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Foliar epidermal anatomy of grasses from Thal desert, district Khushab, Pakistan

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Abstract

Leaf epidermal anatomical studies of 29 grass species belonging to 10 different tribes was carried out in Thal desert, (District Khushab). These leaf epidermal characters are of great value in grass systematics and important for characterization of broad groups within the grasses, particularly subfamilies and tribes. Grass species were evaluated in terms of epidermal features such as length, width and shape of long cells, stomatal apparatus, subsidiary cells, prickles, hooks, microhair, macrohair and silica bodies. Epidermal cells of grasses have a higher degree of variation than in any other family. In this study, largest long cell was found in *Avena fatua* and smallest in *Cynodon dactylon*. Silica bodies of different shapes were observed such as dumb bell, cross, saddle, rounded, square shaped etc. Subsidiary cells of low dome, high dome shape and of parallel sides were observed in different species. Hooks were present in some members of Arundineae, Aristideae, Andropogoneae,Paniceae. Anguler prickles with swollen bases, narrow tips and prickles between the veins are found in almost all the species examined. This taxonomic study will help in species identification and classification into different tribes.

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Introduction

Grasses belong to family Poaceae which is among the largest family of flowering plants. Grasses are classified into about 50 tribes, 660 genera and 10,000 species (Lowe, 1989). Grasses occur on all continents, including Antarctica, because grass species are accustomed to almost every type of environment on planet earth (Kellogg, 1998).The grass family, Poaceae, is noted for its diverse number of characteristics and complexity, so it is complicated to classify the grasses based only on apparent morphological characters (Srivastava, 1978).

The leaf epidermis is generally considered as an important aspect for the classification and delimitation of species and genera, and for sorting out the evolutionary and phylogenetic problems (Stace, 1984; Jones, 1986). It is confirmed to be very crucial in supplying data of taxonomic significance (Ogie-Odia *et al*., 2010) and these leaf epidermal characters have a significance in grass systematics and classification of ambiguous groups which are not properly adjusted within grasses, particularly at subfamily and tribe level (Ahmad *et al*., 2011).

The plant epidermis is a multifunctional tissue playing important roles in water relations, defense and pollinator attraction. This range of functions is performed by a number of different types of specialized cells. These various cells show different degrees of morphological specialization. It is imperative to therefore to attempt a search for epidermal characters that may be of taxonomic utility (Khan *et al*., 2011) It is confirmed that leaf epidermal features can help to elucidate taxonomic relationships at different levels (Prat, 1936; Stebbins,1956; Metcalfe, 1960, Ellis, 1986, Palmer & Tucker,1981, Davila & Clark, 1990; Cai &Wang, 1994; Mejia-Saules & Bisbey, 2003).

As grasses have lot of economic importance so they are being studied in different areas of Pakistan taxonomically. But Thal area of Khushab is quite unexploited in this regard, Although because of its sandy soil, harsh environment and low rain fall, unique species grow there. So there was need to document the species belonging to Poaceae family in the area as they are basic source of fodder for cattle. This study is a step for analyzing the grasses of Thal from anatomical point of view which would serve as the reference point for future experimentation on grasses of this area.

Materials and methods

Study area

Khushab is a region of diverse topography. It consists of mountains, plains and sandy dunes. Khushab district may be divided into the four major physical divisions; one and most important of them is the Thal desert (DCR, 1998).

Fig. 1. Map showing the location of Thal Desert, District Khushab.

The climate of the district is arid to semi-arid sub-tropical continental. The maximum temperature goes up to 45.6 °C in summer; it is recorded as 5.5 °C to 13 °C in winter and the mean annual rainfall in the district is 150 to 350 mm (Rahim and Hasnain, 2010). The desert of Thal adjoining District Khushab has almost disappeared. As far as pedology of the area is concerned, the soils of the huge Thal desert are alluvial in nature with sandy textured dunes covering almost 50 to 60 percent of the area (Khan and Anwar, 1968). These dunes are barren and local people cultivate grams, as this crop resist extreme weather conditions. Trees small shrubs and grasses are distributed between sand dunes and low lying areas (Nadeem *et al*., 2005). Dwarf trees are common in desert area. The importent flora of the thal desert includes Karir (*Capparis aphylla*), Van (*Salvadora abeoides*), Jand (*Prosopis spicigera)* and Malla (*Zizyphus nummularia*) (DCR, 1998).

