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Full Length Article

Significance of Anatomical Markers in Tribe *Paniceae* (Poaceae) from the Salt Range, Pakistan

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Abstract

In this study, leaf anatomical characteristics of 17 species belonging to 9 genera of tribe Paniceae, native the Salt Range, Pakistan, were evaluated. Two *Urochloa* species, *U. deflexa* and *U. ramosa,* were distinguished from the rest of the species by absence of median vascular bundles. Presence of sclerenchyma strands on the abaxial side only makes the genus *Cenchrus* distinct from the remaining species within the tribe. The distribution pattern of bulliform cells proved to be helpful in the differentiation among *Cenchrus* species. Large macrohairs with deep penetration on the adaxial surface are characteristic of *Digitaria nodosa*. The observed diversity in anatomical markers could be used to clarify the status of problematic taxa in tribe Paniceae. Saddle shaped silica bodies, microhairs and bulliform cells deeply penetrating the mesophyll are the prominent characters of this tribe, which justify placing all these species in the same tribe. Anatomical markers of taxonomic importance are the nature and size of macro- and micro-hairs, shape of bulliform cells, arrangement of vascular bundles and amount of sclerification present in the leaves, which may be used for resolving taxonomic problem of problematic genera within tribe Paniceae.

Keywords: Foliar anatomy; Tribe paniceae; Salt range; Pakistan

Introduction

The role of anatomical markers i.e., characteristics specifically used for species identification, has been long recognized since the variations exist within species, genera or a family (Ahmad *et al*., 2010). Anatomical features are of particular significance to scientists who need to identify small scraps of plant material, for example that found in animal's gut, fossil remains or destroyed material (Al-Edany and Al-Saadi, 2012). The most important among them include cuticular deposition, epidermis and epidermal appendages, stomata along with subsidiary cells, and spines (Ellis, 1976; Rudgers *et al*., 2004; Yasmin *et al*., 2009). The significance of anatomical markers in the systematics of many plant groups has been emphasized by Adedeji and Dloh (2004) in *Hibiscus* species, Celka *et al*. (2006) in *Malva alcea*, Zou *et al*. (2008) in *Cercis* species and Ahmad *et al*. (2011) in tribe Eragrostideae (Poaceae).

Grasses under high saline habitat can minimize the detrimental effects of high salinities by showing a series of structural and functional modifications in morphological, anatomical and physiological characteristics. These include reduced growth (Alshammary *et al*., 2004), extensive root system (Hameed and Ashraf, 2008), lignifications in root vascular region (Hameed *et al*., 2013), increased thickness (succulence) of epidermis, midribs and cortical parenchyma (Flowers and Colmer, 2008), increased sclerenchyma in the leaves and roots (Reinoso *et al*., 2004), reduced leaf area (Monteverdi *et al*., 2008), greater density of salt secreting glands and hairs on the leaf surface (Marcum *et al*., 1998), enlarged bulliform cells that help in leaf rolling to avoid water loss (Alvarez *et al*., 2008), increased stomatal density with decreased stomatal size (Hameed *et al*., 2013). Presence of sclerenchyma girders, ribs and furrows, and position of bulliform cells are important characteristics of grasses that depict the affinity among different genera (Dube and Morisset, 1987; Vecchia *et al*., 1998; Ahmad *et al*., 2009).

The tribe Paniceae is extended from tropics to warm temperate regions having about 101 genera all over the world. There are about 450 species of genus *Panicum* that are distributed worldwide and it is one of the largest genera of the tribe Paniceae (Webster, 1988). In Pakistan, this tribe is represented by 15 genera with 73 species (Cope, 1982). Salt Range has a very rich ecological diversity and a treasure of valuable natural resources. The area has a mountainous dry subtropical climate with semi-evergreen forests, typically with *Acacia modesta, Dodonaea viscosa, Olea ferruginea, Reptonia buxifolia and Salvadora*n *oleoides* (Ahmad *et al*., 2012).

Grasses often cause nuisance for taxonomists during identification process as many of the problematic genera (*Dicanthium*, *Saccharum*, *Chrysopogon,* and *Cymbopogon*) are not easy to distinguish on the basis of morphological

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markers. Of all the non reproductive organs, leaf is the most commonly used in plant taxonomy and leaf epidermis is of prime importance in solving taxonomic problems parallel with cytology (Ahmad *et al*., 2011). Anatomical markers have been used successfully to clarify taxonomic status and help in the identification of different species (Gillani *et al*., 2002). Anatomical studies could be an important tool to resolve the taxonomic problems between closely related species or even populations of same species (Naz *et al*., 2009). There are many cases where species nomenclature has been changed based on differences in anatomical features, especially in family Poaceae and Cyperaceae. The most common examples are of C_4, C_3 and many intermediate types that exist in these families (Koteyeva *et al.,* 2011; Sage *et al.,* 2014). The Salt Range is a specific biome characterized by a variety of environmental stresses, and therefore, the native grass species are expected to be well adapted and possess specific structural modifications to survive under harsh environmental conditions. These structural features are the indicators of specific set of environments, and therefore, structural-based mechanism of adaptation is necessary to cope with environmental stresses, in particular high salinity and extreme aridity which is a characteristic of the Salt Range. During the investigation, the main focus was on grasses of Tribe Paniceae, the largest tribe of family Poaceae, which has many problematic genera and species from taxonomic point of view. The present study was therefore, conducted to evaluate foliar anatomical characteristics, which are of great ecological and taxonomic significance.

