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# LEAF EPIDERMAL MORPHOLOGY AND ITS SYSTEMATIC IMPLICATIONS IN POACEAE: INVESTIGATING 15 TAXA FROM WETLAND AREAS

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**Abstract**

Light microscopic study is significant for the identification of taxonomically complex grass family Poaceae. This is the first microscopic study of foliar epidermal characters of 15 species of family Poaceae belonging to waterlogged and marshy (wetland) areas. These 15 species were collected from different localities of Punjab and Khyber Pakhtunkhwa, Pakistan. In current study we compared the morphological features of foliar epidermis characters of leaf by using light microscopy. Variations in qualitative characters have been observed that include epidermal cell shape, pattern of anticlinal wall, types of stomata, micro hair types, silica body shapes, subsidiary cell shape and presence of prickles. The key result of this study showing that type of stomata in all species is paracytic on both upper and lower surfaces. The anticlinal walls are mostly sinuous but some plant species have straight and undulating walls. Mostly epidermal cells are rectangular but in some species they are elongated and irregular in shape. Micro hairs are either Panicoid type or Chloridoid type in some species. The difference in anatomical characters of leaf between the genera and species proved very helpful in the identification and classification. Taxonomic key using micro morphological characters is provided for the distinction among the genera and species.

**Key words:** Foliar epidermal morphology, Light Microscopy, Poaceae, Waterlogged, Marshy.

## Introduction

The Poaceae or Gramineae is considered as one of the most diverse families of flowering plants in terms of their ecology, morphology and economic signaficance. According to (Kellogg, 2015; Soreng *et al*., 2017) the family Poaceae is one of the remarkable family in term of evolutionary perspective comprising (11000-12000) species and (750-770) genera comprising twelve subfamilies; Anomochlooideae, Arundinoideae, Aristidoideae, Bambusoideae, Chloridoideae, Danthonioideae, Micraioideae, Oryzoideae, Panicoideae, Pharoideae, Pooideae and Puelioideae. It is fourth largest family comprising of 700 genera & eleven thousand grass species (Wei *et al*., 2006). In Pakistan this family is represented by 158 genera and 492 species with 26 tribes and five subfamilies (Cope, 1982).

The study of (Clayton & Renvoize, 1986) suggested that approximately 40 species were being discovered per annum. It is estimated that about (40%) of the total earth surface is covered by ecologically dominant species of Poaceae including grasses and bamboo forests (Hilu, 1984). The members of the family are mostly cosmopolitan regarding distribution and considered one of the most successful plant groups among all angiosperms, as they revel in every climatic condition, diverse habitat and distributed in all phyto-geographical regions throughout the globe. In the family Poaceae approximately 14% of grasses are monocots (Oyediran *et al*., 2004), and they are also ecologically dominant, comprising of about twenty percent on the surface of earth (Shantz, 1954).

Plants are identified based on external features of all levels of taxonomic ranks however; several angiosperms cannot be differentiating on external features. Generally, for accurate identification of these species microscopic studies (anatomical) can be used to determine taxonomic status (Gilani *et al.*, 2003; Yousaf *et al*., 2008; Birjees *et al.*, 2021; Khan *et al*., 2021). In previous studies monocots have been identified by using anatomical characters along with the other morphological characters (Nazir *et al*., 2013). It has been observed that grasses are fairly advanced in their evolutionary process as several floristic features are reduced, thus providing inadequate external characters which are taxonomically significant and therefore posing various difficulties in exact identification.

The external layer of the leaf known as epidermal layer is a multi-functional tissue that has significant value in protection and water processes. Different specialized cells are present in plants which perform specific functions. These several cells show diverse steps of external characters specialization. It is vital therefore to attempt a search on foliar epidermal features that may be of taxonomic important (Khan *et al*., 2011). Anatomical characters can provide additional taxonomic evidence and establishing the inter-relationships of different plant taxa (Prat, 1936; Stebbins, 1956; Metcalfe, 1960; Ellis, 1986; Mejia-Saules & Bisbey, 2003; Khan *et al*., 2020).

The anatomical studies of leaf are significant for delimitation, classification and for resolving phylogenetic and evolutionary complications among the taxa (Chaudhary *et al*., 2001). According to a study of Prat (1948), it is reported that foliar anatomy of family Poaceae shows greater diversity of micro morphological characters and give information for systematic deployment of species (Brown & Emery, 1958). Many workers have worked on micro-morphological characters of the family Poaceae including Prat (1932) who studied 101 species of family Poaceae in terms of leaf anatomy, Metcalfe (1960) examined the epidermal anatomy of monocots that included family Poaceae, Renvoize (1982) investigated

leaf-blade anatomy of tribe Andropogoneae containing 86 genera, Hilu (1984) reported different characters of foliar epidermal layer of *Andropogon* sect. *Leptopogon* of family Poaceae. Besides this Dávila & Clark (1990) and Mcwhorter *et al*., (1995) also investigated anatomical structures of 17 species of the genus *Sorghum*. In Pakistan many workers have worked on anatomical features of leaf (Ahmad *et al.*, 2012; Ahmad *et al.*, 2011a,b,c; Chaudhari *et al*., 2013; Chaudhari *et al*., 2014; Gilani *et al.,* 2002; Khan *et al.*, 2017a; Khan *et al*., 2017b; Nazir *et al.*, 2013; Ullah *et al.*, 2011).

