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Systematic implications of foliar epidermis in andropogoneae (poaceae) from Hindukush-himalayas Pakistan

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The study was aimed to investigate systematic potential of foliar epidermal characters in species identification and delimitation. 18 species of summer grasses from tribe Andropogoneae (Poaceae) from Hindukush-Himalayas (HKH) were evaluated for variations in both the adaxial and abaxial epidermis. The results reveal that certain features of the leaf epidermis are significant in differentiating closely related taxa. For instance variations were found in the shape and wall sinuosity of long cells, papillae, micro hairs size and abundance, stomatal size, shape of subsidiaries, presence and abundance of intercostal short and silica cells, arrangement and type of costal silica bodies and the distribution of prickles, among different species. Keys for identification have been provided for authentic identification even at vegetative stages. It was concluded that characters of the foliar epidermis are diagnostic and ensure more authentic identification when coupled with morphological features.

Key words: Andropogoneae, foliar epidermis, micromorpholgy, papillae, poaceae, stomata, systematics.

INTRODUCTION

Grasses constitute the most economically important, larger and cosmopolitan family Poaceae of flowering plants. There are about 700 genera and 11000 species of grasses (Chen et al. 2006), represented by 492 species, 158 genera and 26 tribes in Pakistan (Cope, 1982). The tribe Andropogoneae includes about 87 genera and 1060 species (Barkworth, 2003) distributed throughout the tropics and sub-tropics, extending into warm temperate regions. According to Cross (1980) it is a tropical tribe with centers of diversity in the Zambezian Domain, Deccan Region and Indo-China. In Pakistan the tribe is represented by 36 genera and 67 species (Cope, 1982). The species included in the present investigation are those collected from temperate zone of HKH.

Grasses being evolutionarily advanced exhibit morphological reduction and simplicity, thereby providing few morphological characters of taxonomic utility and hence posing many problems in correct identification for taxonomists. However, this morphological simplicity of grasses has overcome by highest degree of specialization and variation in the foliar epidermis. The foliar epidermis has been the subject of intense investigation in grass systematics since Prat (1932) primarily utilized it for this purpose. According to Prat (1948: 51) leaf epidermal anatomy shows greater diversity in Poaceae than in any other family and provides extensive data for systematic utilization (Brown, 1958; Metcalf, 1960; Ellis, 1979). Since 1930's many workers have contributed to the systematic potential of foliar epidermal micromorphology or anatomy. Instances of major contributions can be made of (Prat, 1932, 1936; Brown, 1958) studied 101 species in 72 genera; Metcalfe (1954, 1960); Renvoiz (1982) examined leaf-blade anatomy of each of the 86 genera of the tribe Andropogoneae except *Polliniopsis* a rare, monotypic genus; Hilu (1984) studied leaf epidermis of *Andropogon* sect. *Leptopogon*; Davila and Clark (1990) examined all 17 species of the genus *Sorghastrum* (Andropogoneae); Whorter et al. (1993) on the genus Sorghum

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(Andropogoneae); Acedo and Llamas (2001) investigated micromorphology of the lemma and palea of *Bromus*; Folorunso and Oeytunji (2007) examined leaf epidermis of *Cymbopogon*; Cristina et al. (2008) and Raole and Desai (2009) studied foliar epidermis of some Andropogoneae from India. Abaxial foliar epidermises of 49 species of North American Stipeae were examined by Barkworth (1981) and concluded that shape of costal bodies and number of costal and intercostals cell files were most useful taxonomic characters. Namaganda et al. (2008) investigated taxonomic potential of leaf epidermal characters in Ugandian species of Festuca and concluded that leaf anatomy should be used with non-anatomical characters before recognizing species. Recently Ahmad et al. (2010) examined 13 species of Andropogoneae for variations in the internal anatomy of blade that can be helpful in species differentiation. However, the species examined by the above workers are mostly different from the present species. The present study is the first ever work on the grasses of Hindukush-Himalayas (HKH) using leaf epidermal features in grass systematics.