Materials and Methods

Frequent field trips were carried out to collect grasses in two seasons, September–October 2011 and May–June 2012. Grasses of Tribe Paniceae (Poaceae) were collected in triplicate from diverse habitats in the study area, which were allotted voucher number, along with the geographical and topographic data like, altitude, locality, soil type, aspect, slope, etc. The specimens were placed in the herbarium of Botany Department after mounted on herbarium sheets for future records. For identification, different available floras, in particular, Flora of Pakistan (Cope, 1982) were consulted.

For anatomical studies, basal portion of flag leaf of the longest tiller of the preserved specimen from herbarium sheet was selected and kept in chloral hydrate solution for 24 h. It was then thoroughly washed with distilled water. For transverse sections of leaves, freezing microtome (Leica CM 1325) was used. At 14°C, about 10–15 µm thick slices of leaves were cut and best sections were used for the preparation of slides.

Leaf sections were then placed on the slide and added one to two drops of safranin, and the extra safranin was removed by pouring few drops of 96% ethanol. After it, one to two drops of fast green were added to the sections, followed by 96% and absolute alcohol, respectively. At the end, one drop of xylene was added. Canada balsam was taken on the cover slip and covered the slide. A microscope was used for observation of slides and microphotographs were taken by a camera mounted on the microscope (Olympus Ax 70). The anatomical characters studied were nature of adaxial and abaxial epidermis, occurrence of sclerenchyma strands and girders, number and position of vascular bundles, arrangement of chlorenchyma cells, nature of keel, and shape and distribution of bulliform cells.

Data were subjected to multivariate (cluster) analysis to investigate similarities and differences among species of tribe Paniceae using Minitab Statistical Software, v. 11 (Minitab, Inc.).

Results

Cenchrus biflorus Roxb

Deep invaginations are present on the upper surface and pointed prickles can be observed on the ribs of upper surface. The prickles are pointed at the tips and wide at the base. The lower surface is without distinct invaginations. Mostly large VBs are present. In large VBs, thick sclerenchymatous strands are present on the upper and lower surface, while sclerenchymatous girders are absent. Chlorenchyma cells are in radial arrangement around the VBs. Keel is broad and round and fairly distinguishable. Large median VB are with three to four small VBs on each side. Bulliform cells are present in furrow region. Near margins, bulliform cells are present in groups. The sheath cells of small VBs are large and bundle sheath is complete and single in all vascular bundles. Sclerification around vascular bundles, microhairs and presence of bulliform cells in groups might be an adaptation of this grass to tolerate severe drought and high salt concentration (Table 1; Fig. 1a).

Digitaria sanguinalis (L.) Scop

On the upper surface, thin and long macrohairs having round and swollen base with deep penetration are observed. Slight ribs are present near the large VBs. Ribs and furrows are absent on the lower surface. On the lower surface, prickles are present. Seven to nine small and median VBs are present between large VBs. Sclerenchyma strands on both surfaces are present near large and medium VBs. The sclerenchymatous strands on the upper surface are much shorter than strands on the lower surface. Sclerenchyma strands are absent near small VBs. Chlorenchyma cells are aggregated abaxially in the mid rib region. Chlorenchyma cells are not found in distinct radial arrangement around the VBs. Keel is prominent, and there are two to three small VBs on each side of median large VB. Mid rib region is partially covered by colorless cells. Bulliform cells are in irregular groups. The sheath cells are complete and in single layer (Table 1; Fig. 1b).

***Digitaria nodosa* Parl**

Ribs and furrows are present on the upper surface, and large ribs are present near large VBs. Stomata and prickles can be seen on slight ribs and furrows present on the lower surface. Lamina is narrow between two VBs. At the borders depositions of sclerenchyma are observed. Sclerenchyma strands on both surfaces are present near the large VBs, helping in retaining the maximum water availability inside the plant. Chlorenchyma cells are in radial arrangement around the VBs. Keel is rounded and prominent. Bulliform cells are present in irregular groups and sheath cells are complete and in single row around the VBs (Table 1; Fig. 1c).

