Foliar Anatomical Traits of Selected Species from the Cyperaceae Family Taxonomic Insights

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Abstract

The current research investigated 17 species from 5 genera of Cyperaceae. It includes *Cyperus*, *Carex*, *Kyllinga*, *Fuirena* and *Fimbristylis* for their foliar epidermal anatomy using light microscopy (LM). The study found that the micromorphological features of the family are important for accurate identification of species. Plant species were collected from different phytogeographical regions of Pakistan and studied for both qualitative and quantitative characteristics. Both the upper and lower epidermis of the leaf were studied, and various micromorphological characters were examined, including the shape of epidermal cells, anticlinal wall pattern, type of stomata, and subsidiary cells. From 5 genera, trichomes were only observed in *Kyllinga* (*Kyllinga brevifolius*). All species have paracytic type of stomata. The research aims to develop taxonomic keys based on foliar epidermal characteristics to enable quick and easy identification of species. The main objective is to study both qualitative and quantitative traits for accurate species identification and delimitation of Cyperaceae. The foliar epidermal anatomy of

the Cyperaceae family provides valuable information for the systematic identification of different species. The current findings regarding the foliar epidermal features are particularly important for plant taxonomists in accurately identifying species within the Cyperaceae family. The micro-morphological features of foliar epidermal anatomy provide novel characteristics for precise taxonomic identification and offer essential information for further study by plant taxonomists.

Keywords: Cyperaceae, Epidermal anatomy, Systematics, Stomata, Bioimaging microscopy

1. Introduction

The Cyperaceae family, commonly referred to as the sedge family, is a globally found herb family displaying an outstanding range of ecological adaptation, spanning from sea level to alpine heights (Batool et al., 2023a). Cyperaceae is the seventh largest family in angiosperm and the third diversified family of monocots. Cyperaceae is herbaceous plant family with around 5700 species organized into 2 subfamilies, 24 tribes, and 90 genera. Of its 90 genera, 79 are members of the early-developing Cyperoideae subfamily and 11 of the late-developing Mapanioideae subfamily (http://legacy.tropicos.org/Name/42000356?projectid=32; (Larridon et al., 2021). Despite its global distribution, this family has a high degree of diversity in the tropics (Silva et al., 2023). Most species are found in Africa and the Neotropics (Kukkonen, 2001). There are 179 species in 22 genera of the family Cyperaceae in Pakistan, the majority of which are weedy species (Kukkonen, 2001).

Cyperaceae species can develop into thick rhizomatous or stoloniferous clusters that are caespitose annual or perennial herbs. They can be found in a range of environments, including dry and waterlogged areas but are absent from the Antarctic mainland. Despite their common misconception of being uniform and grass-like, sedges are highly variable morphologically (Larridon et al., 2022a). It is regarded as a cosmopolitan family and is the most prevalent in the vegetation of wetlands (Bezerra et al., 2023). Habitat of these species varies from grime salinities to hyperregulate water, but most are present in wetlands, or poor soil (Mumtaz et al., 2021). Typically, they are herbaceous, either annual or perennial, frequently having rhizomes, and occasionally stoloniferous. Cyperaceae have reduced flowers and their vegetative anatomy has shown to be helpful in both assessing the presence of similar anatomical traits among various taxa and infrageneric classification of the family (Silva et al., 2019).

Cyperaceae leaves display the usual morphology of monocotyledons, with a leaf blade and sheath that are typically closed in this family (Alves-dos-Santos et al., 2023). The classification of the family Cyperaceae includes, among other things, the spikelet and inflorescence structures. However, it might be difficult to examine because the spikelet is so small and the inflorescence has such a complex structure. Stems are typically trigonous, solitary to densely fascicled, distributed radiately, strong or slender, sturdy or obliquely ascending, and rosette-like in form. Basal, cauline, usually ranking leaves have sheaths or blades. Leaf blades are often concave, broad or narrow, linear, grass-like, and involute. Sheaths can be closed or open. Ligules are frequently seen, occasionally on the leaf blades' opposing sides. There are no petioles or the blades are essentially limited to a pseudopetiole (Xu et al., 2017) few taxa, tribes, and subfamilies are uncertain. In addition to conducting the first systematic study of sedges, C. B. Clarke in 1893 grouped the 449 species of the Flora of British India into 28 genera (Aryal,2023).

