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TAXONOMIC APPLICATION OF FOLIAR ANATOMY IN GRASSES OF TRIBE ERAGROSTIDEAE (POACEAE) FROM SALT RANGE OF PAKISTAN

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Abstract

Foliar anatomical investigations of some members belonging to tribe Eragrostideae (Poaceae) were carried out. In total of 8 species belonging to 6 genera were collected in wild from Salt Range region of Pakistan for anatomical studies. Maximum length of long cells was observed in genus *Eragrostis.* Macrohairs are found absent in all species except *Eragrostis papposa. Acrachne racemosa* is identified by dumb bell shaped or cross shaped silica bodies while saddle shaped silica bodies are present in other species. *Desmostachya bipinnata* is distinct by having a tall girder of bulliform cells, from adaxial to abaxial side. Microhairs with hemispherical distal cell, saddle shaped silica bodies and bulliform cells deeply penetrating the mesophyll are the diagnostic characters, which justify all the species in the same tribe.

Introduction

Tribe Eragrostideae (Poaceae) is represented by about 50 genera in the world, found throughout the tropics. In Pakistan 16 genera and 33 species of this tribe are reported (Cope, 1982). This tribe includes the annual or perennial grasses. Among the perennial *Desmostachya bipinnata* is a robust coarse perennial grass, attaining a height upto one meter, the culms are with stout scaly rhizomes and form large swards. Other perennial grasses are *Octhochloa compressa* having culm whitish wooly at the base. *Eragrostis papposa* is an erect or ascending perennial grass and *Dactyloctinium scindicum* is a stoloniferous perennial grass forming extensive spreading mats and has rooting at the nodes. The annual grasses are *Eleusine indica*, *Dactyloctinium aegyptium*, *Acrachne racemosa* and *Eragrostis cilianensis*.

The leaf epidermal anatomy provides extensive taxonomic data related to grasses. Epidermal traits i.e. epidermal cells, stomata and hairs have proved to be an important tool in delimitation of taxa in many plant families (Uphof *et al*., 1962; Sinclair & Sharma, 1971; Lackey, 1978; Ditsh *et al.*, 1995; Barthlott *et al*., 1998; Stenglein *et al.,* 2003; Naz *et al*., 2009; Hameed *et al.*, 2010; Riaz *et al.*, 2010). It is confirmed that leaf epidermal features can help to elucidate taxonomic relationships at different levels (Prat, 1936; Stebbins, 1956; Metcalfe, 1960, Ellis, 1979, Palmer & Tucker, 1981, Palmer *et al.,* 1985, 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.

Occurrence of sclerenchyma and bundle sheath (Kranz Sheath), the width of sclerenchyma, the indumentum of leaves and length and frequency of epidermal basis are important features that can identify relationship among the genera of Poaceae (Dube & Morisset, 1987; Jarves & Barkworth, 1992; Yousaf *et al*., 2008). 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, 1986). The position of vascular bundles in the blades appears to be a useful diagnostic character above the generic level (Ellis, 1976). Grasses in tribe Eragrostideae look morphologically similar and there is confusion in the identification, differentiation and delimitation of species, genera and the tribe based on morphological description (Hameed *et al.*, 2008). Anatomical studies could be an important tool to resolve the taxonomic problems within the tribe, so the purpose of the present study is to identify and explore the foliar anatomical diversity in the tribe Eragrostideae that may prove helpful in the identification and differentiation at specific and generic level.

Materials and Methods

Plant material was collected from resource based areas of Salt Range, Pakistan during field visits. Plants were identified with the help of the Flora of Pakistan and preserved at Herbarium, Quaid-i-Azam University, Islamabad (ISL).

Leaf epidermal anatomy: 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 method of Cotton (1974) with modified technique of Clark (1960). 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. Abaxial and adaxial epidermis was removed, alongwith 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 digital camera (Meiji CCD Model 00179048, Canada) fitted on Lieca Light Microscope (DM 1000, Germany) for microphotography. The following anatomical characters of both abaxial and adaxial epidermis were studied;

- Length and width of long cells, their shape and types
- Short cells and papillae in intercostal zone

- Number of rows of long cells between two costal zones
- Length and width of stomatal complex
- Shape of guard and subsidiary cells
- Shape and type of microhairs
- Length, width and shape of macrohairs, hooks and silica bodies.
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Transverse sections (T.S.) of leaves: 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. 2-3 drops of water were poured on the block and placed the leaf section vertically and covered with water droplets. When temperature reached at -14^{o}C, water was frozen and 10-15 µm thick slices of leaves were cut by moving the microtome in forward and backwards direction and the best sections were selected for preparing slides. Both stained and unstained slides were prepared. For staining thin leaf sections were stained by following procedure. Leaf sections were placed on the slide and 1-2 drops of safranin were added, and then added a few drops of 96% ethanol to remove the extra safranin. After it poured, 1-2 drops of fast green, then added 96% and absolute alcohol respectively. Finally, one drop of xylene was added. Slides were observed under microscope and microphotographs were taken by Camera mounted on microscope (Olympus Ax 70).

