* and *Tephrosia purpurea.* Trichomes are absent in the other six species. The length of trichomes ranges from (355± 44.3µm) in *Prosopis juliflora* to (67.5± 2.09µm) in *Crotalaria burhia* on the abaxial surface. On the adaxial surface, it ranges from (347± 67.6µm) in *Prosopis cineraria* to (75± 8.83µm) in *Crotalaria burhia*.

**Taxonomic identification keys based on foliar anatomical features**

1. + Trichomes absent epidermal cell polygonal, entire anticlinal wall, stomata paracytic ..... ***Acacia jacquemontii***
   - Trichomes present, epidermal cell irregular ..... **2**
2. + Epidermal cell irregular, anticlinal wall entire, stomata paracytic, trichome absent ..... ***Acacia nilotica***
   - Epidermal cell tetragonal, anticlinal wall entire and sinuate, stomata paracytic ..... **3**
3. + Epidermal cell tetragonal to pentagonal with sinuate anticlinal wall, stomata paracytic abaxial, trichome absent ..... ***Acacia modesta***
   - Epidermal cell rectangular shape, anticlinal wall rounded ..... **4**
4. + Epidermal cell polygonal shape, anticlinal wall sinuate, stomata paracytic ..... ***Astragalus hamosus***
   - Epidermal cell polygonal, anticlinal wall entire ..... **5**
5. + Epidermal cell polygonal isodiametric, abaxial paracytic stomata, trichome non-glandular unicellular ..... ***Crotalaria burhia***
   - Epidermal cell polygonal, stomata paracytic, anticlinal wall wavy ..... **6**
6. + Epidermal cell polygonal, anticlinal wall entire and slightly wavy, stomata paracytic, trichomes absent ..... ***Dalbergia sissoo***
   - Anticlinal wall entire, trichome present ..... **7**
7. + Epidermal cell tetragonal shape, stomata paracytic, trichome unicellular non-glandular ..... ***Prosopis cineraria***
   - Epidermal cell polygonal shape, stomata paracytic ..... **8**
8. + Epidermal cell polygonal, anticlinal wall entire, trichomes unicellular ..... ***Tephrosia purpurea***
   - Stomata paracytic, anticlinal wall sinuate ..... **9**
9. + Epidermal cell wavy, stomata paracytic (abaxial), anticlinal wall sinuate, trichome absent ..... ***Parkinsonia aculeata***
   - Epidermal cell polygonal, stomata anisocytic ..... **10**
10. + Epidermal cell polygonal, stomata anisocytic, anticlinal wall entire, trichome unicellular glandular ..... ***Prosopis juliflora***