This family has diversity in reproductive and seed dispersal structures, and this morphological difference is used to define taxon limits and to observe the taxonomic complexity of genera such as *Cyperus*, *Carex* and a broad range of dispersal vectors of this family such as birds and ants (Larridon et al., 2021a). Cyperaceae fruits can fall under the mother plant to produce long-lasting seed banks, or they can be adapted to disperse by water, wind, insects, birds, or mammals (Leck & Schütz 2005). Cyperaceae family have intrinsic characteristics like high reproductive output, vegetative proliferation, and extended seed dormancy that enhance the spreading and expansion of the population after any disturbance and evolve as colonisers of disturbed habitats (Bryson et al., 2008). Within the Cyperaceae, two major clades that correspond to the subfamilies Mapanioideae and Cyperoideae have some differences, While Cyperoideae is significantly more diversified in terms of species richness, morphology, and ecology, Mapanioideae is primarily composed of broad-leaved tropical forest beneath herbs (Larridon et al., 2021b).

Cyperaceae have achenes that are quite big and typically prominently sculptured, cylindrical spikes that are sometimes broader than the culms, and spikelet scales with 15 or more prominent longitudinal veins (González-Elizondo & Peterson, 1997). A higher order of spicate, paniculate, or umbellate inflorescences are formed from the inconspicuous flowers, which are

grouped into spikelets. Flowers can be perfect or imperfect, and plants become monoecious (or very rarely dioecious) when flowers are imperfect (Cronquist, 1981).

Information regarding the structure of the leaf epidermis is of interest to taxonomists. The diversity of natural habitats or genetic variations may cause the variations in epidermal features amongst species (Hameed et al., 2020). Foliar epidermal characteristics have very important analytical properties such as the size and shape of stomata, guard cell morphology, number of subsidiary cells and their length and width (Hussain et al., 2019) It is valuable both theoretically and practically to observe the leaf epidermis's microstructure. To study leaf growth and function as well as the classification of plant species, the micro-morphology of leaf epidermises is quite useful (Yuan et al., 2020). Utilizing microscopic techniques for taxonomic analysis, LM is used to examine the micromorphology of the foliar epidermal layer. In light microscopy, transmission light is frequently utilized as a light source (Abid et al., 2023) It is difficult to underestimate the significance of anatomical methods in taxonomic research. Taxonomic monographs are insufficient without microscopic details of the epidermal anatomy (Abbas et al, 2022). Identification of any species depends upon the morphology of plants (Ullah et al., 2021; Khan et al., 2023). Various Quantitative and qualitative morphological investigations of many families have been issued (Attar et al., 2019).

The sedge family (Cyperaceae) is well known to be a taxonomically challenging family and can be observed while considering the vegetative organs' anatomical characteristics for taxonomic purposes (Metcalfe, 1971). Though morphological and molecular approaches have been used to study the interactions among its members in extensive detail, the linkages among sedges are still incompletely understood (Bouchenak-Khelladi et al., 2014). Previous research on the anatomy of several Cyperus species and other Cyperaceae genera was conducted (Amini et al., 2008). The literature survey showed that the foliar anatomy of the Cyperaceae has not been comprehensively studied yet..This study aims to examine the leaf anatomy of selected Cyperaceous species using light microscopy to revealed significant taxonomic features. The analysis of leaf structures; epidermal cell morphotypes, and stomatal complex and trichome micromorphology revealed characters to construct taxonomic keys based on epidermal anatomy leads to identification and delimitation of Cyperaceae species. The species were selected for the study based on their diverse ecological niches and taxonomic relevance within the Cyperaceae family, enabling a comprehensive examination of foliar anatomical variations.

