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Taxonomic utilization of anatomical characters in tribe Andropogoneae (Poaceae) based on transverse sections of leaves

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In the present investigations, 13 species of grasses belonging to 10 genera of tribe Andropogoneae (Poaceae) were collected from salt range area of Pakistan. The purpose of the study is to identify and differentiate the grasses on the basis of leaf anatomical characters, at the specific and generic level within the tribe. Variations are observed in different characters of T.S. of leaf (presence and absence of sclerenchyma girders or strands, ribs, furrows and prickles adaxially and abaxially in different species), which are valuable in taxonomy and identification and differentiation of grasses of tribe Andropogoneae. The studies showed that *Dicanthium foveolatum* and *Saccharum spontaneum* are differentiated by anchor shaped abaxial and anchor shaped sclerenchyma girders, while the presence of large air spaces in the mesophyll of *S. spontaneum* and *Vetiveria zizanioides* makes them distinct from other species of the tribe. Bulliform cells in irregular and fan shaped groups and radial arrangement of chlorenchyma cells around the vascular bundles is the characteristic of all the species of the tribe, showing panicoid type of leaf with kranz type of anatomy. It is concluded that anatomical characters of leaves in tribe Andropogoneae are used as taxonomic markers at generic and specific level.

Key words: Taxonomic utilization, anatomical characters, tribe Andropogoneae.

INTRODUCTION

The Andropogoneae is a cosmopolitan tribe of grasses having 87 genera throughout the tropics, extending in warm temperate regions widely distributed and not clearly delimited (Cope, 1982). This tribe is represented by 36 genera and 67 species in Pakistan (Nasir and Ali, 1970 - 2002). From the 'salt range' of Pakistan, 13 species belonging to 10 genera of this tribe are collected and studied. The genus *Saccharum* has 3 species followed by genus *Dicanthium* with two species, while all other genera have one species each. The species of Andropogoneae may serve as fodder for grazing and for storage in winter when there is shortage of fodder (Ahmad et al., 2009). The tribe Andropogoneae is recognized by a pair of spikelets, one sessile and the

other pedicelled. So, this character helps to justify the particular specimen in the tribe.

There are some problematic genera which are not easy to distinguish from each plant on the basis of morphological characters. Genus *Bothriochloa* and *Dicanthium* are very similar to each other and there is always confusion in identification and differentiation between these genera. Similarly, *Saccharum ravennae* and *Saccharum bengalense* are also very similar in external morphology and *S. bengalense* is often confused with *Arundo donax* by non-taxonomists. Other species, that is, *Chrysopogon serrulatus*, *Sorghum halepense* and *Cymbopogon jwarancusa* are also not properly identified merely on the basis of morphological (vegetative and floral) characters. Anatomical studies have been used successfully to clarify taxonomic status and help in the identification of different species (Gilani et al., 2002). In the past, anatomical studies incorporation with morphological studies for the resolution of taxonomic

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problems of monocots has been used. Webster (1988) studied the *Digitaria* anatomy for taxonomic purposes. The aim of the present study is to find out the solution of existing taxonomic problems of species, which overlap in most of their morphological characters and elucidate relationship of the critical taxa by utilization of leaf anatomical characters.

Transverse sections of grass leaves are also helpful in the identification and taxonomic delimitation of grasses. Dual-Jouve (1875) first studied the transverse sections of grass lamina, and used the character of the bulliform cells' position in relation to the vascular bundle for identification purposes. In the genus *Brachypodium*, distribution of sclerenchyma and bulliform cells proved useful at a specific level (Khan, 1984).

Occurrence of sclerenchyma and bundle sheath (Kranz sheath), the width of sclerenchyma, the indumentum of leaves and length and frequency of epidermal basis are features of prime importance that can identify relationship among the genera of Poaceae (Dube and Morisset, 1987; Jarves and Barkworth, 1992). Ellis (1986) pointed out that characters such as the thickness of the leaf, the number and arrangement of vascular bundles might be systematically useful and characters such as the distribution of prickles may be relatively stable or environmentally variable. Ellis (1976) also observed that the position of vascular bundles in the blades appeared to be a useful diagnostic character above the generic level.

The bulliform cells and associated colourless cells are used as a taxonomic character (Metcalfe, 1960; Markgraf - Dannenberg, 1980) and their structural details have been studied in few species by (Tuan et al., 1965; Jane and Chiang, 1991; Vecchia et al., 1998). The results could be used to study the different anatomical adaptations in grasses under different habitats as grasses in this tribe are known to be adapted to mesic and xeric environments. The purpose of the study is to identify and differentiate the grasses on the basis of leaf anatomical characters, at the specific and generic level within the tribe and to explore the diversity within the Andropogoneae.

