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

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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, *Cynodon dactylon* (L.) Pars, *Panicum summatrense* Roeth ex Roem & Schult and *Viteveria zizanoides* (L.) Nash.

Key words: Leaf epidermal anatomy, *Cymbopogon citrates*, *Cynodon dactylon*, *Panicum Summatrense*, *Viteveria 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 (Metcalf, 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. Pharmacognosists (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*, *Cynodon dactylon*, *Panicum summatrense* and *Viteveria 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.* (1994), has reported the nutritive value of *Cynodon dactylon* seeds and their role on pregnancy of rat and littre size in bandi cot rat (*Bandicota bengalensis*). Essential oil of *Cymbopogon citrates* has been reported antibacterial by Onawunmi *et al.* (1984). Similarly, *Viteveria zizanoides* has been included in "Medicinal plants of Utter Perdesh" 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 30-45 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 scalpal 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 = 10μm
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-bell shaped with shallow indentations.

Macro-hair (Mah): Absent

Micro-hair (Mih): 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.

Prickle-hair (Ph): Prickles rather elongated with swollen bases, numerous near the veins.

Papilla (P): Most long-cells are provided with thin walled papillae. Papillae two celled, 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

Species	View	Short cells	Silica Bodies	Macro Hairs	Micro Hairs	Prickle Hairs	Papillae	Stomata	Long Cells
Cymbopogon citrates	Abaxial	Both over veins and between veins	Cross shaped with shallow indentations	Absent	Tapering apex	Elongated with swollen bases	Two cells with swollen base	Dumb-bell shaped, abundant	Fairly short with sinus walls
	Adaxial	Over the veins	Cross or dumbbell shaped	Absent	Absent	Absent	Absent	Dumb-bell shaped in 3 rows	Both over and between the veins with sinus walls
Cynodon dactylon	Abaxial	Both over veins and between veins, abundant	Saddle shaped	Absent	Rounded apex	Rare or absent	Two cells with sunken pits	Subsidiary cells triangular	Intercostal cells with sinus walls and concave ends
	Adaxial	Both over veins and between veins	Saddle shaped rarely dumbbell shaped	Absent	Absent	Dumbell-shaped with blunt ends	Absent	Dumb-bell shaped, small sized subsidiary cells	Intercostal cells with sinus walls and concave end
Panicum summatrense	Abaxial	Both over veins and between veins Abundant	Elongated or irregularly shaped	Abundant moderately long and between veins	Distal cells with tapering apex	With pointed or blunt ends	Absent	With triangular subsidiary cells	Between the veins with thin sinus walls
	Adaxial	Both over veins and between veins Abundant	Cross or dumbbell shaped with sinus walls	Absent	Variable in shape and size	Absent	Absent	With triangular subsidiary Cells	Between the veins with thin sinus walls
Viteveria zizanoides	Abaxial	Both over veins and between veins Paired, abundant	Mostly cross shaped some with distorted appearance	Absent	Flattened with round Apex, Hairs more wide than long	Absent	Absent	With triangular or dumbbell shaped subsidiary Cells	Both over and between veins, fairly long in size
	Adaxial	Both over veins And between veins Paired, abundant	Obliquely placed with cork cells	Absent	Absent	Absent	Absent	2-3 rows Of stomata 3rd layer Scattered	Both over and between veins, fairly long in size

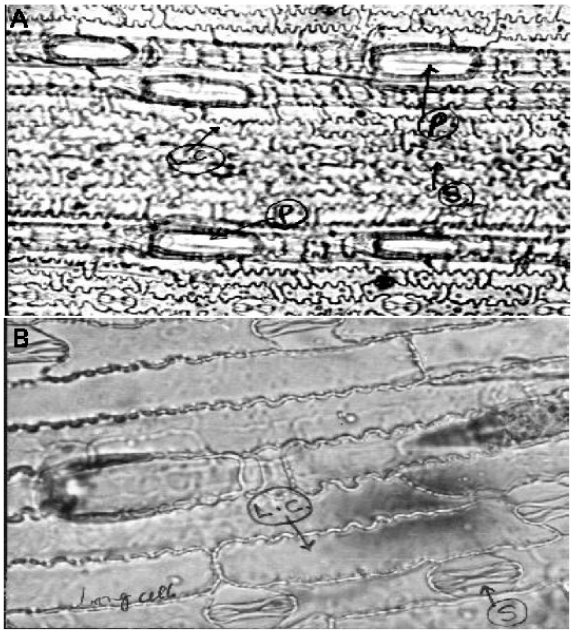

Plate 1: *Cymbopogon citrates*
a) Abaxial view (40x); b) Adaxial view (20x)
Sc = Short cell; Sb = Silica body; Mah = Macrohair;
Mih = Microhair; S = Stoma; Lc = Long cell; Iz = Intercostal zone

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.