Collection of specimen

Several field trips were conducted during different seasons of the year in various resource based sites i.e: Garout chowk, tehsil Joharabad, Tehsil Khushab of District Khushab for collection of plant material. Plants were identified with the help of the Flora of Pakistan and preserved at Herbarium of PMAS Arid Agriculture University Rawalpindi.

Leaf epidermal anatomy

Fresh specimens were used for anatomical studies. Leaf samples were prepared according to the modified method of Clark's (1960) technique.

Sample Preparation

The fresh leaves were placed in a test tube filled with 88% of lactic acid, kept in water bath (Model Memert GmbH+Co.KG D-91\26, Schwabach FRG, Germany) at 100°C for 30-40 minutes. The leaves were then removed from the test tube into a Petri dish. A drop of lactic acid is used to soften the tissue of leaf due to which it's peeling off is made possible.

Slides Preparation

For microscopic study the slides were prepared, both for adaxial and abaxial surfaces. When abaxial epidermis was to be prepared, the leaf was placed on a tile adaxial surface uppermost and flooded with cold lactic acid. By using a sharp blade the adaxial epidermis was peeled off. The epidermis was placed on clean glass slide and mounted in fresh 88% lactic acid. When a preparation of the adaxial side was to be made the leaf was placed abaxial side uppermost with same next procedure. For each of surface three slides were prepare of all the specimens.

Microscopic Analysis

.The micro-graphs of these mounted materials were taken using a digital camera (Meiji CCD Model 00179048, Canada) fitted on the Lieca light microscope (DM 1000, Germany). Terminology, sampling and preparative techniques are followed after Metcalfe (1960), Clifford and Watson (1977) and Watson *et al.* (1988).

Results and discussion

In present study, 29 grasses were collected from the area, belonging to ten tribes. Tribe Paniceae was the largest tribe studied in terms of number of species. Other tribes studied were Arundineae, Aristideae, Chlorideae, Eragrostideae, Pappophoreae, Andropogoneae, Aveneae, Bromeae, Poeae. These grasses were analyzed anatomically for their proper identification.

Table 1. Qualitative leaf epidermal characteristics of grasses collected from Thal Desert, District Khushab.

Botanical Names	Long Cells Margins	Subsidiary Cells	Silica bodies
Aristida adscensionis L.	slightly sinuous	Low dome/parallel sided	Dumb bell
Aristida funiculata Trin. & Rupr.	slightly sinuous	low dome/ parallel	Dumb bell
Arundo donax L.	sinuous margins	Parallel sided	Dumb bell/cross shaped
Avena fatua Linn.	Sinuous margins	Parallel sided	Square shaped
Brachiaria ramosa (Linn.) Griseb.	sinuous margins	low dome shaped	Dumb bell shaped
Bromus pectinatus Thunb.	sinuous margins	Parallel sided	Saddle shaped
Cenchrus biflorus Roxb.	sinuous margins	Triangular	Dumb bell shaped
Cenchrus ciliaris Linn.	sinuous margins	Triangular/ high dome	Dumb bell/cross shaped
Cenchrus pennisetiformis Hochst. & Steud. Ex Steud.	sinuous margins	Triangular/ high dome	Dumb bell
Cenchrus setigerus Vahl	slightly sinuous	Triangular	Dumb bell/cross shaped
Cymbopogon jwarancusa (Jones.) Schult	sinuous margins	high dome shaped	Dumb bell/cross shaped
Cynodon dactylon (Linn.) Pers.	sinuous margins	High dome/ triangular	Saddle shaped
Dactyloctenium aegyptium (Linn.) Willd.	slightly sinuous	low dome	Saddle shaped