2. Materials and Methods

2.1 Plant material, identification, and herbarium deposition

Cyperaceae species selected for this study were chosen based on their representativeness of diverse taxonomic groups within the Cyperaceae family and their occurrence in various ecological habitats across the studied regions. Moreover, the selection was aimed at capturing a broad spectrum of foliar anatomical traits by including species with distinct morphological and ecological characteristics. Sampling sites were strategically chosen from Dera Ismail Khan, Bannu, Lakki Marwat, and Islamabad to ensure geographic and environmental diversity. Cyperaceae samples were collected from various regions of Dera Ismail Khan, Bannu, Lakki Marwat, and Islamabad from March to August 2023 after extensive field work (Table 1). The plant identification was done from online Herbaria database such as The Flora of Pakistan (http://www.efloras.org), Plants of the World Online (https://powo.science.kew.org), and related portals like The Plant Net (https://identify.plantnet.org).. The dried, pressed, mounted, and labelled plant specimens were placed in the Herbarium of the Plant Sciences Department, Faculty of Biology, Quaid-e-Azam University Islamabad (ISL).

2.2 Light microscopy

For the epidermal leaf anatomy, leaves from the fresh and preserved collection were utilized. A modified method was used to prepare the leaf samples given by (Ahmad et al., 2010). Initially, the leaves were put in a test tube with 30% nitric acid and 70% lactic acid carefully. It was then boiled for ten to fifteen minutes when the leaves grew softer, and it was simple to peel off the outer layer. After that, the boiled material was put into a Petri plate containing dilute water where the slides of the adaxial and abaxial epidermis appeared. Before the piece was mounted on a slide using coverslips, the isolated epidermis was cleaned with a droplet of lactic acid. Three to four samples of the adaxial and abaxial surfaces were prepared for every type of plant taxa. After that, the slides were examined under the light microscope for quantitative analysis (Glime & Wagner,2013). At a 40× magnification, 10 to 12 readings of each species were obtained using the Meiji (MT 4300H) light microscope. Microanatomical features such as the length and width of epidermal, subsidiary, guard, and stomatal cells were considered (Abbas et al., 2022) and photography of both adaxial and abaxial was done by the C-ME1 model camera fitted Leica

154 microscope. All the quantitative data such as mean and standard deviation was measured using
155 SPSS software.

156

157 **2.3 Stomatal index determination**

158 The number of epidermal cells and stomata were counted under the same ocular, and an
159 average of five was taken. The stomatal index was determined using the formula of Beerling and
160 Kelly (1997).

161

162 $$S.I = [S \div (S + E)] \times 100$$

163

164 Where, S.I= Stomatal Index

165 S= No. of stomata

166 E= No. of Epidermal Cells

167 **2.4 Statistical Analysis**

168 Different microanatomical features such as the length and width of epidermal cells,
169 stomata, guard cells, subsidiary cells, and stomatal pores were analyzed quantitatively. Five
170 consecutive values were used to calculate the mean and standard error for each character (Zaman
171 et al., 2023). Principal Component Analysis (PCA) was employed to obtain the value of different
172 characters. The statistical software IBM SPSS 16.0 Statistics was used for these calculations.
173 Relationships and comparisons between different species were determined through graphs by
174 using MS Excel.

175 **3. Results**

176 **3.1 Foliar anatomy**

177 By utilizing the light microscope, the foliar epidermal anatomy of 18 species of the
178 Cyperaceae from different regions of Pakistan was investigated. Both the qualitative and

quantitative characteristics including shape, type, pattern of appearance, stomata complex, size, and the number of epidermal cells were observed (Figure 1, 2, 3 & 4; Table 2 & 3)..

3.2 Epidermal Cells Anatomy

The current research was conducted based on the quantitative analysis of both the width and length of the leaf epidermal cells found on the upper (adaxial) and lower (abaxial) surfaces. The qualitative attributes examined epidermal cell appearance, AW pattern, and stomata types on abaxial and adaxial surfaces. However, the measurements showed that mostly the epidermal cells on the lower surface had the greatest length, while the cells on the upper surface had the maximum width. *Cyperus melaccensis* has the largest epidermal cells (93.26±0.74 µm) on the abaxial surface while *Cyperus iria* has the largest epidermal cells (107.32±0.96 µm) on the adaxial surface. *Cyperus iria* has the highest width of epidermal cells in both the abaxial (21.42±0.95 µm) and adaxial surface (48.26±0.79 µm) as shown in Figure 5 & 6 and Table 2. In the abaxial surface, the anticlinal walls of epidermal cells are wavy in *Cyperus rotundus*, *Cyperus nutans var. eleusinoides*, *Carex flacca Schreb*, *Cyperus esculentus*, *Cyperus malaccensis, Kyllinga brevifolius*, *Fuirena pubescens, Cyperus alulatus*. Sinuous-wavy anticlinal walls are found in *Cyperus difformis*, and *Cyperus niveus*. Epidermal cells were present in linear rows with large and a few small cells. Epidermal cells are rectangular to polygonal varies from smaller to larger cells. Only the measurement were taken of the longer cells (Table 3)

