Leaf Epidermal Anatomy of Medicinal Grasses of Islamabad, Attock and Mirpur (Azad Kashmir)

M. Iftikhar Chaudhary, Abdul Samad Mumtaz and Mir Ajaz Khan
Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan

Abstract: Abaxial and adaxial leaf epidermal anatomy of four related species having morphological resemblances have been studied. These taxa showed variation in short and long cells, silica bodies, macro and micro hair, presence or absence of stomata and shape of subsidiary cells. Most of these characters are diagnostic and have been used for making keys. Hence, on the basis of leaf epidermal anatomy we can distinguish among Cymbopogon citrates (DC.) Stapf, Cyanodon dactylon (L.) Parf, Panicum summatrense Roth ex Roem & Schult and Vetiveria zizanoides (L.) Nash.

Key words: Leaf epidermal anatomy, Cymbopogon citrates, Cyanodon dactylon, Panicum Summatrense, Vetiveria zizanoides, medicinal grasses, Poaceae.

Introduction
Leaf epidermal anatomy shows variation in Poaceae. Prat (1948, 1951) stated that the epidermal cells of Poaceae have a higher degree of specialization than in any other family. Therefore, such variations are extensively used in identifying and classifying the grasses. The leaf epidermis anatomy provides extensive taxonomic data on this subject (Metcalfe, 1960; Ellis, 1976). The purpose of present study is to discover leaf epidermal features helpful in identifying the plant material brought to a taxonomist lacking complete morphological identity. Such studies are of particular value for scientists, who need to identify small scraps of plant material e.g. Pharmacognostics (in drugs), Forensic experts (as clues) and (in gut and faeces) for Animal-Dieticians (Stace, 1980).

The present work has deliberately been based on four species due to limited collection period i.e. post monsoon when only Cymbopogon citrates, Cyanodon dactylon, Panicum summatrense and Vetiveria zizanoides could be found in vicinities of Islamabad, Attock and Mirpur (A.K.). Medicinal importance of these species is evident e.g. Hussain et al. (1984), has reported the nutritive value of Cyanodon dactylon seeds and their role on pregnancy of rat and litter size in bandicoot rats (Bandicota bengalensis). Essential oil of Cymbopogon citrates has been reported antibacterial by Onwumere et al. (1984). Similarly, Vetiveria zizanoides has been included in "Medicinal plants of Uttar Pradesh" by Siddique et al. (1994).

Materials and Methods
Leaves of fresh specimens were collected from the study area and those of dried from herbarium specimens, during Autumn 1998, at Plant Anatomy Laboratory, Department of Biological Sciences, Quaid-I-Azam University. Voucher specimens are submitted in University herbarium (ISL), Islamabad.

Before handling the leaves, they were softened and their margins were straightened by placing them in boiling water for 20-45 minutes, while fresh leaves being soft and straight, need not such treatment. Further following Clarke (1960) the softened leaves were subjected to 88% lactic acid, while in a test tube, then kept again in boiling water for 50-60 minutes. When the abaxial epidermis was to be prepared, the leaf was placed on a tile adaxial surface uppermost and flooded with cold lactic acid. Using a sharp scalpel blade, the adaxial epidermis was cut across the leaf and scraped away together with mesophyll cells, until just the abaxial epidermis of the leaf remained on the tile. The epidermis was placed on a clean slide and mounted in cold 88% lactic acid, placing a cover slip, examined under microscope. When a preparation of the adaxial epidermis was to be made, the leaf was placed with abaxial side uppermost and flooded with cold 88% lactic acid. It was then scraped. Preparations of adaxial and abaxial epidermises were photographed using a 35mm camera mounted on the microscope. The measurements of the individual structures were taken with the help of ocular micrometer calibrated with stage micrometer. The values can be tabulated as follows:

At 100x 1 Ocular small division = 10,0μm
At 40x 1 Ocular small division = 5μm
At 20x 1 Ocular small division = 2.5μm

Results
The carefully observed slide information rendered results that have been compiled in a schematic manner for each of the species as follows. Later a key is formulated which helps in identifying the species, followed by two tabulated data sheets providing a complete picture at a glance. Photographs of a part of adaxial and abaxial slides are also given in Plates 1-4.