Desmostachya bipinnata (Linn.) Stapf	slightly sinuous	dumb bell/high dome	Saddle shaped
Dicanthium annulatum (Forssk.) Stapf	sinuous margins	high dome shaped	Dumb bell shaped
Digitaria sanguinalis (Linn.)Scop.	sinuous margins	dome shaped/triangular	Saddle shaped
Eleusine indica (Linn.) Gaertn.	slightly sinuous	high dome	Saddle shaped
Enneapogon shimpranus (Hochst. ex A. Rich) Renvoize	slightly sinuous	low dome/ parallel	Dumb bell/cross shaped
Imperata cylendrica (Linn.) Raeu	sinuous margins	high dome shaped	Dumb bell
Leptochloa chinensis (Linn.) Nees.	sinuous margins	Dome shaped	Saddle shaped
Octhochloa compressa (Forssk.) Hilu.	sinuous margins	Triangular	Saddle shaped
Phalaris minor Retz.	sinuous margins	Parallel sided	Rounded shaped
Poa annua Linn.	non sinuous	low dome/ parallel	elongated in shape
Polypogon fugax Nees ex Steud	sinuous margins	Parallel sided	Dumb bell/ cross shaped
Saccharum ravennae (Linn.) Murr.	sinuous margins	dome shaped	Cross shaped
Saccharum spontaneum Linn.	sinuous margins	Triangular	Dumb bell/cross shaped
Setaria intermedia Roem. & Schult.	sinuous margins	High dome/ triangular	Dumb bell shaped
Setaria verticellata (Linn.) P. Beauv.	slightly sinuous	Dome shaped	Dumb bell/saddle shaped
Sorghum halepense (L.) Pers	sinuous margins	High dome/ triangular	Dumb bell/cross shaped

The following Anatomical characters of both adaxial and abaxial surfaces were studied in all grass species that whether coastal and intercostals zonation conspicuous or not. Papillae absent or present. Long cell shape coastally and intercoastally, long wall thickness coastally and intercoastally, long cell wall sinous, undulating or straight. Microhairs present or absent, microhairs type, shape, form and size, microhairs basal and apical shape and wall thickness. Stomata: common, rare, or absent, guard cell shape and length, subsidiaries shape; whether parallel-sides, dome-shaped, or triangle. Guard cells overlapping or not, intercostals short cells presence, absence, distribution, silica bodies absence, presence, shape; macrohairs presence, absence, size, prickles presence, absence, distribution.

Length and width of long cells is a significant parameter which help in identification and classification of grasses (Elahi and Ashraf, 2002). In *Arundo donex* member of tribe arundineae, long cell length was recorded as 67-118 µm and width is 4-10.5 µm on abaxial side. Parallel sided subsidiary cells were present and saddle or dumb bell shaped silica were found.

Subsidiary cells in both *Aristida* species of tribe Aristideae are mostly low dome shaped, rarely triangular. The subsidiary cells in these species are mostly triangular in shape. (Clifford and Watson, 1977). Silica bodies are found dumb bell shaped in both species. Metclafe (1960) observed variously shaped silica bodies such as oblong, elliptical, lobed, dumb bell shaped in *Aristida* genus.

Almost all epidermal cells in *Cynodon dactylon* are found sinuous or wavy similer to the findings of Ahmad *et al*., (2010). Like almost all grasses stomata in *Cynodon dactylon* are paracytic type earlier proved by Abid *et al*., (2007). According to Metcalfe (1960), Chaudhary *et al.* (2001), Ahmad (2009), silica bodies in *cynodon dactylon* are saddle shaped which are again confirmed by my research. Subsidiary cells in *Cynodon dactylon* found to be triangular at abaxial side and high dome shaped at adaxial side. However previously Chaudhary *et al.* (2001) found subsidiary cells in this species to be only triangular in shape, no variation present. Microhair present either on adaxial, abaxial or both sides. (Freire *et al.*, 2005; Chaudhary *et al*., 2001; Ahmad, 2009). No microhair found in present epidermal study adaxially or

abaxially, which may be due to harsh environmental conditions.