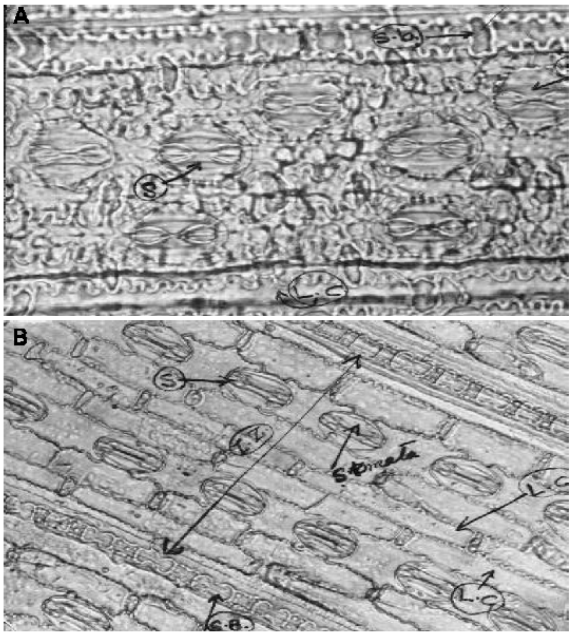

Plate 2: *Cynodon dactylon*
a) Abaxial view (40x); b) Adaxial view (20x)
Sc = Short cell; Sb = Silica body; Mah = Macrohair;
Mih = Microhair; S = Stoma; Lc = Long cell; Iz = Intercostal zone

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.

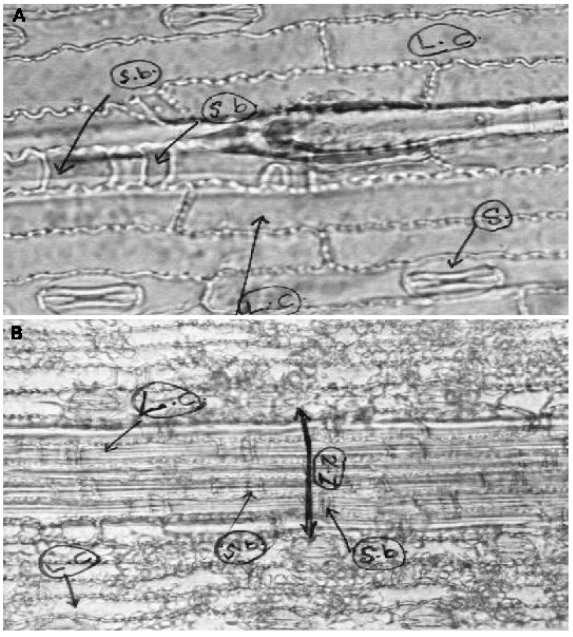

Plate 3: *Panicum summatrense*
a) Abaxial view (40x); b) Adaxial view (40x)
Sc = Short cell; Sb = Silica body; Mah = Macrohair;
Mih = Microhair; S = Stoma; Lc = Long cell; Iz = Intercostal zone

