Chelonian Conservation And Biology

Vol. 19 No. 1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2024.01(1).126.139

MICROMORPHOLOGICAL STUDY OF GLUME DIVERSITY OF CYPERACEAE IN WESTERN HIMALAYAN REGION OF PAKISTAN

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Abstract:

Cyperaceae is cosmopolitan in distribution and widely distributed in western Himalayan regions of Pakistan, but limited studies have been conducted on identification & classification of family at both molecular and morphological perspective. In this study western Himalayan region of Pakistan were explored to collect species previously not studied and submitted to Pakistan Museum of Natural History for future use. 20 species were selected from collection and different quantitative & qualitative parameters were studied. Eight micromorphological features of specimens that includes glume shape, glume length, glume width, presence or absence of arista, glume color, midrib width, midrib color and detailed surface of glume; of these specimens were observed under light microscope and scanning electron microscope. These species showed 12 types of glume shape and great variation in color of both glume and midrib. Similarly, length and width of glume has wide range, and two types (Narrow, Broad) of midrib were observed in this study. Arista was present in most of species and were absent in only few species. On basis of this study, it can be said that glume morphology can be great help in correct differentiation of morphological identical species that looks similar but distinct. These results can play major role in identification of said region species at morphological level.

1 Introduction:

The Cyperaceae is third largest monocot plant family. after Orchidaceae and Poaceae, having around 90 genera and 5500 species and eleventh large angiosperm family which has cosmopolitan distribution (Semmouri et al., 2019). Member of this family are particularly prevalent in moist, rainy or marshy areas of temperate and subarctic zone, where vegetation can be dominated by these species (Heywood, Brummitt, Culham, & Seberg, 2007). By temporarily holding water on slopes and preserving basin water balance, they aid in reducing mountain overflow. Because of their extensive root systems and exceptional capacity to bind soil particles together, they prevent soil erosion. Sedges serve as indicators of specific habitat and wetlands because they thrive in specific pH and salinity levels (Tande & Lipkin, 2003). Some bird species use the fruits and leaves of sedges as food and nesting materials (MacLeod, Blackwell, Moller, Innes, & Powlesland, 2008). Sedges are most easily identified by their trigonous stem and small, primarily wind-pollinated flowers. Despite being monoecious in the majority, flowers in some of the species can also be both unisexual or bisexual (Xu & Zhou, 2017). The ovule, nut, achene, stamen, carpels, and glumes are some of the more typical components of the flower

The identification of Cyperaceae taxa is challenging because of the diminished structure of their flowers and fruits and the remarkably consistent morphological characteristics of their vegetative organs. For taxonomic classification of the family at both generic and specific levels, the microscopic cellular structure has shown to be helpful (Schuyler, 1971). The taxonomic problems among the species can be mostly resolved by using contemporary taxonomical methodologies (Vogler APMonaghan, 2007). By using scanning electron microscope (SEM) and other microscopic techniques, the study of the floral components at the micro-morphological level facilitates the comparative study of the species through visual observations (Friis, Crane, & Pedersen, 2011). The variety and variability in how the floral elements are arranged are better understood using SEM. The scanning electron microscope is especially helpful because it makes it possible to examine cellular detail in tissues that would otherwise be challenging to prepare and observe with a light microscope (Schuyler, 1971). Given the micro-morphological studies of the family, the glume, a membranous to leathery scale-like structure encompassing individual flowers in sedges, is especially significant for differentiating cyperaceae species that appear extremely similar on morphological basis. Considering all the Cyperaceae species, the glume structure exhibits a wide range of variation at microscopic level.

In the western Himalayan region of Pakistan, there are 200 Cyperaceae species which are distributed in a wide range of habitats, from low-lying areas to the highest inhabited altitudes, including both dry and wet environments (Kukkonen, 2001). There has been no comparative investigation of micro-morphological parts among Pakistani species. Distinguishing sedges based on glume structure can be useful in resolving sedge identification disputes. The study's main objective was to assess the diversity of the glumes of the chosen Cyperaceae species.