**Table 4. Quantitative measurements of foliar epidermal anatomical features of selected leguminous species.**

| Leguminous species | Ad/Ab | Epidermal cells (µm) | | Subsidiary cells (µm) | | Stomata (µm) | | Guard cells (µm) | | Stomatal pore (µm) | | Trichome (µm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Min-Max (Mean±SE) | | | | | | | | | | | |
| | | L | W | L | W | L | W | L | W | L | W | L | W |
| *Acacia jacquemontii* Benth. | Ad | 25-35 (27.5±1.9) | 17.5-25 (21.5±1.3) | 15-35 (22.5±3.53) | 15-35 (22.5± 3.5) | 15-20 (16.5±1) | 10-12.5 (11±0.61) | 12.5-17.5 (14±1) | 2.5-5 (4±0.61) | 10-12.5 (11±0.61) | 1.25-2.5 (2.05±0.27) | - | - |
| | Ab | 12.5-25 (19±2.17) | 10-17.5 (13.5±1.3) | 20-27.5 (24.5±1.22) | 10-15 (12±0.93) | 15-17.5 (17±0.5) | 10-15 (12±0.93) | 12.5-15 (13.5±0.61) | 2.5-5 (3±0.5) | 10-12.5 (11±0.61) | 1.25-2.50 (1.7±0.22) | - | - |
| *Acacia nilotica* (L.) Delile | Ad | 22.5-37.5 (31±2.9) | 10-25 (18±2.89) | 12.5-15 (14.1±0.5) | 5-5.5 (5.25±0.1) | 10-15 (12±0.9) | 6-8.7 (7.2±0.49) | 10-15 (12±0.9) | 2.5-3.25 (2.8±0.14) | 6.75-10 (7.8±0.5) | 0.25-1 (0.6 ±0.12) | 117.5-275 (223±28.5) | 10-12.5 (11.2±0.6) |
| | Ab | 17.5-50 (31.5±5.4) | 10-17.5 (14±1.5) | 15-17.5 (16.1±0.57) | 5-5.5 (5.15±0.1) | 14.7-17.5 (15.9±0.6) | 6.7-10 (8.3±0.6) | 14.7-17.5 (15.9±0.6) | 2.5-3 (2.7±0.1) | 7.5-12.5 (9.5±0.9) | 0.25-1 (0.7±0.14) | - | - |
| *Acacia modesta* Wall | Ad | 27.5-50 (36±3.84) | 17.5-22.5 (19.5±0.9) | - | - | - | - | - | - | - | - | - | - |
| | Ab | 17.5-27.5 (22±2.0) | 10-15 (12.5±0.8) | 15-22.5 (19±1.27) | 4.25-7.50 (5.6±0.57) | 15-20 (17±0.93) | 15-20 (17±0.93) | 12.5-17.5 (14.5±0.93) | 2.75-3.25 (2.9± 0.09) | 7.5-12.5 (10±0.79) | 0.75-1.25 (1±0.11) | - | - |
| *Astragalus hamosus* L. | Ad | 25-32.5 (29±1.27) | 10-15 (12.8±0.8) | 15-20 (17.5±1.11) | 5-7.5 (6.25±0.5) | 12.5-13 (12.6± 0.1) | 9.25-10 (9.8±0.14) | 10-13 (12.1±0.53) | 2.5-3.75 (3.35±0.2) | 9.25-10.5 (9.9±0.2) | 0.04-0.25 (0.14±0.04) | - | - |
| | Ab | 15-20 (17.5±1.1) | 12.25-13.75 (13.1±0.8) | 15-17.5 (16.5±0.61) | 6.25-7.75 (7.15±0.3) | 10-15 (13±0.93) | 6.25-8 (7.2±0.3) | 10-12.5 (11.5±0.61) | 2.5-3.5 (2.9±0.2) | 5-10 (7.45± 0.8) | 0.04-0.25 (0.10±0.03) | - | - |
| *Crotalaria burhia* Benth. | Ad | 17.5-30 (25±2.09) | 12.5-25 (20.5±2.1) | - | - | - | - | - | - | - | - | 50-100 (75± 8.83) | 7.5-11.25 (9.25±0.6) |