***Echinochloa colona* (Linn.) Link**

On the abaxial side wide sclerenchyma strands are present. The keel region has VBs with sclerenchyma strands on the lower surface only. Sclerenchyma strands are present opposite to large VBs. Small VBs are without any sclerenchyma strand or girder. Chlorenchyma cells have radial arrangement around the VBs. Keel is prominent and rounded having large median VB, and has three small VBs on each side. Bulliform cells are present in regular groups. Small VBs are with large sheath cells and sheath cells are complete and in single layer (Table 1; Fig. 1d).

***Moorochloa eruciformis* (Sm.) Veldkamp**

There are shallow furrows and small ribs on adaxial surface, while large ribs and furrows are present on the lower surface. Sclerenchymatous cells are observed at the borders only. Mostly, VBs are small while there are a few basic type large VBs. Sclerenchyma strands on the upper and lower surface are present in all VBs. The strands on the lower surface are large than those on the upper surface. Chlorenchyma cells are in radial arrangement. There is a single large VB in the keel region. Bulliform cells are in groups and the middle cell of a group deeply penetrates the middle surface. Bundle sheath is not prominent in small VBs, while sheath cells are of single and complete layer in large VBs (Table 1; Fig. 1e).

***Pennisetum ciliare* (L.) Link**

There is a slight unevenness on the upper surface. Pointed macrohairs are present on ribs but are not frequent. On the abaxial surface furrows are narrower and deeper than upper surface. There are two distinct rows of VBs in the mesophyll, and 5 – 7 small VBs are present between the large VBs. Large VBs are with large and wide sclerenchymatous strands on the upper and lower surface. Small VBs are with prominent sclerenchyma strands on lower surface, but sclerenchyma strands on the upper surface are absent. Sclerenchyma cells are also observed at the margins. Chlorenchyma cells are in radial arrangement around the VBs. There is a continuous strand of chlorenchyma cells throughout the middle surface of leaf, from one VB to the other. Keel is present with large median VB and 3 small VBs are present on each side. Bulliform cells are fan-shaped or in irregular groups. The sheath cells form a single layer and are complete, surrounding all VBs (Table 1; Fig. 1f).

***Pennisetum setigerum* (Vahl) Wipff**

The upper surface is with distinct ribs and furrows and pointed prickles on the ribs are present. There are slight invaginations on the lower surface and prickles are also observed. Large VBs are few in number while most VBs are small. Sclerenchyma depositions are present at the borders to protect the VBs. Large VBs are with sclerenchyma strands on the upper and lower surface and are of basic type. Strands of sclerenchyma on the upper surface are present in small VBs only. There are no sclerenchymatous strands or girders in small VBs at the margins. Chlorenchyma cells are in radial arrangement around the VBs. Keel is rounded, wide and conspicuous with pointed prickles. Median VB is present having three to four small VBs on each side. The mid rib is covered with colorless cells. Bulliform cells form a group and the middle cell in the group is larger than the other cells of the group may play important role in leaf rolling to minimize the water loss and the presence of microhairs at the margins may help to remove the excessive salts (Table 1; Fig. 1g).

***Panicum antidotale* Retz**

There are slight ribs and furrows on the upper surface. Large ribs are present near the large VBs. The ribs over the large VBs are with pointed projections. Abaxial surface is mostly flat, and sometimes ribs are present near the large VBs. On the basis of size there are 3 types of VBs, that are large, medium and small VBs. Thick sclerenchyma strands on both surfaces are present opposite to large VBs. Mostly sclerenchyma stands and girders are absent in small VBs and small strands on upper and lower surface are observed opposite to few small vascular bundles (VBs). Chlorenchyma cells are in more or less radial arrangement around VBs. Keel is not prominent. Bulliform cells are present in irregular groups. There is single and complete sheath around VBs (Table 1; Fig. 1h).

***Panicum maximum* Jacq**

There are slight furrows and ribs on the upper surface and large ribs are present near large VBs. Pointed prickles with rounded base can be observed on the ribs, present on the upper surface near the keel region that may involve in salt excretion. Slight ribs are also present on lower surface opposite to VBs. VBs of large and medium size is of basic type and other VBs are angular and small.

Table 1: Distinguishing characters of different taxa in tribe Paniceae (Poaceae)

