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LEAF MICROMORPHOLOGICAL STUDIES IN SUBFAMILY BAMBUSOIDEAE AND POOIDEAE FROM GUJARAT, INDIA

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ABSTRACT
Leaf is the most widely used vegetative organ for identification in plant taxonomy. Microscopic features such as epidermal cells, stomata, cuticle, surface contours, roughness and ornamentation are in use since the beginning of the last century. Information on foliar micromorphology can shed more light on structural features and their possible functional attributes. In the present study 14 species belongs 4 tribes has been studied from the vegetative characteristic features of the leaves. All the species has been studied for their variations at generic level and tried to utilize for preparation of artificial key. All the characters has been discussed at length and presented in a tabular form.

Key words: Aristideae, Arundineae, Bambuseae, Micromorphology, Oryzeae

INTRODUCTION
Family Poaceae is the most diverse in their morphological features and used since Linnaeus [16] for demarcating different genera and species. Early part of the 19th century anatomical as well as micromorphological characters are also utilized for segregating the lower taxonomic categories. Non reproductive organs are also used for identification and segregation and among them, of which leaf is the most widely used organ in plant taxonomy [18, 39]. Microscopic features such as roughness, venations, epidermal cells, stomata, cuticle, surface contours and ornamentation (hairs, papillae, trichomes) are in use since the work of Avdulov [4] and Prat [27, 28]. Information on foliar micromorphology can shed more light on structural features and their possible functional attributes. It is well established that foliar anatomy and epidermal features are very important in grass systematics and utilized for characterization of broad groups, within subfamilies and tribes [21, 22, 23, 24, 25, 30, 31, 32, 33, 34]. Initially, much of the literature based on the representative taxa of major groups. However, Hilu [8] observed species specific differences in leaf epidermises and suggested that micromorphological variations exist within the genus. Soon after, Clayton & Renvoize [5] used micromorphological features to solve the taxonomic problems. In recent years, anatomy and micromorphology of grasses has gained the importance due to their specificity at tribe, family, generic level. Most of the characters are further described and explained by numerous researchers; such as stomata, trichomes [18], microhairs [3, 42], silica cells [15, 29]. In recent years even micromorphological features of floral bracts of grasses i.e., lemma and palea [1, 37] has also been used to access systematic relationship. Watson & Dallwitz [47] have utilized data of abaxial leaf surfaces only at generic level. They have given the structural diversity as well as measurements of some epidermal characters including cells, nature of the walls, stomata, microhairs, silica bodies and papillae for abaxial leaf surfaces only. But, their voluminous work did not pay any attention to some epidermal ornamentation such as, prickles, hooks, macrohairs and margin, as these characters are helpful for identification of certain genera and species.

Perusal of literature suggests that there are very few reports on the tribe Bambuseae, Aristideae, Oryzeae and Arundineae from Indian subcontinents. Therefore, in the present

work we are making an attempt to explain the micromorphological characteristic pattern observed in 14 species from Gujarat region. By and large, characters which are described and utilized almost all the micromorphological features for these species. At the same time, these characters helped us to demarcate closely related species within the genera.

MATERIALS AND METHODS

All the taxa of were collected from the natural vegetation of Gujarat state, India. Details of the voucher specimens are deposited in BARO herbarium (Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India).
For micro morphological studies, the leaves from the middle of the culms were used throughout the preparation [9]. The epidermal peels were taken out by the standard method and stained it with the Saffranin and phenol to be mounted in glycerin [12]. Observations were carried out with the help of compound microscope. Figures of observed characters were drawn as well as photographed using Leica DME research microscope using ×40 objective. Measurements of all the epidermal components were done by standard micrometry. Terminology and classification for the epidermal cells are followed after Metcalfe [18]. Quantitative and qualitative results of micromorphological studies are summarized in a tabular format and presented. Measurements for cell constituents have been given comprehensively, whenever the major difference were noticed it is indicated as L for lower/abaxial and U for upper/adaxial epidermises.

