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Role of Systematic Foliar Micro-Morphology in Indentification of Grasses

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ABSTRACT

The grass family is one of the largest of all plant families. Extensive survey and micro-morphological study was conducted to explore the diversity and generate a documentation of the family Poaceae (grasses) in north eastern parts of India.The region forms a significant segment of both the Himalayan and the Indo-Burma biodiversity hotspots.Although, floral morphological characters are noteworthy in the identification of all levels of taxonomic ranks as families, tribes, genera, species etc.; but members of the family Poaceae do not flower for a greater part of their life span; therefore foliar epidermal studies can be valuable and worthy in their categorization, completing their systematic position and illustrating their phylogenetic relationship among various taxa. This study can be considered subsequent to cytological enumerations. Systematic variation of foliar epidermis with the aid of Scanning Electron Microscope (SEM) pertains to the epidermal cell size, stomatal types, shape of silica bodies, micro-hairs, macro-hairs, subsidiary cells, prickles, hooks, papillae, short and long cells etc. on abaxial surface. Twenty (20) species of family Poaceae collected from the different localities of north east India were examined under Scanning Electron Microscope (SEM) for detailed micro-morphological variations. The species under investigation were selected on the basis of rarity in distribution in north east India. The present paper encompasses a detailed micro-morphological study along with illustrated key and vivid photographs to delimit allied species unfeasible to separate morphologically and proves to be supportive in determining the taxonomic as well as phylogenetic position of intimately related and complicated taxa.

Key words: Foliar, Identification, Grasses, Morphology, Systematic.

INTRODUCTION

Morphological characters play significant role in the identification of all levels of taxonomic ranks as families, tribes, genera, species etc. The family Poaceae (Gramineae) commonly known as 'grasses', comprises about 700 genera, 11,000 species distributed worldwide (Ullah et al., 2015; Chen & Peterson, 2006; Christenhusz & Byng, 2016). The foliar epidermal studies are important for categorization, delimitation and sorting out the evolutionary and phylogenetic problems in plants (Stace, 1984; Chaudhri et al., 2014; Bibi, 2015). Grasses do not flower for a greater part of their life span, therefore foliar epidermal studies are useful in completing their systematic positions and phylogenetic relationships among various taxa (Ortunez, 2010;Yanis, 2010; Khan, 2017; Yan et al., 2018) and secondly it is significant after cytological studies (Goel et. al., 2011; Nazir et al., 2013; Ullah et al., 2015). Heywood (1971) drew attention towards the microscopic studies in concluding systematic disparity among closely related and alike species (Metcalfe, 1960; Stebbins & Khush, 1961; Chaudharietal., 2014).

Stomatal characters are vital to delineate the diverse taxa in terms of phylogenetic and taxonomic considerations (Shaikh & Potdar, 2018; Soreng et al., 2015). Angiosperm leaves are the most suited and preferable for this purpose (Watson, 1990). Information on foliar micro-morphology can shed more light on structural features and their possible functional

attributes (Perveen, 2006; Desai et al., 2013). Several authors recognized the importance of these stomatal features in recognizing the different taxa (Tateoka, 1959; Stebbins & Khush, 1961; Amarasinghe, 1988). It is well established that foliar anatomy and epidermal features are very important in grass systematics and has been utilized for characterization of broad groups, within sub-families and tribes (Redmann, 1985). Furthermore, the study is imperative to correlate between taxa and intimately associated genera (Vicari, 2002; Wu & Ge, 2012).

The foliar epidermis of grasses is constructed of two distinct types of cells; the long, rectangular cells with blunt ends arranged parallel with the long axis of the leaf and elongate horizontally, constitute slightly less than half of the total epidermal cells and are frequently referred as 'fundamental elements', 'undifferentiated cells', 'ordinary epidermal cells' or simply the 'long cells' (Ellis, 1979). Besides, the other type of cells are the 'short cells' which are cube to rectangular in shape with blunt ends formed as the product of asymmetric division of intercalary meristem which give rise to all cells except the long cells (Kaufman, 1970). The long and short cells together constitute the 'costal (C)' and 'inter costal (IC)' region (Acedo, 2001). These cells produce cork-silica cell (Sb), three types of prickles (Pr), macro hairs (Ma), which may be unicellular or 2-celled with basal cell (Bc) and apical cell (Ac), micro hairs (Mi), stomata (St) with prominent to blur subsidiary cells (Sc). Scanning Electron Microscopic(SEM) studies related to the morphology of the abaxial surface of the leaves of 20 species collected from the different areas of north east India has been worked out.