For microtomy method of Johnson, (1940) with some modifications was followed. Following characters were studied in the T.S. of leaf.

- Nature of adaxial and abaxial surface
- Sclerenchyma girders and strands adaxially or abaxially
- Number, type and arrangement of vascular bundles
- Arrangement of chlorenchyma cells
- Nature of keel
- Shape and distribution of bulliform cells

Results

1. *Acrachne racemosa*

Abaxial intercostal zone: Abaxial intercostal long cells with thin sinuous walls, 50-82.5µm long and 10-15µm wide. 4-8 rows of long cells between two costal zones, 2-4 stomatal rows between two costal zones. Stomatal complex: guard cells dumbbell shaped, subsidiary cells very low dome shaped. Micro and Macro hair absent. Hooks absent.

Costal zone: Silica bodies dumbbell shaped or cross shaped, 15-12.5µm long and 7.5-10µm wide, 1-6 layers of silica bodies. Short cells 20-25µm long and 5-10µm wide. Rounded cells present between silica bodies and short cells, these may be cork cells 10-17.5µm long and 7.5-15µm wide. Prickles absent (Fig. 1A).

Adaxial intercostal zone: Adaxial intercostal long cell with sinuous walls, 40-52.5µm long and 15-27.5µm wide. 3-6 rows of long cells, between two costal zones, 2-3 stomatal rows between two costal zones. Stomatal complex: 15-20µm long and 10-12.5µm wide. Microhairs none seen. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies dumbbell shaped or cross shaped, 2-5 rows of silica bodies. Short cells 20-27.5µm long and 5-7.5µm wide, cork cells or rounded cells 10-12.5µm long and 7.5-12.5µm wide. Prickles 45-55µm long and 15-17.5µm wide (Fig. 1B).

T. S. of lamina: Adaxial and abaxial surface smooth and with no ridges. Mostly vascular bundles small, large vascular bundles of basic type. Small vascular bundles with thin sclerenchyma strands, adaxially and abaxially. Large vascular bundles with prominent abaxial and adaxial sclerenchyma strands. Keel conspicuous, narrow and rounded, having a solitary vascular bundle in the middle, accompanied by two small vascular bundles on the sides of basic type vascular bundles. Chlorenchyma cells radially arranged around vascular bundles. Bulliform and associated colorless cells in fan shaped groups penetrating into the mesophyll. The middle cell larger than the lateral cells. In the mid rib region large number of colorless cells angular in outline, extending from bundle sheath to the adaxial side. Small vascular bundles with a single incomplete sheath, interrupted by large colourless cells, abaxially and adaxially. Large vascular bundles with a double sheath, the inner sheath complete and outer sheath interrupted abaxially (Fig. 2A&B).

2. *Dactyloctenium aegyptium*

Abaxial intercostal zone: Abaxial intercostal long cells with slightly sinuous walls, 62.5-120µm long and 8.75-12µm wide, rounded papillae abundent on the long cells. 4-8 rows of long cells between two costal zones, 2-3 stomatal rows between two costal zones. Stomatal complex: 19-22 µm long and 11-16µm wide, guard cells dumbbell shaped, subsidiary cells low dome shaped. Microhairs bicelled, both cells almost equal or distal cell longer than the basal cell, both cells short and broad, sometimes distal cell broader than basal cell, 22.5-27.5µm long and 11.25-13.75µm wide. Macrohairs absent, Hooks absent (Fig. 1C).

Costal zone: Silica bodies saddle shaped, 10-12.5µm long and 7.5-8.75µm wide. Short cells with slightly sinuous walls, 17.5-25µm long and 6-7.5µm wide. Prickles absent.