RESULTS AND DISCUSSION

The epidermis of grasses is made up of cells of two distinct types. The larger cells are commonly referred as long cells because they elongate horizontally and are parallel with the long axis of the leaf [18]. These cells usually constituted slightly less than half of the total epidermal cells present. Long cells are frequently referred to as fundamental elements, undifferentiated cells, or ordinary epidermal cells [6]. In grasses, short cells produced cork-silica cell pairs, three types of prickles, macrohairs, bicellular trichomes, or stomata [17]. Short cells are products of asymmetric division of intercalary meristem cells [13] which gives rise to all cells except long cells.

Epidermal features of grass leaves are utilized well by Prat [27, 28], Tateoka *et al.* [42], Stebbins and Khush [39] and Jacques-Felix [11] with the help of light microscopy. Later, Palmer [20] described the grass cuticle is also well suited for diagnostic features. During the course of present investigations, authors could get the representative taxa of 4 tribes in natural vegetation and used for their micromorphological characterization with the help of light microscopy. Variations in following characters were observed and recorded:

a. Surface view of the epidermal peel depicts numerous types of intercostal long cells. Size, shape, orientation, nature of anticlinal and periclinal walls is proving to be useful for observation. Numerous variations as well as intermediate types are also noticed and utilized for reorganization purpose.
b. Adaxial surfaces contained many bulliform cells, a specialized type of long cell, which often appear in clusters of 5-15. In few members it is found to be present in the abaxial surface as well. These cells often are much larger than ordinary long cells and always with smooth walled hexagonal-cubical in shape. Sometimes impossible to distinguish from ordinary long cells in surface view.
c. Short cells usually find above the veins but intercostal short cells are also observed. It may be solitary or in pairs (cork-silica pairs) or in 3-5 cells in rows. The distribution of short cells silica bodies along with its shape are important for taxonomic consideration.

d. Macrohairs vary in length, thickness, rigidity, flexibility and bending pattern.
e. Microhairs: two major groups of microhairs are recorded; one celled & two celled. Here shape of the distal cell, their diameter and relative length with proximal cell are useful diagnostic feature.
f. Prickles may be small or large and are varying in shape and degree of infatuation such as angular, hook, unpointed, and interlocking.
g. Papillae are the ornamentation on epidermal long cells, sometimes found to be present on the subsidiary cells of stomata. Their size, shape and structure are also variable.
h. Shapes of the subsidiary cells are responsible for the shape of the stomata. It can be triangular and low-dome type. Mostly single type of stomata is recorded from the single plant or epidermis, but 2-3 are also noticed.

Tribe Bambuseae

Tribe has been reported in the works of Metcalfe [18], Palmer & Tucker [21] and Renvoize [35]. In their works generalized pattern was described, of which 2 are included in the present study, i.e. *Bambusa* and *Dendrocalamus*. The reference to *Bambusa* met with the work of Soderstrom & Ellis [38], and for *Dendrocalamus* Wu [48] and Wang *et al.* [45]. In Renvoize [35] has given the characteristic features of *D. membranaceous* and *B. burmanni*. Soderstrom and Ellis [38] observations for *Bambusa* are not matching with our observations for stomata, microhairs, prickles and hooks; as they have recorded in the present specimens on adaxial epidermises. Over & above, Wang *et al.* [45] described *Dendrocalamus* as amphistomatic but our specimens depict hypostomatic leaves. Recently Yang *et al.* [49] has utilized the papillae in foliar micromorphological characters for understanding the relationships between woody bamboos of Asian tropics. Our observations related to *Dendrocalamus* and *Bambusa* are in accordance with them, i.e. 4 papillae overarching the stomata in *Bambusa* and 8 in *Dendrocalamus* (Figure 1A-F; Table 1).