3.3 Stomatal Complex

The stomatal complex of the 18 studied species was carefully examined using a light microscope. Variations in the width and length of stomatal pores and guard cells were observed on both the upper and lower leaf surfaces in our investigation. All species studied were found to have stomata only on the abaxial surface, and they were paracytic with only two subsidiary cells surrounding them (Table 3). *Cyperus iria* has the stomata and guard cells with the largest length of (53.47±0.88 µm) and (50.08 ±1.01 µm) respectively. *Kyllinga brevifolius* has the highest width of stomata and guard cells with (36.56±1.016 µm) and (14.96±1.03 µm) respectively. *Cyperus nutans* have the smallest length of stomata (28.50±1.51 µm) and guard cells (20.86±1.22 µm). Like all monocots, the members of the Cyperaceae family have dumbbell-shaped stomata (Table

2). Trichomes are only observed in *Kyllinga brevifolius*. This combination of features may serve as a distinguishing characteristic for species within this family. The largest subsidiary cell (67.71±1.59 µm) was observed in *Kyllinga brevifolius* while the widest subsidiary cell (27.38±0.98 µm) was found in *Cyperus iria*. Most of the species have 1-4 rows of stomata in bands on the lower surface in almost all species

3.4 Stomatal pores

In the Cyperaceae family, the stomatal pores have a unique shape, which is commonly referred to as "horseshoe-shaped" or "annular." These pores are curved, resembling a horseshoe, with the guard cells forming a ring around the pore. This shape is distinct to species within the Cyperaceae family and differs from the more common circular or elliptical shapes found in other plant families. The largest stomatal pore (21.77±0.99 µm) was observed in *Furiena pubescens*. *Cyperus alternofolius* have the widest pores (4.83±1.21 µm) whereas, *Cyperus rotundus* has the smallest pore (0.66±0.11 µm). In this study, we have identified consistent anatomical traits in the leaf epidermis of certain species (Table 2 & 3).

3.5 Stomatal index

Cyperus nivius showed the highest stomatal index of (46.15%) on the abaxial surface and *Kyllinga* brevifolius showed the lowest (20%) stomatal index on lower surface.

3.6 Dichotomous Taxonomic Key Based on Cyperaceae Foliar Anatomical Traits

1. Abaxial epidermal cells

- Rectangular…….2
- Polygonal…….8

2. Anticlinal wall pattern of abaxial epidermal cells

- Straight…….3
- Wavy or wavy-undulating……5

3. Number of epidermal cells (abaxial)

- Fewer than 20…….*Cyperus exaltatus* Retz.
- More than 20…….4

4. Number of stomata (abaxial)

- Fewer than 15…….*Cyperus iria* L.

- More than 15…….5

5. Stomata type

- Paracytic……6
- Other types……..*Cyperus malaccensis* Lam.

6. Stomatal pore shape:

- Annular……...7
- Not annular…….*Cyperus esculentus* L.

7. Number of stomata (abaxial)

- Fewer than 20……*Cyperus rotundus* L.
- More than 20…….*Cyperus longus* L.

8. Adaxial epidermal cells

- Rectangular……..9
- Polygonal……11

9. Anticlinal wall pattern of adaxial epidermal cells

- Straight…….10
- Wavy or undulating……..*Cyperus nutans* var. *eleusinoides*

10. Number of stomata (abaxial)

- Fewer than 10……*Cyperus alterniflorus* R.Br.
- More than 10…….*Cyperus nutans* Vahl

11. Number of stomata (abaxial)

- Fewer than 10……*Cyperus alulatus* J.Kern
- More than10……..12

12. Number of stomata (abaxial)

- 16 or more……*Cyperus niveus* Retz.
- Fewer than 16……13

13. Shape of epidermal cells (adaxial):

- Wavy……*Fuirena pubescens* (Poir.) Kunth
- Straight or slightly wavy……..*Kyllinga brevifolia* Rottb.