MATERIALS AND METHODS

Both abaxial and adaxial surfaces of 18 species of Andropogoneae were examined including some taxonomically problematic species. Fresh green leaves were collected from wild populations of grasses in various valleys of Swat and Hazara in HKH ranges. Voucher specimens were deposited in the Herbarium of Pakistan Quaid-I-Azam University Islamabad [ISL] (as given below). For epidermal preparations, representative samples 1 to 2 cm were cut from the midportion of mature foliage leaves. The fresh leaves were placed in a test tube filled with 88% lactic acid, kept in water bath (Memert D-91126-FRG, Germany) and boiled at 100℃ for 60 to 80 min. When abaxial epidermis was to be prepared the leaf was placed on a tile adaxial surface uppermost and flooded with cold lactic acid. By using a sharp blade the adaxial epidermis was scraped along with the mesophyll tissue leaving the abaxial epidermis. The epidermis was placed on clean glass slide and mounted in fresh 88% lactic acid. When a preparation of the adaxial side was to be made the leaf was placed abaxial side uppermost with same next procedure. Some peels were stained in 1% Sudan IV solution for distinction between cork and silica cells. Microhistological photographs of both epidermal surfaces were taken using a camera (Infinity 1-5 C-MEI, Canada) at x20 objective lens of (LEICA DM 1000) microscope.

Preparative techniques were followed after Metcalfe (1960), Clark (1960) and Cotton (1974). Terminology and description format were adopted after Watson and Dallwitz (1988) and Ellis (1979).

Specimens examined for Andropogoneae of Swat, N.W. Pakistan

Apluda mutica L. 125561 (ISL)*, *Arthroxon prionodes* (Steud.) Dandy 125544, *Chrysopogon aucheri* (Boiss.) Stapf 125609**,** *Chrysopogon gryllus* (Nees) T.A. Cope 125546, *Cymbopogon jawarancusa* (Jones) Schult. 125615, *Cymbopogon martinii* (Roxb.) Wats. 125574, *Cymbopogon pospischilii* (K.Schum.) C.E. Hubbard 125578, *Dichanthium annulatum* (Forssk.) 125605, *Eulaliopsis binata* (Retz.) C.E. Hubbard 125621, *Hemarthria compressa* (Linn.f.) R. Br.125625**,** *Heteropogon contortus* (Linn.) P. Beavu. ex Roem. and Schult. 125622, *Hyparrhenia hirta* (Linn.) Stapf 125577, *Imperata cylindrica* (Linn.) Raeuschel 125584, *Ischaemum rugosum* Salisb. 125576, *Rottboellia exaltata* Linn. f. 125565, *Saccharum spontaneum* Linn. 125604, Sorghum *halepense* (Linn.) Pers 125563, *Themeda anathera* (Nees ex Steud) Hack. 125619.

* ISL= Herbarium of Pakistan, Quaid-I-Azam University, Islamabad.

RESULTS

This study was confined to the foliar epidermal micromorphology in 18 summer grasses of the tribe Andropogoneae found above 1300 m in various valleys of swat and Hazara in Hindukush-Himalayan ranges. The genera studied include *Apluda, Arthraxon, Chrysopogon, Cymbopogon, Dichanthium, Eulaliopsis, Hemarthria, Heteropogon, Hyparrhenia, Imperata, Ischaemum, Rottboellia, Saccharum, Sorghum and Themeda.* Micromorphological features studied include, shape and wall morphology of long cells costally and intercostally; presence, absence, arrangement and distribution of short cells costally and intercostally; shape and arrangement of silica bodies over the veins; presence, absence, type and size of micro hairs and its apical and basal cells; size of stomatal complex, shape of subsidiary cells and absence, presence and type of papillae and distribution of prickles and macro hairs. Details of micro morphological features have been provided in (Tables 1 and 2). Keys for identification has been provided for distinguishing different species using foliar epidermal features. Light micrographs of abaxial epidermis have been provided for each species (Figures 1A-L and 2M-R).

Key to the species of andropogoneae

1a. Papillae present--2
1b. Papillae absent---6
2a. Papillae in the form of several finger like projections--Themeda anathera*
2b. Papillae in the form of oblique swellings---3
3a. Papillae in the form of single oblique swelling per cell------------------------------ 4
3b. Papillae in the form of several oblique swellings per cell---*Ischaemum rugosum*
4a. Papilla single per cell, subsidiaries non-papillate-------------------------------------5
4b. Papilla single per cell, subsidiaries papillate--- *Hyparrhenia hirta*
5a. Subsidiaries non-papillate, microhair basal cell less than 30 µm long---*Heteropogon contortus*

Table 1. Summary of qualitative characters of the foliar epidermis in tribe andropogoneae.