MATERIALS AND METHODS

In the present investigations, 13 species of grasses belonging to 10 genera of tribe Andropogoneae were collected from 'salt range' area of Pakistan in different seasons of the year. The investigated species include *Saccharum bengalense* Retz., *Saccharum ravennae* (Linn.) Murr., *Saccharum spontaneum* Linn., *Dicanthium annulatum* (Forssk.) Stapf, *Dicanthium foveolatum* (Del.) Roberty, *Bothriochloa bladhii* (Retz.) S.T. Blake, *Chrysopogon serrulatus* Trin., *Cymbopogon jwarancusa* (Jones.) Schult., *Eulaliopsis binata* (Retz.) C.E. Hubbard, *Heteropogon contortus* (Linn.) P. Beauv. ex Roem. and Schult., *Imperata cylindrica* (Linn) Raeuschel, *Sorghum halepense* (L.) and *Viteveria zizanoides* (Linn.) Nash. The plants of each species were collected in triplicate, and voucher specimen was preserved in the herbarium of Quaid-i-Azam University Islamabad. In this experiment, 2 - 3 cm long sections of dried

leaves were kept in Chloral hydrate solution for 24 hours and washed with distilled water before section cutting. Freezing microtome (Leica CM 1325) was used for T. S. of leaves. About 2 - 3 drops of water were poured on the block, which was placed on the leaf section vertically and was covered with water droplets. When temperature reached at -14°C, water was frozen and 10 - 15 µm thick slices of leaves were cut by moving the microtome in forward and backward direction and selected the best sections for preparing slides. Both stained and unstained slides were prepared for studies. For staining thin leaf, sections were stained by the following procedure.

The leaf section is placed in the slide and 1 - 2 drops of safranin were added. Then, a few drops of 96% ethanol were added to remove the extra safranin. After it was poured, 1 - 2 drops of fast green and 96% of absolute alcohol was added respectively. Finally, one drop of xylene was added. Take Canada balsam on the cover slip and cover the slide. Slides were observed under microscope and the microphotographs were taken by a camera mounted on the microscope (Olympus Ax 70). Method of Johnson (1940) was followed with modifications for microtomy. Various anatomical characters were used as taxonomic markers in tribe Andropogoneae.

RESULTS

Bothriochloa bladhii (Retz.) S.T. Blake

Adaxial surface (almost flat) with no ribs and furrows has glandular structure that is present abaxially. Sclerenchyma is deposited near the margins of lamina. Large vascular bundles of basic type (with large meta xylem vessels) have adaxial and abaxial sclerenchyma girders, except the median vascular bundle present in the mid rib region that has only abaxial sclerenchyma girders. Small vascular bundles have small abaxial strands. Chlorenchyma cells are radially arranged around the vascular bundles. Keel conspicuously rounded a median vascular bundle of basic type, with about half of the mid rib occupied by the small and large colourless cells of irregular shapes. Keel vascular bundle, with abaxial sclerenchyma girders and adaxial sclerenchyma strands, is present above the mid rib region. The phloem in the median vascular bundle is covered by sclerenchyma cells. Bulliform cells are in fan shaped groups, while the middle cell largely and deeply penetrates the mesophyll. Most part of the adaxial surface is covered by bulliform cells. Small vascular bundles are found with single and complete sheath. The large vascular bundles have complete sheath or are interrupted adaxially and abaxially by sclerenchyma girders, except the large median vascular bundle that has complete sheath adaxially and interrupted abaxially (Figure 1a and b).

Chrysopogon serrulatus Trin

Adaxial surface (mostly with no ribs and ridges) has ribs at certain regions. Thick macrohairs are present adaxially. Abaxial surface is with no ribs and furrows. Median vascular bundle has a sclerenchyma girder, while

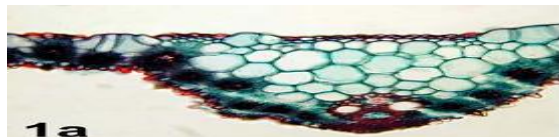


Figure 1a. *Bothriochloa bladhii*, T.S. of leaf showing midrib.



Figure 1b. T.S. of leaf closer to the margin.



Figure 2a. *Chrysopogon serrulatus*, T.S. of leaf showing midrib.