2 Material and Methods:

2.1 Sample Collection and Tagging:

Plant specimens were collected from Pakistan's lowlands and northern regions. The position of each of these specimens was recorded and was later used to GPS tag them. The samples were brought to the Plant Systematic and Evolution Laboratory at the Atta-ur-Rahman School of Applied Biosciences, National University of Science and Technology Islamabad, Pakistan. In the same facility, each sample was meticulously conserved and mounted on herbarium sheets. These sheets were then taken to the Pakistan Museum of Natural History in Islamabad for taxon identification numbers. The flora of Pakistan was used to identify each of the samples based on their morphology and molecular data (Kukkonen, 2001). Specimens are listed in Table 1 along with voucher number and GPS readings.

2.2 Light and Scanning Microscopy:

The collected specimens were studied micromorphologically using a stereomicroscope of IRMECO (Model: IM-900, 21943 Schwarzenbek / Germany). The spikelet was dissected with forceps and a needle to study the inflorescence. Glume was observed under 10X lens and micrographs were taken using ISCapture software equipped with Tucsen camera. (Fig 1 & 2). For SEM analysis sample was placed on copper stub with help of double side carbon tape. These samples were sputter-coated of 10nm gold and studied by using JEOL JSM 6490A scanning electron microscope. The SEM analysis was performed partly at NUST and in a lab of University of California, Davis (Fig 3, 4 & 5)

2.3 Qualitative and Quantitative Analysis:

Five qualitative parameters that were observed in this study include glume color, glume shape, presence of arista, midrib width, midrib color under stereomicroscope and detailed surface of glume through SEM. Two quantitative parameters included in this study were length and width of glume. ImageJ software (Rasband, 1997) was used to record these measurements and their standard deviations. Terminologies for glume shape were adapted from study of (Gaglioti, Severin, & Wooller, 2010; Nobis, 2013; Nobis, Klichowska, Nowak, Gudkova, & Rola, 2016).

Table 1. Taxon table containing the location of species along with voucher numbers.

Sr. No.	Taxa	Voucher number	Locality	Gps readings
1	*Bolboschoenus maritimus*	PMNH 00046228	Kurram	33°54′29"N-70°05′12"E
2	*Carex canescens*	PMNH 043883	Mansehra	34°19′54"N-73°11′52"E

3	*Carex distans*	PMNH 00046448	Nagar	36°14′26"N-74°23′52"E
4	*Carex nubigena*	PMNH 043873	Abbottabad	34°10′10"N-73°13′17"E
5	*Carex pseudobicolor*	PMNH 00046447	Nagar	36°14′26"N-74°23′52"E
6	*Carex pseudolaxa*	PMNH 00046449	Nagar	36°16′40"N-74°43′10"E
7	*Carex remota*	PMNH 043866	Abbottabad	34°10′10"N-73°13′17"E
8	*Carex satigera*	PMNH-043862	Abbottabad	34°10′10"N-73°13′17"E
9	*Carex stenocarpa*	PMNH 00046450	Nagar	36°14′26"N-74°23′52"E
10	*Carex viridula*	PMNH 00046229	Parachinar	33°54′08"N-70°02′59"E
11	*Cyperus alopecuroides*	PMNH 042232	Kallar kahar	32°46′17"N-72°42′19"E
12	*Cyperus difformis*	PMNH 043869	Islamabad	33°40′06"N-73°02′42"E
13	*Cyperus flavidus/Pycreus flavidus*	PMNH 042312	Wah Gardens	33°48′09"N-72°41′55"E
14	*Cyperus iria*	PMNH 042315	Khanpur	33°48′16"N-72°55′37"E
15	*Cyperus niveus*	PMNH 041726	Islamabad	33°38′33"N-72°59′33"E
16	*Fimbristylis bisumbellata*	PMNH 042319	Khanpur	33°48′16"N-72°55′37"E
17	*Fimbristylis litoralis*	PMNH 044051	Mirpur	33°02′08"N-73°38′52"E

18	*Kyllinga brevifolia*	PMNH 042316	Haripur	33°59′40"N-72°54′38"E
19	*Schoenoplectus juncoides*	PMNH 042322	Haripur	33°59′44"N-72°54′34"E
20	*Schoenoplectus litoralis*	PMNH 042233	Kallar kahar	32°46′17"N 72°42′19"E