MATERIAL AND METHODS

North East India (262,179 km² area including 8 states) forms a significant part of both the Himalayan and the Indo-Burma biodiversity hotspots. SEM studies of the abaxial surface of the leaves of 20 species were carried out. The species were selected on the basis of their scarcity of distribution in this study area along with those species having difficulty in identification with allied taxa. All the samples were taken from live specimens collected during the survey and are housed at CAL herbarium. Collection and preservation of specimens were done following Smith (1971), Jain and Rao (1977), Van Balgooy (1987), Rao and Sharma (1990) and Singh & Subramaniam (2008). Specimens were morphologically studied under Olympus SZ2-ILST Microscope' and 'Nikon Stereo Zoom Microscope' and live photographs were taken with 'Canon Power Shot SX50HS (50X optical zoom) camera'. Literature consultation were done following standard Floras, monographs and revisions (Bor 1940, 1960; Sreekumar and Nair 1991; Shukla 1996; Moulik 1997; Veldkamp 1999; Kabeer and Nair 2009), studying authentic herbarium specimens housed at ASSAM, ARUN, CAL (herbaria acronyms according to Thiers 2015) and matching with digital images of specimens including type materials available at different herbarium databases (https://science.mnhn.fr, http://apps.kew.org/herbcat,https://plants.jstor.org,http://linneanonline.org/linnaean_herbarium.html). For SEM study, the specimens were mounted on 'metallic stubs' with the help of 'double coated scotch tape' and then dried in the 'desiccator' without the use of any chemicals (Jeffree & Read (1991); Hartola, 2015; Butterfield 2017; Mohammed & Abdullah, 2018). The stubs were coated with gold ion in the 'Gold Coater' (Karnovsky, 1965; Baghaei, 2007; Murphy , 2010; Butterfield, 2017) and scanned at different magnifications in the 'HITACHI-S530 Scanning Electron Microscope, Japan', housed in the University of Burdwan.

RESULT

Characterization of the abaxial epidermis of leaf:

1. *Briza media:* Costal (C) and inter costal (IC) region distinct; IC region formed of thick walled, long cylindrical to tubular cells; prickle (Pr) 2- celled, basal cell (Bc) cylindrical, apical cell (Ac) acute, distributed only in the IC region; stomata (St) not frequent. *S. Pathak* 48474 (CAL). (Pl.1. figs. A & B).
2. *Bromus catharticus:* Costal (C) and inter costal (IC) region distinct; IC zone formed of tubular, cylindrical cells; silica bodies (Sb) rectangular, arranged in parallel rows; macro

hairs (Ma) unicellular, base of the hairs round to bilobed; stomata (St) scarce. *S. Pathak* 48570 (CAL). (Pl. 1. figs. C & D).