In tribe Eragrostidae, Smallest long cells found in *Desmostachya bipinnata* starting from 30 µm and largest found in *Eleusine indica* reaching to 112 µm in length. Saddle shaped silica bodies found in all four species as (Renvoize, 1983) stated that saddle shaped bodies present in 60% genera of this tribe however he observed a variety of silica bodies in this tribe. *Dactyloctenium aegyptium* has low dome shaped subsidiary cells. *Desmostachya bipinnata* have both triangular and dome shaped cells while *Octhochloa sp.* has triangular subsidiary cells so a variety in subsidiary cells shape is observed. Microhair found in *D. aegyptium* and *Desmostachya bipinnata* while absent in *Octhochloa sp.* and *Eleusine indica.*

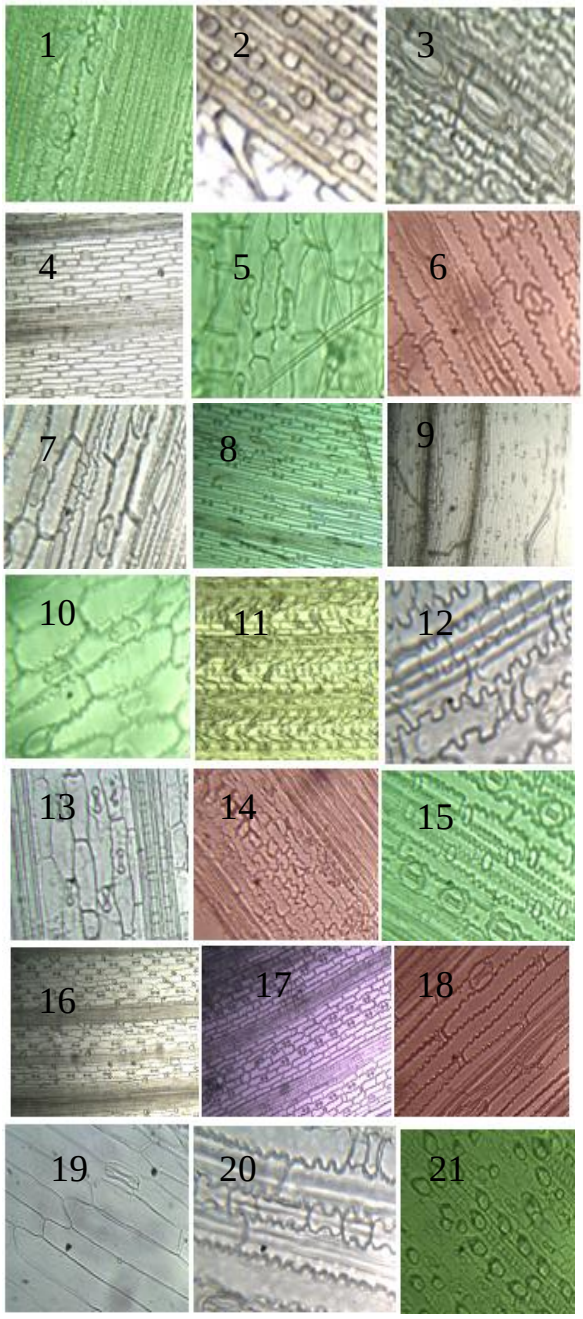
Table 2. Quantitative leaf epidermal characteristics of grasses collected from Thal Desert, District Khushab.