Adaxial intercostal zone: Adaxial intercostal long cells with slight sinuous walls, 82.5-105µm long and 11.25-12.5µm wide. 8-10 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones. Stomatal complex: 20-21.25µm long and 11.25-15µm wide, guard cells 2.5-3.75µm wide and subsidiary cells 5-6.25µm wide, guard cells dumbbell shaped and subsidiary cells low dome shaped. Microhairs bicelled, both cell almost equal, 17.5-20µm long and 11.25-13.75µm wide, almost spherical in shape. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 10-12.5µm long horizontally and vertical diameter is 11.25-12.5µm. Short cells, 8.75-10µm wide and 21.25-22.5 µm long. Prickles absent (Fig. 1D).

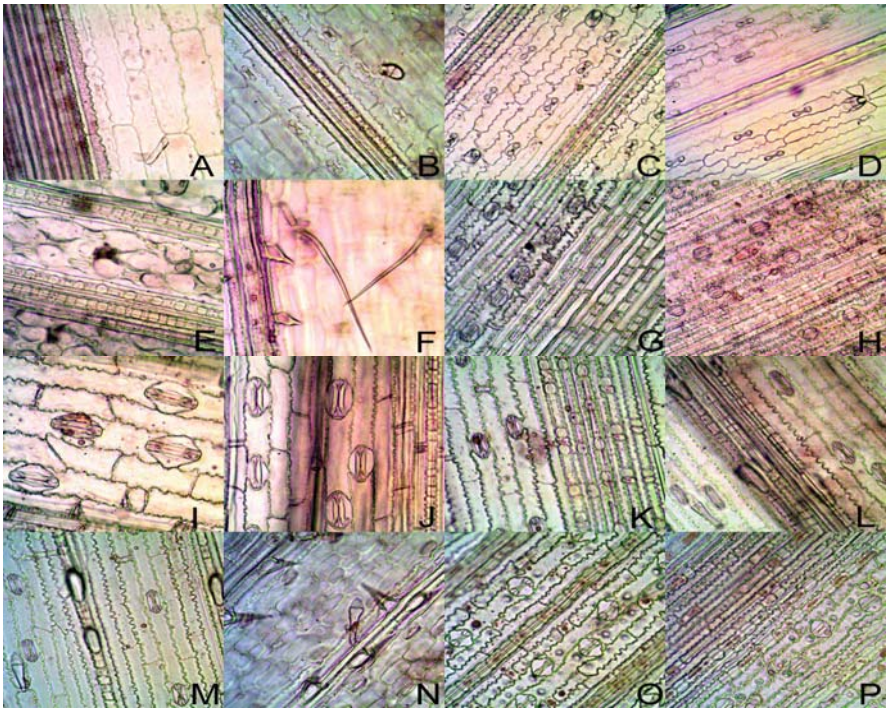

Fig. 1. A. *Acrachne racemosa*,. Leaf abaxial surface, B. Leaf adaxial surface, C. *Dactyloctinium aegyptium*, Leaf abaxial surface, D. Leaf adaxial surface, E. *Dactyloctinium scindicum*, Leaf abaxial surface, F. Leaf adaxial surface, G. *Desmostachya bipinnata*, Leaf abaxial surface, H. Leaf adaxial surface, I. *Eleusine indica*, Leaf abaxial surface, J. Leaf adaxial surface, K.*Eragrostis cilianensis*, Leaf abaxial surface,L. Leaf adaxial surface, M. *Eragrostis papposa*, Leaf abaxial surface, N. Leaf adaxial surface,O. *Octhochloa compressa*, Leaf abaxial surface, P. Leaf adaxial surface.