Figure 2b. T.S. of leaf closer to the margins.

the sclerenchyma cells form a cup-like structure around the phloem of median vascular bundle. The other vascular bundles have adaxial and abaxial sclerenchyma girders. However, abaxial girder is larger than the adaxial girder. A small layer of sclerenchyma is deposited at the margins. Chlorenchyma cells are radially arranged around the vascular bundles. Keel is very conspicuous and v shaped, containing one large vascular bundle of basic type, accompanied by 2 - 3 small vascular bundles on each side. Almost half of the mid rib is occupied by colorless cells and thick macrohairs on the adaxial surface in the mid rib region. Adaxial surface in the mid rib region is accompanied by bulliform cells. A continuous row of colourless cells is found on the adaxial side, just under the adaxial surface. Bulliform cells in irregular groups are close to the keel region. Small colourless cells are also present abaxially, but not continuously. Vascular bundles are with a single sheath. Large vascular bundles with incomplete sheath are interrupted abaxially or both adaxially and abaxially. Small vascular bundles have complete sheath (Figure 2a and b).

***Cymbopogon jwarancusa* (Jones.) Schult**

Adaxial surface has slight ribs and furrows. Macrohairs and glandular structures present on the adaxial side are curved at the margins. On abaxial side, ribs and furrows are more prominent. Six to seven small vascular bundles are found between large vascular bundles of basic type. Large vascular bundles are found with adaxial and abaxial sclerenchyma girders, except for the median one. Chlorenchyma cells radiate the vascular bundles. Keel is conspicuous and rounded. Keel contains one large vascular bundle of basic type, accompanied by 1 or 2 small vascular bundles on each side. Mid rib region is covered by large bulliform cells present in irregular groups adaxially and middle cells longer than other cells of the group. On abaxial side, bulliform cells are smaller and are present in continuous rows and almost all cells are of same size. Vascular bundles have a single sheath. Small vascular bundles are found with a complete sheath,



Figure 3a. *Cymbopogon jwarancusa*, T.S. of leaf showing midrib.

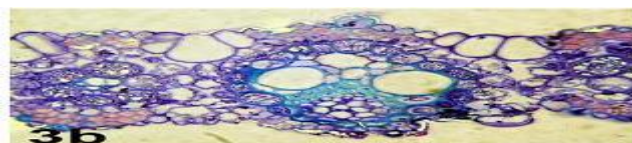


Figure 3b. T.S. of leaf showing prickles.

while in large vascular bundles, sheath is interrupted abaxially (Figure 3a and b).

***Dicanthium annulatum* (Forssk.) Stapf**

Adaxial surface with slight ribs and furrows has thick and short triangular spines that are present on the adaxial side. Abaxial surface with no ribs and furrows, have glandular structures that are present on the abaxial side. Five to six small vascular bundles are found between large vascular bundles of basic type. The margins of lamina are found with sclerenchyma depositions. Consequently, large vascular bundles of basic type are found with abaxial or adaxial girders or only with abaxial girders, while small vascular bundles are found with short abaxial sclerenchyma strands. As a result, chlorenchyma cells are radially arranged around the vascular bundles. Keel (which is conspicuous with a median large vascular bundle of basic type) has 2 or 3 small vascular bundles on each side. Almost half of the mid rib region occupied by colorless cells are angular in outline. The median keel vascular bundle is found with abaxial girders only, whereas bulliform cells are in fan shaped groups. The



Figure 4a. *Dicanthium annulatum*, T.S. of leaf showing mid rib.



Figure 4b. T.S of leaf showing bulliform cells.

large and smaller cells found in the middle and at the sides, respectively, are narrower towards the adaxial surface, while vascular bundles are found with a single and incomplete sheath. The median large vascular bundle is interrupted abaxially by sclerenchyma girders and adaxially by colourless cells. Small vascular bundles have single complete sheath, while other large vascular bundles are interrupted adaxially and abaxially (Figure 4a and b).

***Dicanthium foveolatum* (Del.) Roberty**

Adaxial surface with no ribs and furrows has long macrohairs that are present adaxially, which is deeply rooted with rounded base and some with swollen base. Flat abaxial surface, with no ribs and ridges, has abaxial surface with glandular structures. A few very long macrohairs are swollen at the base. Six small vascular bundles are found at the margins, while 5 - 6 small vascular bundles are found between large vascular bundles. Sclerenchyma depositions are found at the leaf margins, while the large vascular bundles have abaxial and adaxial sclerenchyma girders. The abaxial sclerenchyma girder is larger and anchor shaped. The small vascular bundles are found without sclerenchyma girders and strands or with only abaxial sclerenchyma strands. The median vascular bundle has abaxial sclerenchyma girder, while the sclerenchyma strands (with one layer of cells) are found adaxially in the mid rib region. Extra layer of 5 cells present abaxially are close to the median vascular bundle, so that sclerenchyma girders are not connected directly to the sheath.