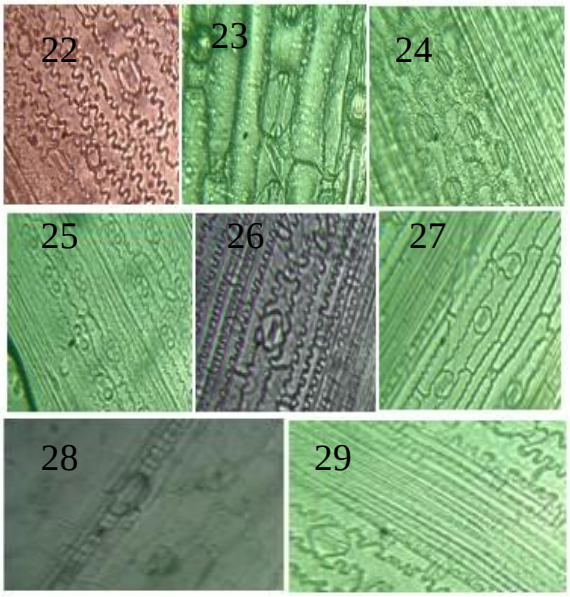
BotanicalNames	Long Cells	Stomata	Prickles	Hooks	Microhair	Silica Bodies
Aristida adscensionis L.	77-121.5	22-27.5		29.5-36.25	20-27	13.5-19
A. funiculata Trin. & Rupr.	44-82.75	20-23.75	16.5-22	22.25-27	-	12.5-14.75
Arundo donax L.	67-118	26-32.5	46-52.5	17.25-21.5	26.5-38	13-19.5
Avena fatua Linn.	120.5-330	40-48.75	35-39.5	-	45.5	8-13.5
Brachiaria ramosa (Linn.) Griseb.	88-112.5	22-26.5	-	-	27-32.5	13.25-16.75
Bromus pectinatus Thunb.	89-233.75	49.5-58	86-97.5	-		49-61.5
Cenchrus biflorus Roxb.	57-114.5	27-35.5	-	-	38.5-40.25	12-16.75
Cenchrus ciliaris Linn.	43-101.25	25.25-33.75	35-39.5	16.5-22	-	9.5-17.5
Cenchrus pennisetiformis Hochst. & Steud. Ex Steud.	61.5-143.5	28.5-36.75	43-47.5	12.5-17.75	26-30	10.75-14.5
Cenchrus setigerus Vahl	57-89	28-30.5	-	18.25-23	18-23.5	12-16.5
Cymbopogon jwarancusa (Jones.) Schult	63-90.5	24.5-30.5	-	-	28.6-34	10-18.25
Cynodon dactylon (Linn.) Pers.	27.2-48	13-15	25-34.5	-	-	4-6.5
Dactyloctenium aegyptium (Linn.) Willd.	47-103.5	17-19.5	-	-	24-26	13.5-16
Desmostachya bipinnata (Linn.) Stapf	45.5-63	20.5-23.5	76-98.75	-	-	6-8.
Dicanthium annulatum (Forssk.) Stapf	17.5-125	22-24.25	-	-	25.5-32.5	15.75-18
Digitaria sanguinalis (Linn.)Scop.	50-68.5	19.5-22.5	-	14-18.75	-	13.75-16.5
Eleusine indica (Linn.) Gaertn.	49.5-93	21-24.5	-	-	46-52.25	6.25-7.75
Enneapogon shimpranus (Hochst. ex A. Rich) Renvoize	63-95	30.5-33.25	48-51.25	-	23-27.25	11- 12.5
Imperata cylendrica (Linn.) Raeu	29-42.25	17.25-20.5	24-29.5	18-21.5	18-23.5	9-11.5
Leptochloa chinensis (Linn.) Nees.	41-62.25	20-22	27-28.5	-	-	4-6.5
Octhochloa compressa (Forssk.) Hilu.	36-89.75	25-26.5	21-24.5	-	-	8-13.75
Phalaris minor Retz.	78-173.75	43-47.5	38.5-43	-	-	43-48.5
Poa annua Linn.	42-47.5	36-40.25	25.25-32	-	-	
Polypogon fugax Nees ex Steud	98.75-169	33-37.5	12-14.5	42.3-56.6	-	18-23.5
Saccharum ravennae (Linn.) Murr.	48-105.5	28-33.5	48.5-65.25	-	15-21.5	16-19
Saccharum spontaneum Linn.	55-117.75	23-26.5	41.5-50.5	-	39-43.5	14-17.5
Setaria intermedia Roem. & Schult.	87.25-136	19.5-23.25	43.25-66.75	15.75-19.25	16-19.5	13-17.75
Setaria verticellata (Linn.) P. Beauv.	73-157.75s	22-24.5	27.25-36.5	-	13-16.6	16-21.5
Sorghum halepense (L.) Pers	95.5-123	25-34.5	-	15-21.5	17-19.5	19.75-25

In *Enneapogon shimpranus* belonging to tribe Pappophoreae long cells found to be 63-95 µm in length, subsidiary cells found to be low dome shaped or parallel sided. Silica bodies observed to be cross or dumb bell in shape. Micro hair was present but macrohair were not found. Earliar Ahmad (2009) recorded macro hair with glanduler heads in *E. persicus*.