3. *Calamagrostis emodensis:* Costal (C) and inter costal (IC) region indistinct; prickles (Pr) present, bicellular, basal cell (Bc) cylindrical, apical cell (Ac) sharply acute, arranged in rows in the costal region; stomata (St) scanty. *S. Pathak* 48584 (CAL) (Pl. 1. figs. E & F).
4. *Capillipedium parviflorum* (R. Br.) Stapf: Costal (C) and inter costal (IC) region almost indistinct; tubular, hypha-like deposition seen; silica bodies (Sb) globular,arranged irregularly all over the surface; stomata (St) arranged in rows; subsidiary cells(Sc) distinct, lanceolate with blunt ends. *S. Pathak* 48566 (CAL). (Pl. 1. figs. G & H).
5. *Cenchrus prieurii* (Kunth) Mair: Costal (C) and inter costal (IC) region distinct; ICregion made of narrowly, cylindrical cells with wavy walls; silica bodies (Sb) obscure; prickles (Pr) sharp, present at the edge of the IC region; stomata (St) shrouded deepwithin the costal region; guard cells swollen, helps in reducing transpiration; subsidiarycells (Sc) unclear. *S. Pathak* 48521 (CAL) (Pl. 1. figs. I & J).
6. *Cynodon barberi* Rang. & Tadul.: Costal (C) and inter costal (IC) region distinct;silica bodies (Sb) prominently butterfly shaped; macro hairs (Ma) unicellular, present inthe costal region; stomata (St) present in 2 to 3 rows in the costal area; subsidiary cells(Sc) indistinct. *S. Pathak* 48543 (CAL) (Pl. 2. figs. A & B).
7. *Enteropogon dolichostachyus* (Lag.) Keng: Costal (C) and inter costal (IC) regionalmost distinguishable; silica bodies (Sb) numerous, prominently clubb shaped, present throughout the entire surface; prickles (Pr) unicellular, frequent, present at the edges ofinter costal zone; micro (Mi) and macro hairs (Ma) not seen; stomata (St) deep seatedwithin the silica bodies, exhibiting xerophytic character. *S. Pathak* 48504 (CAL). (Pl. 2. figs. C & D).
8. *Eulalia quadrinervis* (Hack.) Kuntze: Costal (C) and inter costal (IC) region indistinct;inter costal (IC) region made of rectangular cells, arranged in rows with prominentlysinuous wall; silica bodies (Sb) prominent, ovoid to globose, scattered individually or inclusters of 2 to 4; stomata (St) arranged in rows separated by 1 to 2 epidermal cells;subsidiary cells (Sc) distinct. *S. Pathak* 48554 (CAL). (Pl. 2. figs. E & F).
9. *Holcus lanatus: Costal* (C) and inter costal (IC) region prominent; silica bodies (Sb) prominent, ovoid, restricted within the inter costal region; macro hairs (Ma) present, having prominently bulbous base and curved to hooked apices, distribution restricted in the costal region; stomata (St) arranged in rows in the costal area, separated by a singleepidermal cell; subsidiary cells (Sc) distinct. *S. Pathak* 48474 (CAL). (Pl. 2. figs. G & H).
10. *Iseilema anthephoroides Hack.:* Costal (C) and inter costal (IC) region distinct; cells of C area cylindrical, walls almost sinuous; silica bodies (Sb) markedly globose,arranged in rows in the costal area, also noticed to be present at both ends of stomata;micro hairs (Mi) bicellular, present costally; stomata (St) arranged in rows in the costalarea, separated by a single epidermal; cell; subsidiary cells (Sc) distinct. *S. Pathak* 48471 (CAL). (Pl. 2. figs. I & J).
11. *Leptochloa chinensis* (L.) Nees: Costal (C) and inter costal (IC) region distinct; ICregion narrow, cells of the this area are regular, cylindrical, walls definitely sinuous;silica bodies (Sb) clubb shaped, irregularly scattered generally in the costal area;prickles (Pr) sharp, found at the edge of IC region; stomata (St) arranged in a single rowin the costal area; subsidiary cells (Sc) distinct. *S. Pathak* 48421 (CAL). (Pl. 3. figs. A & B).
12. *Leptochloa panicea* (Retz.) Ohwi: Costal (C) and inter costal (IC) region markedlydistinct; silica bodies (Sb) prominently butterfly shaped, arranged in rows in the intercostal region; prickles (Pr) present, minute, with sharp pointed apices, in the inter costalregion; macro hairs (Ma) present, unicellular; stomata (St) present in 2 to 3 rows

in thecostal area; subsidiary cells (Sc) indistinct. *S. Pathak* 48409 (CAL). (Pl. 3. figs. C & D).

13. *Miscanthus nepalensis* (Trin.) Hack.: Costal (C) and inter costal (IC) region almostdistinct; cells of the costal region cylindrical, rectangular; silica bodies (Sb) distinctlyirregular shaped, scattered; stomata (St) present along the edge of IC region; subsidiarycells (Sc) indistinct. *S. Pathak* 48401 (CAL). (Pl. 3. figs. E & F).

14. *Ophiuros exaltatus* (L.) Kuntze: Costal (C) and inter costal (IC) region notprominently distinct; silica bodies (Sb) few in number, butterfly shaped, arranged inrows in the IC region; stomata (St) present in rows in the costal area; subsidiary cells(Sc) distinct. *S. Pathak* 48454 (CAL). (Pl. 3. figs. G & H).

15. *Phalaris arundinacea* L.: Costal (C) and inter costal (IC) region distinct; silica bodies(Sb) minute, irregular, restricted in IC zone; prickles (Pr) present, unicellular, arrangedin rows in the IC region, apex sharp pointed, base prominently forked; stomata (St)scarce. *S. Pathak* 48470 (CAL). (Pl. 3. figs. I & J).