3 Results and Discussion:

Morphological characters of glume are of great importance in correct identification of species. In this study 20 species were collected from different regions of Pakistan for morphological study of glume. These species showed great diversity in context of shape, presence of arista, glume length and width. The micrographs of glume taken through stereomicroscope are illustrated in Figure 1 and 2. Glume of some species were also observed under scanning electron microscope and these micrographs are illustrated in Figure 3. To observe the surface of glumes samples were studied under scanning electron microscope and micrographs are illustrated in Figure 4 and 5. The qualitative and quantitative readings are summarized in Table 2.

Species showed great variety in glume color under stereomicroscope. *Bolboschoenus maritimus* has bright orangish brown, *Carex canescens* has light green, *Carex distans* has whitish, *Carex nubigena* has white with brown lines, *Carex pseudobicolor* has greenish brown, *Carex pseudolaxa* has yellowish green, *Carex remota* has light greenish, *Carex satigera* reddish brown with light green boundary, *Carex stenocarpa* has brownish green, *Carex viridula* has pale brown to reddish brown, *Cyperus alopecuroides* has dull green with dark green stripes and margins yellowish or pale, *Cyperus difformis* and *Schoenoplectus juncoides* has green brownish, *Cyperus flavidus*/*Pycreus flavidus* has yellowish brown with reddish margins, *Cyperus iria* has yellowish brown, *Cyperus niveus* has white green at base and brown at tips, *Fimbristylis bisumbellata* has brown, *Fimbristylis litoralis* has chestnut brown, *Kyllinga brevifolia* has yellowish with brown lines, and *Schoenoplectus litoralis* has brownish shade (Table 2).

Shape of glume ranges from ovate, ovate keeled, ovate membranous, barely ovate & acute, oblong ovate, oblong, obovate, Cymbiform, deeply Cymbiform, Cymbiform acute with scarious sides, keeled, and acute with eroded margins. Detail of every species is mentioned in Table 2.

Length of glume ranges from 0.57mm to 6.77mm and glume width from 0.42mm to 4.10mm. *Cyperus difformis* had smallest length and width, while *Bolboschoenus maritimus* had largest glume length while *Carex pseudolaxa* had largest width in species observed in this study.

Talking about presence or absence of arista, 13 species out of 20 had arista and remaining 7 do not possess arista. Midrib of glume has two varieties either narrow or broad. 14 species had

narrow midrib and other 6 had broad type. All species have wide variety in color of midrib that is mentioned in Table 2.

Morphological studies done in past were mostly focused on achene, perigynium and leaf morphology (Hoffmann & Gebauer, 2016; Jimenez-Mejias, Benitez-Benitez, Fernandez-Mazuecos, & Martin-Bravo, 2017; Jin et al., 2014; LAMIAA & Gazer, 2015; Larridon et al., 2011; Molina, Chung, & Hipp, 2015; Semmouri et al., 2019; Vrijdaghs, Goetghebeur, Smets, & Muasya, 2006; Zhang, 2004). Most recently, a study was done by Semmouri et al., in which embryo morphology was used to know the phylogeny and evolution of Cyperaceae (Semmouri et al., 2019). There are not more than one or two published studies reported in which glume/pistillate scales were studied in context of diversity. A study was done by (Strack, Zahid, Hipp, Rabia, & Hayat, 2019) to know the diversity of pistillate scales on basis of micromorphological and molecular inferences. Present study thus focused on possible use of glume diversity for identification of Cyperaceae species. The species observed in this study showed great degree of variation that can be a potential source for identification purpose.