| | Ab | 17.5-37.5 (27±3.39) | 12.5-25 (20.5±2.2) | 15-22.5 (20±1.36) | 5-7.5 (6.4±0.4) | 15-22.5 (19±1.27) | 7-8.25 (7.6±0.20) | 12.5-20 (16.5±1.27) | 2.50-3.25 (2.85±0.2) | 10-15 (12±0.93) | 0.5-1 (0.75±0.11) | 62.5-75 (67.5±2.09) | 7.5-12.5 (10.05±0.8) |
| *Dalbergia sissoo* DC. | Ad | 37.5-50 (47.5±2.5) | 25-37.5 (27.5±2.5) | 25-62.5 (42±6.49) | 10-17.5 (14±1.5) | 22.5-25 (24±0.6) | 17.5-22.5 (20.5±0.9) | 22.5-25 (24±0.6) | 6.25-7.5 (7.05±0.3) | 12.5-17.5 (14±1) | 3.75-5 (4.5±0.3) | - | - |
| | Ab | 12.5-50 (30±6.37) | 20-25 (23.5±1) | 25-50 (40±4.67) | 10-17.5 (13±1.2) | 24.7-25 (24.9±0.06) | 17.25-17.5 (17.4±0.05) | 24.7-25 (24.9±0.06) | 7.25-7.5 (7.45±0.05) | 15-17.5 (16.9±0.5) | 2.5-5 (3.9±0.59) | - | - |
| *Prosopis cineraria* (L.) Druce | Ad | 22.5-37.5 (28±2.6) | 12.5-20 (16±1.27) | 15-22.5 (19±1.5) | 5-7.5 (6.5±0.6) | 12.5-17.5 (15.5±0.8) | 7.5-8 (7.65± 0.1) | 12.5-17.5 (15.05± 0.79) | 2.5-3 (2.85± 0.1) | 10-15 (12.5±0.8) | 1.25-2.5 (1.85±0.2) | 225-525 (347±67.6) | 12.5-15 (13.5±0.6) |
| | Ab | 25-62.5 (38.5±6.35) | 12.5-27.5 (21±3.02) | 15-25 (18±1.8) | 5-7.5 (6.5±0.6) | 12.5-17.5 (14.5±0.9) | 5-10 (7.5±0.79) | 12.5-17.5 (14.5±0.9) | 2.5-3.25 (2.8±0.15) | 10-12.5 (11.5±0.6) | 1-1.5 (1.25±0.1) | 150-550 (335±79.7) | 5-17.5 (12.5±2.1) |
| *Prosopis juliflora* (Sw.) DC. | Ad | 23-47 (35.5±3.09) | 10-22.5 (14±2.17) | 10-15 (12.5±1.11) | 5-6.25 (5.35± 0.3) | 7.7-10.2 (8.8±0.58) | 5.7-7.3 (6.5±0.59) | 7.2-10.1 (8.6±0.66) | 2.8-3.4 (3.1±0.23) | 5.3-6.2 (5.9±0.76) | 0.56-2.2 (1.7±0.32) | 180-320 (252±45.8) | 15-30 (19±2.80) |
| | Ab | 24.5-52 (36.3±3.27) | 11.3-26 (16±2.31) | 9.7-16.3 (12.7±0.87) | 5.4-7.3 (6.1±0.82) | 7.4-9.3 (8.2±0.54) | 5.3-7.2 (6.4±0.39) | 7-10.3 (8.8±0.58) | 2.4-3.3 (2.7± 0.21) | 5-7.3 (6.4±0.41) | 1-1.5 (1.3±0.09) | 225-450 (355±44.3) | 25-50 (38.5±4.1) |
| *Parkinsonia aculeata* L. | Ad | 10-30 (21.5±3.5) | 7.5-17.5 (12.5±1.7) | - | - | - | - | - | - | - | - | - | - |
| | Ab | 15-37 (24±3.9) | 7.5-15 (12±1.2) | 12.5-25 (19.5±2.42) | 5-7.5 (6.5±0.6) | 15-22.5 (18.5±1.5) | 7.5-10 (8.5±0.6) | 12.5-20 (16±1.5) | 3.75-5 (4.5±0.27) | 7.5-15 (11.5±1.3) | 1.5-2.5 (2.05±0.2) | - | - |
| *Tephrosia purpurea* (L.) Pers. | Ad | 12.5-22.5 (17.5±1.76) | 12.5-17.5 (14±1) | - | - | - | - | - | - | - | - | 95-142.5 (111±8.38) | 10-15 (11.5±1) |
| | Ab | 22.5-45 (35±4.13) | 12.5-22.5 (17.5±1.7) | 12.5-22.5 (17.5±1.76) | 5-10 (8±0.93) | 10-17.5 (14±1.5) | 7.5-10 (8.5±0.61) | 10-17.5 (14±1.5) | 2.75-3.25 (2.9±0.09) | 7.5-12.5 (10.5±0.9) | 1-1.75 (1.35±0.12) | - | - |