Cymbopogon citrates (DC.) Stapf.
Abaxial side: (Plate 1-a)
Short-cell (Sc): Over the veins, paired/solitary in short or long rows. Cells between veins mostly solitary or paired, infrequent between veins.
Silica-body (SB): Cross-shaped or intermediate to cross and dumb-ball shaped with shallow indentations.
Macro-hair (Mah): Absent
Micro-hair (Mh): Length 44-54μ (mostly 44-48μ): basal cells 28-38μ (mostly 28-34μ); distal cells 15-24μ (mostly 15-18μ); basal cell somewhat inflated and much wider than distal cell, later tapering to a pointed apex.
Prickles (Ph): Prickles rather elongated with swollen bases, numerous near the veins.
Papilla (P): Most long-cells are provided with thin walled papillae. Papillae two cells, each with a spherical, probably glandular head and short-stalked cell.
Stoma (S): Confined to abaxial surface, abundant; usually dome-shaped, occasionally with triangular subsidiary cells.
Long cell (Lc): Mostly fairly short and approximately rectangular, with thin, slightly sinuous wall, inter-stomatal cells may be short with concave ends.
Table 1. Comparative quantitative characters of the four species

<table>
<thead>
<tr>
<th>Species</th>
<th>View</th>
<th>Short cells</th>
<th>Silica Bodies</th>
<th>Malpighi Hairs</th>
<th>Micro Hairs</th>
<th>Stomata</th>
<th>Long Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbopogon citratus</td>
<td>Abaxial</td>
<td>Both over veins and between veins</td>
<td>Cross shaped, shallow indentations</td>
<td>Absent</td>
<td>Tapering apex</td>
<td>Absent</td>
<td>Dumb-bell shaped, abundant</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Over the veins</td>
<td>Cross or dumbbell shaped</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Dumb-bell shaped in 3 rows</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Felted short, with sinuous walls</td>
</tr>
<tr>
<td>Cynodon decaryon</td>
<td>Abaxial</td>
<td>Both over veins and between veins, abundant</td>
<td>Saddle shaped</td>
<td>Absent</td>
<td>Rounded apex</td>
<td>Rare or absent</td>
<td>Subsidiary cells, triangular</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Both over veins and between veins</td>
<td>Saddle shaped, rarely dumbbell shaped</td>
<td>Absent</td>
<td>Absent</td>
<td>Dumb-bell shaped, thin walled</td>
<td>Intrasporal cells with sinuous walls</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Concave and between veins, thin walled</td>
</tr>
<tr>
<td>Panzeria sericea</td>
<td>Abaxial</td>
<td>Both over veins and between veins</td>
<td>Elongated, irregularly shaped</td>
<td>Absent</td>
<td>Distal cells with tapering apex</td>
<td>With pointed or blunt ends</td>
<td>With triangular subsidiary cells</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Both over veins and between veins, abundant</td>
<td>Cross or dumbbell shaped with sinuous walls</td>
<td>Absent</td>
<td>Variable in shape and size</td>
<td>Absent</td>
<td>Between the veins, with thin walled</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>With triangular subsidiary cells</td>
</tr>
<tr>
<td>Vetiveria zizanioides</td>
<td>Abaxial</td>
<td>Both over veins and between veins, Paried, abundant</td>
<td>Mostly cross shaped, some with pointed appearance</td>
<td>Absent</td>
<td>Flattened with round apex, hairs more than long</td>
<td>Absent</td>
<td>Both over veins and between veins, long in size</td>
</tr>
<tr>
<td></td>
<td>Adaxial</td>
<td>Both over veins and between veins, Paried, abundant</td>
<td>Obliquely placed with cork cells</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>2-3 rows of convex or concave, 3rd layer interrupted near the veins, with triangular subsidiary cells</td>
</tr>
</tbody>
</table>

Plate 1: Cymbopogon citratus
a) Abaxial view (40x); b) Adaxial view (20x)
Sc = Short cell; Sb = Silica body; Mh = Malpighi hair; Mn = Microhair; S = Stoma; Lo = Long cell; Iz = Intercostal zone

Adaxial side, Plate 1-b
Short cell: Over the veins, mostly solitary, sometimes paired, occasionally in rows of 3-5 cells.
Silica-body: Cross shaped, dumb-bell shaped or intermediate between the two.

