FOLIAR EPIDERMAL STUDIES AS AN AID TO THE IDENTIFICATION OF GRASSES OF TRIBE ANDROPOGONEAE (POACEAE) FROM POTOHAR REGION OF PAKISTAN

ABDUL NAZIR¹, MIR AJAB KHAN¹, FAROOQ AHMAD³, KIFAYAT ULLAH¹ AND AMIN SHAH⁴

¹Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan ³Department of Botany, University of Agriculture Faisalabad, Pakistan ⁴Department of Botany, University of Sargodha, Sargodha Pakistan *E-mail of corresponding author: nazir_malik1@yahoo.com

Abstract

In the present investigations, 13 species of grasses belonging to 10 genera of tribe Andropogoneae (Poaceae) were collected from the Potohar region of Pakistan and their leaf epidermal studies were carried out. The leaf epidermal studies showed that all the species have paracytic stomata, with dumb bell shaped guard cells, except *Heteropogon contortus* and *Cymbopogon jwarancusa* in which guard cells are straight in the middle. Different types of subsidiary cells such as high dome shaped or low dome shaped subsidiary cells are observed. The difference in shape of subsidiary cells can be used to differentiate problematic species such as genus *Bothriochloa* from *Dicanthium*, as these genera look similar morphologically. Diversity in shapes of silica bodies is observed in the species of this tribe, which is valuable for identification. Silica bodies are cross shaped, dumb bell shaped or intermediate between cross and dumb bell shaped. Bicelled microhairs of panicoid type are present in all the species except *Euloliopsis binata* in which microhairs are absent. Microhairs are rounded in all species except *Cymbopogon jawarancusa* and *Heteropogon contortus*. The studies revealed that different leaf epidermal characters such as shape of subsidiary cells, silica bodies, presence or absence of microhairs, macrohairs and rounded papillae are valuable in the identification of grasses at the specific and generic level of the tribe.

Introduction

This tribe has 87 genera throughout the tropics, extending in the warm temperate regions. From Pakistan, 36 genera and 67 species of this tribe are reported. From the Potohar region of Pakistan, 13 species belonging to 10 genera of 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. This is the second largest tribe after Paniceae studied in this area.

Morphological characters provide useful information for the identification of all levels of taxonomic ranks (families, tribes, genera and species, etc) but many taxa of flowering plants cannot be distinguished on the basis of morphological characters only.

Anatomical studies can be used to determine taxonomic status and also assist in the identification and differentiation of different taxa (Gilani *et al.*, 2002, Yousuf *et al.*, 2008). In the past anatomical studies along with morphological studies for solving taxonomic problems of monocots have been used (Gilani *et al.*, 2003 & 2003a). Anatomy of Digitaria species was studied by Webster, (1983) for the taxonomic purposes. The present study is aimed to find out the solution of taxonomic problems at species and generic level, which overlap in most of their morphological characters and to show relationship between different taxa of tribes by utilization of leaf epidermal characters.

The foliar epidermal anatomical studies provide sufficient taxonomic data related to Poaceae. Different epidermal characters such as length and shape of epidermal cells, stomata and hairs have proved to be an important tool in delimitation of taxa in many plant families (Metcalfe & chalk, 1950-1989, Ditsh *et al.*, 1995; Barthlott *et al.*, 1998; Stenglein *et al.*, 2003). It is confirmed that different leaf epidermal features can help to elucidate taxonomic affinities and relationships at different levels (Davila & Clark, 1990, Cai & Wang, 1994; Mejia-Saules & Bisbey, 2003) and these leaf epidermal characters are of great value in grass systematics and characterization of broad groups within the grasses, particularly subfamilies and tribes.