Keywords: (L) = Length, (W) = Width, (Ad) =Adaxial, (Ab) = Abaxial, (Min) = Minimum, (Max) = Maximum, (SE) = Standard error, (µm) = Micrometer

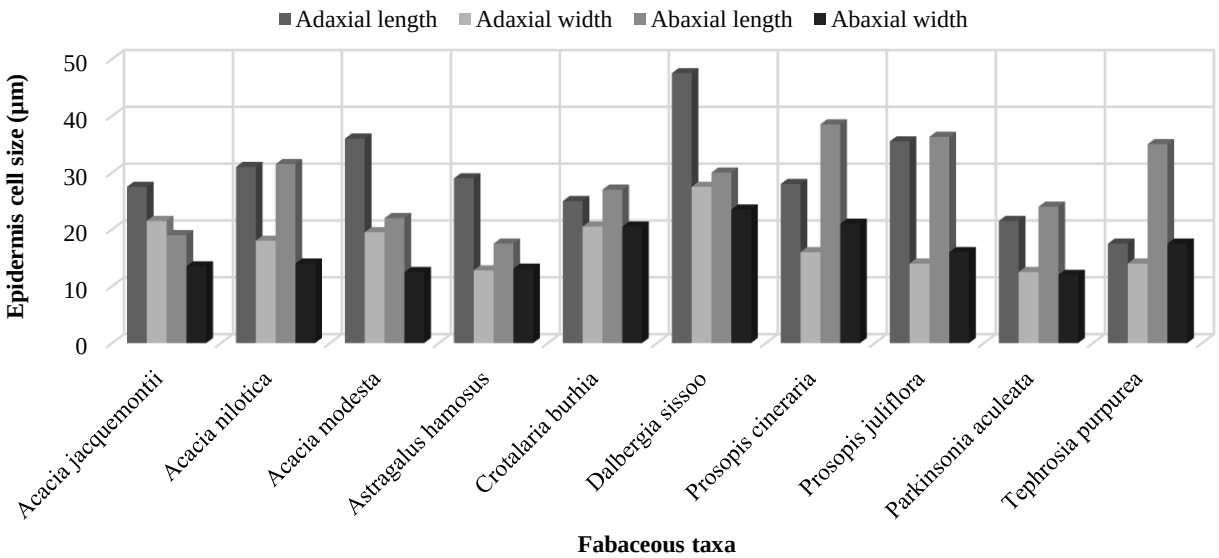


Fig. 5. Epidermal cell size variations along adaxial and abaxial surface among leg7890uminous species.

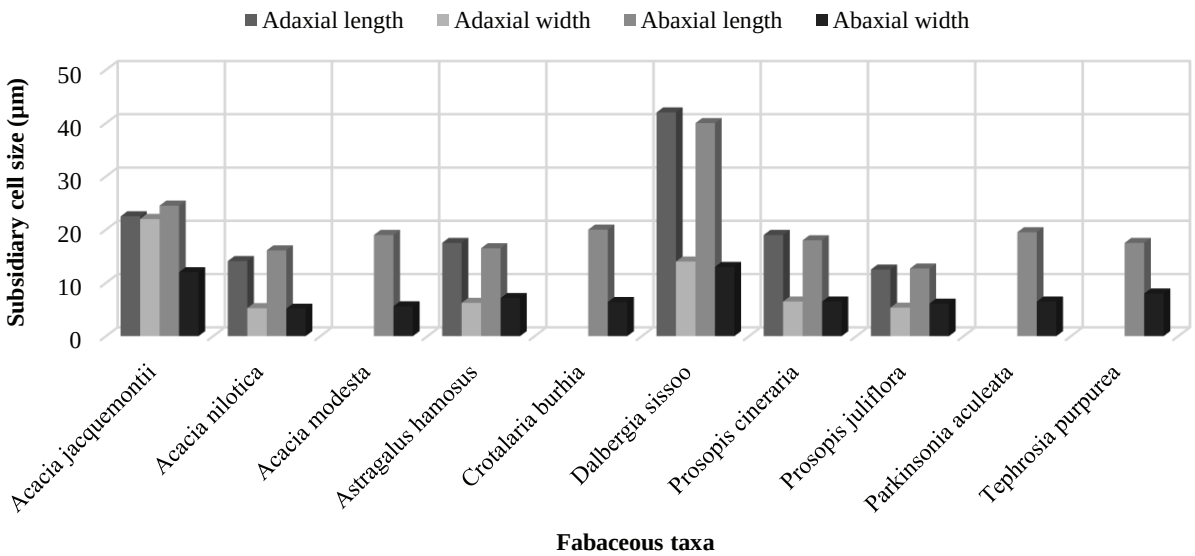


Fig. 6. Subsidiary cell size variations along among leguminous species.