Tribe Oryzeae

Metcalfe [18] and Zhang et al. [50] has given the generalized pattern for the tribe and genera, while recent studies are based on silica bodies/phytolith for genus *Oryza* [26, 46]. Previous works of Nwokeocha [19] Palmer and Tucker [21] was on the leaf micromorphology of *O. punctata* only. Terrell *et al.* [43] described the spikelet micromorphology of *Oryza* only. On the basis of epidermal cell shape, papillae and microhairs both the genera can be segregated. In *Hygroryza* epidermal cells are uniform, mostly cubical, irregularly arranged with compactly encircled papillae on adaxial and hexagonal with regular arrangement in files on abaxial surfaces with uniformly scattered papillae. Microhairs mostly bicellular, chloris type observed intermittently (Figure 3 A-D). Earlier report of Tateoka [41] described absence of microhairs in *Hygroryza*. Prickles are found only on veins and of sporadic in nature. Silica cells are of crescent and oryza type on intercostals and costal zones respectively (Figure 2; Table 2). Whereas in *Oryza*, two types of epidermal cells i.e. rectangular and hexagonal were recorded on adaxial surface and only rectangular shape are recorded on abaxial surface are in accordance with Renvoize [32]. Papillae of various sizes are also noticed in 1-2 rows. Present observations for the papillae are differing from the report of Zhang *et al.* [50] wherein large papillae were not recorded from the epidermises of *O. latifolia*. Loudetia type of microhairs is noticed on both the surfaces. Small and pointed prickles present on the midvein while hooked on intercostals zone. Stomata are with triangular-low domed subsidiary cells in all the species. Mostly they are overarched by 4 papillae, but in the present record 6-8 papillae overarching the stomata were noticed in *O. latifolia*, and in *O. sativa* 6-8 were reported against 4 by Zhang *et al.* [50]. Silica cells are of oryza type i.e. vertical dumbbell. Nodular

type of silica cells and trichomes are noticed only from the epidermal peels of *O. latifolia.* Lower leaf surface of *O. rufipogon* and *O. sativa* depict the presence of 3 sizes of papillae (Figure 2 G-L; Table 2).

Typical silica short cell silicification did not differ due to leaf positions and sections, probably because these cells are predetermined to be silicified and the silica deposition occurred very early in leaf development [10]. Moreover, bulliform cells lose water more rapidly than ordinary long cells to facilitate leaf folding during periods of water stress and accumulate higher amount of silica than the other cells [36, 44]. Silicon which is taken up by the plant from the soil is beneficial as it improves pest and pathogen resistance, drought, heavy-metal tolerance, crop quality and yield of the plants. One function is that of providing support to the leaf other than that due to the presence of cellulose and lignin in the cell walls [14, 40]. This support function afforded by *Oryza*, in which silicified bulliform cells and dumbbell shaped short silica bodies found in the vertical alignment (Figure 2), in contrast to other taxa; and due to that leaves are more erect.

Tribe Arundineae

Tribe includes 2 genera viz. *Arundo* and *Phragmites*. Both are quite different from one another at epidermal long cells and microhair types. Previous reports for the tribe are found in Metcalfe [18], Palmer & Tucker [21, 22], Renvoize [34] and Ahmed [2]. In the Ahmed's report [2] microhairs are found in *Arundo* only, and absent in *Phragmites*, but during the present investigations arundo type of microhairs recorded in both the genera, which was also reported by Prat [28] (Figure 1 G-O; Table 1).