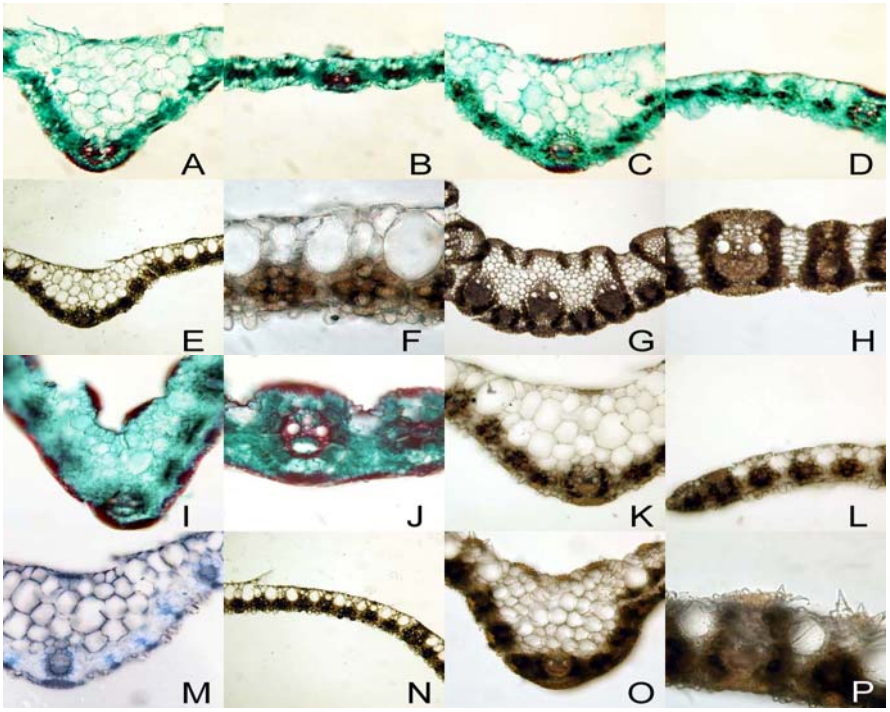

Fig. 2. A. *Acrachne racemosa*,T.S. of leaf showing mid rib, B. T.S. of leaf near margins C. *Dactyloctinium aegyptium*, T.S. of leaf showing mid rib, D. T.S. of leaf near margins E. *Dactyloctinium scindicum*, T.S. of leaf showing mid rib, F. T.S. of leaf near margins G. *Desmostachya bipinnata*, T.S. of leaf showing mid rib, H. T.S. of leaf near margins I. *Eleusine indica*, T.S. of leaf showing mid rib, J. T.S. of leaf near margins K. *Eragrostis cilianensis*, T.S. of leaf showing mid rib, L. T.S. of leaf near margins , M. *Eragrostis papposa*, T.S. of leaf showing mid rib, N. T.S. of leaf near margins O, *Octhochloa compressa*, T.S. of leaf showing mid rib, P. T.S. of leaf near margins.

T. S. of lamina: Adaxial surface flat, glandular structures present on the abaxial side, protruding from the abaxial epidermis. Three to four small vascular bundles present between two large vascular bundles. Phloem of the vascular bundles less or more sclerised.Vascular bundles with sclerenchyma girder on the abaxial side. Mostly vascular bundles with adaxial sclerenchyma strands. Keel rounded, conspicuous, having a large vascular bundle of basic type in the middle, accompanied by 3 small vascular bundles on each side. Chlorenchyma cells radially arranged around the vascular bundles. Bulliform and associated colourless cells in fan shaped groups, penetrating deeply into the mesophyll. Bulliform cells not present at the margins. In the mid rib region, large number of colourless cells, slightly angular in outline, extending from bundle sheath to adaxial surface. Large vascular bundles with double sheath, the outer sheath winged, sheaths complete or interrupted on the adaxial side due to colorless cells. In small vascular bundles it is not clear, that there is single or double sheath, however the outer sheath is winged. The inner sheath, if present not prominent (Fig. 2C&D).

3. *Dactyloctinium scindicum*

Abaxial intercostal zone: Abaxial intercostal long cells with slightly sinuous walls, 58.5-115µm long and 7.5-12.7µm wide. 5-7 rows of long cells between two costal zones, 2-4 stomatal rows between two costal zones. Stomatal complex, 18-22.5µm long and 10.5-15.75µm wide, guard cells dumb bell shaped, subsidiary cells triangular or high dome shaped. Microhairs, macrohairs and hooks absent.

Costal zone: Silica bodies saddle shaped, 11.5-12.5µm long and 8.75-10µm wide, 2-3 layers of silica bodies in costal zone. Short cells with sinuous walls 20-25µm long and 6-8.75µm wide (Fig. 1E).

Adaxial intercostal zone: Adaxial intercostal long cells with non sinuous or slightly sinuous walls near the costal zone, 40-90.5µm long and 8-11.5µm wide. 4-8 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones. Stomatal complex: 17.5-24µm long and 9-13.5µm wide, Microhairs, macrohairs absent, hooks absent.

Costal zone: Silica bodies saddle shaped or cross shaped in a single or two rows, 9-12µm long and 7-9.75µm wide. Short cells with sinuous walls, 19.5-22.5µm long and 7-10.5µm wide prickles absent (Fig. 1F).

T. S. of lamina: Adaxial surface with slight ribs and furrows, abaxial surface flat. Mostly vascular bundles with adaxial sclerenchyma strands.Chlorenchyma cells radially arranged around the vascular bundles. Keel conspicuous and rounded with single median vascular bundle, having 2-3 small vascular bundles on each side. Bulliform and associated colorless cells in fan shaped groups. Large vascular bundles, with double sheath, complete or interrupted on the adaxial side, due to colorless cells. In small vascular bundles, the inner sheath not prominent (Fig. 2E&F).