Taxon	Bulliform Cells	Chlorenchyma Cells	Keel and Bundle Sheath	Sclerenchyma Strands and Girders
Cenchrus biflorus Roxb.	Fan-shaped in furrows near margin	Radial	Fairly conspicuous, wide, rounded	Girders absent
Digitaria sanguinalis (L.) Scop.	Irregular	Radial	Conspicuous, rounded	Girders absent
Digitaria nodosa Parl.	Irregular	Inconspicuous	Conspicuous	Girders absent
Echinochloa colona (L.) Link	Irregular	Radial	Conspicuous, rounded	Girders absent
Moorochloa eruciformis (Sm.) Veldkamp	Fan-shaped, deeply penetrating	Radial	Conspicuous, basic-type solitary vascular bundle	Abaxial strands thicker than adaxial
Pennisetum ciliare (L.) Link	Irregular or fan-shaped	Radial, continuous strands	Fairly conspicuous	Girders absent
Pennisetum setigerum (Vahl) Wipff	Fan-shaped, middle cell larger	Radial	Conspicuous, with pointed prickles	Girders absent
Panicum antidotale Retz.	Mostly irregular	Semi-radial	Inconspicuous	Girders absent
Panicum maximum Jacq.	Irregular	Semi-radial	Conspicuous	Adaxial and abaxial girders
Paspalum distichum L.	Fan-shaped, middle cell larger	Radial	Inconspicuous	Girders absent
Paspalidium flavidum (Retz.) A. Camus	Fan-shaped, only in midrib	Radiating vascular bundles	Very prominent, V-shaped	Girders absent
Pennisetum orientale Rich.	Irregular or inflated	Radial	Conspicuous	Adaxial and abaxial girders
Urochloa deflexa (Schumach.) H. Scholz	Fan-shaped	Inconspicuous	Conspicuous, rounded	Adaxial and abaxial strands
Urochloa distachya (L.) T.Q. Nguyen	Small, fan-shaped, sometimes penetrating mesophyll	Radial	Inconspicuous, with single median vascular bundle	Adaxial and abaxial strands
Urochloa panicoides P. Beauv.	Fan-shaped	Radial	Fairly conspicuous	Adaxial & abaxial girders
Urochloa ramosa (L.) T.Q. Nguyen	Fan-shaped	Inconspicuous	Conspicuous, rounded abaxially	Adaxial and abaxial strands
Urichloa reptans (L.) Stapf	Fan-shaped	Inconspicuous	Fairly conspicuous	Mostly adaxial and abaxial strands

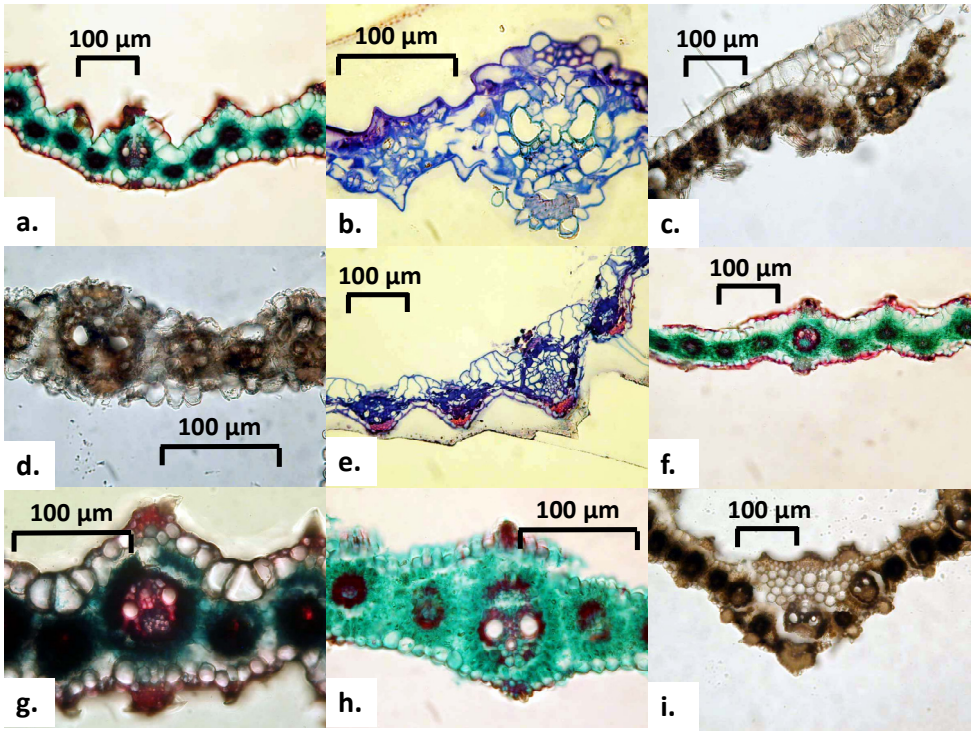

Fig. 1: Leaf transverse sections of some grasses of tribe Paniceae from the Salt Range Pakistan
a. Midrib of *Cenchrus biflorus* showing trichomes on adaxial surface, b. Midrib of *Digitaria sanguinalis*, c. *D. nodosa* showing trichomes adaxially, d. midrib of *Echinochloa colona*, e. Midrib of *Moorochloa eruciformis* showing bulliform cells adaxially and sclerenchyma girders abaxially, f. *Pennisetum. ciliaris* showing a continuous row of bulliform cells adaxially, g. *P. setigerum,* h. Midrib of *Panicum antidotale* showing mid rib and adjacent vascular bundles, i. Midrib of *Pa. maximum*