16. *Sacciolepis indica* (L.) Chase: Costal (C) and inter costal (IC) region markedly distinct;IC region narrow, cells with wavy walls; silica bodies (Sb) faintly butterfly shaped,arranged in rows in the costal region; prickles (Pr) present, unicellular with curved tipin the IC region; stomata (St) present costal area; subsidiary cells (Sc) linear. *S. Pathak* 48522 (CAL). (Pl. 4. figs. A & B).

17. *Schizachyrium delavayi* (Hack.) Bor: Costal (C) and inter costal (IC) region faintlyseparable; cells cylindrical, with prominent undulated wall; silica bodies (Sb) butterflyshaped, arranged in rows in the costal region; prickles (Pr) present, 2- celled, basal cell(Bc) cylindrical, terminal cell (Tc) with acute, sharp apex; stomata (St) present, withindistinct subsidiary cells (Sc). *S. Pathak* 48454 (CAL). (Pl. 4. figs. C & D).

18. *Sporobolus coromandelianus* (Retz.) Kunth: Costal (C) and inter costal (IC) regionmarkedly distinguishable; prominent tubular structures present at the junction of C andIC region; silica bodies (Sb) irregular, scattered; hairs and prickles (Pr) not seen;stomata (St) present in rows of 1 to 2 in the IC zone; subsidiary cells (Sc) distinct. *S. Pathak* 48412 (CAL). (Pl. 4. figs. E & F).

19. *Stenotaphrum dimidiatum* (L.) Brongn.: Costal (C) and inter costal (IC) regionmarkedly distinguishable; C region made of square to rectangular rows of cells; ICnarrow, made of irregular to rectangular cells; silica bodies (Sb) irregular, scattered;hairs and prickles (Pr) not seen; stomata (St) present in costal area; subsidiary cells (Sc) distinct. *S. Pathak* 48432 (CAL). (Pl. 4. figs. G & H).

20. *Vulpia bromoides* (L.) Gray: Costal (C) and inter costal (IC) region distinct; IC madeof rectangular cells with prominently undulated wall; silica bodies (Sb) irregular, scattered in IC; prickles (Pr) present at the edge of IC, with sharp, curved apices; threadlike deposition seen all over the entire surface; stomata (St) scarce. *S. Pathak* 48424 (CAL). (Pl. 4. figs. I & J).

11. *Leptochloa chinensis* (L.) Nees: Costal (C) and inter costal (IC) region distinct; ICregion narrow, cells of the this area are regular, cylindrical, walls definitely sinuous;silica bodies (Sb) clubb shaped, irregularly scattered generally in the costal area;prickles (Pr) sharp, found at the edge of IC region; stomata (St) arranged in a single rowin the costal area; subsidiary cells (Sc) distinct. *S. Pathak* 48421 (CAL). (Pl. 3. figs. A & B).

12. *Leptochloa panicea* (Retz.) Ohwi: Costal (C) and inter costal (IC) region markedlydistinct; silica bodies (Sb) prominently butterfly shaped, arranged in rows in the intercostal region; prickles (Pr) present, minute, with sharp pointed apices, in the inter costalregion; macro hairs (Ma) present, unicellular; stomata (St) present in 2 to 3 rows in thecostal area; subsidiary cells (Sc) indistinct. *S. Pathak* 48409 (CAL). (Pl. 3. figs. C & D).

13. *Miscanthus nepalensis* (Trin.) Hack.: Costal (C) and inter costal (IC) region almostdistinct; cells of the costal region cylindrical, rectangular; silica bodies (Sb) distinctlyirregular shaped, scattered; stomata (St) present along the edge of IC region; subsidiarycells (Sc) indistinct. *S. Pathak* 48401 (CAL). (Pl. 3. figs. E & F).

14. *Ophiuros exaltatus* (L.) Kuntze: Costal (C) and inter costal (IC) region notprominently distinct; silica bodies (Sb) few in number, butterfly shaped, arranged inrows in the IC region; stomata (St) present in rows in the costal area; subsidiary cells(Sc) distinct. *S. Pathak* 48454 (CAL). (Pl. 3. figs. G & H).

15. *Phalaris arundinacea* L.: Costal (C) and inter costal (IC) region distinct; silica bodies(Sb) minute, irregular, restricted in IC zone; prickles (Pr) present, unicellular, arrangedin rows in the IC region, apex sharp pointed, base prominently forked; stomata (St)scarce. *S. Pathak* 48470 (CAL). (Pl. 3. figs. I & J).