4. *Desmostachya bipinnata*

Abaxial intercostal zone: Abaxial intercostal long cells with sinuous walls, 40-70µm long and 10-12.5µm wide, short cells and long cells in a row, short cells 15-16.25µm long and 10-12.5µm wide. 6-9 rows of long cells between two costal zones, 1-3 stomatal rows between two costal zones. Stomatal complex, 22-25µm long and 15-17.5µm wide, guard cells dumb bell shaped and subsidiary cells low dome shaped or triangular in shape, guard cells, 4-5µm wide and subsidiary cells, 5-6.25µm wide. Microhairs bicelled, distal cell shorter than basal cell, 37.5-40µm long and 7.5-8.75µm wide. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 10-12µm wide, horizontally, and vertical diameter 10-15µm, 2-5 layers of silica bodies. Short cells with sinuous walls, 12.5-22.5µm long and 8.75-11.25µm wide. Prickles 105-125µm long and 22-25µm wide (Fig. 1G).

Adaxial intercostal zone: Adaxial intercostal long cells with sinuous walls, 37.5-55µm long and 10-11.25µm wide. 8-10 rows of long cells between two costal zones, 1-3 stomatal rows between two costal zones. Microhairs absent. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 10-11.25µm wide, horizontally and vertical diameter is 12.5-13.75µm. Short cells and long cells with slightly sinuous walls, 17.5-25µm long and 10-11.25µm wide. Prickles 30-43.75µm long and 10-11.25µm wide (Fig. 1H).

T. S. of lamina: Adaxial surface with wide ribs separated by short furrows. Prickles present on the adaxial side between two ribs. Abaxial surface not flattened but ribs are not prominent as on adaxial side.Large vascular bundles of basic type, vascular bundle and phloem highly sclerised.Vascular bundles with adaxial and abaxial girders or with thick sclerenchyma strands on adaxial side and abaxial girders, especially in keel vascular bundles, sclerenchyma girders abundant on the abaxial side and also between the vascular bundles. Keel conspicuous and widely rounded, with 6-7 vascular bundles of different size. Chlorenchyma cells angular in outline.A tall girder of bulliform and associated colourless cells extending from the adaxial side to the abaxial side, between two vascular bundles. Bundle sheath double, outer sheath interrupted abaxially in large vascular bundles. Small vascular bundles complete (Fig. 2G&H).

5. *Eleusine indica*

Abaxial intercostal zone: Abaxial intercostal long cells with thin to moderately thick sinuous walls, 75-107.5µm long and 12.5-15µm wide. 3-7 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones. Stomatal complex: 25-27.5µm long and 21.5-27.5µm wide, guard cells dumb bell shaped, subsidiary cells triangular in shape. Guard cells 5-7.5µm wide and subsidiary cells 7.5-10µm wide. Microhairs absent. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 7.5-8.75 µm wide, horizontally and 10-11.25 µm wide vertically, 2-7 layers of silica bodies. Long cell over the veins with straight walls, 62.5 – 87.5 µm long and 7.5 – 8.75 µm wide. Prickles absent (Fig. 1I).

Adaxial intercostal zone: Adaxial intercostal long cells with slightly sinuous walls, 61.25-137.5µm long and 8.75-20 µm wide. 3-5 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones. Stomatal complex: guard cells dumb bell shaped and subsidiary cells triangular, 25-26.25µm long and 15-17.5µm wide, guard cells 6-6.5µm wide and subsidiary cells 6.5-7.5µm wide. Microhairs absent. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies **s**addle shaped, 7-8.75µm horizontally and 7-8.75µm wide**.** Long cells with sinuous walls along with silica bodies, 35-87.5µm long and 6.25-10µm wide. Prickles absent (Fig. 1J).

T. S. of lamina: Adaxial surface with wide rounded ribs, separated by shallow V shaped furrows. 2-3 small vascular bundles between two large vascular bundles of basic types. Large vascular bundles angular in outline. Vascular bundles having sclerenchyma strands, on abaxial and adaxial surface. Large vascular bundles having large sclerenchyma adaxial and abaxial strands. The leaf margins also sclerised, sometimes abaxial girders present with bundles sheath. Keel conspicuous and V shaped, containing a solitary vascular bundle. Chlorenchyma cells are radially arranged around the vascular bundles. Bulliform and associated colorless cells are in narrow groups penetrating in the mesophyll, between two consecutive vascular bundles. In large vascular bundles outer sheath prominent, with large cells, complete, inner sheath not prominent (Fig. 2I&J).