Foliar epidermal anatomical characters plays significant role in taxonomy of grasses and are important in the classification of extensive groups, within sub families, tribe and delimitation of species. The current research is necessary for emphasizing the essential internal features of grasses species of waterlogged and marshy area as the region has diversity of grass vegetation which has been unknown related to foliar epidermal anatomy. Furthermore, current research is also required for knowing that how grass species are linked to one another and how foliar epidermal anatomy is important in identifying the taxa or species or generic level.

## Materials and Methods

**Taxon sampling and identification:** Fifteen species of family Poaceae were collected from different localities of Punjab and Khyber Pakhtunkhwa province during the month of March 2017 to May 2017. The detailed account of specimen is mentioned in (Table 1, Fig. 1) along with their habitat, altitude and voucher specimen numbers. Identification of these grasses was carried out by comparing samples with the specimens of herbarium kept in Herbarium of Pakistan in Quaid I Azam University ISL, Pakistan. Furthermore identification was confirmed by comparing their morphological characters with the Flora of Pakistan and those used previously by (Nakaike & Malik, 1992). Correct botanical names and their families were validated using TPL (www.theplantlist.org). After identification these specimens were submitted to Herbarium of Pakistan, Quaid-i-Azam University, Islamabad for future reference.

**Light microscopic investigation of leaf epidermal characters:** Fully dried and matured leaves of plant species have been examined under the light microscope. All of these samples were prepared according to the technique mentioned by (Clarke, 1960) with some modifications. A small part of leaf was placed in a test tube having 75 percent lactic acid and 25 percent nitric acid and boiled until the chloroplast present in the leaves evaporated and epidermis became clearly visible. This transparent section was then placed into petri-plate afterwards containing water in it. Further the upper and lower surfaces of epidermis were removed and then placed on a glass slide. In order to make peeling easy and to soften tissues of plant only a droplet of lactic acid was used. A slide of both upper & lower surfaces was prepared and then protected with the help of cover slip. About 9–10 slides were prepared for both surfaces of each species. Epidermal cells and stomata were observed following the technique proposed by Bondada *et al*., (1994). Describing stomatal types different terminologies were used following that of Metcalfe (1960) and Van Cotthem, (1970). The samples were studied under light microscope (Model: XSP-45LCD) on different epidermis parameters. Light microscopic photographs were taken using LEICA-DM-1000 microscope (Tokyo, Japan) having Meiji infinity DK- 5000 camera.

**Quantitative analysis:** Under microscope we observed size of epidermal cell, size of stomata, size of micro hairs, size of subsidiary cells and size of guard cells, epidermal cells number, stomata number, epidermal cells shape, and shape of silica bodies. All the qualitative and quantitative characters have been given in (Tables 2 and 3). Statistical analysis had been carried out by SPSS software 16. The measurements of epidermal cells have been carried out in the resolution of 40 x in light microscope. Cells of epidermis on both abaxial and adaxial surfaces were examined. Along with total number of epidermal cells per unit area was also observed.

**Stomatal index:** For stomatal index number of epidermal cells and stomata number on the surface area from 5 different ocular for adaxial and abaxial surfaces were counted.

$$\text{S.I} = \frac{S}{E+S} \times 100$$

Here, S= No. of stomata per unit area, S.I=stomatal index, E= No. of epidermal cell per unit area.

## Results

**Epidermal cell characters:** In this study, 3 categories of epidermal cells for example elongated, rectangular & sometimes slightly irregular in shape typified by thick, undulating and sinuous anticlinal walls are observed (Figs. 2, 3 & 4). Epidermal cells of upper and lower surfaces exhibit same sizes yet adaxial epidermal cells were recorded to be larger than abaxial ones. Shape of epidermal cells is separated in to 3 groups; (i) species with rectangular epidermal cells (*Arundo donax*, *Brachiaria reptans*, *Cynodon dactylon*, *Polypogon monospeliensis*, *Saccharum spontanum*, *Dicanthium annulatum*, *Glyceria notata*, *Echinochloa crus-galli*, *Setaria pumila*, *Paspalum paspalode*), (ii) species with elongated epidermal cells (*Echinochloa colona*, *Polypogon fugax*, *Coix lacraymajobi*, *Phragmites karka*) and (iii) species with irregular epidermal cells (*Phragmites australis*) (Figs. 2, 3 & 4). Epidermal cells are also separated on the basis of anticlinal wall i.e. sinuous (SI), Undulating (UN) and straight anticlinal walls (ST). Epidermal cells thickness was highest in *Setaria pumila* (Fig. 5). Diverse variation is found among the size of epidermal cells as these sizes differ in both the surfaces. The maximum average length of epidermal cell on adaxial surface was noted for *Setaria pumila* (172.1µm) while highest average length on abaxial surface was observed for *Polypogon monspeliensis* (131.1µm) (Fig. 5). Lowest average epidermal cells length on abaxial side was observed in *Saccharum spontaneum* (11.6µm).