S.No	Species	Papillae present/a bsent	Intercostal long cells shape	Long cells wall morphology	Micro hairs type	Stomata	Subsidiaries shape	Intercostal short cells	Distribution of costal short cells	Costal silica bodies	Prickles
1	*A. mutica*	Absent	Rectangular, in 2-4 rows	Markedly sinous	panicoid	Common	High dome-shaped	Rare, single, absent from adaxial surface	Long rows as well as paired	Butterfly to dumb-bell	Common over the veins
2	*A. prionodes*	Absent	Short rectangular to hexagonal to irregular	Undulating or straight walled	Panicoid with apical cell often collapsing	Common abaxially, rare or absent adaxially	Dome to low dome shaped	Very rare, often approached by prickles	In long rows	Butterfly to dumb-bell	Very common intercostaly
3	*C. aucheri*	Absent	Rectangular with rounded ends	Markedly sinous, adaxial undulating	Panicoid 1-celled or 2-celled	Common	Low dome and triangular to triangular	Common mostly in pairs	paired	Cross to dumb-bell	Common
4	*C. gryllus*	Absent	Rectangular with rounded ends ,a few fusiform	Markedly sinous to undulating	Panicoid	Common	Low dome and triangular	Common, paired	paired	Cross to dumb-bell	Common, short cells modified to prickles
5	*C. jawarancusa*	Absent	Rectangular abaxially, hexagonal to short rectangular adaxially	Markedly sinous	Panicoid apical cell often collapsing	Common	Dome or high dome and triangular	Common mostly in pairs few solitary	long rows few solitary	Dumb-bell or butter fly	Present
6	*C. martini*	Absent	Rectangular, a few fusiform, cuboidal Adaxially	Markedly sinous	Panicoid, often singlecelled	Common	Dome or high dome and triangular	Very common, solitary	Long rows or pairs	Cross/ butterfly/ dumb-bell	Absent
7	*C. pospischili*	Absent	Rectangular. Adaxially short rectangular to hexagonal	Abaxial markedly sinous, adaxial straight walled	Panicoid, rare	Common on abaxial, rare on adaxial surface	High dome and triangular	Common, single or in pairs	Long rows	Cross/butterfly/ dumb-bell	Present
8	*D. annulatum*	Papilla a single oblique swelling per cell	Rectangular	Markedly sinous	Panicoid, clearly two celled	Common	Low dome to high dome to triangular	Common, paired or not	Long rows	Rounded/cross/ dumb-bell	Common on veins and margins

Table 1. Contd.

9	*E. binata*	absent	Rectangular, markedly different costaly and intercostaly	Markedly sinous	Panicoid ostensibly two-celled	Common	High dome	Common, single	Long rows	Butterfly/dumb-bell	Numerous adaxially, absent adaxially
10	*H. compressa*	absent	Rectangular, markedly different costally and intercostaly	Markedly sinous	Panicoid, 2-celled, adaxially absent	Common	Dome to high dome	Common, paired	paired	Butterfly/dumb-bell	Only on abaxial surface
11	*H. contortus*	Papilla a single oblique swelling per cell	Rectangular	Markedly sinous	Panicoid one or two celled	Common	Triangular	Common, paired or single or in short rows	Long rows	Cross to dumb-bell	Present
12	*H. hirta*	Papilla single oblique swelling per cell	Rectangular/ adaxial short cuboidal to irregular	Markedly sinous	Panicoid, clearly two-celled	Common	Subsidiaries papillate and triangular to dome and triangular	Rare, solitary modified to prickles	Long rows	Butterfly/dumb-bell/cross	Prickles present, short macrohairs common
13	*I. cylindrical*	absent	Rectangular, adaxial short rectangular with rounded ends	Markedly sinous, adaxial with undulating walls	Panicoid ostensibly two-celled	Common	Triangular	Common, paired or single	Long rows	Dumb-bell/nodular /cross/butterfly	Not seen
14	*I. rugosum*	Papillae many rounded swellings, in 1-2 rows	Long or short rectangular	Markedly sinous	Panicoid somewhat spherical or balloon shaped	Common	Subsidiaries non-papillate, dome to high dome triangular	Absent or rare	Long rows, few paired	Cross/ dumbbell /butterfly	Absent
15	*R. exaltata*	absent	Rectangular	Markedly sinous	Panicoid, apical cell often collapsing	Common	Dome to high dome	Common, single	Pairs or short rows	Cross/ dumbbell/ butterfly	Costal prickles present
16	*S. spontaneum*	Absent	Rectangular	Markedly sinous	Panicoid clearly 2-celled	Common	Low dome to triangular, high dome	Common, single	Paired or short rows	Cross/saddle/b utterfly s/dumb-bell	Not seen

Table 1. Continued.