Sclerenchyma girders on the both surfaces are present opposite to large VBs. Strands or girders of sclerenchyma are not present in some small VBs opposite to the bulliform cells, while strands on upper and lower surface or strands on lower surface are present in other small VBs. Chlorenchyma cells are in more or less radial arrangement around the VBs. Keel is prominent and there are slight ribs and furrows on the lower surface. There are three VBs on each side of the median vascular bundle. Middle part in the mid rib region is covered by angular colorless cells. Bulliform cells are not found in regular groups. Single and complete sheath is present around VBs and sometimes the large VB in the mid rib region is interrupted by sclerenchyma girders on the lower surface (Table 1; Fig. 1i).

***Paspalum distichum* L.**

Slight ribs and furrows are present on the upper surface while lower surface is smooth and flat, having no ribs and furrows. Sclerenchymatous cells are observed at the borders and bulliform cells are present opposite to small VBs. Sclerenchyma strands are present opposite to large VBs. Strands on upper and lower surface are also observed opposite to medium sized VBs. These strands or girders are found absent opposite to small VB. Chlorenchyma cells are in radial arrangement around the VBs. Keel is not very prominent. Two to three small VBs are observed on both sides of median VB. Sclerenchyma strands are present on the upper surface in the mid rib region. Bulliform cells form a group in which middle cell is larger than the remaining one. Bundle sheath is single and complete around VBs (Table 1; Fig. 2a).

***Paspalidium flavidum* (Retz.) A. Camus**

Bulliform cells are narrow towards the epidermis and present on the upper surface opposite to the mid rib region. The lower surface is almost smooth. Between large VBs, 6 to 7 small VBs are observed. Thick abaxial strand is present opposite to median VB in the keel region. Sclerenchyma strands on the lower surface are absent in small VBs. Chlorenchyma cells form radial arrangement around the VBs, and a continuous strand is formed from one VB to the other. Keel is very prominent and V shaped, having median VB along with one small VB on each side. Bulliform cells are present only in the mid rib region. Bundle sheath cells are almost equal in size and sheath cells are complete and in single layer. Small VBs are surrounded by large sheath cells (Table 1; Fig. 2b).

***Pennisetum orientale* Rich**

Slight ribs are present over the large vascular bundles and upper surface is smooth. Slight ribs and furrows are also present on the lower surface. Mostly VBs are angular in outline and are small, in which xylem and phloem is not differentiated easily. Strands of sclerenchyma on both surfaces are absent in most of the small Vbs and small strands on the upper surface are present only in few small VBs. Strands or girders on the both surfaces and girders on the lower surface are present opposite to large VBs. Keel is prominent and 3–4 small VBs on each side of the median VB are present. Chlorenchyma cells form radial arrangement around the VBs. Bulliform cells are in the form of irregular groups or inflated. A single complete sheath is present in small VBs. A double sheath is present in large VBs (Table 1; Fig. 2c).

***Urochloa deflexa* (Schumach.) H. Scholz**

The adaxial leaf surface is with inconspicuous ribs and furrows, while lower surface is flat. Large vascular bundles (VBs) are of basic type, where most of the bundles are small in cross-sectional area. Sclerenchyma cells are totally absent around small VBs. Strands of sclerenchyma cells are present near large VBs on both adaxial and abaxial surface in order to protect them. Large median VB is absent. Keel is prominent and rounded with four to five small VBs. Middle bulliform cells are larger and deeply penetrate the surface that may involve in leaf rolling, an adaptation of drought resistant grasses. Sheath cells are in single row and complete, while chlorenchyma cells are not prominent. Presence of microhairs/spines on both abaxial and adaxial surface may involve in the salt secretion under high salinity levels (Table 1; Fig. 2d).

***Urochloa distachya* (L.) T.Q. Nguyen**

Adaxial and abaxial leaf surfaces are flat, somtimes slightly uneven due to ribs and furrows. The large VBs are few in number, while mostly small VBs are present that are not angular. Sclerenchyma girders or strands are generally absent in small VBs, while small strands of sclerenchyma on both surfaces are observed in large VBs. Keel is not prominent and has a solitary median VB. Chlorenchyma cells are found in radial arrangement around the VBs. The middle cell of bulliform penetrates the mesophyll, and mostly found on adaxial leaf surface. The sheath cells are found in single and complete layer in small VBs, while double sheath is present in large vascular bundles (Table 1; Fig. 2e).

***Urochloa panicoides* P. Beauv**

Upper surface is uneven opposite to the VBs. Slight ribs are present on the lower surface. At the borders small VBs are present having no sclerenchyma cells opposite to them. Minute strands are present opposite to small VBs on upper and lower surface. Sclerenchyma strands or girders on upper and lower surface are present opposite to large VBs. Keel is round and fairly prominent, with a solitary median VB. Bulliform cells are present in group and the middle cell of the group deeply penetrates the surface. VBs are with a single complete sheath and sheath cells are not equal in size (Table 1; Fig. 2f).