Chlorenchyma cells are radially arranged around the vascular bundles. Keel is conspicuous and rounded, but not high. Median vascular bundle is accompanied by 5 - 6 small vascular bundles at each side. Bulliform cells are in irregular groups of 3 - 5 cells. Vascular bundles with a single sheath, have large vascular bundles (with complete sheath or interrupted abaxially) and small vascular bundles (with complete sheath) (Figure 5a and b).



Figure 5a. *Dicanthium foveolatum*, T.S of leaf showing midrib.



Figure 5b. T.S. of leaf showing prickles with bulbous base.



Figure 6a. *Eulaliopsis binata*, bulliform cells.



Figure 6b. T.S. of leaf showing prickles adaxially.

***Eulaliopsis binata* (Retz.) C.E. Hubbard**

Adaxial surface with wide ribs and furrows along with thick and long pointed macrohairs are deeply embedded in the epidermis, rounded at the base. Abaxial surface that is flat has no ribs and furrows. Three to five small vascular bundles are present between large vascular bundles of basic type. At margins 2 - 3, small vascular bundles are found between large vascular bundles. Large vascular bundles are in the middle and small vascular bundles are more towards the abaxial side. Chlorenchyma cells are radially arranged around the vascular bundles, especially around the small vascular bundles, while chlorenchyma cells are restricted around the small vascular bundles. Large vascular bundles are found with wide and high adaxial and abaxial sclerenchyma girders. Small vascular bundles are found without sclerenchyma strands or girders, while a few small vascular bundles are found with abaxial girders only. Keel is not conspicuous. Bulliform cells are in irregular groups of 10 - 12 cells and are present in the furrows. Vascular bundles with single sheath have large vascular bundles interrupted adaxially and abaxially. Small vascular bundles with single complete sheath have sheath with thick walled cells, equal in size (Figure 6a and b).



Figure 7a. *Heteropogon contortus*, T.S. of leaf showing midrib.



Figure 7b. T.S. of leaf closer to the margins.

***Heteropogon contortus* (Linn.) P. Beauv.ex Roem. and Schult.**

Adaxial surface is with slight ribs, present opposite to large vascular bundles. Abaxial surface is also with slight ribs and furrows. In small vascular bundles, xylem and phloem can not be differentiated. Vascular bundles are of 3 types with respect to their sizes, that is, small vascular bundles, medium size and large vascular bundles. Large vascular bundles of basic type have large metaxylem with small protoxylem on each side, while small vascular bundles are not accompanied by sclerenchyma. Medium size vascular bundles have small adaxial and abaxial girders, while large vascular bundles have adaxial and abaxial strands. Keel (which is conspicuous and rounded) is found with a single median vascular bundle that has 2 - 3 small vascular bundles on each side. Bulliform cells are in irregular groups or fan shaped groups with some cells penetrating into the mesophyll. Single bundle sheath, all small vascular bundles with complete sheath and the large vascular bundles having incomplete sheath are interrupted abaxially (Figure 7a and b).

***Imperata cylindrica* (Linn.) Raeuschel**

Adaxial surface are found with slight ribs and furrows. Abaxial surface is smooth and flat and has a thick cuticle on both surface of the epidermis. Vascular bundles of different sizes that have two very small vascular bundles on both sides of the large vascular bundles of basic types are present near the abaxial side. Three different sizes of vascular bundles were observed and they are large vascular bundles of basic types, medium and small vascular bundles. The bundle sheaths of medium vascular bundles (having elongated cells adaxially) are also pointed adaxially. The small vascular bundles are angular in outline, while the chlorenchyma cells are radially arranged around the vascular bundles. The large vascular bundles of basic type and the medium sized

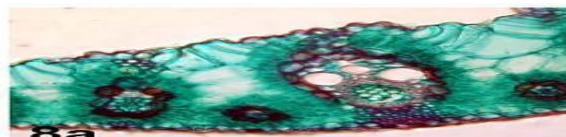


Figure 8a. *Imperata cylindrica*, T.S. of leaf showing bulliform cells.