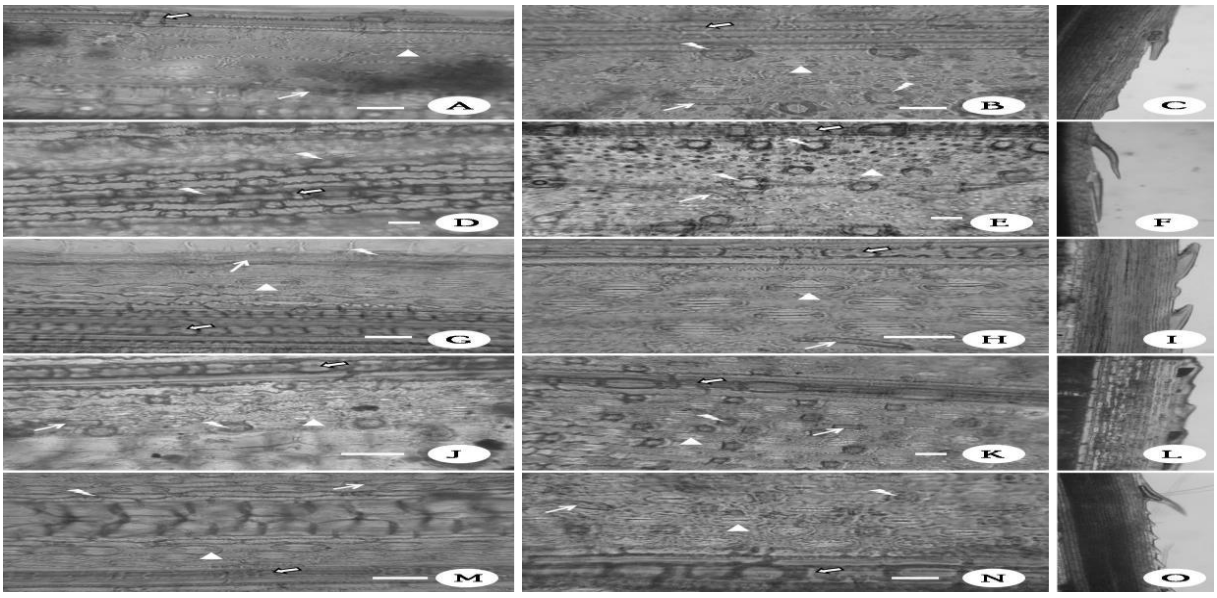

Figure 1: A-C *Bambusa arundinacea***; D-F** *Dendrocalamus strictus***; G-I** *Arundo donax***; J-K** *Phragmites australis***; M-O** *Phragmites karka.* A, F, J, M, P: Adaxial surface; B, G, K, N, Q: Abaxial surface; C, H, L, O, R: Margin (Bar=30 µ). (Sign in the figure indicate-Triangle= Stomata, White arrow= Microhairs, Black arrow= Papillae, Zigzag arrow= Prickles/hooks, Solid arrow with black outline= Silica cells)