***Urochloa ramosa* (L.) T.Q. Nguyen**

Slight ribs and shallow furrows are on adaxial and abaxial leaf surfaces. Most VBs are small and not angular. Sclerenchymatous strands and girders may be absent on lower and upper surface or may have small sclerenchyma strands on the upper surface. Keel is distinguishable and rounded. Large median VB is absent except a few small VBs. Chlorenchymatous cells are not in radial arrangement around the VBs. Bulliform cells are found on the adaxial surface only. Sheath cells surround the large VBs and sometimes it is extended towards the girders on the lower surface (Table 1; Fig. 2g).

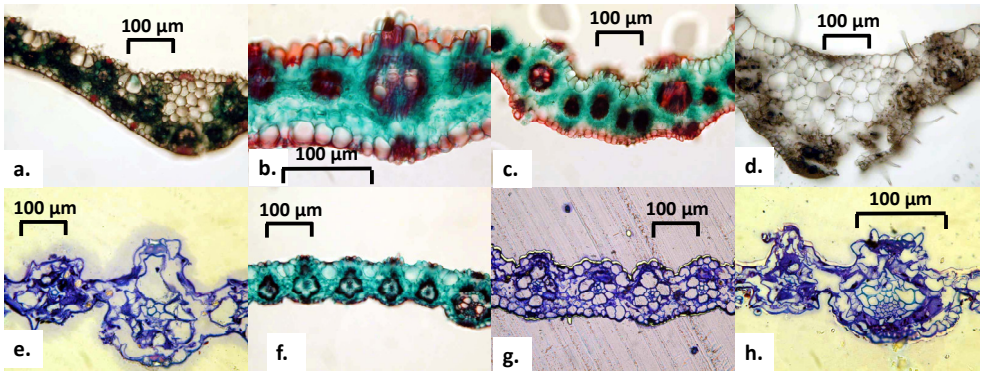

Fig. 2: Leaf transverse sections of some grasses of tribe Paniceae from the Salt Range Pakistan
a. Midrib of *Paspalum distichum*, b. *Paspalidium flavidum* showing U shaped mid rib region, c. Midrib of *Pennisetum orientale*, d. Midrib of *Urochloa deflexa*, e. *U. distachya* showing arrangement of bulliform cells and sclerenchyma girders, f. Midrib of *U. panicoides,* g. *U. ramosa* showing bulliform cells near margins, h. *U. reptans*

***Urichloa reptans* (L.) Stapf**

Surface is smooth adaxially and abaxially, and mostly VBs are small. There are a few large VBs and the strands of sclerenchyma on the upper and lower surfaces have a few cells opposite to small and large VBs, while these strands are absent in few small VBs. Keel has a large median vascular bundle and is fairly conspicuous. Single complete sheath is present in small VBs while large VBs are with double-layered complete sheath, sometimes there is interruption in the outer layer of sheath cells on the abaxial side (Table 1; Fig. 2h).

Multivariate Analysis

Multivariate (cluster) analysis showed very distinct clustering on the basis of leaf anatomical characteristics (Fig. 3). All the species of genus *Urochloa* except *U. distachya* formed a separate cluster. *Urochloa distachya,* however clustered with *Paspalum distychum* and *Cenchrus biflorus*. A major cluster of *Panicum, Pennisetum, Echinochloa* and *Digitaria* species was observed, but all these species formed isolated sub-clusters showing less similarity in leaf characteristics.

Discussion

Anatomical studies have been used successfully to clarify taxonomic status and to help in the identification of species. Anatomical studies showed differences in size and shapes of prickles, short cells, silica bodies, micro hairs with basal and distal cells, hooks, stomates and long cells of Paniceae species.

Panicoid characters in grasses of tribe Paniceae inhabiting highly saline habitat is of prime importance, bringing unique leaf anatomical characters such as increased sclerification, high density of salt glands, microhairs and large number of buliform cells on the adaxial surface (Table 2).

Variations in the number of VBs in the midrib region and the position of vascular bundles are important diagnostic characteristic of many grasses (Ellis, 1986). Median vascular bundle is absent in *U. deflexa* and *U. ramosa*, in the keel region and there are four to five small VBs in the midrib region. Bulliform cells are found in all monocots including Poaceae, except the order Helobiae. There is single layer root epidermis with thick cuticle, polyarch vascular bundle and radial with exarch xylem. Vascular bundles are scattered in ground tissue with endarch xylem. Leaves have abundant paracytic type of stomata (Dwari and Mondal, 2011). The structure of bulliform cells is an important character from taxonomic point of view in *Urochloa,* i.e., *U. reptans* and *U. deflexa* species (Alvarez *et al*., 2008).