16. *Sacciolepis indica* (L.) Chase: Costal (C) and inter costal (IC) region markedly distinct;IC region narrow, cells with wavy walls; silica bodies (Sb) faintly butterfly shaped,arranged in rows in the costal region; prickles (Pr) present, unicellular with curved tipin the IC region; stomata (St) present costal area; subsidiary cells (Sc) linear. *S. Pathak* 48522 (CAL). (Pl. 4. figs. A & B).

17. *Schizachyrium delavayi* (Hack.) Bor: Costal (C) and inter costal (IC) region faintlyseparable; cells cylindrical, with prominent undulated wall; silica bodies (Sb) butterflyshaped, arranged in rows in the costal region; prickles (Pr) present, 2- celled, basal cell(Bc) cylindrical, terminal cell (Tc) with acute, sharp apex; stomata (St) present, withindistinct subsidiary cells (Sc). *S. Pathak* 48454 (CAL). (Pl. 4. figs. C & D).

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19. *Stenotaphrum dimidiatum* (L.) Brongn.: Costal (C) and inter costal (IC) regionmarkedly distinguishable; C region made of square to rectangular rows of cells; ICnarrow, made of irregular to rectangular cells; silica bodies (Sb) irregular, scattered;hairs and prickles (Pr) not seen; stomata (St) present in costal area; subsidiary cells (Sc) distinct. *S. Pathak* 48432 (CAL). (Pl. 4. figs. G & H).

20. *Vulpia bromoides* (L.) Gray: Costal (C) and inter costal (IC) region distinct; IC madeof rectangular cells with prominently undulated wall; silica bodies (Sb) irregular, scattered in IC; prickles (Pr) present at the edge of IC, with sharp, curved apices; threadlike deposition seen all over the entire surface; stomata (St) scarce. *S. Pathak* 48424 (CAL). (Pl. 4. figs. I & J).

KEY TO THE SPECIES BASED ON SEM CHARACTERS

1a. Costal and intercostals region distinct 2

1b. Costal and intercostals region indistinct 15

2a. Prickles present 3

2b. Prickles absent 4

3a. Prickle unicellular ..... *Enteropogon dolichostachyus*

3b. Prickle 2- celled ..... *Briza media*

4a. Silica bodies prominent 5

4b. Silica bodies obscure ..... *Cenchrus prieurii*

5a. Silica bodies ovoid, rectangular to irregular, scattered, rarely in rows 6

5b. Silica bodies butterfly shaped, arranged in rows 14

6a. Marco hairs present, unicellular 7

6b. Marco hairs absent 9

7a. Base of macro hair bilobed ..... *Bromus catharticus*

7b. Base of macro hair not lobed 8

8a. Base of the macro hair prominently bulbous .....*Holcus lanatus*

8b. Base of the macro hair not bulbous .....*Cynodon barberi*

9a. Thread like deposition present all over the entire surface.....*Vulpia bromoides*

9b. Thread like deposition absent 10

10a. Walls of the cells of the costal or inter costal regions definitely sinuous.....11

10b. Walls of the cells of the costal or inter costal regions straight.....5

11a. Silica bodies markedly present at both tips of the stomata.....*Iseilema anthephoroides*

11b. Silica bodies present scattered irregularly.....12

12a. Silica bodies globose to club shaped .....*Leptochloa chinensis*

12b. Silica bodies irregular in shape .....*Miscanthus nepalensis*

13a. Tubular cells present at the junction of costal and inter costal region..... .....*Sporobolus coromandelianus*

13b. Tubular cells absent ..... *Stenotaphrum dimidiatum*

14a. Subsidiary cells distinct ..... *Ophiuros exaltatus*

14b. Subsidiary cells indistinct ..... *Leptochloa panicea*

15a. Tubular, network like deposition present in the entire surface..... *Capillipedium parviflorum*

15b. Tubular, network like deposition absent 16

16a. Walls of the cells of the costal or inter costal regions definitely sinuous.....17