17	*S. halepense*	Absent	Rectangular	Markedly sinous	panicoid	Common	Dome and triangular	Common, solitary	Long rows	Cross or dumb-bell	Present
18	*T. anathera*	Papillae: several finger like projections	Rectangular	Markedly sinous to gently undulating	panicoid	Common, rare on adaxial surface	Subsidiaries papillate, low dome to triangular	Common, paired or solitary	Long rows	Cross, dumb-bell, nodular	Present

Table 2. Summary of quantitative characteristics of the species of tribe andropogoneae.

S. No	Species	Microhairs length (µm)	Apical cell length (µm)	Basal cell length (µm)	Stomata length (µm)
1	*A. mutica*	32-36	12.5-20	16-20	13-17.5
2	*A. prionodes*	33-41	15-25	13-27	30-35
3	*C. aucheri*	62.5-75	28-37	25-38	43-47.5
4	*C. gryllus*	40-55	25-30	20-25	36-45
5	*C. jawarancusa*	35-53	17.5-25	28-38	25-33.5
6	*C. martini*	30-44.5	13-25	17-23	29-36
7	*C. pospischili*	39-53	18-29	20-26	23-25
8	*D. annulatum*	60-80	35-40	40-45	35-40
9	*E. binata*	45-58	25-33	25-30	39-50
10	*H. compressa*	30-40	15-24	13-18	27-33
11	*H. contortus*	60-80	22-40	20-30	40-44
12	*H. hirta*	54-73	25-34	20-30	30-38
13	*I. cylindrica*	40-54	17-21.5	25-35	25-27
14	*I. rugosum*	15-25	4-9	9-15	26.5-33.5
15	*R. exaltata*	85-124	40-80	30-39	35-40
16	*S. spontaneum*	40-53	24-33	18-27	30-36
17	*S. halepense*	30-70	18-30	8-16	28-40
18	*T. anathera*	35-68	13-19.5	30-46	24-30

5b. Subsidiaries non-papillate, microhairs basal cell more than 40 µm long--*Dichanthium annulatum*

6a. Intercostal long cells in more than 4 rows---7

6b. Intercostal long cells in 2-4 rows--*Apluda mutica*

7a. Intercostal short cells common, mostly in pairs, few solitary, prickles present

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Cymbopogon jawarancusa

7b. Intercostals short cells very common and always solitary, prickles absent-- *Cymbopogon martini*

8a. Midintercostal long cells rectangular to fusiform with markedly sinous walls, stomata common --9

8b. Midintercostal long cells irregular, cubical or short fusiform with walls always straight or gently undulating but never sinous, adaxial stomata absent ---

Arthraxon prionodes

9a. Intercostals short cells common and paired--Chrysopogon aucheri*

9b. Intercostals short cells modified to prickle hairs--*Chrysopogon gryllus*

10a. Microhairs present both adaxially and abaxially-------------------------------11

10b. Microhairs absent adaxially---*Hemarthria compressa*

11a. Microhairs less than 80 µm long, prickles absent---------------------------------12

11b. Microhairs up to 124 µm long, costal prickles present --- *Rottboellia exaltata*

12a. Both adaxial and abaxial long cell's walls markedly sinous, subsidiaries low dom-triangular and intercostals short cells always single---

Saccharum spontaneum

12b. Adaxial long cells with only gently undulating walls, subsidiaries triangular and intercostals short cells often in pair--

Imperata cylindrica

13a. Long cells similar in shape and wall thickness costally and intercostally, microhairs ostensibly two celled, prickles present only adaxially--

-*Euolaliopsis binata*

13b. Long cells markedly different in shape and wall thickness costally and intercostally, microhairs clearly two-celled, prickles present on both sides---

Sorghum halepens.