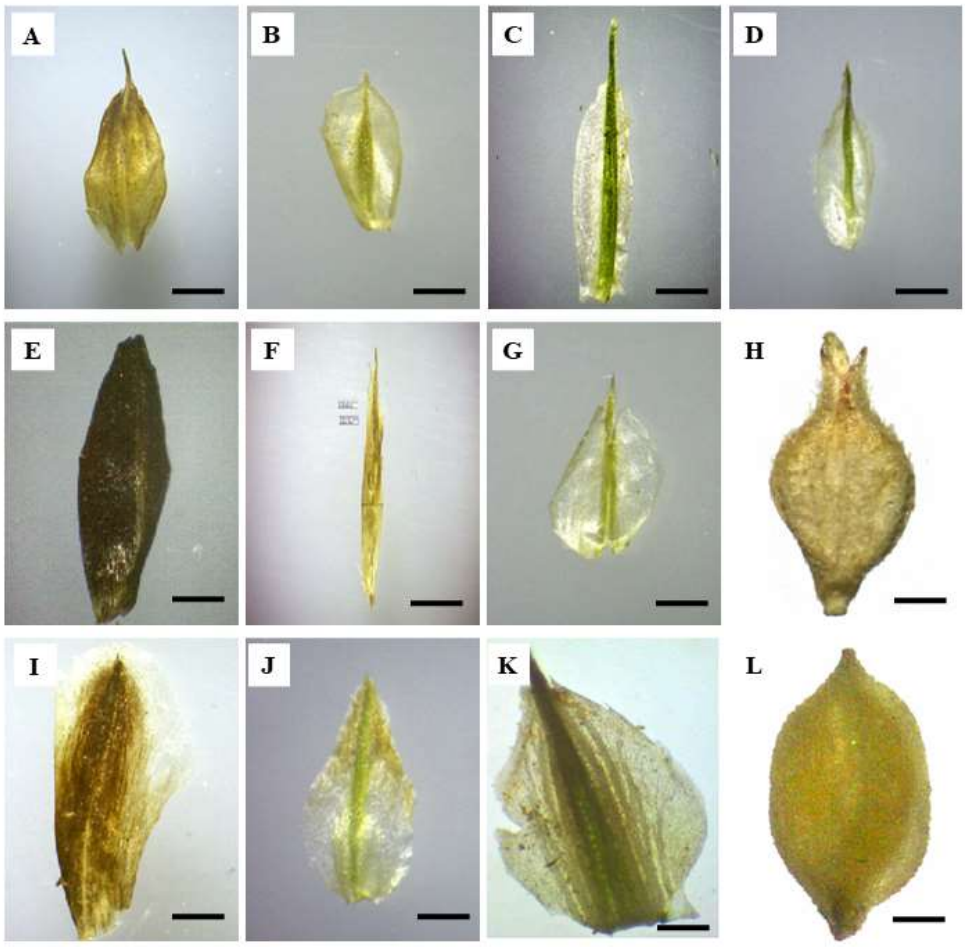

Figure 1: Micrographs of different species observed under light microscope. A: *Bolboschoenus maritimus*, B: *Carex canescens*, C: *Carex distans*, D: *Carex nubigena*, E: *Carex*

pseudobicolor, F: *Carex pseudolaxa*, G: *Carex remota*, H: *Carex satigera*, I: *Carex stenocarpa*, J: *Carex viridula*, K: *Cyperus alopecuroides*, L: *Cyperus difformis*. Scale bar: 5µm (A-L).