The upper leaf surface of *Cenchrus* species has pointed prickles; however *Pennisetum setigerus* is distinguished by having abundant prickles with curved tips on the lower surface. The presence or absence of prickles may be relatively constant character or varies with different environmental factors (Ellis, 1986). The presence or absence of prickles may be significant in identification and differentiation of species (Ahmad *et al*., 2012). Thick sclerenchyma strands on the upper and lower surface are present in all large VBs while sclerenchyma strands on the lower surface only, are present opposite to small VBs which differentiate the genus *Cenchrus* from other genera of the tribe. Moreover, presence of this anatomical diversity within *C. ciliaris* and *C. setigerus* also enable them to play a vital role for fixation and reclamation of sand dunes in areas of low rainfall (Yusafzai and Gandhi, 1999). The keel region in *P. setigerus* has few prickles and in the group of bulliform cells, middle cell is larger than the remaining cells, while the occurrence of bulliform cells at the borders differentiates *C. biflorus* from other *Cenchrus* species. Distribution pattern of bulliform cells is a valuable taxonomic character in differentiation of this species. Enlarged bulliform cell are very crucial under moisture limited environments as these are responsible for

Table 2: Habitat ecology of different grasses of tribe Paniceae from the Salt Range Pakistan

Plant species	Locality	Habitat ecology
Cenchrus biflorus	Kallar Kahar, Kala Bagh, Soon Sakesar	Foothill region; sandy clay
Digitaria sanguinalis	Soon Sakesar, Narwari, Mardwal, Khabaki, Sodhi, Khewra, Dhok Seela, Chakwal	Agricultural fields, rare on mountains; clay and red clay soil
Digitaria nodosa	Sakesar, Mardwal, Sodhi, Narwari	Rare on mountains, commonly at foothill region; red sandy clay and clay soil
Echinochloa colona	Soon Sakesar, Choa Saiden Shah, Dhok Seela, Chakwal	Common weed of fields and wetlands; clay and moist clay soil
Moorochloa eruciformis	Kallar Kahar, Kala Bagh, Soon Sakesar	Common on mountains; stony clay and clay soil
Pennisetum ciliare	Kallar Kahar, Choa Saidan Shah, Dhok Seela,Chakwal	Common near agricultural fields; sandy clay and sandy soil
Pennisetum setigerum	Sakesar, Mardwal, Khabaki,	Common weed and ranked among top 10 in the world, common on mountains; stony clay and clay soil
Panicum antidotale	Choa Saidan Shah, Kallar Kahar, Khabaki, Sodhi	Banks of agricultural fields and slopes of foothill region; clay and stony clay soil
Panicum maximum	Soon Sakeser, Kallar Kahar	Common on banks of fields and on slopes; stony clay soil
Paspalum distichum	Kallar Kahar, Choa Saidan Shah, Dhok Seela, Chakwal	Common along margins of ponds, ditches and water channels; clay and sandy clay soil
Paspalidium flavidum	Choa, Saidan Shah	Common along the road at foothill region, restricted to limited area due to over grazing; clay soil
Pennisetum orientale	Kallar Kahar	Common on mountains; stony clay and clay soil
Urochloa deflexa	Mardwal, Kanhati garden, Kallar Kahar,	Common near agricultural fields; sandy clay and dry sandy soil
Urochloa distachya	Sakesar, Mardwal, Khabaki, Narwari, Chakwal	Rare, mostly under shady trees; moist clay and wet organic black soil with litter
Urochloa panicoides	Soon Sakesar, Choa Saidan Shah, Dhok Seela,Chakwal.	Common near agricultural fields and shady places, rare in crevices of rocks; clay, sandy clay and wet clay soil
Urochloa ramosa	Kallar Kahar, Choa Saidan Shah, Sakesar, Sodhi.	Common in waste places and in agricultural fields; clay, dry and wet sandy clay
Urichloa reptans	Kallar Kahar, Narwari	Common along water courses, under shade of bushes and near agricultural fields; wet clay soil

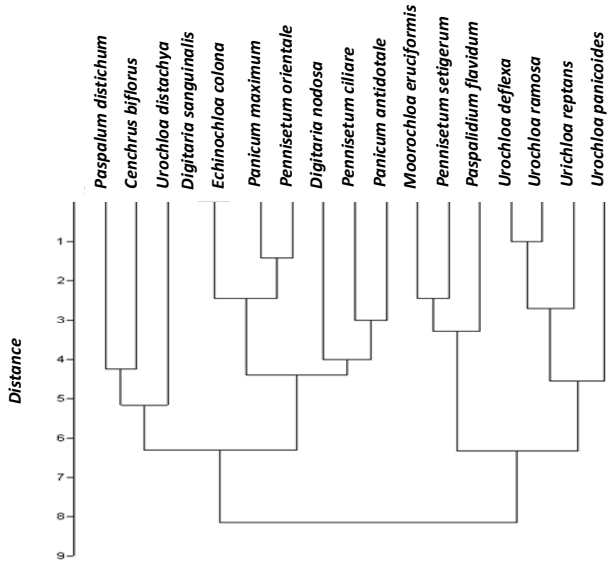

Fig. 3: Dendrogram based on leaf anatomical characteristics of grasses of tribe Paniceae

leaf curling, and ultimately checking water loss through leaf surface (Abernethy *et al*., 1998; Alvarez *et al*. 2003).