DISCUSSION

We observed considerable variation in micro morphological features between different genera and species. Shape and wall morphology of long cells, papillae morphology, micro hairs and stomata size and subsidiaries shape were found to be most significant structures for generic and specific delimitation at least in the presently studied species. Papillae were found to possess significant taxonomic potential due to their specific shape, arrangement and number per cell. Many workers like (Metcalfe, 1960; Watson and Dallwitz, 1988) has already emphasized role of papillae in grass taxonomy. Papillae were observed only in 5 species. In *Themeda anathera* papillae are in the form of several finger like projections, with the subsidiary cells also are papillate (Figure R). In *Dichanthium annulatum* (Figure H), *Hyparrhenia hirta* (Figure L) and *Heteropogon contortus* (Figure K) papillae are in the form of single oblique swelling per cell. In *D. annulatum* the papillae are overarching the stomata. In *Ischaemum rugosum* (Figure N) several oblique swellings are present per cell, in one or two rows.

The epidermal long cells are oriented parallel to the long axis of the leaf surface and varied from rectangular to hexagonal to squarish to fusiform to irregular. The long cells in *Arthraxon prionodes* (Figure B) are hexagonal to irregular to squarish on both the epidermises and the epidermis apparently looks like that of dicots. This may be due to lanceolate and relatively broader leaves rather than linear. In *E. binnata, H. compressa and A. prionodes* the long cells are markedly different costally and intercostally, the costal short, narrow and thick walled, the intercostals broader, long and thin walled. Similar findings were also observed by (Watson and Dallwitz, 1999). The wall morphology (degree of sinuosity) has also been found as an important systematic feature. Inmost species we studied that walls of long cells are markedly sinuous, as (Rinvoize, 1982) has also described that 80% Andropogoneae possess sinuous (convolute) walls. However in *A. prionodes* (Figure B) the cell walls are undulating to straight, and in *C. aucheri, C. gryllus, H. hirta, C. pospischili, I. cylindrica and T. anathera* the adaxial long cells possess undulating or straight walls.

Micro hairs are bicellular trichomes (Tateoka et al., 1959; Metcalfe, 1960) universally present in all subfamilies except Pooideae (Watson and Dallwitz, 1999). Our findings reveal panicoide type (with long and thin walled apical cell) of micro hairs for all the species investigated, as stated by (Amarasinghe and Watson, 1990). *C. aucheri* and *C. gryllus* are taxonomically problematic; micro hairs are 62 to 75 µm long in the former case, while in the later they are 40 to 55 µm long. In *I. rugosum* (Figure N) micro hairs are unique in the sense that they are balloon-shaped and very small (15 to 25 µm long).

Stomata and the associated subsidiaries exhibit variations in their shapes and pattern of arrangement in longitudinal cell files. In majority of the species stomata were common and similar stomatal densities were observed in both the epidermises. In *A. prionodes, C. pospischili and T. anathera* stomata are very rare or often