3.7 Dendrogram and PCA clustering tool analysis

Cluster analysis based on Euclidean distance was performed based on analytical pollen data of selected Cyperaceaespecies. Two major associations were formed, named Association C1 and

Association C2. Association C1 consists of ten species. These species have similarities based on colpi length and width (Figure 7). PCA performed with the microanatomical quantitative data from Cyperaceous taxa. PCA, principal component analysis; ECL, epidermal cell length; ECW, epidermal cell width; SL, stomatal length; SW, stomatal width. Association C2 consists of seven species and these associations have similarities based on epidermal cell length, epidermal cell width, stomatal length, stomatal width (). PCA analysis was used on the foliar epidermal quantitative mean data of Cyperaceous species to determine the variance among different variables in the context of eigenvariables. PCA illustration showed that the length of epidermal cell on the PC1 axis of *C. esculentus* and width of epidermal cell of *C. alulatus* was characterised by a higher values, whereas on PC2 a higher value in terms of epidermal cell length was observed with reference to *C. longus* (Table 4 and Figure 8).

3. Discussion

This research systematically investigated both qualitative and quantitative characteristics of the family Cyperaceae. Qualitative features such as the shape of epidermal cells, anticlinal wall, types of stomata, and trichomes on both adaxial and abaxial surfaces were studied. Quantitative features included measurements of the length and width of leaf epidermal cells, stomata, stomatal pores, subsidiary cells, and trichomes, along with the calculation of stomatal indexes for both adaxial and abaxial surfaces. The findings provide valuable insights into the taxonomic identification of plant species in the family Cyperaceae. The foliar epidermal characters analyzed in this study are significant for identification and species delimitation within the family Cyperaceae. Furthermore, the study revealed anatomical features are constant, adaptable, and not subject to change with environmental variations (Ullah et al., 2018).

Under a light microscope, the cells appear elongated with varying thicknesses and outlines, ranging from irregular to rectangular and even polygonal. The shape of the anticlinal wall may be straight, slightly wavy, or strongly undulated, which is similar to the previous work of Ahmad et al., (2010). According to Bercu (2019), sinuous epidermal anticlinal walls were observed in *Cyperus alternifolius* but straight anticlinal walls were observed in this study. The epidermal cells exhibited variation in size between the adaxial and abaxial surfaces, with the cells on the adaxial surface being significantly larger than those on the abaxial surface and this observation by Hameed et al. (2012 aligns with the findings of the current study.

According to the findings of Odedeji & Adedeji,. (2015) the paracytic type of stomata, where guard cells are surrounded by two subsidiary cells, has been found in all the species studied. All species are hypostomatic, meaning they have stomata only on the lower surface. Mumtaz et al (2021) observed stomata both on the upper and lower surfaces *in Cyperus rotundus, Cyperus iria, Cyperus longus, Cyperus diformis, Cyperus alternifolius, Cyperus nutans and Cyperus esculentus* but in this study no stomata were observed in these species in the upper surface. The same results were observed by Oh & Park, (1997) for the *Fimbristylis* species. According to Parvaz et al., (2023), in *C. nivius* the upper epidermal cells are larger than the lower epidermal cells and stomata were only observed on the lower surface, similar to this study. Epidermal and stomatal cells are observed in linear rows oriented parallel to the major veins, which is similar to the previous work of Rudall (2023). Hameed et al., (2012) observed no stomata in *Cyperus* species on the abaxial surface which is opposite to the current results as stomata were only observed on the abaxial surface just like observed by Zarinkamar (2006). If we talk about *Carex*, the results involving stomata and epidermal cells length and shape were constant with the previous work of Bugg C. et al., (2013). In a study conducted by Odedeji & Adedeji, (2015) on the Cyperaceae family, it was found that trichomes were only present in *Kyllinga* species and not in any Cyperus species and a similar result was observed in the current study. Trichome micro-morphology provides important information about species, genera, tribes, and subfamily relationships. The length and width of stomata, guard cells, epidermal cells and subsidiary cells of the studied Cyperus species vary greatly from those observed by Hamid & Al-Garaawi, (2023).