Materials and Methods

Dried leaves were placed in boiling water by using water bath, to soften the leaves until they become unfolded and were used for epidermal scraping. Fresh leaves were used directly for anatomical studies. Leaf samples were prepared according to the modified method of Cotton, (1974) who followed Clark (1960) technique. The fresh or dried leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath for about 50-60 minutes. Lactic acid softens the leaf tissues so that its peeling is made possible. The abaxial and adaxial epidermis was removed, along with the mesophyll cells by using scalpel blade, until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed on the slide and mounted in clean 88% lactic acid. The micro photographs of the mounted materials were taken by using a camera mounted on Leica light microscope. Different anatomical observations were made on the different species of grasses collected from Potohar region of Pakistan. The following anatomical characters of both abaxial and adaxial epidermis were studied.

Length and width of long cells, their wall shape whether sinuous, slightly sinuous or nonsinuous was noted. It was examined that short cells and papillae in intercostal zone are present or absent. No of rows of long cells between two costal zones, length and width of stomatal complex and shape of guard and subsidiary cells was studied. Subsidiary cells, low or high dome shaped or triangular, length, width and shape of microhairs, bicellular or unicellular and shape of distal and basal cells present or absent were considered. Length and width of macrohairs and hooks and their shape, their presence or absence was noted. Length, width and shape of silica bodies and short cells and their distribution in costal zone was observed.

Results

1. Bothriochloa bladhii

Abaxial intercostal zone: Abaxial intercostal long cells are with sinuous walls. A few short cells are present between long cells. Number of rows of long cells between two costal zones is 3-7. Number of stomatal rows between two costal zones is 2-3. Guard cells are dumb bell shaped while subsidiary cells are dome shaped. Microhairs are bicelled, distal cell is thin walled, longer or equal to basal cell and tapering and rounded at the tip. Macrohairs and hooks are absent.

Costal zone: Silica bodies are dumb bell shaped and short cells are with slight sinuous walls. Prickles are at the margins of costal zone (Fig. 1).

Adaxial intercostal zone: Adaxial intercostal long cells are with irregular sinuous walls and short cells are present between long cells. Number of rows of long cell between two costal zones is 1–6. Number of stomatal rows between two costal zones is 1-2. Microhairs are bicelled, distal cell is thin walled and tapering towards apex. Macrohairs are absent and hooks are with long pointed beak.

Costal zone: Silica bodies are dumb bell shaped. Short cells are present while prickles are absent (Fig. 2).

2. Chrysopogon serrulatus Trin

Abaxial intercostal zone: Abaxial intercostal long cells are with thick sinuous walls. Number of rows of long cell between two costal zones is 3-18. Number of stomatal rows between two costal zones is 2-6. Guard cells are dumb bell shaped; subsidiary cells are low dome shaped or triangular in shape. Microhairs are knife like, distal cell is thin walled and not prominent. Macrohairs are absent. Hooks are tapering towards the tip and broad at the base.

Costal zone: Silica bodies are cross shaped. Short cells are with sinuous walls present between silica bodies. Prickles are absent (Fig. 3).

Adaxial intercostal zone: Adaxial intercostal long cells are with thick sinuous walls. Number of rows of long cells between two costal zones is 5-9. Number of stomatal rows between two costal zones is 2-4. Guard cells are dumb bell shaped while subsidiary cells are triangular in shape. Stomata are not found frequently on the adaxial side. Microhairs are present. Macrohairs are abundent on adaxial side. Hooks are rounded at the base, pointed at one side.

Costal zone: Silica bodies are cross and dumb bell shaped. Short cells are rarely present. Prickles are present at the margins (Fig. 4).

3. Cymbopogon jawarencusa

Abaxial intercostal zone: Abaxial intercostal long cells are with sinuous walls, mostly long cells are with large papilla at one side of long cell and short cells are present between long cells. Numbers of rows of long cells, between two costal zones are 3-6. Numbers of stomatal rows between two costal zones are 1-2. Guard cells are dumb bell shaped with thickness in the middle and subsidiary cells are high dome shaped. Microhairs are present between two long cells and distal cell is shorter than basal cell. Macrohairs and hooks are not seen.

Costal zone: Silica bodies are intermediate between cross and dumb bell shaped or cross shaped while silica bodies and prickles are present in a row. Short cells are present in rows. Prickles are in abundance in the costal zone (Fig. 5).