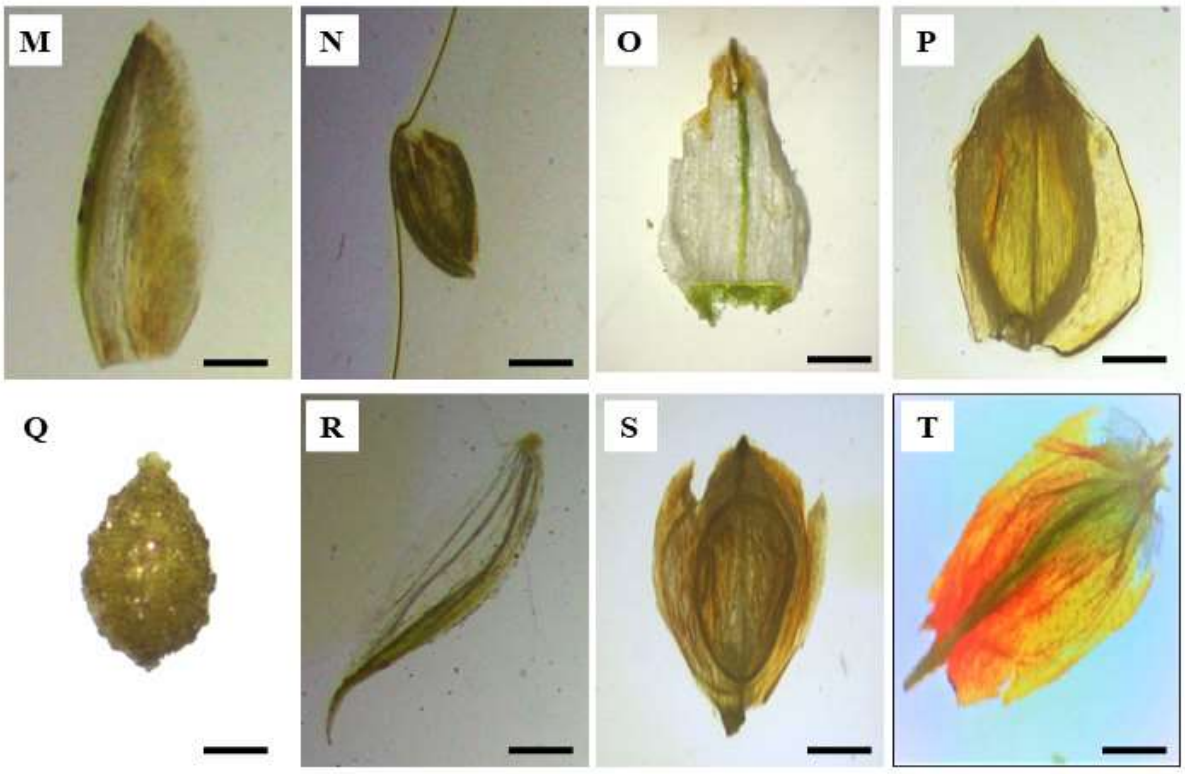

Figure 2: Micrographs of different species observed under light microscope. M: *Cyperus flavidus*/*Pycreus flavidus*, N: *Cyperus iria*, O: *Cyperus niveus*, P: *Fimbristylis bisumbellata*, Q: *Fimbristylis litoralis*, R: *Kyllinga brevifolia*, S: *Schoenoplectus juncoides*, T: *Schoenoplectus litoralis*. Scale bar: 5µm (M-T).

Table 2. Qualitative and Quantitative parameters of species using light microscopy.

Sr. No.	Taxa	Glume color	Shape	Arista	Length (mm)	Width (mm)	Width of midrib	Midrib color
1	*Bolboschoenus maritimus*	Bright orangish brown	Oblong ovate	+	6.77	3.5	Narrow	Yellowish brown
2	*Carex canescens*	Light green	Cymbiform, acute,	+	1.88	1.12	Broad	Dark green

			sides scarious					
3	*Carex distans*	Whitish	Ovate	+	4.5	2.1	Broad	Green
4	*Carex nubigena*	White with brown lines	Ovate	+	2.8	1.8	Narrow	Dark Green
5	*Carex pseudobicolor*	Greenish brown	Cymbiform	-	1.8	0.9	Broad	Light green
6	*Carex pseudolaxa*	Yellowish green	Oblong	+	5.5	4.1	Narrow	Light greenish
7	*Carex remota*	Light greenish	Acute, margins eroded	+	1.94	0.79	Narrow	Dark Green midrib
8	*Carex satigera*	Reddish brown with light green boundary	Barely ovate and acute	-	3.63	1.31	Broad	Dark green
9	*Carex stenocarpa*	Brownish green	Obovate	+	4.4	2.3	Narrow	Light green
10	*Carex viridula*	Pale brown to reddish brown	Ovate	-	3.22	1.77	Narrow	Green
11	*Cyperus alopecuroides*	Dull green, with dark green stripes, margins yellowish or pale	Cymbiform	+	2.3	0.8	Broad	Dark green