Plate 2: Cynodon decaryon
a) Abaxial view (40x); b) Adaxial view (20x)
Sc = Short cell; Sb = Silica body; Mh = Malpighi hair; Mn = Microhair; S = Stoma; Lo = Long cell; Iz = Intercostal zone

Macro-hair: Absent
Micro-hair: Absent
Stoma: Usually in 3 rows but 3rd layer interrupted near the veins, usually dome shaped, but with triangular subsidiary cells.

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Plate 3: *Panicum sumatrense*

a) Abaxial view (40x);

So = Short cell; Sb = Silica body; Mh = Macrohair;

Mh = Microhair; Sc = Stoma; Lc = Long cell; Iz = Intercoastal zone

Plate 4: *Vitreveria zizanoides*

a) Abaxial view (40x);

Sc = Short cell; Sb = Silica body; Mh = Macrohair;

Mh = Microhair; Sc = Stoma; Lc = Long cell; Iz = Intercoastal zone

Key to the species (based on anatomical characters):

1a. Macro hair slender or stiff with sunken bulbous base or some times surrounded with specialized epidermal cells ———————————— 2

1b. Macro hair absent ———————————— 3

2a. Long cells cuticle with sinuous walls. Micro hairs with distal cells slightly tapering. Short cells in pairs found between veins ————— *Panicum sumatrense*

2b. Long cells rectangular with concave ends. Micro hairs inflated and wider, short cells solitary over veins ——— Cymbopogon cinctus

3a. Silica bodies cross-shaped. Micro hairs båluniform. Stomata subsidiary cells triangular. Short cells solitary over veins ——— *Vitreveria zizanoides*

3b. Silica bodies saddle shaped. Micro hairs spherical with two equal size short cells. Stomata subsidiary cells dumbbell shaped. Short cells solitary between the veins ——— Cyanodon dactylon

**Long cell:** Cells between veins are relatively short, thin and non-sinus or with slight sinus walls. Cells besides veins longer with thin walls slightly sinus, inter-stomatal cells rather long, with long sinus walls and concave ends.

**Prickles-hair:** Absent

**Cynodon dactylon (L.) Pers.**

**Abaxial side.** (Plate 2-a)

**Short cell:** If between veins are solitary, tall and narrow, but seldom containing conspicuous silica bodies. If over veins are paired, mostly in rows of 5 or more cells, abundant.

**Silica-body:** Over the veins, saddle-shaped.

**Micro-hair:** Length: 15-22 µ; basal cells 6-9 µ; distal cells inflated, hemispherical, with a rounded apex.

**Macro-hair:** Absent.

**Prickles-hair:** Absent or sparse if present.

**Papilla:** Small, variously shaped, circular warts, abundant over the long cells. Shorty stalked, 2 celled papillae sunk into the pits.

**Stoma:** Confined to abaxial surface with triangular subsidiary cells.

**Long-cell:** Cells shorter with thin sinus walls. Intercoastal cells much sinus with concave ends.

**Adaxial side.** (Plate 2-b)

**Short cell:** Abundant, both over and between the veins.

**Cynodon dactylon (L.) Pers.**

**Abaxial side.** (Plate 2-a)

**Short cell:** Between the veins, mostly solitary; those over veins are paired, mostly in rows of more than 5 cells. Short cells between the veins tall and narrow, seldom containing conspicuous silica bodies.

**Silica-body:** Rarely dumb-bell shaped but mostly saddle shaped.

**Macro-hair:** Absent

**Prickles:** Dumb-bell shaped with blunt ends.

**Stoma:** Small stomata with dumb-bell shaped subsidiary cells.

**Long-cell:** With thin sinus walls, the cell being shorter, intercoastal cells very sinus in outline and with concave ends.

**Panicum sumatrense Roth ex Roem & Schult.**

**Abaxial side.** (Plate 3-a)

**Short-cell:** Between veins paired those over the veins in rows of 3 or more cells, abundant.

**Silica-body:** Between the veins, horizontally elongated, or irregular in shape; those over the veins are cross-shaped, more uniform in outline or intermediate between cross and dumbbell shape with sinus walls.