Figure 8b. T.S. of leaf showing margins.

vascular bundles are found with adaxial and abaxial girders (5 layers thick), while the very small vascular bundles are found with no sclerenchyma or 1 or 2 layers thick short strand. Deposition of sclerenchyma is found at the margins, whereas keel is not observed in the material. Bulliform cells are in fan shaped groups or in a group tapering towards the base. Bulliform cells along with colourless cells deeply penetrate the mesophyll. Sometimes, the colourless cells made girder towards the abaxial side. Large vascular bundles of basic type with complete double or outer sheath are interrupted abaxially by sclerenchyma girders. Small vascular bundles with a single complete sheath are of almost equal sized cells (Figure 8a and b).

***Saccharum bengalense* Retz.**

Adaxial surface with slight ribs and furrows has macrohairs that are seen on the adaxial surface. A rounded extension is adaxially present in the mid rib, opposite to the large vascular bundle of basic type. Abaxial surface has flat, but not ridged macrohairs. Vascular bundles are of different sizes such as, large vascular bundle of basic type, medium size and small vascular bundles. Medium size and small vascular bundles are angular in outline. Large vascular bundles and medium size vascular bundles are found with adaxial and abaxial sclerenchyma girders. Chlorenchyma cells are radially arranged around vascular bundles, whereas keel is not seen. Bulliform cells are in fan shaped groups. The middle cell is longer in size and narrower towards the base. Associated colourless cells deeply penetrate the mesophyll and sometimes the colourless cells making girders towards the abaxial side. All vascular bundles are with single sheath and the small bundles are with complete sheath (cells of sheath equal in size). Mostly, medium and large vascular bundles with incomplete sheath are interrupted adaxially and abaxially. The sclerenchyma girders that penetrate the bundle sheath are surrounded by the xylem and phloem (Figure 9a and b).



Figure 9a. *Saccharum bengalense*, T.S. of leaf closer to the mid rib.



Figure 9b. T.S. of leaf closer to the margins.

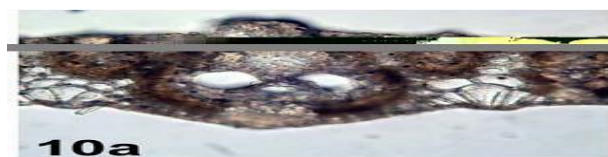


Figure 10a. *Saccharum ravennae*, T.S. of leaf showing mid rib.

***Saccharum ravennae* (Linn.) Murr.**

Adaxial and abaxial surfaces are found with slight ribs and furrows, while macrohairs are present on the adaxial side. Vascular bundles are categorized into three groups on the basis of their size. They are: small vascular bundles, medium sized and large vascular bundles. Large and medium size vascular bundles are found with adaxial and abaxial sclerenchyma girders, while chlorenchyma cells are radially arranged around the vascular bundles. Keel is conspicuous and rounded, whereas bulliform cells are in fan shaped groups. The associated colourless cells penetrate deeply into the mesophyll. Consequently, all vascular bundles are found with a single sheath, while the small vascular bundles are found with a complete sheath and the cells forming sheath are equal in size. Mostly, medium and large vascular bundles with incomplete sheath are interrupted adaxially and abaxially (Figure 10a and b).

***Saccharum spontaneum* Linn.**

Adaxial and abaxial surface, found with slight ribs and furrows, is flat and smooth. Mostly, the vascular bundles (small) have three types of vascular bundles with respect to size, that is, large, medium and small vascular bundles. Large vascular bundles are present in the middle of the mesophyll, while the medium and small vascular bundles are closer to the abaxial side, especially



Figure 10b. T.S. of leaf closer to the margins.

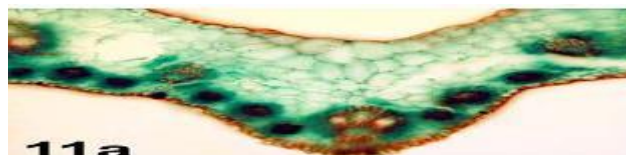


Figure 11a. *Saccharum spontaneum*, T.S. of leaf showing mid rib.