12	*Cyperus difformis*	Green brownish	Deeply Cymbiform	-	0.57	0.42	Narrow	Dark greenish brown
13	*Cyperus flavidus/Pycreus flavidus*	Yellowish brown with reddish margins	Cymbiform	-	2.04	0.61	Broad	Dark green with reddish tint
14	*Cyperus iria*	Yellowish brown	Ovate	-	1.40	0.83	Narrow	Reddish
15	*Cyperus niveus*	White, green at the base, brown at tips	Keeled	-	3.64	1.23	Narrow	Green mid nerve
16	*Fimbristylis bisumbellata*	Brown	Ovate, keeled	+	2.55	1	Narrow	Green
17	*Fimbristylis litoralis*	Chestnut brown	Ovate and membranous	+	1.453	0.9	Narrow	Green
18	*Kyllinga brevifolia*	yellowish with brown lines	Keeled	+	2.56	0.69	Narrow	Dark green
19	*Schoenoplectus juncoides*	Green brownish	Cymbiform	+	3.28	2.31	Narrow	Greenish
20	*Schoenoplectus litoralis*	Brownish	Cymbiform	+	3.7	1.1	Narrow	Green
	*- Absent, + Present							

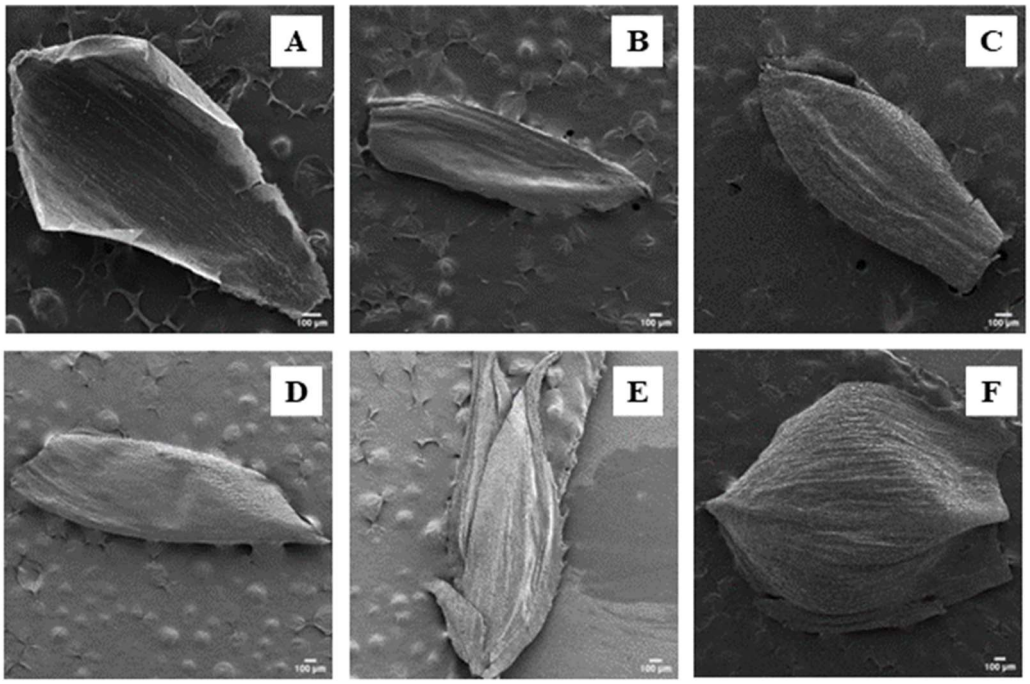

Figure 3: SEM Micrographs of Different Species. A: *Carex remota*, B: *Cyperus flavidus*, C: *Cyperus iria*, D: *Fimbristylis bisumbellata*, E: *Kyllinga brevifolia*, F: *Schoenoplectus juncoides*. Scale bar: 100µm (A-F).