**Table 1. Selected species of Poaceae used for epidermal morphology: habitat, voucher and other information.**

| Sr. No. | Species | Voucher | Locality | Altitude (m) | Flowering season | Habitat |
|---|---|---|---|---|---|---|
| 1. | *Arundo donax* L. | ASK-154 | Ghandi Khan Khel (Lakki Marwat, Khyber Pakhtunkhwa/K.P.K) | 287.122 | June - December | Common in waterlogged areas and found near the banks of rivers, ditches, ponds |
| 2. | *Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb. | ASK-186 | Ahmed Khel (Lakki Marwat, Khyber Pakhtunkhwa/K.P.K) | 462.0768 | June-October | Found in moist and shady places |
| 3. | *Coix lacryma-jobi* L. | ASK-134 | Teri (Karak, K.P.K) | 648.9192 | August-October | Found on the banks of ponds and streams |
| 4. | *Cynodon dactylon* (L.) Pers. | ASK-113 | Mitha khel (Karak, K.P.K) | 363.3216 | Throughout the year | Moist and shady places |
| 5. | *Dichanthium annulatum* (Forssk.) Stapf | ASK-164 | Zarobi (Swabi, K.P.K) | 319.4304 | March-November | Found in moist areas mostly at the edges of streams and water courses |
| 6. | *Echinochloa colonum* (L.) Link | ASK-161 | Kaddi (Swabi, K.P.K) | 320.9544 | May -September | Weed and it is found in moist lands |
| 7. | *Echinochloa crus-galli* (L.) P. Beauv. | ASK-196 | Isa Khel (Mianwali, Punjab) | 206.959 | June-October | Commonly found on shady and wet places |
| 8. | *Glyceria notata* Chevall | ASK-147 | Daud khel (Mianwali, Punjab) | 215.189 | July-September | Commonly found in aquatic environment |
| 9. | *Paspalum distichum* L. | ASK-118 | Tamman, Talagang (Chakwal, Punjab) | 366.3696 | April-May and again August-September | Common along margins of ponds, ditches, and water |
| 10. | *Phragmites australis* (Cav.) Trin. ex Steud. | ASK-112 | Talagang (Chakwal, Punjab) | 369.5612 | July-October | Commonly found in waterlogged and marshy areas |
| 11. | *Phragmites karka* (Retz.) Trin. ex Steud. | ASK-169 | Basharat Town (Chakwal, Punjab) | 893.9784 | April-November | Along stream banks, wet rocky places near streams |
| 12. | *Polypogon fugax* Nees ex Steud | ASK-177 | Chak Bhon (Chakwal, Punjab) | 516.0264 | May-August | Found on moist, shady places and marshy areas |
| 13. | *Polypogon monspeliensis* (L.) Desf. | ASK-183 | Gujar Khan (Rawalpindi, Punjab) | 456.2856 | March and July | Common in moist, marshy places, along water courses, near sanitary pipes and channels |
| 14. | *Saccharum spontaneum* L. | ASK-156 | Darya Khan (Bhakkar, Punjab) | 175.26 | July-September | Found on the banks of ponds and streams |
| 15. | *Setaria pumila* (Poir.) Roem. & Schult. | ASK-159 | Kallurkot (Bhakkar, Punjab) | 192.024 | June-October | Common on moist soil, near water and waterlogged soils |


Fig. 1. Field photographs of *Saccharum spontaneum* (A), *Arundo donax* (B), *Brachiaria reptans* (C), *Coix lacryma-jobi* (D), *Cynodon dactylon* (E), *Paspalum distichum* (F), *Phramites australis* (G), *Setaria pumila* (H), *Polypogron fugax* (I).

**Stomata and Guard cells:** In the leaves of all mentioned species both upper and lower surfaces contains stomata. Paracytic stomatal type have been commonly noted in all the species. Variations were found in measurement of the size of stomata. Longest stomata were seen in *Setaria pumila* (54.2µm) on upper surface while on lower surface largest stomata was seen in *Polypogon fugax* (35.6µm) (Table 3) (Fig. 6). Smallest stomata were found in *Glyceria notata* (18.1 µm on upper surface while on lower surface, *Polypogon monspeliensis* (16.1) has the smallest stomatal length. Variation in stomatal size was quite diverse as they differ among the species and maximum size of stomata was found on lower surface than upper (Table 3). Maximum stomatal index was observed in *Echinochloa crus-galli* (89%) while lowest was observed in *Glyceria notata* (51%) on abaxial surfaces (Fig. 6).

Sizes of guard cells of stomata also vary. Guard cells size ranges from (31.1 µm) in *Phragmites australis* to (8.7 µm) in *Saccharum spontanum* on abaxial surface of leaves. Length of guard cells of upper surface ranged between maximum (35.1 µm) in *Polypogon fugax* to minimum (6.5 µm) in *Saccharum spontanum*.