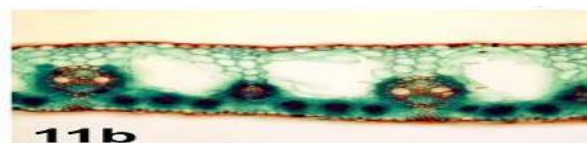


Figure 11b. T.S. of leaf showing air cavities.

the small vascular bundles which are located near the abaxial surface. Apparently, the large vascular bundles are found with wide and tall anchor shaped abaxial sclerenchyma girders, while the medium size vascular bundles are found with short sclerenchyma abaxial girders and the small vascular bundles with no abaxial girders and sclerenchyma depositions at the margins. Adaxial sclerenchyma strands are present opposite to the large and medium sized vascular bundles. A major part of the mesophyll is covered by large air spaces (Aerenchyma cells). These air spaces are absent at the margins and are closer to the margins opposite to the vascular bundles on the adaxial side, while the mesophyll is occupied by colourless cells. Chlorenchyma cells are restricted to narrow zones around the vascular bundles and are radiating around the vascular bundles. Keel is conspicuous and rounded, thereby having a median large vascular bundle with 2 or 3 small vascular bundles on each side. Most of the mid rib region is covered by colorless cells. Bulliform cells are present on the adaxial side opposite to the keel region, while only large cells are present in the middle and smaller cells on the sides (in fan shaped groups). Large vascular bundles are found with double sheath (both complete inner and outer sheaths) or are interrupted at the abaxial side by the girders (Figure 11a and b).

***Sorghum halepense* (L.) Pers.**

Adaxial surface with prickles or macrohairs are slightly



Figure 12a. *Sorghum halepense*, T.S. of leaf showing mid rib.



Figure 12b. T.S. of leaf closer to the margins.

opposite to the large vascular bundle of the basic type. Adaxial surface that is smooth or with slight ribs and furrows, has 4 - 5 small vascular bundles present between the large vascular bundles of basic type. Chlorenchyma cells clearly radiate the vascular bundles. Large vascular bundles of basic type are found with adaxial and abaxial girders, while small vascular bundles are found without sclerenchyma strands or girders. Keel is conspicuous and is occupied by the median large vascular bundle of basic type rounded at the tip. Most parts of the mid rib are occupied by colorless cells and some cells angular in shape. About 2 or 3 outer layers of epidermal cells are found on the adaxial surface in the mid rib region. Bulliform cells are in irregular group of 2 - 5 cells. Large vascular bundles with single sheath are interrupted abaxially by abaxial sclerenchyma girders. Also, small vascular bundles are found with a single sheath and sheath cells are almost equal in size (Figure 12a and b).

***Vetiveria zizanoides* (Linn.) Nash**

Adaxial surface is smooth with no ribs and furrows, whereas abaxial surface is smooth, with slight ribs and furrows. Large vascular bundles of basic type have small vascular bundles embedded in the assimilatory tissues. Sclerenchyma depositions are found at the margins. Large vascular bundles are found with wide and thick abaxial sclerenchyma girders and short adaxial strands. Most of the small vascular bundles do not have girders or strands, but some vascular bundles have abaxial strands or girders. The adaxial strands are connected to the bundle sheath by girders of large colorless cells and are separating the different intercellular cavities from each other. Chlorenchyma cells are in narrow strips towards the abaxial epidermis. These strips extend towards the middle, thereby covering the right and left sides of large vascular bundles of basic type. Most part of the lamina covered by large intercellular cavities extends from the assimilatory tissue to just below the adaxial epidermis. Girders of colorless cells are present between



Figure 13a. *Vetiveria zizanoides*, T.S. of leaf showing mid rib.



Figure 13b. T.S. of leaf showing air cavities.

intercellular cavities. These girders extend from vascular bundles to adaxial surface, while about 6 - 7 intercellular cavities are present on either side of the mid rib. Intercellular cavities are absent at the margins, whereas keel is conspicuous. The lamina is narrower in the mid rib region, and at the margins, abaxial projection is at the mid rib region. Bulliform cells are in a single group along the adaxial side and also in a single row abaxially.

Vascular bundles are found with a single sheath. While large vascular bundles are interrupted abaxially, small vascular bundles are found with a complete sheath (Figure 13a and b).

DISCUSSION

Studies regarding the transverse section of lamina may provide information to resolve the systematic problems surrounding a particular taxa (Macfarlane and Watson, 1980). Variations are observed in the outline of lamina, that is, the presence or absence of ribs and furrows on the adaxial and abaxial side. According to Metcalfe (1960), the absence of ribs is of taxonomic value. In *Chrysopogon serrulatus* and *Dicanthium foveolatum*, the absence of ribs and furrows are observed on the adaxial and abaxial surface; however, macrohairs are present on the adaxial surface of these species' lamina. Macrohairs are thick in *Chrysopogon serrulatus* (Figure 2b), while long macrohairs are present in *D. foveolatum* (Figure 5b). The presence and distribution of prickles or macrohairs may be important in differentiating and identifying different species. According to Ellis (1986), the distribution of prickles might be relatively stable or environmentally variable.