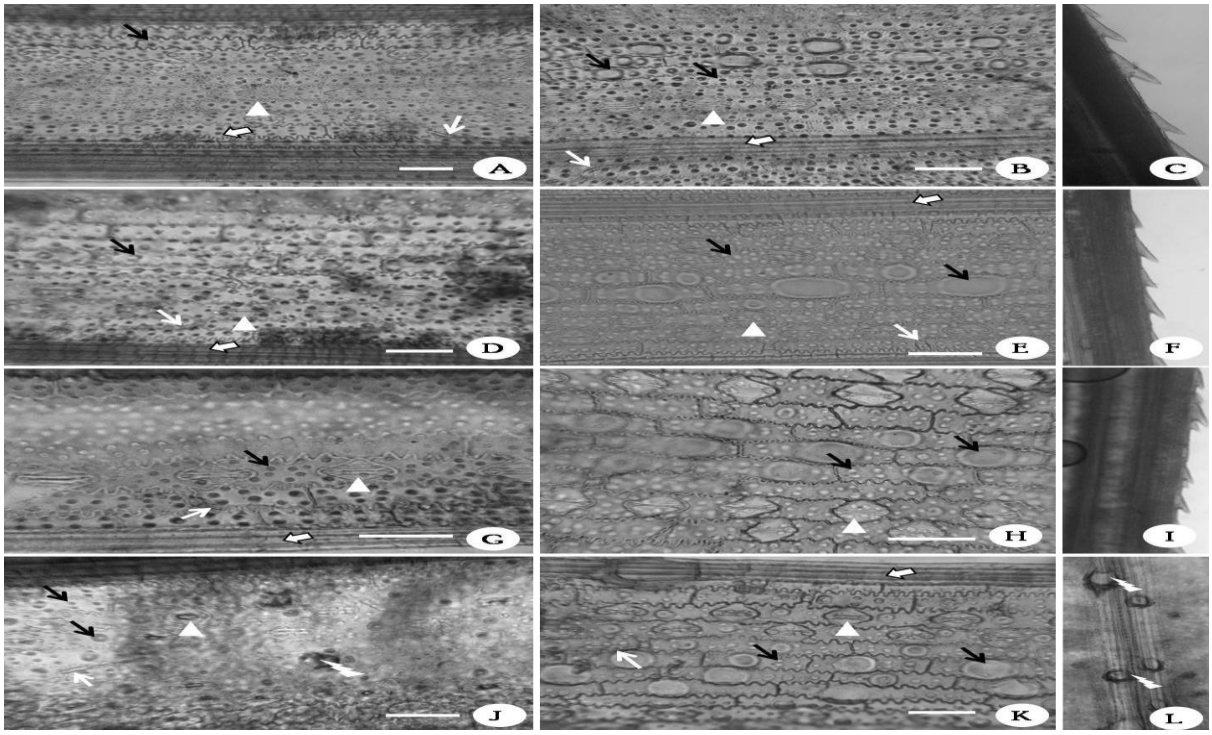

Figure 2: A-C *Oryza glaberrima***; D-F** *Oryza latifolia***; G-I** *Oryza rufipogon***; J-L** *Oryza sativa*. A, E, I, M, Q: Adaxial surface; B, F, J, N, R: Abaxial surface; C, G, L, P: Margin; D, S: Prickles; T: Prickle base; K, O: Silica bodies (Bar=30 µ)

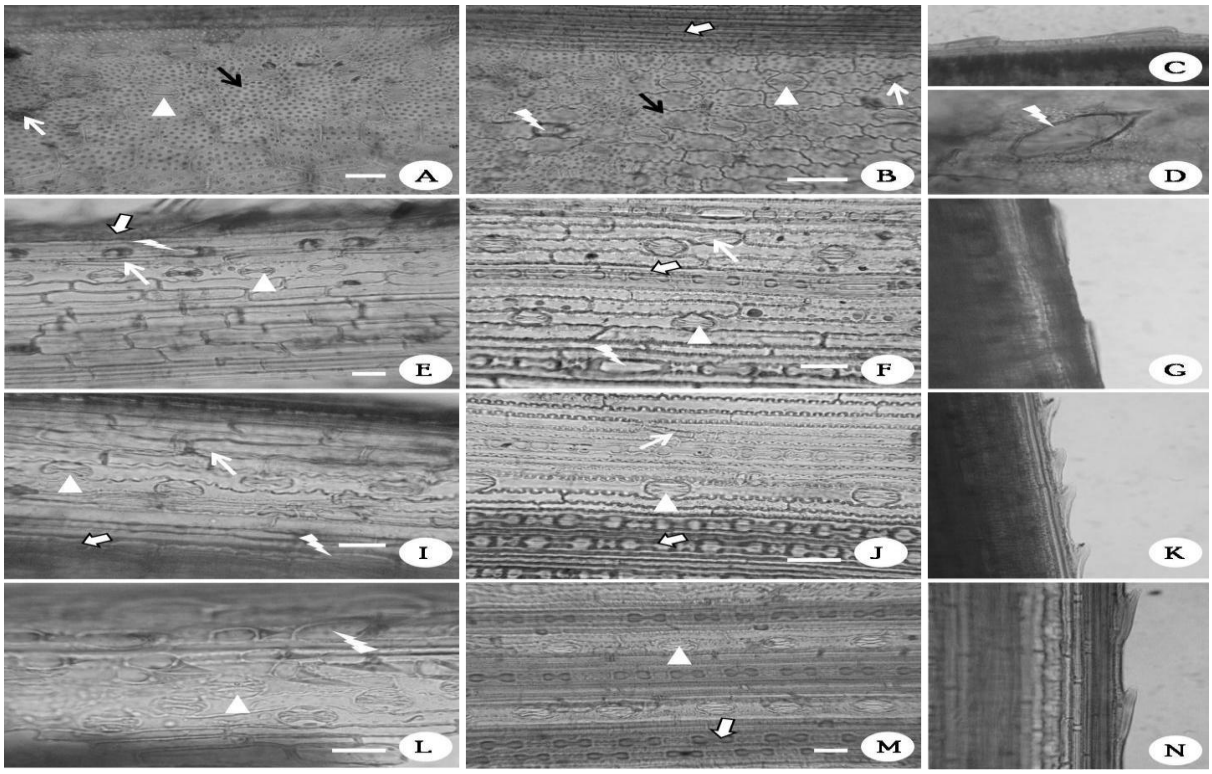

Figure 3: A-D *Hygrorhiza aristata***; E-G** *Aristida adscensionis***; I-K** *Atistida funiculata***; L-N** *Aristida setacea.* A, E, I, L: Adaxial surface; B, F, J, M: Abaxial surface; D, G, K, N: Margin; C, H: Prickles (Bar=30 µ)