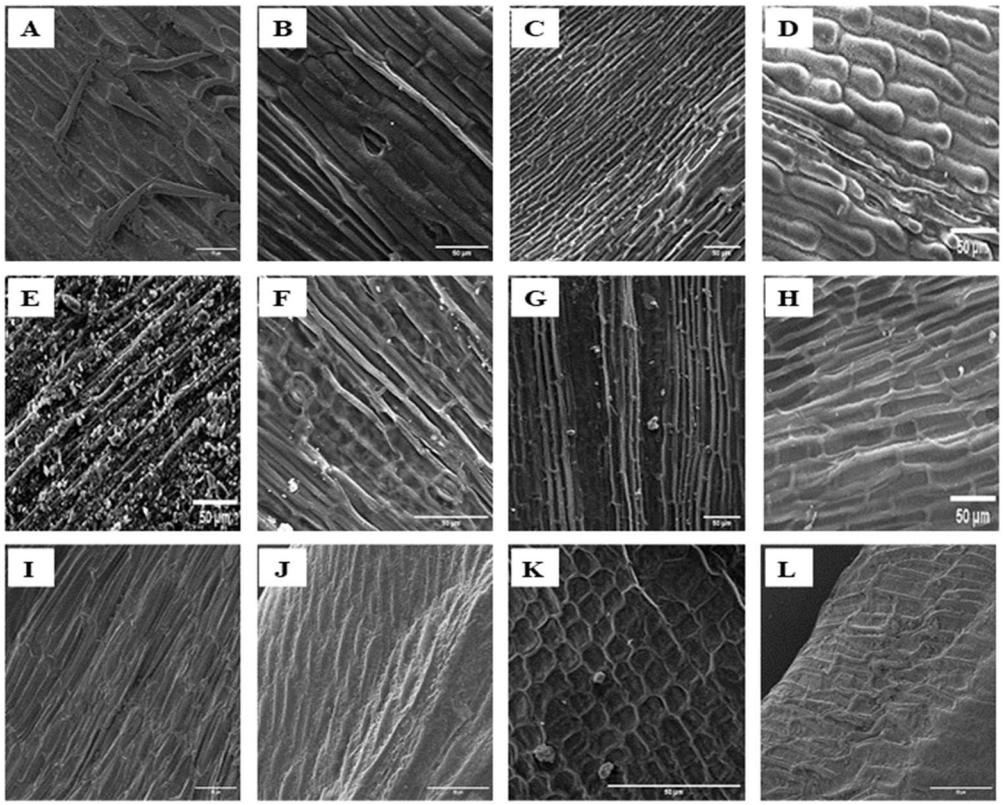

Figure 4: SEM Micrographs of Glume Surface. A: *Bolboschoenus maritimus*, B: *Carex canescens*, C: *Carex nubigena*, D: *Carex pseudobicolor*, E: *Carex pseudolaxa*, F: *Carex remota*, G: *Carex satigera*, H: *Carex stenocarpa*, I: *Carex viridula*, J: *Cyperus alopecuroides*, K: *Cyperus difformis*, L: *Cyperus flavidus*. Scale bar:50µm (A-L).