16b. Walls of the cells of the costal or inter costal regions straight.....19

17a. Silica bodies found in clusters..... .....*Eulalia quadrinervis*

17b. Silica bodies individually present 18

18a. Prickles unicellular ..... *Sacciolepis indica*

18b. Prickles 2- celled ....*Schizachyrium delavayi*

19a. Base of prickle prominently forked ....*Phalaris arundinacea*

19b. Base of prickle not forked..... .....*Calamagrostis emodensis*

DISCUSSION

Grasses are considered one of the major prevalent plant type which plays a precious role as source of food and energy for all sorts of wildlife and organics. Leaves of grasses have been analyzed traditionally under optical microscope. But recently the use of SEM in the study of leaf epidermal surface sturdily supports their taxonomic implications, which can portray structures more visibly, revealing distinct aspects of critical features. The resent investigation is aimed at studying their foliar morphology under SEM which exposed several interesting facts as, the morphological characters of the stomata of each species under analysis, which are vital to correlate and demarcate among the different taxa in terms of phylogenetic and taxonomic point of view when reproductive features are unavailable. The epidermal cells, their wall traits, nature and distribution of macro and micro hairs, along with stomatal features are suitably applied here which have separated the species, effectively and accurately, applying which a key has been prepared. Furthermore, the study contributed in thriving and lucratively establishing a relationship among distant taxa and closely associated ones, facilitating the identification of the species and its derivatives.

CONCLUSION

Consequently, it can be evidently accomplished that, such exercise of examination of foliar stomata should be considered helpful in determining the taxonomic as well as phylogenetic position of intimately related and complicated taxa. The pragmatic micro-morphological characters proved to be vital in identification and delimitation of closely related taxa which has been segregated with the aid of 'taxonomic keys'. Furthermore, the study was very helpful in detection and classification of taxa at the species levels where reproductive features lacked. Consequently, it can be evidently accomplished that, such exercise of examination of foliar micro-morphological features should be considered supportive in determining the taxonomic as well as phylogenetic position of intimately related and complicated taxa.

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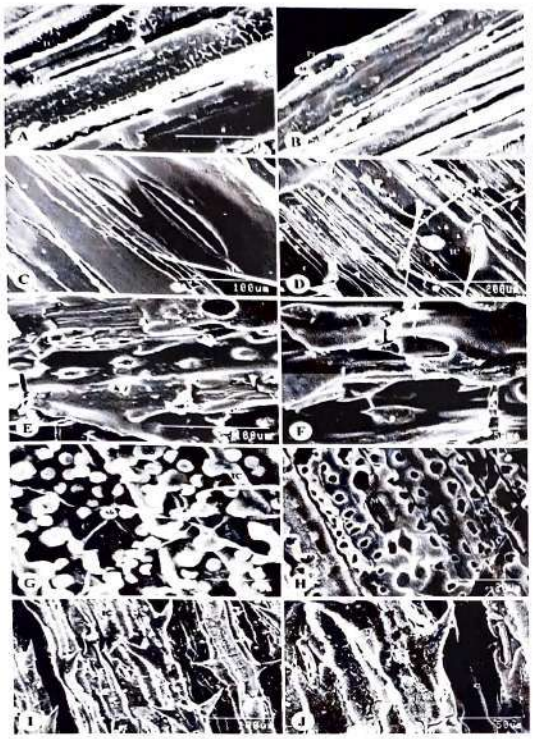

Plate 1. Scanning Electron Micrographs of abaxial leaf surfaces. A & B: *Briza media*; C & D: *Bromus catharticus*; E & F: *Calamagrostis emodensis*; G & H: *Capillipedium parviflorum*; I & J: Cenchrus prieurii. [Costal region (C); Inter costal region (IC); Silica body (Sb); Micro hairs (Mi); Macro hairs (Ma); Prickle (Pr); Basal cell (Bc); Apical cell (Ac); Stomata (St)].