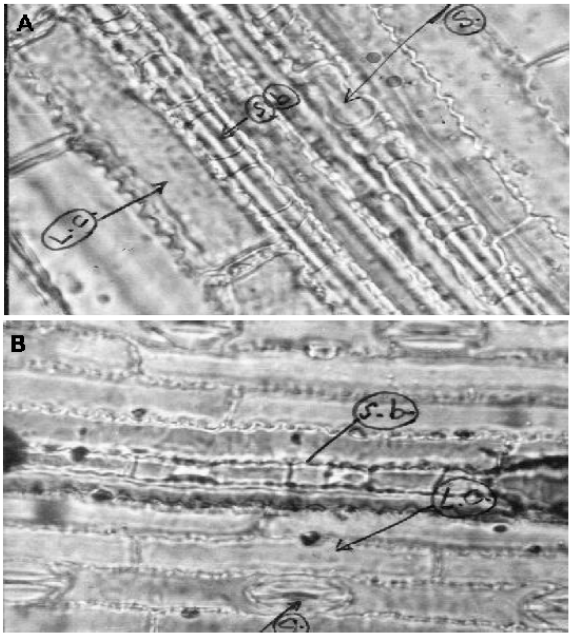

Plate 4: *Viteveria zizanoides*
a) Abaxial view (40x); b) Adaxial view (40x)
Sc = Short cell; Sb = Silica body; Mah = Macrohair;
Mih = Microhair; S = Stoma; Lc = Long cell; Iz = Intercostal zone

Key to the species (based on Anatomical characters):

1a- Macro hairs slender or stiff with sunken bulbose base or some times surrounded with specialized epidermal cells ----------2
1b- Macrohairs absent ----------3
2a- Long cells cubical with sinuous walls. Microhairs with distal cells slightly tapering. Short cells in pairs found between veins -------- *Panicum summatrense*
2b- Long cells rectangular with concave ends. Microhairs inflated and wider, short cells solitary over veins ---------- *Cymbopogon citrates*
3a- Silica bodies cross-shaped. Microhairs balanoform. Stomatal subsidiary cells triangular. Short cells solitary over vein----------*Viteveria zizanoides*
3b- Silica bodies saddle shaped. Microhairs spherical with two equal size short cells. Stomatal subsidiary cells dumbell shaped. Short cells solitary between the veins ---------- *Cynodon dactylon.*

Long cell: Cells between veins are relatively short, thin and non-sinuous 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.
Prickle-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.
Prickle-hair: Absent or sparse if present.
Papilla: Small, variously shaped, circular warts, abundant over the long cells. Shortly stalked, 2 celled papillae sunken into the pits.
Stoma: Confined to abaxial surface with triangular subsidiary cells.
Long-cell: Cells shorter with thin sinuous walls. Intercostal cells much sinus with concave ends.

Adaxial side. (Plate 2-b)
Short cell: Abundant, both over and between the veins. 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
Prickle: Dumb-bell shaped with blunt ends.
Stoma: Small stomata with dumb-bell shaped subsidiary cells.
Long-cell: With thin sinuous walls, the cell being shorter, intercostal cells very sinus in outline and with concave ends.

***Panicum summatrense* Roth ex Roem & Schult.**
Abaxial side. (Plate 3-a)
Short-cell: Between veins paired those over the veins in rows of 5 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 dumb-bell 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 38-58μ (mostly 42-54μ); base cells 15-18μ distal cell 26-44μ (mostly 26-34μ); 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 sinus 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 dumb-bell shaped with sinus walls.
Macro-hair: Absent.
Micro-hair: Length 38-58μ (mostly 42-54μ), base cells 15-18μ, distal cell 26-44μ (mostly 26-34μ); distal cells slightly tapering towards apices, with variation in shape and size.
Stoma: With triangular subsidiary cells.
Long cell: Between veins with thin sinus 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.

***Viteveria 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 cork cells, some of them having distorted appearance.
Macro-hair: Absent.
Micro-hair: Length 39-54 μ, basal cells 14-22 μ, distal cells 24-33 μ, hair often balanoform, the distal cells frequently flatten with round apices; hair more wide than long.
Prickle-hair: Absent.
Stoma: 2 or 3 rows with triangular dumb-shaped subsidiary cells.
Long-cell: Both over and between veins with moderately thick sinus 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 cork 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 sinus 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 dumb-bell shape or in 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 (Twiss *et al.*, 1969; Brown, 1984; Mulholland, 1989). Piperno and Pearsall (1998) studied the silica bodies of Tropical American grasses and discussed their taxonomic implications. Thomasson (1984) noted that micro-morphological characters of the leaf and provided information on the fossil's phylogeny 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 citrates*, the long cells are rectangular with concave ends, micro hair are 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 summatrense*.
In *Viteveria zizanoides*, silica bodies are cross-shaped, micro hair balanoform, subsidiary cells triangular and short cells solitary over veins. Other wise, micro hairs 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 taxonomy 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.

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