Table 1: Foliar micromorphological features of Tribe Bambuseae and Arundineae

Plant name	***Bambusa arundinacea*** (Retz.) Willd.	***Dendrocalamus strictus*** (Roxb.) Nees	***Arundo donax*** L.	***Phragmites australis*** (Cav.) Trin. ex Steud.	***Phragmites karka*** (Retz.) Trin. ex Steud.
Long cell					
Size (µ)	75-120×16-22	70-100×13-17	70-180×6-18	74-160×10-18	68-172×6-15
Shape	Rectangular	Rectangular	Rectangular	Rectangular	Rectangular
Short cell / Silica cell					
Intercostal	L:Absent U:Elongate-saddle	Absent	Elliptical	Crescent	Crescent
Costal :Type & Size(µ)	Saddle 7-15×15-23	Saddle 7-15×15-23	Dumbbell 15-22×12-18	Saddle 12-20×4-14	Saddle 12-20×4-14
Si-Cr pairs	Present	Present	Absent	Absent	Absent
Prickle					
Costal(µ)	L:35-50×20-30	30-100×17-35	L:17-32×16-20	L: 36-50×18-24	L:30-40×16-20
Intercostal(µ)	35-50×20-30	30-40×15-20	Absent	Absent	Absent
Hooks	Absent	Absent	U:Present	Present	Present
Macrohair	Absent	Absent	Absent	Absent	Absent
Microhair					
No. of cells	Bicellular	L:Bicellular	Bicellular	Bicellular	Bicellular
Type	Bambusoid	Bambusoid	Arundo	Arundo	Arundo
size (µ)	200-270×8-10	180-240×7-10	48-55×5-8	24-28×4-5	20-22×3-4
Papillae/cell	L: 4-10,1-2 row	L:3-7, 1-row	Absent	Absent	Absent
Stomatal complex					
Subsidiary cell type	Triangular-low dome	L:Triangular-low dome	Triangular-low dome	Low dome-triangular	Low dome-triangular
Size(µ)	30-40×20-35	26-35×14-17	28-36×15-22	32-35×14-22	30-35× 10-20
Papillae overarching the stoma	Covered by 4 papillae(each in 2 lobe)	Covered by 8 finger like papillae	Absent	Absent	Absent
Margin	Long angular prickled	Long angular prickled	Angular prickled	Short angular prickled	Long and short angular prickled

Table 2: Foliar micromorphological features of Tribe Oryzeae

Plant name	***Hygrorhiza aristate*** (Retz.) Nees ex W.&A.	***Oryza glaberrima*** Steud.	***Oryza latifolia*** Desv.	***Oryza rufipogon*** Griff.	***Oryza sativa*** L.
Long cell					
Size (µ)	21-35×15-30	30-80×14-18	30-50×12-15	35-45×12-15	30-50×15-20
Shape	L:Rectangular U:Hexagonal	Rectangular	Rectangular	Rectangular	Rectangular
Short cell / Silica cell					
Intercostal	Tall narrow	Absent	Absent	Absent	Absent
Costal :Type & Size(µ)	Oryza 9-11×16-25	Oryza 10-12×20-28	Oryza-Nodular 6-10×12-16	Oryza 6-10×10-14	Oryza 6-10×10-14
Si-Cr pairs	Absent	Absent	Absent	Absent	Absent
Prickle					
Costal(µ)	L: Absent U: 40-70×30-35	70-85×24-32	L: 72-80×20-28 U:Absent	L: Absent U: 40-50×14-20	40-55×12-18
Intercostal(µ)	Absent	U:9-11×16-25	Absent	20-24×10-12	22-26×10-12
Hooks	Absent	Present	Present	Present	Present
Macrohair	Absent	Absent	U:Present	Absent	Absent
Microhair					
No. of cells	Biicellular	Bicellular	Bicellular	Bicellular	Bicellular
Type	Chloris	Loudtia	Loudtia	Loudtia	Loudtia
Size (µ)	6-8×2-4	30-35×6-9	30-35×6-9	32-35×3-5	32-35×3-5
Papillae/cell	Numerous L: Aggregated	Numerous L: Variable size	Numerous L: Variable size	Numerous L: Variable size	Numerous L: Variable size
Stomatal complex					
Subsidiary cell type	Triangular	Triangular	Triangular	Triangular	Triangular
Size(µ)	20-26×15-20	15-20×12-15	15-20×12-15	21-24×18-20	18-22×14-18
Papillae overarching the stoma	Absent	6	6-8	4-6	4
Margin	Angular prickled	Angular prickled	Angular prickled	Angular prickled	Angular prickled