As far as epidermal features in Andropogoneae are concerned, In *Cymbopogon jwarancusa, Imperata cylendrica, Saccharum ravennae* subsidiary cells found to be high dome shaped. In *Dicanthium annulatum, Saccharum spontaneum, Sorghum halepense* both high dome and triangular shaped subsidiary cells are found. In *Cymbopogon*, silica bodies are found which are mostly cross shaped

intermediate b/w dumb bell and cross shaped, cross shaped silica bodies is an identification character in Genus *Cymbopogan* (Bibi *et al.*, 2007). In *Dicanthium* dumb bell shaped, In *Imperata*, saddle or dumb bell shaped, *Saccharum ravennae* (Linn.) has cross shaped while *Saccharum spontaneum* and *Sorghum halepense* has both dumb bell and cross shaped silica bodies. So there are a variety of silica bodies found in different genera of this tribe. Similer conclusions are made by group of scientists working on tribe Andropogoneae from different areas such as (Bibi *et al*, 2007; Ullah *et al*, 2011; Ahmad *et al.*, 2010).





Anatomically in tribe Paniceae, *Brachiaria ramosa* has low dome shaped subsidiary cells, silica bodies dumbbell shaped, microhair recorded on abaxial surface, macrohair found on both abaxial and adaxial surfaces. Earlier Ahmad (2009) recorded the presence of macrohair only on adaxial side. In *Cenchrus* genus all species collected have rectangular long cells with sinuous walls. In *C. Ciliaris* and *C. pennisetiformis* subsidiary cells are high dome or triangular in shape while in *Cenchrus setigerus* Vahl and *C. biflorus* subsidiary cells are only triangular in shape. In *C. Ciliaris* and *C. pennisetiformis* subsidiary cells are high dome or triangular in shape while in *Cenchrus setigerus* Vahl and *C. biflorus* subsidiary cells are triangular in shape. Silica bodies are found dumb bell or cross shaped or intermediate b/w dumb bell and cross shaped. Sharma and Kaplia (1985) stated that microhairs are found in the costal and intercostal zones of both surfaces of epidermis in majority of species of the genus *Digitaria*. However in present studies microhair are found only on abaxial surface ranging in length from 46 to 52 µm. Saddle shaped silica bodies are found on both surfaces unlike Gilani *et al.*, (2002) who observed cross shaped silica bodies.In *S. verticellata* dumb bell and cross shaped silica bodies are found. In genus *Setaria* shape of silica bodies is very important in identification and classification upto species level (Shaheen *et al.*, 2011).

In tribe Aveneae, long cells in *Avena fatua* are largest

in size ranging from 120-178 µm and the smallest size is recorded in *Polypogon fugax*. Similerly the broadest cells are of *Avena fatua*. All species of oat tribe anatomically observed from Thal desert have parallel sided subsidiary cells. Unlike observation made by Ahmad *et al*. (2011) who also observed dome shaped cells in *Polypogon fugax*. Silica bodies non sinuous in outline however Ying *et al*. (2006) observed silica bodies with sinuous walls in oat tribe. In *avena* cubical, in *Phalaris sp*. Silica bodies with rounded ends are present and in *Polypogon fugax,* dumb bell shaped silica bodies are present similer diversity is noted by Ahmad *et al*. (2011) in tribe Aveneae.

Ahmad *et al*., 2010 concluded that *Poa annua* of tribe Poeae has long cells with wavy margins but in present studies they are recorded as straight walled. Guard cells found to be low dome shaped or parallel sided similer to the previous results obtained by (Metclafe, 1960; Peterson *et al*., 2006; Ahmad, 2009). Several features including silica bodies are significant in identification of grasses. Metcalfe (1960) and Ellis (1986) figured out that Silica bodies have no defined shape in *poa sp*. they are found to be elongated in shape in present study.

Conclusion

Different parameters of leaf epidermal anatomy are helpful in identification and classification of species like long cells shape and size, shape of silica bodies and subsidiary cells. In present research study, these variable features like silica bodies of different shapes such as dumb bell, cross, saddle, rounded, square shaped etc; subsidiary cells of low dome, high dome shape and of parallel sides; bicelled microhair, prickles, hooks, macrohair presence, absence, variable size in different species of grasses were significant to specify the position of grass species at tribal and subfamily level.

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