Adaxial intercostal zone: Adaxial intercostal long cells are cubical and with slightly sinuous or straight walls. Numbers of rows of long cell between two costal zones are 2-6. Numbers of stomatal rows between two costal zones are 1-2. Guard cells are dumb bell shaped and subsidiary cells are high dome shaped. Microhairs are bicelled; distal cell is thin walled, shorter than basal cell and tapering towards the apices. Macrohairs and hooks are not present.

Costal zone: Silica bodies are cross shaped or intermediate between dumb bell and cross shaped. Short cells are with sinuous walls. Angular prickles are present at the margins of costal zone (Fig. 6).

4. Dicanthium annulatum (Forssk.) Stapf.

Abaxial intercostal zone: Abaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells between two costal zones are 5-8. Numbers of stomatal rows between two costal zones are 1-2. Guard cells are dumb bell shaped and subsidiary cells are triangular. A micro hair is bicelled, distal cell almost equal to basal cell and is thin walled. Hooks are pointed at one end.

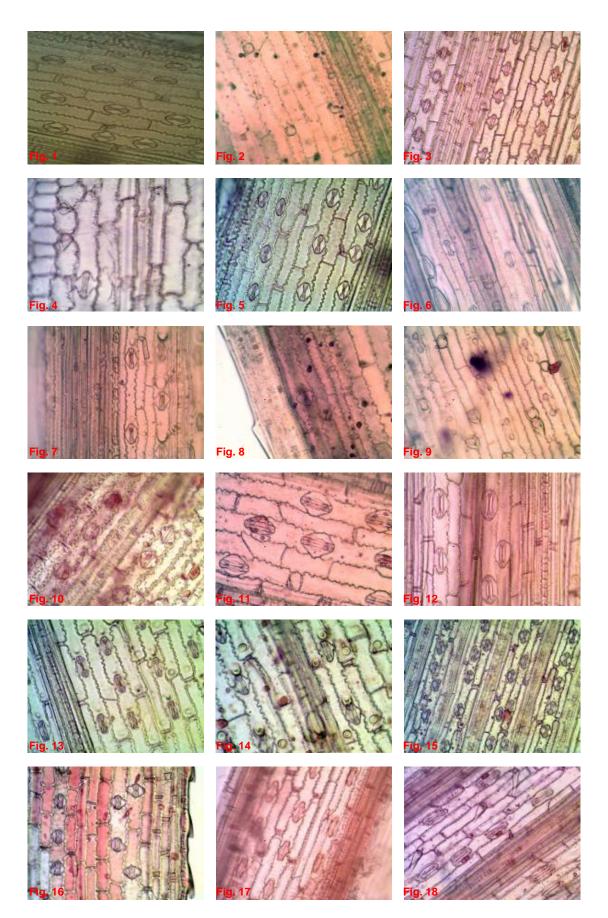
Costal zone: Silica bodies are dumb bell shaped with 1-3 layers. Short cells are present. Prickles are swollen and pointed at one end (Fig. 7).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells, between two costal zones are 4-9. Numbers of stomatal rows between two costal zones are 1-2. Guard cells are dumb bell shaped and subsidiary cells are triangular. Microhairs are bicelled and distal cell is almost equal to basal cell. Macrohairs are not seen.

Costal zone: Silica bodies are dumb bell shaped. Short cells are with sinuous walls. Prickles are present at the margins (Fig. 8).

5. Dicanthium foveolatum (Del.) Roberty

Abaxial intercostal zone: Abaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells between two costal zones are 4-9. Numbers of stomatal rows between two costal zones are 1-2. Guard calls are dumb bell shaped and subsidiary cells are somewhat triangular. Microhairs are bicelled, distal cell is very thin walled and not prominent. Macrohairs are none seen. Hooks are swollen at the base and pointed towards the tip.