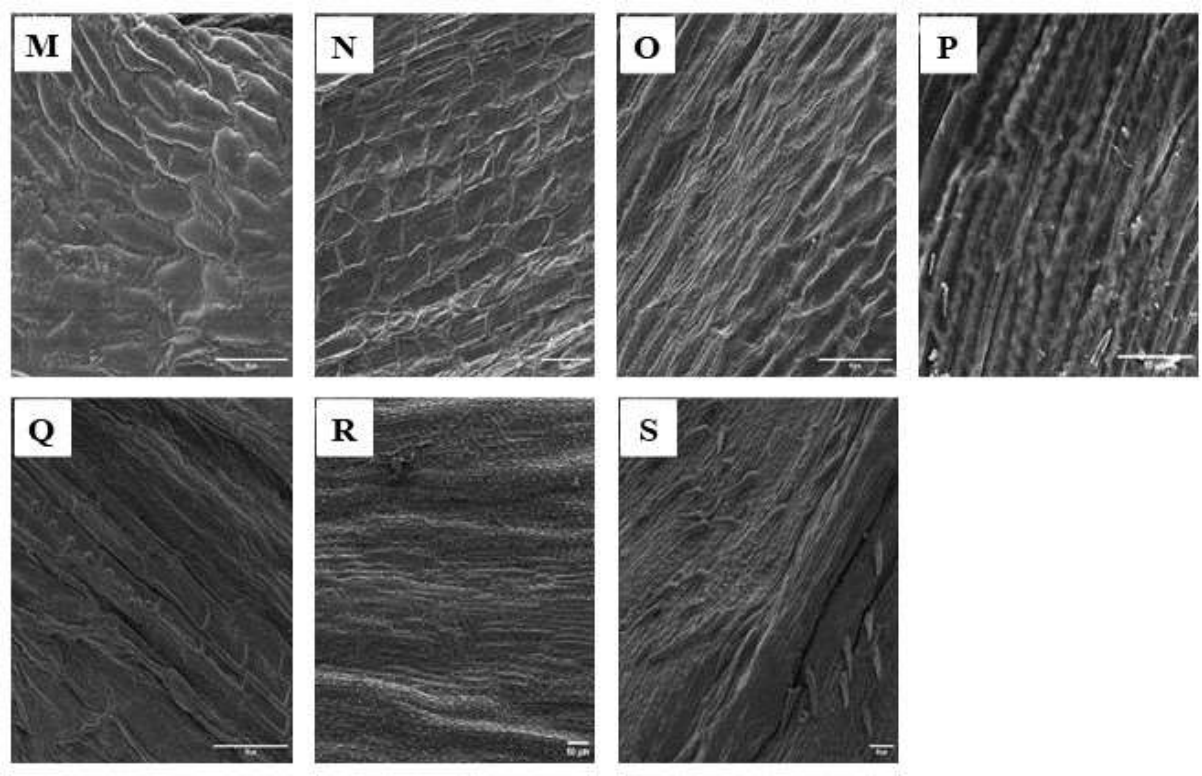

Figure 5: SEM Micrographs of Glume Surface. M: *Cyperus iria*, N: *Cyperus niveus*, O: *Fimbristylis bisumbellata*, P: *Fimbristylis litoralis*, Q: *Kyllinga brevifolia*, R: *Schoenoplectus juncoides*, S: *Schoenoplectus litoralis*. Scale bar:50µm (M-S).

Table 3. Glume characters observed under Scanning Electron Microscope

Serial.no.	Taxa	Scale surface	Hyaline margins	Cell type of epidermis
1	*Bolboschoenus maritimus*	Prickles, hooks	-	Elongated interlocking rectangular cells
2	*Carex canescens*	Trichomes	+	Rectangular interlocking cells
3	*Carex nubigena*	Smooth	+	Elongated rectangular cells

4	*Carex pseudobicolor*	Hooks	-	Interlocking cells
5	*Carex pseudolaxa*	Trichomes, papillae	-	Elongated cells
6	*Carex remota*	Trichomes	+	Elongated rectangular cells
7	*Carex satigera*	Trichomes	+	Elongated rectangular cells
8	*Carex stenocarpa*	Trichomes	-	Elongated rectangular cells
9	*Carex viridula*	Trichomes, papillae	+	Elongated interlocking rectangular cells
10	*Cyperus alopecuroides*	Papillae, trichomes,	+	Rectangular interlocking cells
11	*Cyperus difformis*	Papillae	+	Elongated interlocking rectangular cells
12	*Cyperus flavidus/Pycreus flavidus*	Trichomes, papillae	-	Small interlocking rectangular cells
13	*Cyperus iria*	Papillae	-	Small interlocking somewhat rectangular cells
14	*Cyperus niveus*	Papillae	+	Small square and rectangular cells
15	*Fimbristylis bisumbellata*	Prickles, hooks, papillae	+	Elongated interlocking rectangular cells
16	*Fimbristylis litoralis*	Trichomes, papillae	-	Elongated rectangular cells
17	*Kyllinga brevifolia*	Hooks, trichomes	+	Rectangular cells
18	*Schoenoplectus juncoides*	Hooks, trichomes	+	Elongated interlocking rectangular cells

19	*Schoenoplectus litoralis*	Trichomes, papillae	-	Elongated rectangular cells

*- Absent, + Present

4 Research Highlights/Conclusion:

Cyperaceae species observed in this study has huge diversity in shape, length, width, and midrib width & color. This study had explored new morphology data that will help in identification of Cyperaceae in specified region and our study can be leading in further research of glume diversity in Cyperaceae for accurate identification.

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