6. *Eragrostis cilianensis*

Abaxial intercostal zone: Abaxial intercostal long cells with thin sinuous walls, 100-150µm long and 15-20µm wide. 5-7 rows of long cells between two costal zones, 2-4 stomatal rows between two costal zones. Stomatal complex: 25-30µm long and 15-17.5µm wide. Mircohairs bicellular, basal cells longer than the distal cells, distal cells rounded at the apices, 50-60µm long and 6.25-10µm wide. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 10-15µm long and 7.5-10µm wide, 1-5 layers of silica bodies.Short cells with sinuous walls, 30-50µm long and 5-7.5µm wide. Rounded cells present between silica bodies and short cells, 10-12.5µm long and 8-10µm wide. Prickles 30-35µm long and 10-12.5 wide (Fig. 1K).

Adaxial intercostal zone: Adaxial intercostal long cells with sinuous walls, 90-160µm long and 10-15µm wide. 5-6 rows of long cells between two costal zones, 2-3 stomatal rows between two costal zones. Stomatal complex: 25-32.5µm long and 15-17.5µm wide, guard cells dumb bell shaped, subsidiary cells low dome shaped. Mircohairs 50-60µm long and 7.5-10µm wide, distal cell hemisphenical or rounded at the apices. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies, saddle shaped, 10-12.5µm long and 7.5-10µm wide. Number of rows of silica bodies, 1-6. Short cells, 17.5-47.5µm long and 5-7.5µm wide, cork cells rounded 10-15µm long and 7.5-10µm wide. Prickels 32.5-40 µm long and 7.5-12.5µm wide (Fig. 1L).

T. S. of lamina: Adaxial surface with ribs and furrows. The regions of the lamina, having vascular bundles are wider and narrower, and regions between two vascular bundles making wide grooves. Macrohairs or prickles are prominent on adaxial side. Large vascular bundles with adaxial and abaxial sclerenchyma girders. Small vascular bundles with adaxial girders or strands. Keel not conspicuous. Chlorenchyma cells not radially arranged around vascular bundles. Bulliform cells in irregular groups. Large vascular bundles with double sheath, the outer sheath and inner sheath interrupted adaxially and abaxially (Fig. 2K&L).

7. *Eragrostis papposa*

Abaxial intercostal zone: Abaxial intercostal long cells with thin sinuous walls, 90-165µm long and 12.5-17.5µm wide. 5-9 rows of long cells between two costal zones, 1-3 stomatal rows between two costal zones. Stomatal complex: 22.5-30µm long and 18-20µm wide, guard cells dumb bell shaped and subsidiary cells low to high dome shaped. Mircohairs bicellular, 50-55µm long and 8-10µm wide, basal cells longer than the distal cells, distal cell with a rounded apex, hemispherical or dome shaped. Macrohairs, 160-175µm long, tapering towards the tip, broad at the base. Hooks absent.

Costal zone: Silica bodies saddle shaped, 15-17.5µm long and 10.5-12.5µm wide. Short cells with sinuous walls, 20-27.5µm long and 5-7.5µm wide. Rounded cells (cork cells), present between short cells and silica bodies, 10-12.5µm long and 6-7.5 µm wide. Prickles pointed at the tip, 32.5-40µm long and 10-15µm wide (Fig. 1M).

Adaxial intercostal zone: Adaxial intercostal long cells with sinuous or non sinuous walls, 60-90µm long and 15-17.5µm wide. 4-9 rows of long cells, between two costal zones, 1-3 stomatal rows between two costal zones. Stomatal complex**:** guard cells dumbbell shaped, subsidiary cells low dome shaped, 20-25µm long and 15-17.5µm wide. Mircohairs bicelled, basal cell longer than distal cell, distal cell rounded at the tip or hemispherical. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 4-5 rows of silica bodies, 15-10µm long and 09-10µm wide. Short cell with sinuous walls, 15-27.5µm long and 6-7.5µm wide, rounded bodies (cork cells) present between short cells and silica bodies, 10-12.5µm long and 7.5-10µm wide. Prickels present at the margins of costal zone, 40-65µm long and 7.5-10µm wide (Fig. 1N).