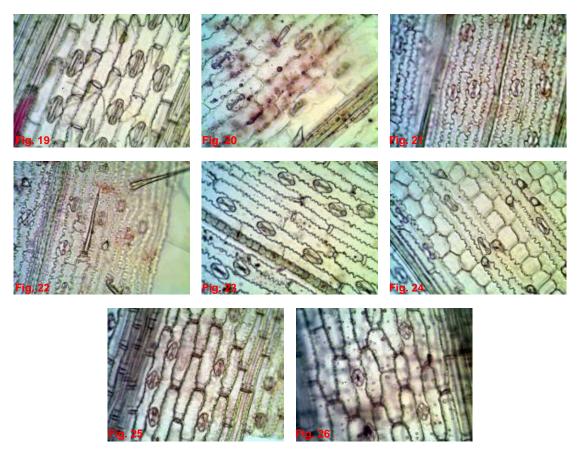


Fig.1. Bothriochloa bladhii. abaxial leaf epidermis. Fig.2. Bothriochloa bladhii, showing adaxial leaf epidermis. Fig.3. Chrysopogon serrulatus, leaf abaxial surface showing costal and intercostals zone. Fig.4. Chrysopogon serrulatus, leaf adaxial surface. Fig.5. Cymbopogon jawarncusa, abaxial leaf epidermis. Fig. 6. Cymbopogon jawarencusa, adaxial leaf epidermis, showing hooks in the costal zone. Fig. 7. Dicanthium annulatum, leaf abaxial epidermis, showing bicellular microhairs. Fig. 8. Dicanthium annulatum, showing leaf abaxial epidermis. Fig. 9. Dicanthium foveolatum, leaf abaxial surface, showing pointed hooks in intercostal zone. Fig. 10. Dicanthium foveolatum, leaf adaxial surface. Fig.11. Eulaliopsis binata, leaf abaxial surface. Fig.12. Euloliopsis binata, leaf adaxial surface showing silica bodies in costal zone. Fig.13. Heteropogon contortus, leaf abaxial surface. Fig.14. Heteropogon contortus, leaf adaxial surface showing rounded papilla in intercostals zone. Fig.15. Imperata cylindrica, leaf adaxial epidermis. Fig.16. Imperata cylindrica, leaf adaxial epidermis. Fig.17. Saccharum bengalense, leaf abaxial epidermis. Fig. 18. Saccharum bengalense, leaf adaxial epidermis. Fig. 21. Saccharum spontaneum, leaf adaxial epidermis. Fig. 22. Saccharum ravennae, leaf adaxial epidermis, showing long marcohairs. Fig. 23. Sorghum halepense, leaf abaxial epidermis, Fig. 24. Sorghum halepense, leaf adaxial epidermis. Fig. 25. Vetiveria zizanoides, leaf adaxial epidermis. Fig. 26. Vetiveria zizanoides, leaf adaxial epidermis.

Costal zone: Silica bodies are intermediate between cross and dumb bell shaped. Short cells are present. Prickles are abundantly present on the costal zone (Fig. 9).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells between two costal zones are 4-12. Numbers of stomatal rows between two costal zones are 2-3. Guard cells are dumb bell shaped and subsidiary cells are low to high dome shaped or triangular. Microhairs are bicelled. Macrohairs and hooks are present.

Costal zone: Silica bodies are cross shaped. Short cells are with sinuous walls. Prickles are also present (Fig. 10).

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

Abaxial intercostal zone: Intercostal long cells are with sinuous walls. Short cells are present between long cells.

Five to seven rows of long cells are present between two costal zones. One to five stomatal rows are present between two costal zones. Guard cells are dumb bell shaped while subsidiary cells somewhat triangular in shape or dome shaped. Microhairs are not observed while macrohairs are very long with rounded base. Hooks are absent.

Costal zone: Silica bodies are cross shaped. Short cells are present between silica bodies. Prickles are absent (Fig. 11).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Numbers of rows of long cell between two costal zones are 5-7. Numbers of stomatal rows between two costal zones are 3-4. Guard cells are dumb bell shaped while subsidiary cells are dome shaped or somewhat triangular in shape. Microhair, macrohairs and, hooks are not observed. **Costal zone:** A large number of dumbbells shaped and cross shaped silica bodies are present in a row. Short cells are rectangular with sinuous walls. Prickles taper towards the tips (Fig. 12).