Table 3: Foliar micromorphological features of Tribe Aristideae

Plant name	***Aristida adscensionis*** L.	***Atistida funiculata*** Trin. & Rupr.	***Aristida setacea*** Retz.
Long cell			
Size (µ)	90-140×8-15	120-200×10-16	80-120×6-12
Shape	Rectangular	Rectangular	Rectangular
Short cell / Silica cell			
Intercostal	Absent	Crescent-Elongate	Elongate
Costal : Type & Size(µ)	Dumbbell 16-27×7-10	Dumbbell 18-30×7-12	Dumbbell 14-22×6-10
Si-Cr pairs	Absent	Present	Absent
Prickle			
Costal(µ)	L:50-56×10-12 U:45-70×12-20	L:Absent U:58-80×15-20	L:Absent U:58-90×15-25
Intercostal(µ)	Absent	Absent	Absent
Hooks	Absent	Absent	Absent
Microhair			
No. of cells	Bicellular	Bicellular	Bicellular
Type	Loudetia	Loudetia	Loudetia
Size (µ)	17-20×5-7	19-21×3-5	15-18×4-6
Macrohair	Absent	Absent	Absent
Papillae /cell	Absent	Absent	Absent
Stomatal complex			
Subsidiary cell type	Triangular- low dome	Triangular- low dome	Triangular- low dome
Size(µ)	25-30×18-20	28-32×18-22	30-36×20-24
Papillae overarching the stoma	Absent	Absent	Absent
Margin	Small angular prickled	Small angular prickled	Small angular prickled

Tribe Aristideae

Previously it has been worked out by Metcalfe [18], Tateoka *et al.* [42], Palmer *et al.* [25] Renvoize [33] and Ahmed [2]. During the present study only loudetia type of microhairs, low dome-triangular stomata and dumbbell shaped silica bodies were recorded in all the 3 studied species of *Aristida* (Figure 3 E-N; Table 3). From the present work only *A. adscensionis* had been earlier studied by Gill and Mehash [7] and Ahmed [2], rest other is studied for the first time. In recent years, usage of micromorphological characters has gain lot of importance not only in taxonomic studies but even in molecular taxonomic studies. Because of the necessity of numerous data sets micromorphological data is profoundly used in demarcating and assimilating different genera from one another. Therefore, according to us the presently described micromorphological characters will be quite useful for further studies. By and large, qualitative and quantitative micromorphological characters have been utilized to prepare a key to segregate different species of the genera and different genera of tribe.

Subfamily: Bambusoideae Asch. & Graeb.
Tribe: Bambuseae Nees.
1. Papillae over arching stomata are 8 *Bambusa arundinacea*
1. Papillae overarching stomata are 4 *Dendrocalamus strictus*

Tribe: Oryzeae Dumort.
1. Microhairs unicellular, papillae numerous & scattered *Hygrorhiza aristata*
1. Microhairs bicellular, papillae of variable sizes & 1-2 rows
2. Costal prickles only on single epidermis
3. Costal prickles only on upper epidermis *Oryza rufipogon*
3. Costal prickles only on lower epidermis *Oryza latifolia*
2. Costal prickles on both epidermises
4. Papillae overarching stomata 6 *Oryza glaberrima*
4. Papillae overarching stomata 4 *Oryza sativa*

Subfamily: Pooideae Macfarlane & Watson
Tribe: Arundineae Dumort.
1. Costal silica cells Dumbbell shaped *Arundo donax*
1. Costal silica cells Saddle shaped
2. Margin with only short angular prickles *Phragmites australis*
2. Margin with long and short angular prickles *Phragmites karka*

Tribe: Aristideae Hubb.
1. Costal prickles present on both epidermises, Intercostal silica cells absent *Aristida adscensionis*
1. Costal prickles present only on upper epidermis, Intercostal silica cells present
2. Silica-Cork pairs present *Atistida funiculata*
2. Silica-Cork pairs absent *Aristida setacea*

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