**Micro hairs and Silica bodies:** Two types of micro hairs were present in the species of grasses i.e. Panicoid and Chloridoid. Chloridoid type of micro hairs were observed in *Cynodon dactylon* while Panicoid type of micro hairs were observed in *Brachiaria reptans*, *Saccharum spontanum*, *Dicanthium annulatum*, *Echinochloa colona*, *Echinochloa crus-galli*, *Setaria pumila*, *Paspalum paspalode*. Variations were seen in size of micro hairs (L×W) (Fig. 8) (Table 3). The highest hair length was found in *Arundo donax* (70.2µm) on adaxial surface while smallest hair was seen in *Saccharum spontaneum* (3.2µm) (Table 3). In most species of grasses these micro hairs were absent (Table 2).

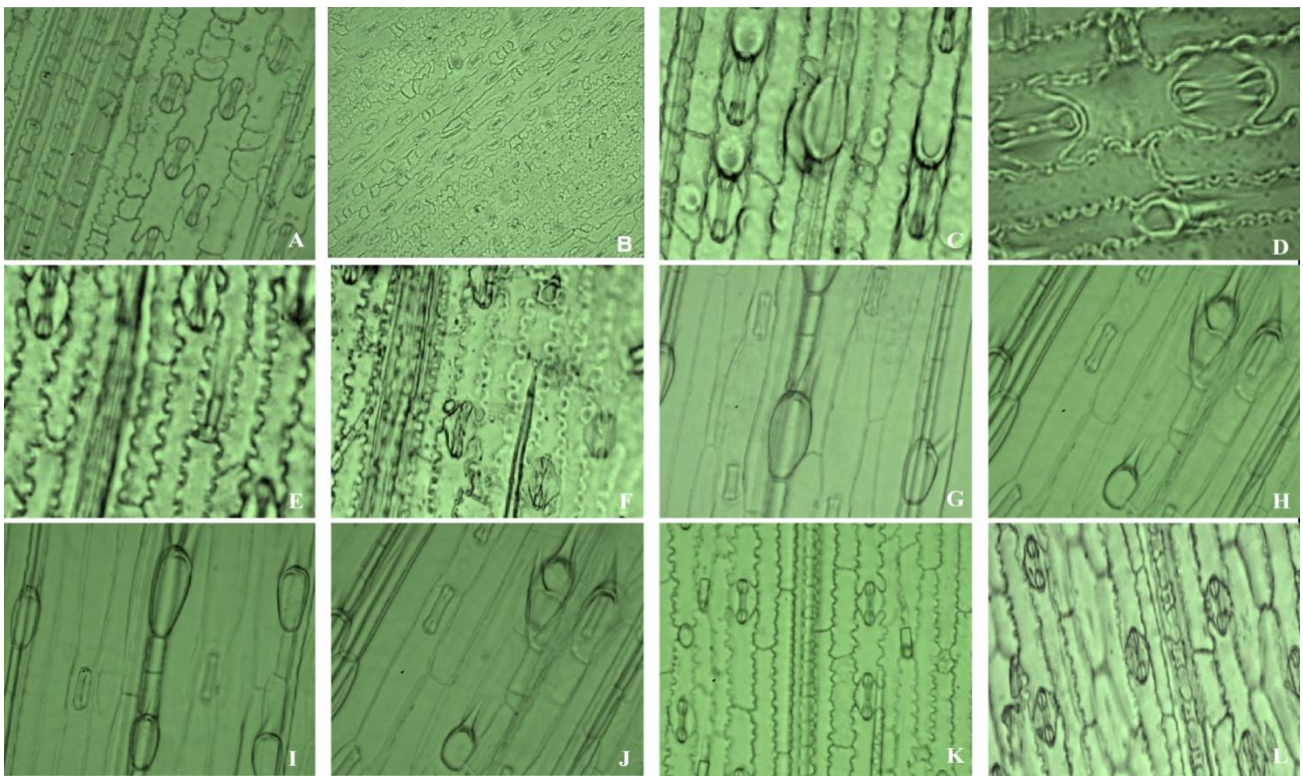


Fig. 2. Abaxial and adaxial leaf surface of *Arundo donax* (A-B), *Dicanthium annulatum* (C-D), *Saccharam spontanum* (E-F), *Polypogon fugax* (G-H), *Polypogon monspeliensis* (I-J), *Brachiaria reptans* (K-L).