7. Heteropogon contortus

Abaxial intercostal zone: Abaxial intercostal long cells are with coarsely sinuous walls, one rounded papilla is present at one side of each long cell; Short cells are present between long cells. Numbers of rows of long cells between two costal zones are 6-11. Numbers of stomatal rows between two costal zones are 2-4. Guard cells are swollen in the middle and subsidiary cells are low dome shaped and subsidiary cells are curved from the inner side. Microhairs are bicelled and distal cell is thin walled and not prominent, knife like and is shorter than basal cell. Macrohairs are absent and hooks are present between two adjacent cells, pointed at the tip.

Costal zone: Silica bodies are dumb bell shaped and somewhat lobed in the middle with 2-4 layers. Short cells are sinuous. Prickles are present in the marginal costal zone (Fig. 13).

Adaxial intercostal zone: Adaxial intercostal long cells are with deep sinuous walls and each cell is with a single papilla on one side. Numbers of rows of long cells between two costal zones are 2-6. Numbers of stomatal rows between two costal zones are 1-4. Guard cells are swollen in the middle and subsidiary cells are low dome shaped. Microhairs are bicelled and distal cell is thin walled and not distinct, both cells are almost equal or distal cell is shorter than basal cell, 50-55 μ m long and 5-7.5 μ m wide. Macrohairs are present and hooks are present.

Costal zone: Silica bodies are dumb bell shaped while short cells are with sinuous walls. Prickles are present at the margins (Fig. 14).

8. Imperata cylindrica (L.) Raeuschel

Abaxial intercostal zone: Abaxial intercostal long cells are with thin sinuous walls. Numbers of rows of long cells, between two costal zones are 3-6. Numbers of stomatal rows between two costal zones are 1-2. Guard cells are dumb bell shaped, while subsidiary cells are triangular. Microhairs are bicelled, distal cell is thin walled and not prominent and tapering to pointed apices. Macrohairs and hooks are absent.

Costal zone: Silica bodies are dumb bell shaped and in one row. Short cells and prickles are absent (Fig. 15).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells between two costal zones are 10-16. Numbers of stomatal rows between two costal zones are 2-3. Guard cells are dumb bell shaped while subsidiary cells are triangular. Microhairs, macrohairs and hooks are absent. **Costal zone:** Silica bodies are saddle shaped. Long cells are present with sinuous walls. Prickles are present (Fig. 16).

9. Saccharum bengalense Retz.

Abaxial intercostal zone: Abaxial intercostal long cells are with thin sinuous walls and inter stomatal cells are with concave ends. Numbers of rows of long cells between two costal zones are 4- 10. Numbers of stomatal rows between two costal zones are 2-4. Guard cells are dumb bell shaped and subsidiary cells are low dome shaped or triangular. Microhairs are present, basal cells are longer than distal cells, distal cells taper to the pointed apices. Hooks are not observed.

Costal zone: Silica bodies are cross shaped or intermediate between cross and dumb bell shaped. Short cells are in the row of 2-5 cells. Angular prickles are present at the margins (Fig. 17).

Adaxial intercostal zone: Adaxial intercostal long cells are sinuous. Macrohairs and hooks are absent. Microhairs are bicellular and basal cell is longer than distal cell, basal cell tapers to the apices. Microhairs are present.

Costal zone: Silica bodies are intermediate between cross and dumb bell shaped and short cells are frequent. Angular prickles are present at margins (Fig. 18).

10. Saccharum ravennae (Linn.) Murr

Abaxial intercostal zone: Intercostal long cells are with thick sinuous walls. Long cells between two costal zones are present in 4-9 rows. Two to four stomatal rows between two costal zones are present. Microhairs are bicelled and distal cells tapering towards the apices. Macrohairs and hooks are not observed.