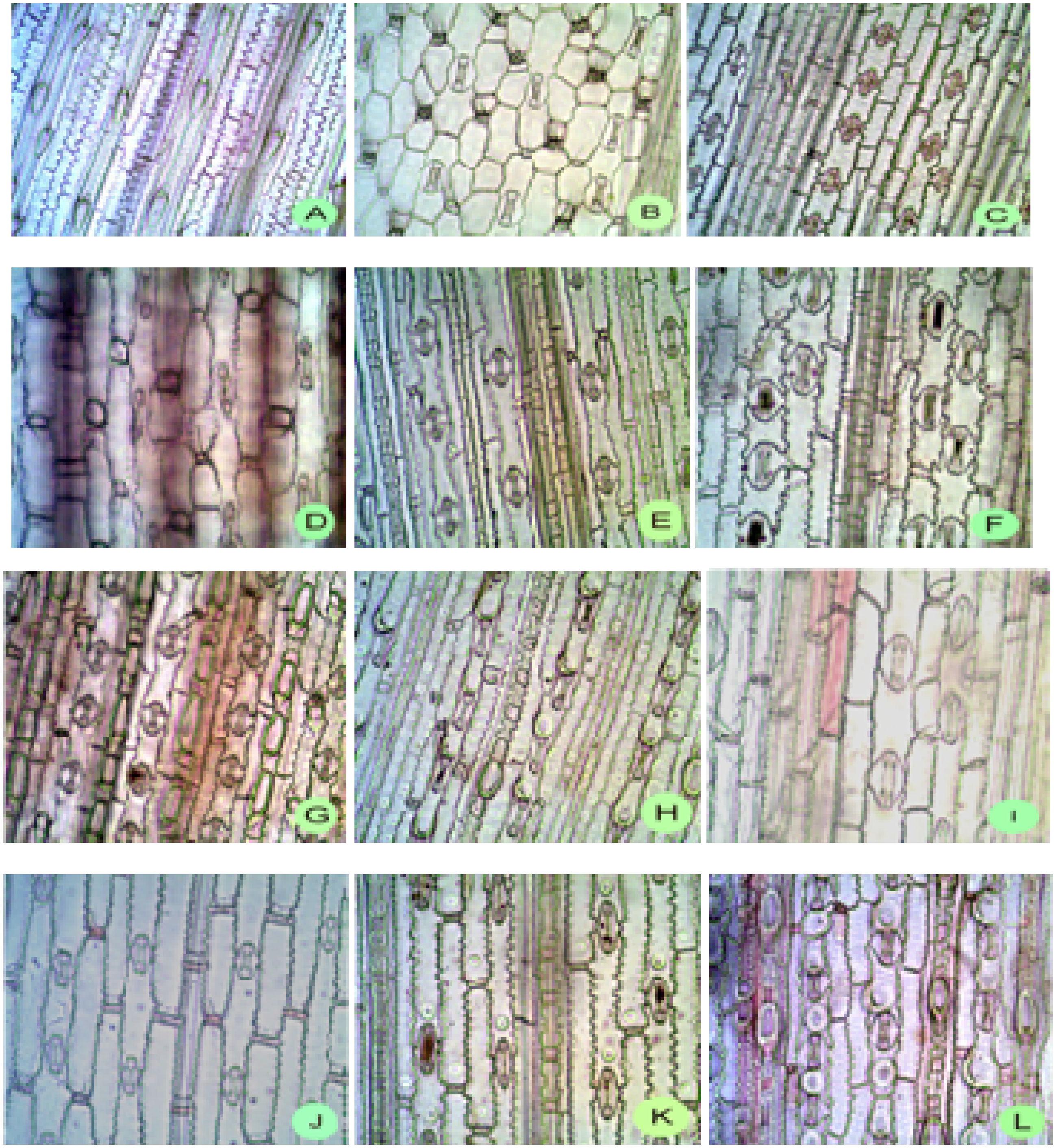

Figure 1A-L. Light micrographs of abaxial epidermises: (A) *A. mutica* (B) A. prionodes (C) *C. aucheri* (D) *C. gryllus* (E) *C. jawarancusa* (F) *C. martini (G) C. pospischili* (H) *D.* annulatum (I) *E. binata* (J) *H. compressa* (K) *H. contortus* (L) *H. hirta*

absent from adaxial surface. Stomata are absent from adaxial surface in *C. aucheri* (Zarinkamar, 2006) but our results are in contrast to him as we have observed equal distribution of stomata on both surfaces. The length of stomata varied from 13 to 47.5 µm, smallest stomata were observed in *A. mutica* (13 to 17.5 µm) while longest stomata were observed in *C. aucheri* (43 to 47.5 µm). In *H. contortus* (Figure K), *I. cylindrica* (M), and *S. halepense* (Q) the subsidiaries were strongly triangular, while in the rest of the species subsidiaries ranged from high dome to low dome and triangular in shapes. Only in *H. hirta* (Figure L) and *T. anathera* (Figure R) papillate