In *Bothriochloa bladhii*, ribs and furrows are absent adaxially, while glandular structures are present abaxially (Figure 1a). *Dicanthium annulatum* is with slight ribs and furrows on the adaxial side, while glandular structures are observed abaxially but not pronounced. As a result, thick short and triangular spines are present on the adaxial side. *Eulaliopsis binata* is differentiated from other species by the presence of wide ribs and furrows adaxially and long pointed macrohairs deeply embedded in the

epidermis (Figure 6a). According to Metcalfe (1960), it is important to note whether the base of hairs is sunken between or below the general level of adjacent cells. The presence of sunken hairs is a characteristic of grasses from warm regions, while more superficial types are common in temperate grasses. In grasses, the basic leaf anatomy can be used to find out if a grass has the C₃ or C₄ photosynthetic pathway. The two basic types of leaf anatomy are kranz and non-kranz patterns. In kranz type, one or two bundle sheaths are present around the vascular bundle and 2 - 4 chlorenchymatous cells are present between adjacent vascular bundles radiating from or towards the bundle sheath (Chaudhary, 1989). According to Clayton and Renvoize (1986), the tribe Andropogoneae has kranz type of leaf anatomy. In the present investigations, in all the species of this tribe, chlorenchyma cells are radially arranged around the bundle sheath, showing the kranz type of leaf anatomy (that is, the character of panicoid type leaf) (Gould, 1968). Reynolds (1959) who studied leaf anatomy of Andropogoneae, for a greater detail, pointed out significant differences at species level which could be helpful in the classification of Andropogoneae. This study of Reynolds, though lacking in the synthesis of demonstrating relationships, was a great step forward in pointing out the fact that inter specific anatomical differences in the leaf do exist among the members of the tribe Andropogoneae.

A major part of mesophyll is covered by large air spaces in *Saccharum spontaneum* (Figure 11b). These air spaces are absent at the margins of lamina. In *Vetiveria zizanioides*, intercellular cavities are also present covering most part of the lamina (Figure 13b). Presence of air cavities, which develop in different organs of the plant while their number and size varies with the age and nature of the organ, is the characteristic of hydrophytous or amphibious species (Sculthorpe, 1967). Both of these species are mostly found near water, and as a result, the presence of air cavities is the adaptation of these species to grow in water saturated soil.

Bernstein (1958) reported that species like *Saccharum spontaneum* can grow better in saline conditions with moisture. According to Chaudhary (2009), *Vetiveria zizanioides* has a strong deep penetrating aerenchymatous fast growing system and physiologically, it has the ability to grow both as a xerophyte and hydrophyte. Bulliform cells are in fan shaped or irregular groups in all the genera of tribe.

In *Chrysopogon serrulatus* and *Cymbopogon jwarancusa*, bulliform cells are in the midrib region (Figures 2a and 3a). In *Heteropogon*, these cells are in fan shaped or irregular groups (Figure 7a and b) and in *Imperata cylindrica*, bulliform cells are in fan shaped groups or in a group tapering towards the base (Figure 8a). In *Vetiveria*, bulliform cells are in a single group adaxially and also present in a single row abaxially (Figure 13b), while Metcalfe (1960) studied *Vetiveria zizanioides* and observed that bulliform cells are confined to a single large group on

the mid rib of adaxial surface only.

Median vascular bundle is solitary and of basic type in *Bothriochloa bladhi* and *Sorghum halepense* (Figures 1 and 12a). Faruqi (1969) studied *Bothriochloa intermedia* and found in cross sectional studies that there are three primary bundles in the keel. The number of bulliform bands between two primary bundles of the lamina is usually two and the number of intercalary bundles is 3 - 4. In other species, median vascular bundle is of basic type and is accompanied by 1 - 6 cells on either side. In *Eulaliopsis binata*, large vascular bundles are in the middle, while small vascular bundles are on the abaxial sides with wide and high adaxial girders (Figure 6b). In most of the species, adaxial and abaxial strands and girders are observed opposite to the medium and large vascular bundles and with no sclerenchyma strands or girders opposite to the small vascular bundles.