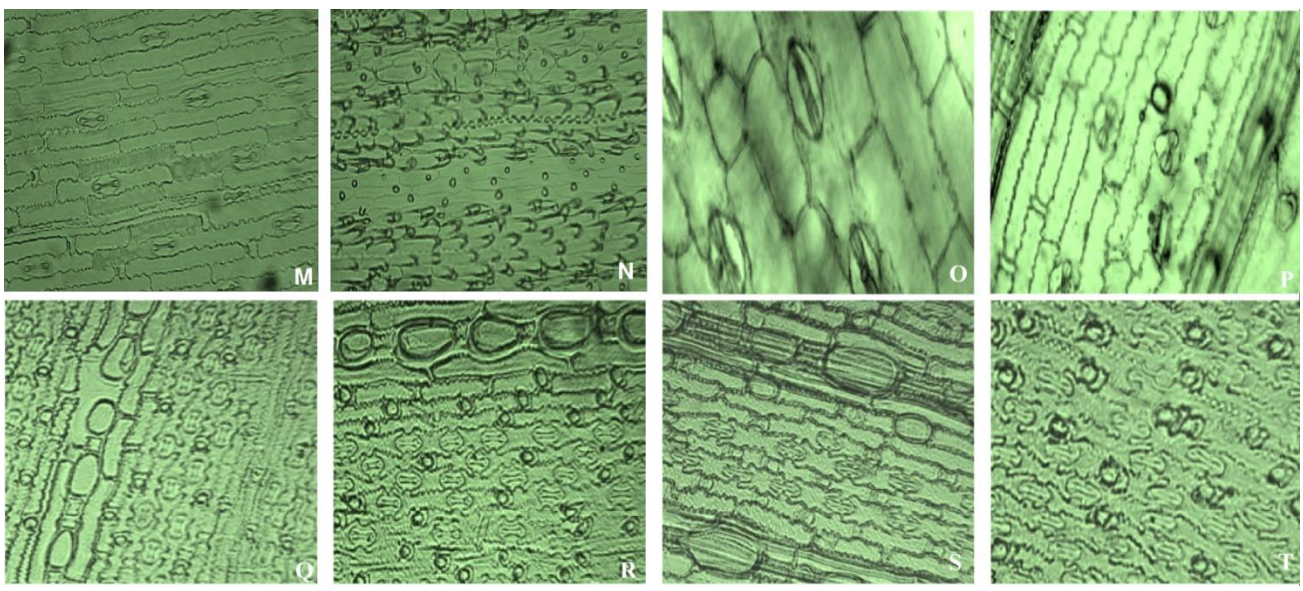


Fig 3. Abaxial and adaxial leaf surface of *Glyceria notata* (M-N), *Setaria pumila* (O-P), *Phragmites australis* (Q-R), *Phragmites karka* (S-T), (LM 100X).

Presence of silica bodies is the distinguishing feature of grass family i.e. Poaceae. Three forms of silica bodies were examined dumb bell shaped, saddle shaped and cross shaped (Table 2). Cross shaped were found in (*Brachiaria reptans*, *Echinochloa colona*, *Echinochloa crus-galli*, *Paspalum paspalode*), Saddle shaped were examined in (*Setaria pumila*, *Cynodon dactylon*, *Phragmites karka*, *Arundo donax*) and dumb bell shaped were observed in (*Phragmites australis*, *Polypogon monospeliensis*, *Saccharum spontanum*, *Dicanthium annulatum*, *Coix lacraymajobi*, *Glyceria notata*).

**Subsidiary cells:** Among the members of family Poaceae shape of subsidiary cells also vary within species. In present research 4 kinds of subsidiary cells have been noticed in grass species which are; triangular shaped, dome shaped, rectangular shaped, elongated shaped (Table 2). Triangular shaped subsidiary cells were present in (*Paspalum paspalode*, *Saccharum spontanum*, *Phragmites australis*, *Arundo donax*), dome shaped subsidiary cells were present in (*Phragmites karka*, *Brachiaria reptans*, *Cynodon dactylon*, *Dicanthium annulatum*, *Coix lacraymajobi*, *Echinochloa colona*, *Echinochloa crus-galli*,

*Setaria pumila*), rectangular shaped in (*Polypogon monospeliensis*) and elongated shaped in (*Glyceria notata*, *Polypogon fugax*). Great variations were observed in size of subsidiary cells. The maximum length of subsidiary cells were present on abaxial surface by *Arundo donax* (95.4µm) while smallest one was observed in *Polypogon fugax* (23.4 µm). On the other hand in adaxial surface largest subsidiary cell length was seen in *Arundo donax* (87.9 µm) and smallest was seen in *Polypogon fugax* (17.8 µm) (Fig. 7).

## Discussion

Members of family Poaceae are growing widely in different waterlogged and marshy areas of Punjab and Khyber Pakhtunkhwa. These grasses have been selected from different ecological zones (Table 1). This research paper is the foremost article on the microscopic study of leaf epidermal characters of the Poaceae family from different waterlogged and marshy soils of Punjab and Khyber Pakhtunkhwa. In previous studies many reports have been found regarding anatomical characters of leaves but not a single study explore the micro-morphological characters of foliar epidermis of grasses on this particular kind of habitat. In present article, experimental work has been done using light microscopy for a perfect understanding of micro morphological features and potential link between the species and different genera of the family Poaceae. Results attained through present investigation found significant and can be utilized as a foundation of taxonomic data in family Poaceae.

Leaf epidermal anatomy is very essential in terms of classification (Ashfaq *et al*., 2019). For differentiation of the various taxa, foliar micro-morphology plays an important role. The previous studies also revealed the implication of foliar epidermal micromorphology in recognizing the complex taxa (Hameed *et al*., 2020). Leaf micro morphological characters of different taxa exhibited noteworthy taxonomic features in the identification of taxa.