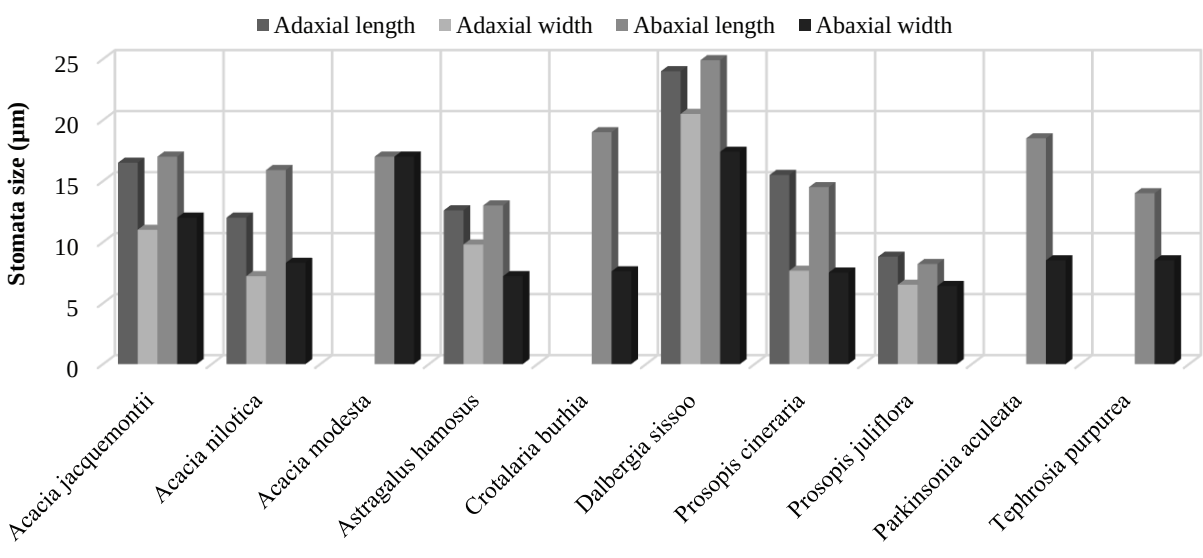


Fig. 7. Stomatal size variations along adaxial and abaxial side among leguminous species.