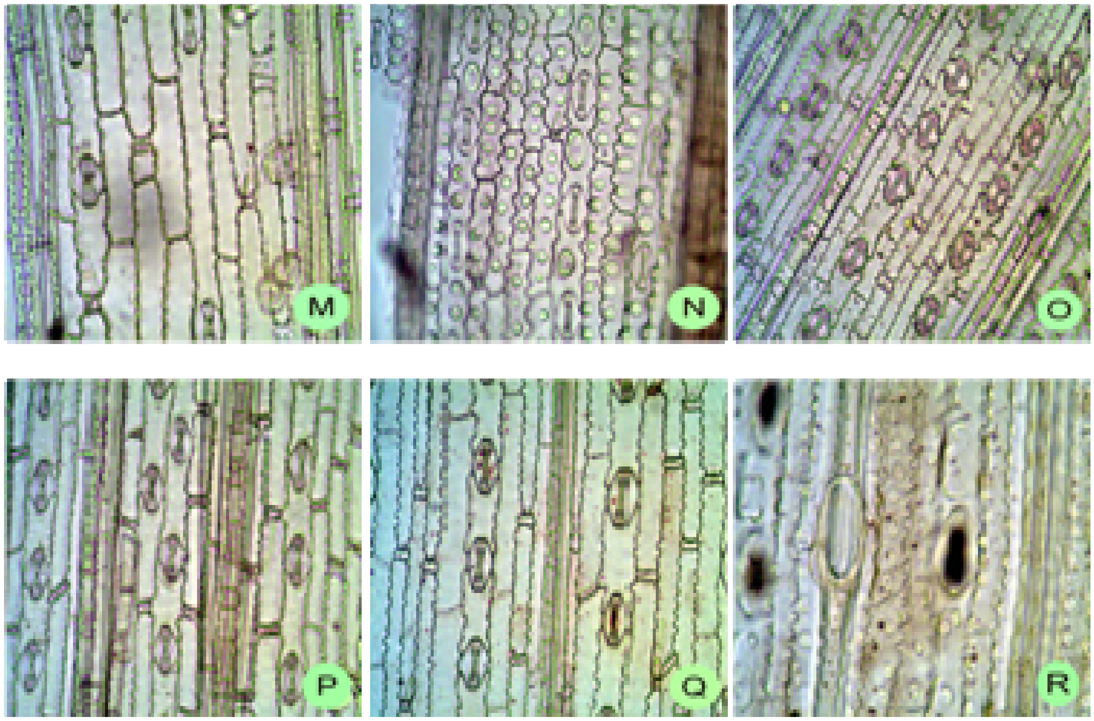

Figure 2M-R. Light micrographs of abaxial epidermises: (M) *I.cylindrica* (N) *I. rugosum* (O) *R. exaltata* (P) *S. spontaneum* (Q) *S. halepense* (R) *T. anathera.*

subsidiaries were observed.

The silica bodies are a type of phytolith that accumulate in the leaf tissue of grasses particularly epidermis and assume various shapes that are very significant in grass taxonomy. This silica perform various functions like mechanical stability, pathogen, insect, herbivore and drought resistance, facilitatation of light and alleviation of nutrient deficiency (Ma, 2004; Epstein and Bloom, 2005; Motomura et al., 2006). They are recognized as distinctive to Poaceae (Prat, 1960). Silica bodies and their associated epidermal pattern can be very useful in grouping of Poaceae to tribes and genera (Hubbard, 1948). We have found different kinds of silica bodies such as dumbbell, butterfly-shaped, saddle-shaped, cross-shaped, rounded, long and narrow and some intermediate types. The evolutionary trend in silica bodies is from dumb bell to cross shaped (Shouliang et al., 1996). In *Cenchrus, Sorghum* and *Chrysopogon* they are mostly cross shaped or butterfly shaped. In *Arthraxon, Cymbopogon, Eulaliopsis, Heteropogon* and *Hyparrhenia,* silica bodies are mostly dumb bell shaped. Still other forms of silica bodies like tall, narrow and rounded are found in *Saccharum*.

Conclusion

Results of the present investigation reveal that leaf epidermal characters show variations with in and among different genera. These inter-generic and intra-generic variations broaden the spectrum of available characters for systematic purposes and hence ensure more authentic identification. As exact identification of a taxon provide base for its detailed assessment for other useful works, therefore in systematic biology data from different fields are integrated to generate more accuracy concerning identification. Grasses of the higher altitudes in particular are mostly known morphologically only; additional characters such as that of leaf epidermis, leaf blade anatomy and palynology are required to be investigated for more reliable determination. Features of both the foliar epidermises are of substantial taxonomic value for further authentication, correct identification and classification of grasses. It is therefore suggested that morphological features must be supported with anatomical information especially when identifying taxonomically problematic taxa.

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