**Macro-hair:** Moderately long, thick-walled, each with a constricted base surrounded by specialized epidermal cells, abundant between the veins.

**Micro-hair:** Length 38-58 µ (mostly 42-54 µ); base cells 15-18 µ; distal cell 26-44 (mostly 26-34 µ); distal cells slightly tapering towards apices.
Prickle-hair: With pointed or blunt ends.  
Stoma: With triangular subsidiary cells.  
Long cell: Between the veins, with thin sinuses walls some cells being rather short.  

Adaxial side. (Plate 3-b)  
Short-cell: Between the veins paired those over veins in rows of 5 or more cells, abundant.  
Silica body: Between the veins, horizontally elongated, may have distorted shape, those over veins cross-shaped and less distorted in out line or intermediate between cross and dumbbell shaped with sinus walls.  
Macro-hair: Absent.  
Micro-hair: Length 38-55 μ, mostly 42-54 μ, base cells 15-18 μ, distal cell 20-44 μ, mostly 20-34 μ, distal cells slightly tapering towards apices, with variation in shape and size.  
Stoma: With triangular subsidiary cells.  
Long cell: Between veins with thin sinuses walls, some cells being rather short.  
Intercostal zone (Iz): 5 rows of cells, four continuous and one discontinuous in intercostal zone: a peculiar feature of the species.  

Vitexia zizanoides (L.) Nash.  
Abaxial side. (Plate 4-a)  
Short-cell: Both over and between the veins, paired, abundant.  
Silica body: Mostly cross-shaped but vertically placed with cortical cells, some of them having distorted appearance.  
Macro-hair: Absent.  
Micro-hair: Length 39-64 μ, basal cells 14-22 μ, distal cells 24-33 μ, hair often balsamiform, the distal cells frequently flattened with round apices; hair more wide than long.  
Prickle: Absent.  
Stoma: 2 or 3 rows with triangular dumb-shaped subsidiary cells.  
Long cell: Both over and between veins with moderately thick sinaxes walls and fairly long size.  

Adaxial sides. (Plate 4-b)  
Short-cell: Both over and between the veins, paired, abundant.  
Silica body: Vertically/obliquely placed and coupled with cortical cells.  
Macro-hair: Absent.  
Prickle: Absent.  
Stoma: 2 or 3 rows of stomata. 3rd layer scattered.  
Long cell: Both over and between the veins with moderately thick sinaxes walls fairly long.  

Discussion  
The present work dates back to Metcalf (1960), who provided the leaf epidermal anatomy of members of family Poaceae. He considered both abaxial and adaxial sides and recorded observations for the silica bodies as having cross or dumbbell shape or between; prickles over the veins; stomata close to veins and in a single row; etc. Silica bodies are a type of phytolith in specialized epidermal cells of grass leaves. Various workers have considered silica bodies to be diagnostic for the family Poaceae (Truss et al., 1966; Brown, 1984; Mulholland, 1989). Piperno and Peasall (1998) studied the silica bodies of Tropical American grasses and discussed their taxonomic implications. Thomasson (1964) noted that micro-morphological characters of the leaf and provided information on the fossil’s phytology and taxonomic relationships. The present work not only confirms the findings of Metcalf (1960) and other workers in the field but also adds something new to it. Therefore, seven characters were studied and found diagnostic for the four species under study. For example, silica bodies in Cynodon dactylon are diagnostic, as their saddle shape does not exist in any of the other three species. In Cymbopogon citratus, the long cells are rectangular with concave ends, micro hair is inflated and wider, short cells solitary over veins are proved diagnostic. On the other hand, long cells cubical, micro hair with tapering margins, short cells in pairs and found between veins are being diagnostic to Panicum sumatrense. In Vitexia zizanoides, silica bodies are cross-shaped, micro hair balsamiform, subsidiary cells triangular and short cells solitary over veins. Other wise, micro hair are usually spherical, subsidiary cells are dumb-bell shaped and short cells are solitary between veins, diagnostic to Cynodon dactylon. Hence the present work remained successful in the sense that it not only widens the spectrum and scope of taxonimony but also provides an efficient approach to identify the plant scraps which otherwise would have required the complete information on the specimen plant for its correct identification. By “complete information” one means the total morphology including roots, stem, leaves and above all the inflorescence.  

References  