Digitaria nodosa is differentiated from *D. sanguinalis* by the presence of long and thin macrohairs, that deeply penetrate the upper surface and chlorenchyma cells surround the VBs on the lower surface, in the mid rib region. The average length of microhairs is also an important character in identification, which is also in conformation with the findings of Webster (1983) and Shouliang *et al.* (1996). However, the comparative lengths of basal and distal cells, shorter, longer or equal to each other, were also useful in delimiting *Digitaria spp.* (Gillani *et al*., 2002).

The presence of prickles with rounded base in *Panicum maximum* makes it, different from *Panicum antidotale* in which pointed projections are present on the upper leaf surface. *Panicum antidotale* has sclerenchyma strands on the both surfaces, that are opposite to large VBs and sclerenchyma girders are not found in these species, however, the sclerenchyma girders are present in *Panicum maximum*, which distinguish it from *P. antidotale*.Presence or absence of sclerenchyma is an important feature that helps in identification of species (Jarves and Barkworth, 1992). In both *Panicum* species, chlorenchyma cells form radial or non radial arrangement around the VBs. (Metcalfe, 1960) pointed out that in most *Panicum* species, distinct chlorenchyma cells in radial arrangement are present, but in some species chlorenchyma cells can be inconspicuously radiate.

Urochloa is distinguished by solitary median VB. In most species of paniceae, two to three small VBs are observed on both sides of median VB. Rarely, single small VB is present as in *Paspalidium flavidum,* so this character is useful to identify different species. Fabbri *et al*. (2006) carried out anatomical studies on *Paspalum* and concluded that in moist conditions, air cavities are developed in these species, and this ability of air cavities formation shows a constant character, however in the present investigations air cavities are found absent in *Paspalum distichum*, and it reveals that in *Paspalum*, presence of air cavities is not a permanent character.

All the species have complete and single bundle sheath around the VBs except the median vascular bundle in which sometimes there are interruptions in sheath on the lower surface. Few species have large VBs with double sheath as found in *U. reptans, U. distachya, P. orientale* and *U. panicoides*. *P. orientale* is often confused with *P. Ciliaris,* and can be distinguished by the presence of large VBs with double sheath.

Grasses can minimize the detrimental effects of high salinities and drought by showing a series of structural and functional modifications in morpho-anatomical and physiological characteristics (Hameed *et al*., 2013).

Different species such as *U. deflexa, Pennisetum ciliaris and D. nodosa* have adapted to dry habitat by developing large bulliform cells that help in leaf rolling to avoid water loss (Alvarez *et al*., 2008). Epidermal and bulliform cell area become greater with the increase in salinity of habitats. Thick epidermis with dense cuticle and large bulliform cells is a characteristic feature of most desert plants (Jianjing *et al*., 2012), and it can be distinctively considered as an important adaptation against physiological drought. Greater density of macrohairs and salt glands is observed in *U. deflexa, Pennisetum setigerus, D. nodosa and C. biflorus* on adaxial leaf surface that seems significant for their survivel in the saline and dry habitat. According to Marcum *et al*. (1998) and Hameed *et al*. (2009) salt glands and hairs on the leaf surface are the significant adaptations of these grasses to withstand against drought and salinity. Greatly increased vesicular hairs at high salinities may play a crucial role in its adaptability and successful survival under harsh climates (Naz *et al*., 2009). Moreover, increased density of trichomes is also critical for checking undue water loss through leaf surface (Dolatabadian *et al*., 2011), and it is more significant under hot arid and saline desert environment. Sclerenchyma girders are present in almost all of the grasses present in dry habitat. The survival of the species in such conditions may depend on increased sclerification that has been reported to be a characteristic feature of most salinity and drought tolerant species (Schreiber *et al*., 2009).

Conclusion

Anatomical markers may be an important tool to resolve the taxonomic problems within the tribe. All the species exhibit the panicoid characters, which justify them in the same tribe. Anatomical markers like nature and size of macro- and micro-hairs, shape of bulliform cells, arrangement of vascular bundles and amount of sclerification present in the leaves are specific at species level and can effectively be used for resolving taxonomic problem of problematic genera within tribe Paniceae.

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