Costal zone: Silica bodies are mostly intermediate between cross and dumb bell shaped, occasionally cross shaped. Short cells are present between the silica bodies and with slightly sinuous walls. Prickles are present in the costal zone repeating after 3-5 silica bodies and short cells (Fig. 19).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Three to ten rows of long cells between two costal zones. There are one to three stomatal rows between two costal zones. Microhairs are bicelled and basal cell is longer than distal cell. Macrohairs and hooks are absent.

Costal zone: Silica bodies are cross shaped or intermediate between cross and dumb bell shaped. Short cells are in short rows. Prickles are present (Fig. 20).

11. Saccharum spontaneum Linn

Abaxial intercostal zone: Intercostal long cells are with thin sinuous walls. Four to eight rows of long cells are present between two costal zones. Two to three stomatal rows are present between two costal zones. Microhairs are bicelled and basal cells are longer than distal cells, distal cells vary in shape and diameter. Mostly tapering to pointed apices. Subsidiary cells are triangular but not prominent, because of overlying prickles. Macrohairs and hooks are not observed.

Costal zone: Silica bodies are dumb bell shaped or intermediate between cross and dumb bell shaped; short cells are frequent in the costal zone. Prickles are present (Fig. 21).

Adaxial intercostal zone: Adaxial intercostal long cells are with thick sinuous or straight walls. Numbers of rows of long cells between two costal zones are 4-10. Numbers of stomatal rows between two costal zones are 1-3. Stomatal complex: 28-32.5 μ m long and 13-21 μ m wide, subsidiary cells triangular or low dome shaped, bicelled microhairs frequent in the inter costal zone, basal cells bent and longer than distal cells, 51-75 μ m long. Macrohairs and hooks none seen.

Costal zone: Silica bodies cross shaped or intermediate between cross and dumb bell shaped, 7.5-22 μ m long and 4-7.5 μ m wide, angular prickles present at the margins (Fig. 22).

12. Sorghum halepense (L.) Pers.

Abaxial intercostal zone: Intercostal long cells are with thin sinuous walls. Number of rows of long cells between two costal zones, are 5-26. Number of stomatal rows between two costal zones, are 2-13. Guard cells are dumb bell shaped and subsidiary cells are somewhat triangular. Microhairs are bicelled and distal cells tapers to apices and is slightly longer than basal cell. Hooks are rounded and present at the junction of two long cells.

Costal zone: Silica bodies are dumb bell shaped or intermediate between cross and dumb bell shaped with 1-5 layers. Short cells are present. Prickles are not observed (Fig. 23).

Adaxial intercostal zone: Adaxial intercostal long cells are with thick sinuous walls. Numbers of rows of long cells between two costal zones are 8-15. Numbers of stomatal rows between two costal zones are 3-4. Guard cells are dumb bell shaped, and subsidiary cells are triangular in shape. Microhairs are bicelled. Macrohairs are not observed. Hooks are rounded and are present at the junction of two long cells.

Costal zone: Silica bodies are dumb bell shaped; silica bodies and short cells are present in pairs with 1-4 layers. Short cells and prickles are present (Fig. 24).

13. Vetiveria zizanoides (Linn.)

Abaxial intercostal zone: Long cells are with moderately thick sinuous walls. Short cells are abundant between the long cells. Numbers of rows of long cells between two costal zones are 4-13. Numbers of stomatal rows between two costal zones are 2-4. Guard cells are dumb bell

shaped while subsidiary cells are triangular. Microhairs are bicelled and distal cells with rounded apices. Macrohairs and hooks are absent.

Costal zone: Silica bodies are cross shaped with 2-5 layers and sinuous cells are present between cross shaped silica bodies. Short cells are present. Prickles are not observed (Fig. 25).

Adaxial intercostal zone: Adaxial intercostal long cells are with sinuous walls. Numbers of rows of long cells between two costal zones are 8-16. Numbers of stomatal rows between two costal zones are 2-4. Microhairs, macrohairs and hooks are absent.