The current research work is proven very successful and resulted in study of intra-specific and inter-generic diversity in the family. In previous reports foliar epidermal micro morphological studies were done by many workers (Ahmad *et al*., 2009; Chaudhary *et al*., 2001; Keshavarzi & Seifali, 2005; Khan *et al*., 2017a; Khan *et al*., 2017b; Khan *et al*., 2017c; Reimer & Cota-Sánchez, 2007; Sharma & Kalia, 1983). While studying *Setaria pumila* foliar epidermal characters paracytic stomata was found with rectangular epidermal cells, subsidiary cells with dome appearance, silica bodies with saddle appearance, panicoid type hairs and also prickles are found similar to the finding of Ahmad *et al*., (2011c). Studies on *Arundo donax* revealed the presence of rectangular epidermal cells, paracytic stomatal type, saddle shaped silica bodies and triangular subsidiary cells which is slightly different from the studies conducted on grasses by Khan *et al*., (2017b) having dumb bell shaped silica bodies. A research conducted by (Desai & Raole, 2013; Palmer & Gerbeth Jones, 1986) on genus *Phragmites* and reported the occurrence of paracytic stomatal type along with triangular subsidiary cells and dumb bell shaped silica which differ from current study results having dome shaped subsidiary cells and rounded silica bodies. Studies carried out on *Cynodon dactylon* revealed the occurrence of paracytic stomatal type, subsidiary cells with dome appearance, silica bodies with saddle appearance and presence of chloridoid type micro hairs while (Ahmed, 2009; Khan *et al*., 2017b) reported the absence of micro hairs. Leaf epidermal characters of *Dichanthium annulatum* and *Saccharum spontaneum* showed the presence of paracytic stomata, dome shaped subsidiary cells, dumb bell shaped silica bodies, panicoid type micro hairs and presence of prickles similar to the study conducted by many workers (Nazir *et al*., 2013; Ullah *et al*., 2011). *Echinochloa colona* have rectangular epidermal cells in our finding while (Jattisha & Sabu, 2015) studies showed that the species had wavy epidermal cells. According to a study conducted by (Thompson & Estes, 1986) on foliar epidermal anatomy of Poaceae in which they determined that *Brachiaria reptans* lack silica bodies while in our findings the species have panicoid type of silica body.

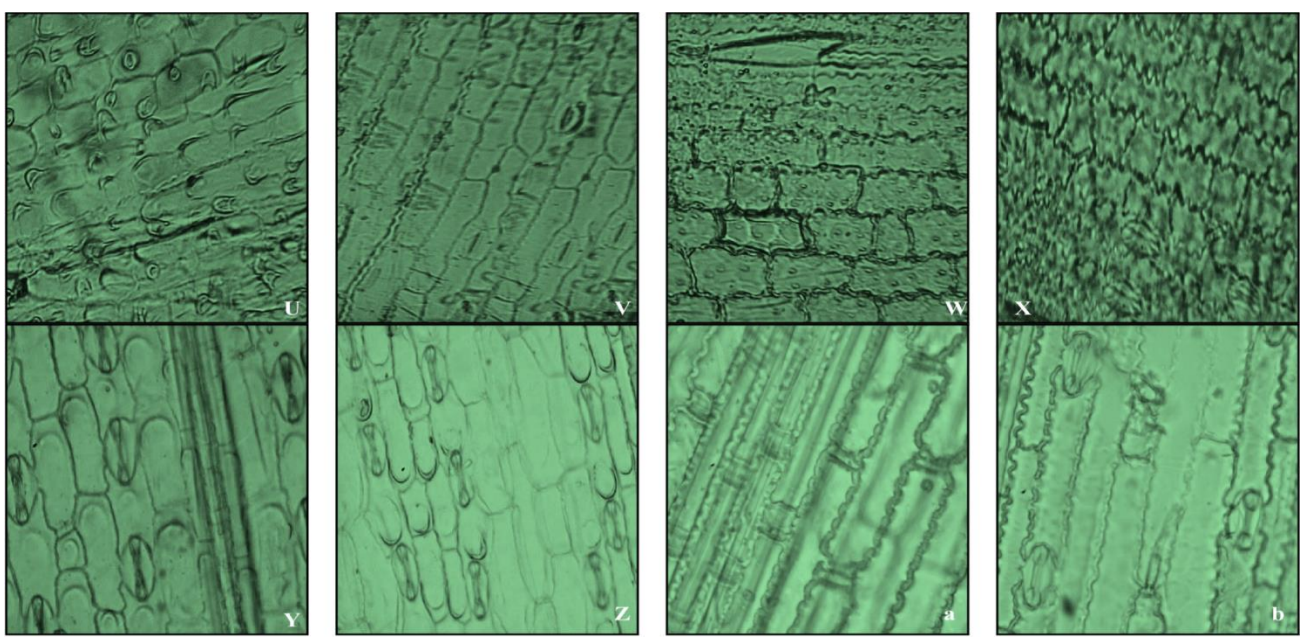


Fig. 4. Abaxial and adaxial leaf surface of *Coix lacryma* (U-V), *Cynodon dactylon* (W-X), *Echinochloa colonum* (Y-Z), *Echinochloa crus-galli* (a-b), (LM 100X).