T. S. of lamina: Adaxial and abaxial surface with slight ribs and furrows. The diameter of the lamina not uniform i.e. the regions of lamina having vascular bundles are

wider. Prickles present adaxially but not frequent, small vascular bundles with no sclerenchyma girders or strands or with scherenchyma strands only. Large vascular bundles with adaxial and abaxial sclenechyma girders and strands. Keel not conspicuous. Chlorenchyma cells radially arranged around the vascular bundles. Bulliform cells in irregular groups and middle cell of the group deeply penetrating into the mesophyll. Large vascular bundles with double and incomplete sheath, the inner sheath not prominent in small vascular bundles (Fig. 2M&N).

8. *Octhochloa compressa*

Abaxial intercostal zone: Abaxial intercostal long cells with thin sinuous walls, 50-112.5µm long and 11.25-15µm wide and short cells, 12.5-15µm long and 11.25-12.5µm wide. 3-5 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones. Stomatal complex: guard cells dumb bell shaped and subsidiary cells triangular in shape, 22.5-27.5µm long and 23.75-27.5µm wide. Mircohairs absent. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 1-8 rows of silica bodies, 10-11.25µm wide horizontally and vertical diameter is 11.25-17.5µm. Short cells with sinuous walls, 25-35µm long and 10-11.25µm wide. Prickles 47.5-52.5µm long and 12.5-18.75µm wide (Fig. 1O).

Adaxial intercostal zone: Number of rows of long cells between two costal zones, 5-7. Number of stomatal rows between two costal zones, 1-2. Stomatal complex: 23-25µm long and 22-23.75µm wide, guard cells, 4-5µm wide and subsidiary cells, 8-10µm wide. Mircohairs absent. Macrohairs absent. Hooks absent.

Costal zone: Silica bodies saddle shaped, 10-13.75µm wide horizontally and 15.25-17.5µm wide vertically. Short cell with sinuous walls, 22.5-27.5µm long and 10-12.5µm wide. Prickles 50-57.5µm long and 16.25-25µm wide (Fig. 1P).

T. S. of Lamina: Adaxial surface is almost smooth, not ridged, pointed prickles or spines with pointed tips are present. Abaxial surface with glandular structures. Five small vascular bundles, present on each side of large median vascular bundle of the keel. Four to five small vascular bundles between two large vascular bundles.Large vascular bundles with abaxial and adaxial sclerenchyma girders.The large vascular bundles in the keel region having abaxial girders only. Mostly the small vascular bundles with adaxial and abaxial strands only. Keel prominent and rounded, having a median large vascular bundle. Chlorenchyma cells not clear in the material. Bulliform and associated colourless cells in fan shaped groups penetrating deeply into the mesophyll. In the mid rib region a large number of colourless cells extending from the bundle sheath to the adaxial epidermis. Colourless cells angular in outline. Outer sheath of the large vascular bundle in the keel region slightly interrupted from the adaxial side. The inner sheath not prominent (Fig. 2O&P).

Discussion

Different genera in the tribe Eragrostideae e.g., *Acrachne*, *Eleusine* and *Dactyloctinium*, look morphologically similar but anatomical studies are helpful in their differentiation and identification, when correlated with their morphological characters. Intercostal long cells in all the species of different genera in the tribe are with thin sinuous or moderately thick sinuous walls. Long cells in genus *Eragrostis* have maximum length ranging from 90-165µm (Fig. 1K&M). *Dactyloctinium aegyptium* is different from all others species by having abundant rounded papilla, on the long cells of abaxial side (Fig. 1C). Metcalfe, (1960) also observed that long cells are obscured by papillae in this species. All species have dumb bell shaped guard cells as it is the characteristic of grasses, while subsidiary cells are very low dome shaped, low dome shaped, high dome shaped or triangular in shape as reported by Prat & Vignal, (1968) and Sanchez, (1971). Microhairs of grasses are characteristic bicellular trichomes, commonly found on the leaves but also occurring elsewhere in the plant (Scholz, 1979; Terrel & Wergen, 1981). They are lacking in the subfamily Pooideae but almost universally present in other subfamilies and their presence or absence is widely used as a taxonomic character (Watson *et al*., 1986; Watson & Dalwitz, 1988). The genus *Eragrostis* is recognized by bicellular microhairs with hemispherical distal cell (Fig. 1 L,M&N). In *Dactyloctinium aegyptium* microhairs are with distal cell and basal cell equal in length or distal cell longer than basal cell while in *D. bipinnata*, distal cell is shorter than basal cell having microhairs, 37.5- 40 µm long. Friere *et al*., (2005) studied two species of Eragrostideae viz. *Eragrostis cilianensis* and *Eleusine indica* and found that in *E. cilianensis,* microhairs are with hemispherical cell that are similar to our findings, but he reported that *Eleusine indica* is characterized by pear like microhairs but in our observations, *E. indica* is found to have no microhairs (Fig. 1I& J). As silica bodies are saddle shaped in all the species except *Acrachne racemosa* in which dumb bell shaped or cross shaped silica bodies are found. *Eragrostis papposa* and *Dactyloctinium scindicum* is distinct from other species by the presence of macrohairs on the adaxial surface, tapering towards the tip having length 160-175µm, (Fig. 1F&N) while macrohairs are absent in other species. *Eragrostis cilianensis* and *Eragrostis papposa* are peculiar in having microhairs with hemispherical distal cells and rounded cork cells are present between silica bodies and short cells over the veins (Fig. 1L,M&N). Bibi *et al*., (2007) reported that silica bodies and marcohairs are absent in *Eleusine indica* but *Eleusine indica* is observed to have saddle shaped silica bodies. According to Prat, (1936) types of mircohairs and silica bodies are very useful in systematic studies as found in this tribe to have saddle shaped silica bodies and microhairs with hemispherical distal cell. Leaf anatomy in this tribe is helpful at specific and generic level. *Dactyloctinium aegyptuim* and *D. scindicum* are characterized by the presence of glandular structures on its abaxial side and protruding abaxially (Fig. 2D&F). Metcalfe, (1960) studied this species but did not mention this character. Adaxial surface is flat in *Dactyloctinium aegyptium* while