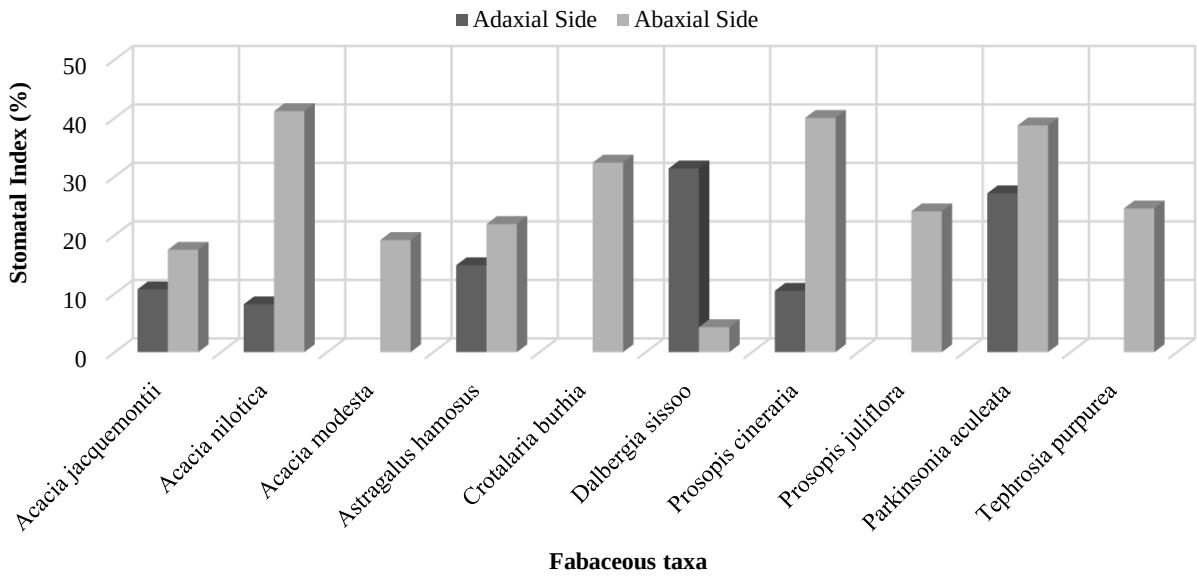


Fig. 8. Stomatal index along adaxial and abaxial surface among leguminous species.

## Discussion

The present study was based on a microscope visualization tool to observe the microstructural characteristics of the leguminous species from the different localities of the Takkar Wildlife sanctuary. The micromorphological features are useful for the identification of plant species at various taxonomic levels. In the current research, the plant species were examined on the basis of epidermal cell size and shape, stomatal presence and absence along with stomatal type and size and presence and absence of trichomes and their size and type.

In the taxonomy of angiosperms, the use of leaf epidermal features is expanding and has been used for many years. The use of leaf epidermal traits in systematic botany is becoming more frequent, as is the use of other markers like DNA sequences and chemical compositions (Hameed *et al*., 2020). Foliar epidermal characters are valuable tools for anatomical studies. Although considerable work has been conducted on wood anatomy and gross morphology for identification purposes but without foliar epidermal morphology identification criteria would be incomplete (Endress *et al*., 2000). One of the significant features of taxonomic classification is anatomical leaf study that is of boundless position from taxonomical assessment. That is why most families have research on the basis of anatomical leaf study (Shaheen *et al*., 2010). Countless stress has been laid on leaf epidermal micro-morphology for the purpose of classification (Metcalfe & Chalk, 1979).

Leaf epidermis is an important taxonomic feature and taxonomic analysis of many families is done with the help of leaf epidermis (Shaheen *et al*., 2009). The use of microscopic imaging tools can be quite useful in examining foliar epidermal micromorphology and quantifying the idea that glandular trichome density reduces with increasing aridity. The impact of the microenvironment on plants can be seen at the morpho-structural level of trichomes as well as the stomata (Belmonte *et al*., 2022). The majority of morphological and anatomical adaptations made by wildlife sanctuary habitats include thinner cuticles, smaller leaves, fewer stomata per unit leaf area, higher succulent, and wax deposition. Foliar epidermal micromorphology is particularly important, and earlier reports highlight its importance in the identification of diverse plant groups (Esfandani-Bozchaloyi & Zaman, 2018; Attar *et al*., 2019; Kandemir *et al*., 2019). The anatomical properties of the Fabaceous species and their significance for the taxonomic classification have been presented by some earlier studies. (Çİldİr *et al*., 2017) analyzed the leaf anatomical and micromorphological implications of *Lathyrus* species using light and scanning microscopy. Variations were observed in the epidermal cell shape of the selected plant species of Fabaceae. Polygonal, tetragonal, pentagonal, and wavy-shaped epidermal cells were reported in the present study. Duarte and Wolf (Duarte & Wolf, 2005) reported polygonal epidermal cells in *Acacia* species which is in accordance with the present study. Variations were also recorded in epidermal cell size with the largest epidermal cell size reported in *Prosopis cineraria* with epidermal cell length (38.5± 6.35µm) and the smallest in *Astragalus homosus* (17.5± 1.11µm) on abaxial surface. While the abaxial surface largest and smallest epidermal cell (47.5± 2.5µm) (17.5± 1.76µm) in *Dalbergia sissoo* and *Tephrosia purpurea,* respectively. In Fabaceous taxa: *Acacia nilotica* and *Acacia modesta* observed epidermal cell shape is polygonal and tetragonal to pentagonal which was not accordance with the (Saini *et al*., 2008). Epidermal cell shape in *Dalbergia sissoo* is polygonal and irregular which was accordance with the previous findings.