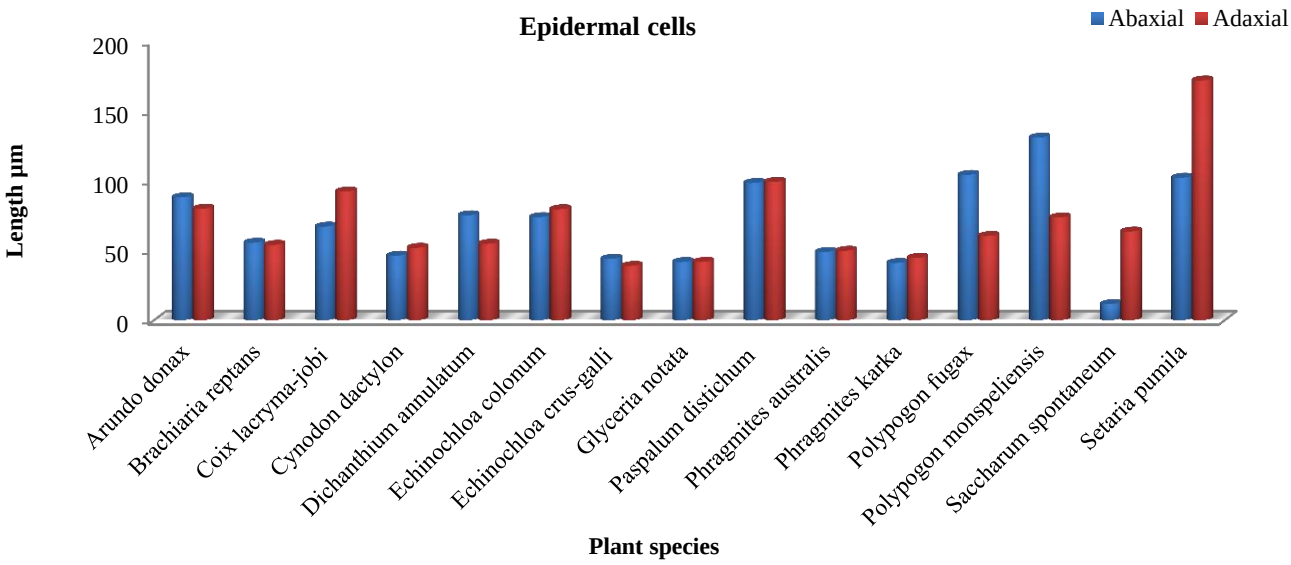


Fig. 5. Quantitative variation in leaf epidermal cells on both adaxial and abaxial surfaces of Family Poaceae.

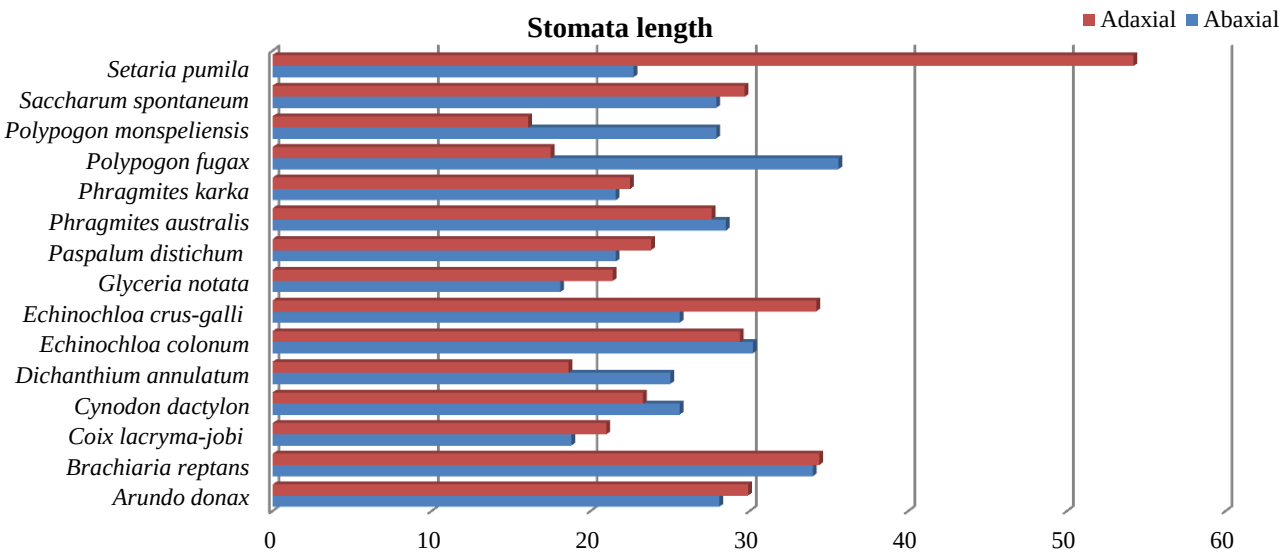


Fig. 6. Length of Stomata on adaxial and abaxial surfaces of Family Poaceae.