in *Dactyloctinium scindicum* adaxial surface is with slight ribs and furrows. Sabnis, (1921) described the leaf structure of *D. scindicum* and found its surface not grooved and sclerenchyma forming adaxial and abaxial girders with the long vascular bundles, while adaxial strands and slight abaxial girders were present opposite to the small vascular bundles. It is observed in the present studies that abaxial surface is not grooved in *D. scindicum* (Fig. 2E&F), it appears that this character varies in the same species with different environmental conditions and habitat, while other observation as the presence of adaxial and abaxial girders opposite to large vascular bundles and slight girders or strands are present opposite to small vascular bundles are similar with the observation of Sabnis, (1921).

When two species of *Eragrostis* were compared on the basis of T.S. studies, they showed a clear difference as in *E. ciliansis,* macrohairs or prickles were prominent on the adaxial side while in *E. papposa*, macrohairs or prickles were not observed or rarely present (Fig. 2N). The previous studies show that macrohairs or prickles are found in different species of *Eragrostis* as described by Gunzel, (1912) and Breakwell, (1915) but found absent in different species studied by Metcalfe, (1960). Nicora, (1941) has reported the occurrence of multicellular glands (extra floral nectaries) on the leaves and floral parts of certain species of *Eragrostis*. In the present investigations glandular structures are also seen in both species of this genus (Fig. 2O). Glandular structures are also recorded in genus *Dactyloctinium* and *Octhochloa compressa*. Sclerenchyma can present several patterns of distribution occurring in the form of sub epidermal layers, sheath extensions or in the leaf margins (Ellis, 1976). In the present studies large vascular bundles, mostly have adaxial and abaxial sclerenchyma girders, while sclerenchyma strands are observed opposite to the small vascular bundles. In some species such as *D. aegyptium* phloem of vascular bundles is less or more sclerised (Fig. 2C) and in *E.indica* leaf margins are sclerised and in *D. bipinnata*, basic type vascular bundles and phloem is highly sclerised. Adaxial paranechyma cells are observed in *Octhochloa compressa*, *Acrachne racemosa* and *Dactyloctinium aegyptium*.

According to Ellis, (1986) adaxial parenchyma in keel region is rarely found in Chloridoideae, and never been found in Pooideae. In most species keel is round and conspicuous but *Eleusine indica* differs by having V.shaped keel (Fig. 2I). Chlorenchyma cells are radially arranged around the vascular bundles and bulliform cells are in fan shaped or irregular groups deeply penetrating into the mesophyll, sometimes a tall girder of bulliform cells extending from adaxial to abaxial side as in *Desmostachya bipinnata* is observed (Fig. 2G&H) and it is the typical character of chloridoid leaf as described by Gould, (1968).

The studies showed that different species exhibit variations in different anatomical characters which are valuable in their identification and differentiation, while there are some characters which are similar in all species of the tribe, e.g., saddle shaped silica bodies, microhairs with hemispherical distal cell and bulliform cells deeply penetrating the mesophyll are the characteristic of this tribe, which justify to place all these species in the same tribe.

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