Conclusion

This research described the examination of foliar leaf anatomical traits of selected Cyperaceae species collected from diverse ecological sites using light microscopic tool. Both qualitative and quantitative micro-morphological epidermal traits of Cyperaceae species play a significant role in describing and defining taxonomic correct identification. Furthermore, developed taxonomic keys based on epidermal micromorphology of Cyperaceae species were clearly differentiate the species and distinguished each studied species characters to used them in future for their further systematic classification and visualizing the leaft traits using scanning electron microscopy.

Declarations

Consent to Publish

The data or findings resulting from your participation may be used in publications, presentations, or other scholarly activities.

Competing interests

The authors has to declare the no potential competing interest.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Captions

Figure 1. Field collection and foliar anatomical micrographs (Author: Arfeen Zahra) of Epidermal cells (EC), Stomata (St), Anticlinal walls AW of Cyperaceae species; (A-C) *Cyperus rotundus*(A) Field photograph (B) abaxial surface (C) adaxial surface (D-F) *Cyperus nutans* var. eleusinoides (D) Field photograph (E) abaxial surface (F) adaxial surface (G-I)

457 *Cyperus alterniflorus* (G) Field Photograph (H) abaxial surface (I) adaxial surface (J-L)
458 *Cyperus longus* (J) Field Photograph (K) abaxial surface (L) adaxial surface.

459 **Figure 2.** Field collection and foliar anatomical micrographs (Author: Arfeen Zahra) of Epidermal
460 cells (EC), Stomata (St), Anticlinal walls AW of Cyperaceae species; (A-C) *Carex flacca*
461 (A) Field Photograph) (B) abaxial surface (C) adaxial surface (D-F) *Fimbristylis*
462 *bisumbellata* (Forssk.) Bubani (D) Field Photograph (E) abaxial surface (F) adaxial surface
463 (G-I) *Cyperus nutans* (G) Field Photograph (H) abaxial surface (I) adaxial surface (J-L)
464 *Cyperus esculentus*(J) Field Photograph (K) abaxial surface (L) adaxial surface.

465 **Figure 3.** Field collection and foliar anatomical micrographs (Author: Arfeen Zahra) of Epidermal
466 cells (EC), Stomata (St), Anticlinal walls AW of Cyperaceae species; (A-C) *Cyperus*
467 *esculentus* (A) Field Photographs (B) abaxial surface (C) adaxial surface (D-F) *Cyperus*
468 *exaltatus* (D) Field Photographs(E) abaxial surface (F) adaxial surface (G-I) *Cyperus*
469 *malaccensis* (G) Field Photograph(H) abaxial surface (I) adaxial surface (J-L) *Cyperus iria*
470 (J) Field Photograph(K) abaxial surface (L) adaxial surface.

471 **Figure 4.** Field collection and foliar anatomical micrographs (Author: Arfeen Zahra) of Epidermal
472 cells (EC), Stomata (St), Anticlinal walls AW of Cyperaceae species; (A-C) *Cyperus*
473 *difformis* (A) Field Photograph(B) abaxial surface (C) adaxial surface (D-F) *Fuirena*
474 *pubescens* (D) Field Photograph (E) abaxial surface (F) adaxial surface (G-I) *Cyperus*
475 *niveus* (G) Field Photograph (H) abaxial surface (I) adaxial surface (J-L) *Cyperus*
476 *flavescens* (J) Field Photograph (K) abaxial surface (L) adaxial surface (M-O) *Cyperus*
477 *alulatus* (M) Field Photograph (N) abaxial surface (O) adaxial surface.

478 **Figure 5.** Epidermal cells length of selected taxa from family Cyperaceae.

479 **Figure 6.** Epidermal cells width of selected taxa from family Cyperaceae.

480 **Figure 7.** Dendrogram clustering showing the relationship among different Cyperaceous taxa.

481 **Figure 8.** PCA performed with the pollen quantitative data from Cyperaceous taxa. PCA, principal
482 component analysis; ECL abax, Epidermal cell length abaxial surface; ECW adax,
483 Epidermal cell width adaxial surface; SW, Stomatal width; SL, Stomatal length

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