The stomatal frequency are very most importantly use in taxonomy (Krishnamurthy & Kannabiran, 1970). The foliar anatomical remaining persistent no change of environmental stress according to (Davis & Heywood, 1963). In *Acacia nilotica* the straight anticlinal wall and paracytic stomata are observed and anticlinal wall straight are also observed by (Sahreen *et al*., 2010). In *Acacia nilotica* paracytic type of stomata were observed while (Baretta-Kuipers, 1981) observed both paracytic and

anomocytic type of stomata. Stomatal frequency, size, and distribution are regarded as important tools in phylogeny and taxonomy (Albert & Sharma, 2013). (Gill *et al*., 1982) studied 21 species of Fabaceae and reported paracytic type stomata. In the order of Legume family, paracytic, anisocytic and anomocytic stomata were examined and discovered stomatal complex on both the adaxial and abaxial side of *Astragalus*. The *Astragalus homosus* was also found to have amphistomatic and polygonal epidermal cells with deeply sinuate anticlinal walls in the current study.

The majority of Fabaceae members have paracytic stomata as their primary stomata type, according to previous studies (Ju, 2020) whereas *Crotalaria* species have stomata that are paracytic, anisocytic, diacytic, or have one subsidiary cell (Ekeke & Agogbua, 2020). Paracytic stomata were reported in all the species except *Prosopis juliflora.* (Martínez Quesada, 1997) reported anisocytic stomata in *Prosopis juliflora* that does not coincide with the present study. Epidermal cells shape observed irregular in *Prosopis juliflora* were coincide with the findings of (Shaheen *et al*., 2020). In order to assess the efficacy of the features defining the botanical identity (Robertson *et al*., 2010) proposed microscopic standards to distinguish the leaves of *Prosopis cineraria* with petiole micromorphological sectioning.

Saeed *et al.,* (2019) reported that epidermal cells in *Tephrosia* species reveal that the abundance of epidermal cells varies from the leaf of one species to another within genera and that the size of stomata is significant taxonomic character. However our study examined *Tephrosia purpurea* stomata complex type was paracytic, which conflicts with the previous observations. The stomatal index varied accordingly to the number of epidermal cells and stomata on both leaf surfaces. The maximum stomatal index was observed in *Acacia nilotica* (41.2%) on abaxial side while the along adaxial surface (31.4%) was observed for *Dalbergia sisso*. (Nazish *et al*., 2022) reported a significant decrease in stomata number per unit leaf area when they grow in more arid land habit.

Presence of trichomes is an additional astonishing character in leaf epidermal micromorphology which have significance role in classification of various taxa. Trichomes are an important taxonomic tool and can be used to delimit taxa. Unicellular trichomes were observed in *Crotalaria burhia* while glandular trichomes were seen in *Prosopis juliflora* and *Tephrosia purpurea.* (Bijauliya *et al*., 2017) reported simple uniseriate trichomes that coincides with our study.

Our findings indicate that plants flourishing in Wildlife sanctuary conditions may improve their leaf functions by changing morphological and histochemical traits, which are significant adaptive capabilities to external conditions of low soil moisture and highly intense light. It is suggested that functional traits are frequently linked to anatomical plasticity.

## Conclusion

This is foremost study that deals with the leaf micromorphology of some leguminous species growing in the Takkar Wildlife sanctuary. The current findings reveal that foliar anatomical features, including the diversity of epidermal cells, trichome morphology, and variation of stoma types, are potentially useful to delimit the taxa at a specific level. Fabaceous species can easily be differentiated on account of their epidermal cell shape. The size of epidermal cells, their shape, and the stomata type of these species were considerably variable and different from each other. The diversity in the foliar trichomes at species level also served as a useful taxonomic tool. Each trait has its own systematic importance in the delimitation of taxa. Furthermore, it is concluded that light microscopic character identification provide key diagnostic description of leguminous species proved to be significant in classification.

**Conflict of interest:** The authors declare no potential conflict of interest regarding publication of this research work.

## Acknowledgement

The authors would like to thank the Herbarium of Pakistan, Quaid-i-Azam University (ISL) for identification of plant specimens and providing lab facilities. We are also grateful to Higher education commission of Pakistan for funding under project No NRPU-7837.

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(Received for publication 12 August 2022)