Costal zone: Silica bodies are cross shaped. Long cells are with sinuous walls present between silica bodies and short cells are with sinuous walls. Prickles are not observed (Fig. 26).

Discussion

All the species have paracytic stomata (guard cells accompanied by two subsidiary cells), with dumb bell shaped guard cells, except Heteropogon contortus and Cymbopogon jwarancusa in which guard cells are straight in the middle. Fahn. (1965) also reported that dumb bell shaped guards cells in gramineae are present however some variations may be observed in shape of subsidiary cells. Abid et al., (2007) observed the paracytic type of stomata in 3 species belonging to 3 different genera of andropogoneae. Bothriochloa and Dicanthium can be differentiated on the basis of difference in shape of subsidiary cells, as these genera cannot be distinguished morphologically. Subsidiary cells in Bothriochloa bladhii are dome shaped while these are triangular in Dicanthium annulatum and D. foveolatum (Fig.8 & Fig.9). Metcalfe, (1960) also observed that in genus Dicanthium mostly the stomata are triangular in shape. In genus Saccharum, mostly the subsidiary cells are low dome shaped or triangular and silica bodies are intermediate between cross and dumb bell shaped. According to Metcalfe, (1960) mostly the panicoid grasses have subsidiary cells with triangular shape.

Cross shaped silica bodies are present in Vetiveria zizanoides, Chrysopogon serrulatus & Euloliopsis binata. Chaudhary, (2001) also observed that mostly the silica bodies are cross shaped in Vetiveria zizanoides. Silica bodies are dumb bell to intermediate between cross and dumb bell shaped in D. foveolatum, Sorghum halepense and Genus Saccharum, while silica bodies are mostly cross shaped or intermediate between dumbbell and cross shaped in Cymbopogon jwarancusa. Same observations were recorded by Folorunso et al., (2007) who observed cross shaped silica bodies in two species of Cymbopogon, that is the characteristic of most of the species of this tribe. In Heteropogon contortus, Imperata cylindrica and Dicanthium annulatum, dumb bell shaped silica bodies were observed by Bibi et al., (2007) but in the present investigations dumb bell shaped silica bodies, somewhat lobed in the middle are found in *H. contortus* while dumb bell shaped in other two species. According to Metcalfe, (1960) mostly the dome shaped silica bodies are present in H. contortus, and middle portion of these bodies are long. So diversity in silica bodies shape is found in the species of this tribe as Clayton, (1981) noted a great complexity and diversity of silica bodies in Andropogoneae. A great variety in shape of silica bodies is a valuable character (Clifford & Watson, 1977). All the species have bicelled microhairs of panicoid type, except Eulaliopsis binata in which microhairs are absent. This is the only species with very long macrohairs, so the absence of microhairs and the presence of macrohairs are the characters which make this species distinct from other genera of the tribe. Rounded papillae at one side of long cell are present in only two species i.e. Cymbopogon jwarancusa and Heteropogon (Plate 26C & D), while found absent in other genera. So this character is also helpful in the identification of these species in the tribe.

Anatomical studies could be an important tool to resolve the taxonomic problems within the tribe (Ahmad *et al.*, 2011). The studies revealed that diversity and variations in leaf anatomical characters may be valuable and helpful in the identification at the specific and generic level of the tribe.

References

- Abid, R., S. Sharmeen and A. Perveen. 2007. Stomatal types of monocots within Flora of Karachi, Pakistan. Pak. J. Bot., 39(1): 15-21.
- Ahmad, F., M.A. Khan, M. Ahmad, M. Hameed, R.B. Tareen, M. Zafar and A. Jabeen. 2011.
- Barthlott, W., C. Neinhuis, D. Cutler, F. Ditsch, I. Meusel and H. Wilhelmi. 1998. Classification and terminology of epicuticular waxes. J. Linn. Soc. Lond .Bot., 126: 237-260.
- Bibi, F., M.A. Khan, M. Ahmad and M. Zafar. 2007. Leaf epidermal anatomy of some grasses. *Pak. J. Sci.*, 59(1-2): 47-51.
- Cai, L.B. and S.J. Wang. 1994. Studies on the evolutionary trends and mechanism of the constituent cells of the leaf epidermis in Poaceae. *Acta Biologia Plateau Sinica.*, 12: 13-27.
- Chaudhary, A.A., M. Hameed, R. Ahmad and A. Hussain. 2001. Phyto-sociological studies in Chhumbi Surala Wild life sanctuary, Chakwal, Pakistan. Species Diversity L. Int. J. Agric. Biol., 3(4): 369-374.