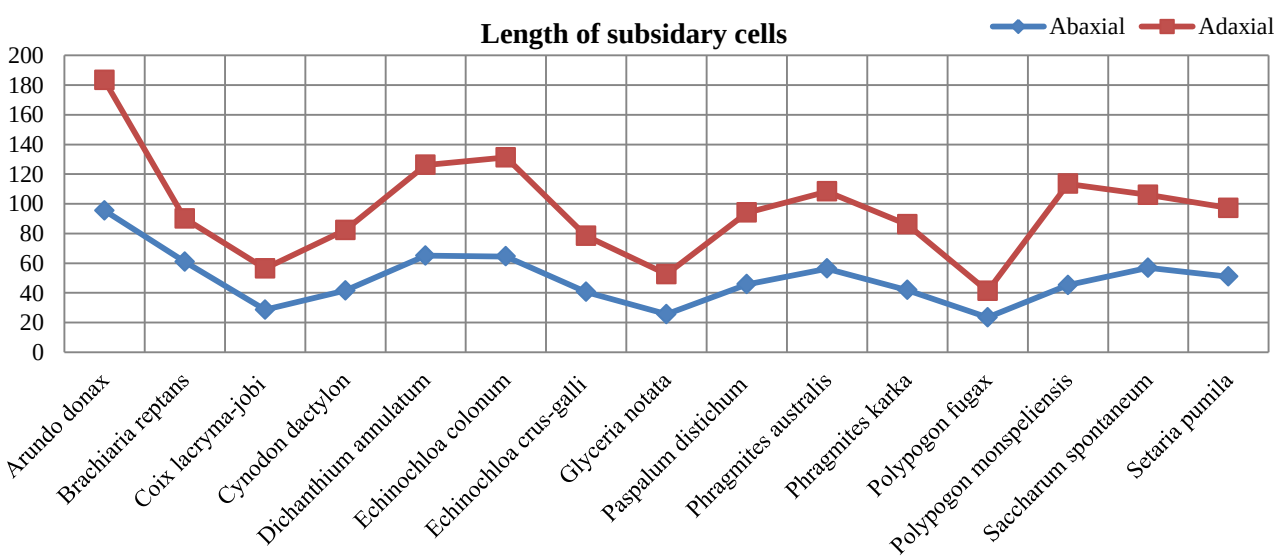


Fig. 7. Showing variation in the length of subsidiary cells on both surfaces of Family Poaceae.

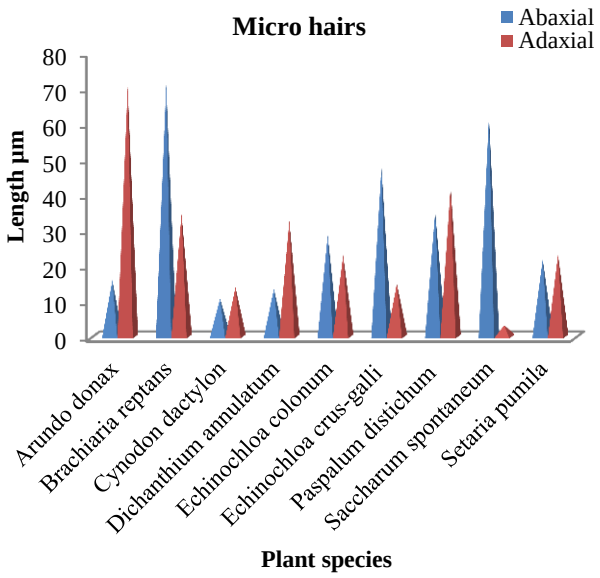


Fig. 8. Showing variation in the length of micro hairs on both surfaces of Family Poaceae.

Qualitative characters of leaves anatomy such as epidermal cells, anticlinal walls, stomata type, silica bodies, hairs, subsidiary cells and prickles provide extensive taxonomic data. Recently it is used as an essential tool in delimitation of grasses taxa, specifically sub-families, tribe and species (Metcalfe, 1960). The current research is the first most detailed study on waterlogged and marshy area grasses that implies which has been done using of Light microscopy to construct taxonomic keys using foliar epidermal features for correct identification. The current results inspire taxonomists to practice light microscopic investigations for the correct identification & comprehensive phylogeny of grass members.

## Conclusion

In total, fifteen species of Poaceae were collected from wetland (waterlogged and marshy) areas and examined for leaf epidermal anatomy. Leaf epidermal anatomy is valuable for the accurate identification of the species. The characters of the anatomized leaf epidermis revealed significant anatomical variation in both quantitative and qualitative characters. In both cases clear differences were observed on the epidermal upper and lower surfaces. The present research work has been compared with previous study and it revealed and confirmed that the leaf epidermal light microscopy is of very important value for the identification of members of family Poaceae. These characters have great information and possess important information for the correct identification of plants. In this research work we adopted taxonomic keys on the basis of qualitative characters like, micro hairs types, silica bodies, epidermal cells shape, stomata type, prickles etc. These micro morphological characters play significant part in accurate identification and delimitation of difficult species. It is thus suggested to study foliar anatomical characters together with the external morphological characters to recognize taxonomically challenging species. Comparative studies of leaf epidermal traits demonstrated great diagnostic variation for the correct identification of the Poaceous species in forth coming studies at tribe, subfamily and genus and species level.

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