In *Eulaliopsis binata*, abaxial girder is observed opposite to few small vascular bundles. Abaxial strands are present in *Bothriochloa bladhi* and *Dicanthium foveolatum*, while adaxial and abaxial strands are observed in *Imperata cylindrica* and *Vetiveria zizanioides*. Grasses from arid areas have well developed sclerenchyma tissue, while many tropical grasses often have a high proportion of the smaller bundles not accompanied by sclerenchyma (Ellis, 1976). Rarely in some species, does sclerenchyma lie between the vascular bundles (Metcalfe, 1960). Sclerenchyma may penetrate the bundle sheath on one or both sides to connect with the sclerenchyma of the vascular bundles (Gould, 1968). As in *Bothriochloa bladhi*, the phloem in the median vascular bundle is covered by sclerenchyma cells, while in *Chrysopogon serrulatus*, sclerenchyma cells make a cup like structure around the phloem of median vascular bundle (Figure 3a). In *S. bengalense*, sclerenchyma girders penetrate the bundle sheath and surround the xylem and phloem.

Mostly in all species of the tribe, bundles sheath is single in small and large vascular bundles and complete in the small vascular bundles except the *Imperata cylindrica* and *Saccharum spontaneum* in which large vascular bundles are with double sheath. In panicoid type leaves, mostly, the bundles sheath is single as observed in this tribe and the inner or mesotome sheath is absent or inconspicuous. According to Metcalfe (1960), large vascular bundles are sometimes with double sheath in some species. Same is observed in this tribe in which two species, *Imperata cylindrica* and *Saccharum spontaneum* have large vascular bundles with double sheath. The studies showed that *Dicanthium foveolatum* and *Saccharum spontaneum* are peculiar in having both anchor shaped abaxial and sclerenchyma girders (Table 1), while *S. spontaneum* and *Vetiveria zizanioides* are differentiated by large air spaces in their mesophyll, which explains their adaptation to the aquatic habitat. Bulliform cells in irregular and fan shaped groups is the characteristic of all the species of the tribe. It is concluded that various characters are helpful in the

Table 1. Diagnostic characters in T.S. of leaves of grasses in tribe Andropogoneae.

S/ No.	Taxon	Bulliform cells	Chlorenchyma cells	Keel	Sclerenchyma strands and girders	Bundle sheath
1	<i>B. bladhii</i>	In fan shaped groups	Radially arranged	Conspicuous and rounded	Present	Single and complete
2	<i>C. serrulatus</i>	In irregular groups	Radially arranged	Very conspicuous and V shaped	Abaxial girder larger than adaxial girders	Single and complete or incomplete
3	<i>C. jwarancusa</i>	In irregular groups	Radially arranged	Conspicuous and rounded	Strands absent	Single and complete or incomplete
4	<i>D. annulatum</i>	In fan shaped groups	Radially arranged	Conspicuous	Girders present only in large vascular bundles	Single and complete or incomplete
5	<i>D. foveolatum</i>	In irregular groups	Radially arranged	Conspicuous and rounded	Abaxial sclerenchyma girders are large and anchor shaped	Single and complete or interrupted abaxially
6	<i>E. binata</i>	In irregular or fan shaped groups	Radially arranged	Not conspicuous	Wide and high scl. girders in large vascular bundles	Single and complete or interrupted abaxially
7	<i>H. contortus</i>	In irregular or fan shaped groups	Radially arranged	Conspicuous and rounded	Girders absent in large vascular bundles	Single and complete or interrupted abaxially
8	<i>I. cylendrica</i>	In fan shaped groups	Radially arranged	Not conspicuous	Large vascular bundles with adaxial and abaxial girders	Single or double
9	<i>S. bengalense</i>	In fan shaped groups	Radially arranged	Not conspicuous	Large vascular bundles with adaxial and abaxial girders	Single and complete or interrupted abaxially
10	<i>S. ravennae</i>	In fan shaped groups	Radially arranged	Conspicuous and rounded	Large vascular bundles with adaxial and abaxial girders	Single and complete or interrupted abaxially
11	<i>S. spontaneum</i>	In fan shaped groups	Radially arranged and restricted to narrow zones	Conspicuous and rounded (mesophyll covered by large air spaces)	Abaxial scl. girders, wide and tall anchor shaped	Double in large vascular bundles
12	<i>S. halepense</i>	In irregular groups	Clearly radiating the vascular bundles	Conspicuous and rounded at the tip	Sclerenchyma girders interrupted abaxially in large vascular bundles	Single, complete or interrupted abaxially

Table 1. Contd.

13	<i>V. zizanoides</i>	In a single row	In narrow strips	Conspicuous (large intercellular cavities present in lamina)	Wide and thick abaxial sclerenchyma girders in large vascular bundles	Single, complete or interrupted abaxially
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identification and differentiation of species and are used as taxonomic markers for delimitation of tribe Andropogoneae.

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