- Clark, J. 1960. Preparation of leaf epidermis for topographic study. *Stain Technol.*, 35-39.
- Clayton, W.D. 1981. Evolution and distribution of grasses. Ann. Missouri Bot. Gard., 68: 5-14.
- Clifford, H.T. and L. Watson. 1977. Identifying grasses data methods and illustrations. St. Luica, Queensland Australia. Univ Queensland Press.
- Cotton, R. 1974. Cytotaxonomy of the genus Vulpia. Ph.D Thesis, Univ. Manchester, USA.
- Davila, P. and L.G. Clark. 1990. Scanning electron microscopy survey of leaf epidermis of *Sorghastrum* (Poaceae) Andropogoneae. *Americ. J. Bot.*, 77: 499-511.
- Fahn, A. 1965. Plant anatomy, 3rd ed. Oxford: Pergamon Press, 115-119.
- Folorunso, A.E. and O.A. Oyetunji. 2007. Comparative foliar epidermal studies in *Cymbopogon citratus* (Stapf) and *Cymbopogon giganteus* (Hochst) Chiov. In Nigeria. Not. Bot. Hort. Agrobot., 35(2): 7-13.
- Gilani, S.S., M.A. Khan, Z.K. Shinwari and A. Nasim. 2003a. A new subspecies of *Digitaria sanguinalis* from Pakistan. *Pak. J. Bot.*, 35(3): 279-282
- Gilani, S.S., M.A. Khan, Z.K. Shinwari, F. Hussein and Z. Yousaf. 2003.Taxonomic relationship of the genus *Digitaria* in Pakistan. *Pak. J. Bot.*, 35(3): 261-278.
- Gillani, S.S., M.A. Khan, Z.K. Shinwari and Z. Yousaf. 2002. Leaf epidermal anatomy of selected *Digitaria* species, Tribe Paniceae, Family Poaceae of Pakistan. *Pak. J. Bot.*, 34: 257-273.
- Meija, T. and F.A. Bisbey. 2003. Silica bodies and hooked papillae in lemmas of *Melica* species (Gramineae: Pooideae). *Bot. J. Linn. Soc.*, 141: 447-463.
- Metcalfe, C.R. 1960. Anatomy of the monocotyledons. L. Gramineae. Clarendon Press, Oxford at the series 13. HMSo, 389.
- Metcalfe, C.R. and L. Chalk. 1950. Anatomy of dicotyledons, Vol. 1 & 2. Clarendon Press, Oxford.
- Stenglein, S.A., M.N. Colares, A.M. Arambarri, M.C. Novoa, C.E. Vizcaino and L. Katinas. 2003. Leaf epidermal microcharacters of the old world species of Lotus (Leguminoseae: Loteae) and their systematic significance. *Austr. J. Bot.*, 51: 459-469.
- Taxonomic application of foliar anatomy in grasses of tribe Eragrostideae (Poaceae) from Salt Range of Pakistan. *Pak. J. Bot.*, 43(5): 2277-2284.
- Webster, R.D. 1988. Genera of the North American Paniceae (Poaceae: Panicoideae). Syst. Bot., 13: 576-609.
- Yousaf, Z., Z.K. Shinwari, R.A. Qureshi and A. Perveen. 2008. Leaf epidermal anatomy of selected Allium species, family Alliaceae from Pakistan. Pak. J. Bot., 40(1): 77-90

(Received for publication 1 September 2012)