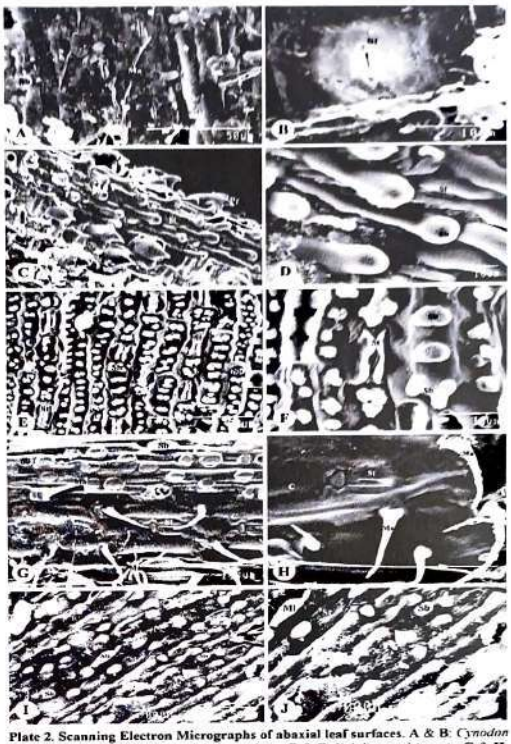

Plate 2. Scanning Electron Micrographs of abaxial leaf surfaces. A & B: *Cynodon barberi*; C & D: *Enteropogon dolichostachyus*; E & F: *Eulalia quadrinervis*; G & H: *Holcus lanatus*; I & J: *Iseilema anthephoroides*. [Costal region (C), Inter costal region (IC); Silica body (Sb), Micro hairs (Mi); Macro hairs (Ma); Prickle (Pr); Basal cell (Bc), Apical cell (Ac); Stomata (St)].

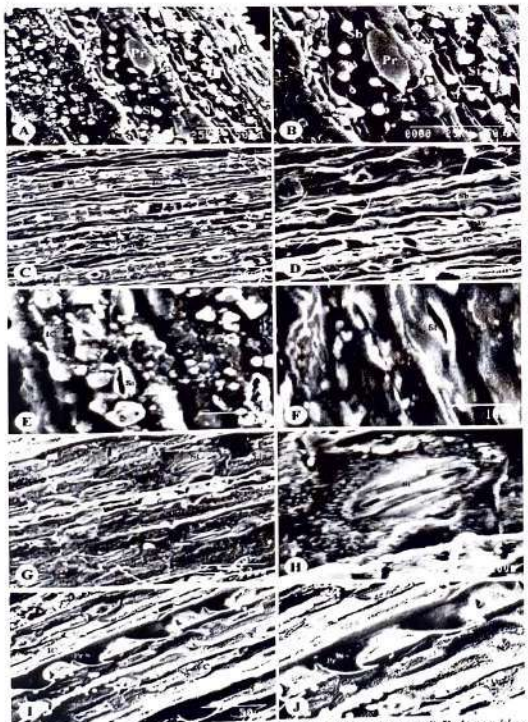

Plate 3 Scanning Electron Micrographs of abaxial leaf surfaces. A & B: *Leptochloa chinensis*; C & D: *Leptochloa panicea*; E & F: *Miscanthus nepalensis*; G & H: *Ophiuros exaltatus*; I & J: *Phalaris arundinacea*. [Costal region (C); Inter costal region (IC), Silica body (Sb), Micro hairs (Mi), Macro hairs (Ma); Prickle (Pr); Basal cell (Bc); Apical cell (Ac); Stomata (St)].

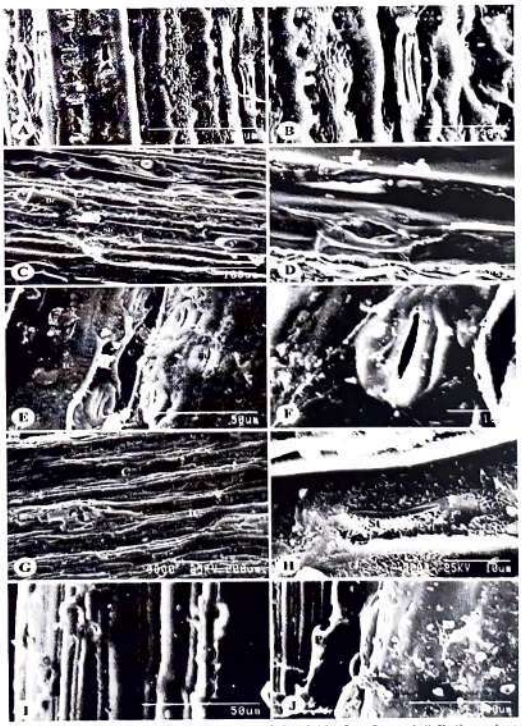

Plate 4. Scanning Electron Micrographs of abaxial leaf surfaces. A & B: *Saccolepis indica*; C & D: *Schizachyrium delavayi*; E & F: *Sporobolus coromandelianus*; G & H *Stenotaphrum dimidiatum*; I & J: *Vulpia bromoides*. [Costal region (C), Inter costa region (IC); Silica body (Sb); Micro hairs (Mi); Macro hairs (Ma); Prickle (Pr); Basa cell (Bc), Apical cell (Ac); Stomata (St)].