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Original Article

Taxonomic implication of nut diversity in selected Cyperaceae species of Pakistan

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Abstract

Cyperaceae, commonly known as a sedge family, is widely distributed and is cosmopolitan, it is the third largest among the monocots. It has great ecological and ethnobotanical value but the taxonomic classification of the family at both morphological and molecular level is not much studied. The species resemble a lot and hence are very difficult to differentiate without proper identification. For the taxonomic implication of nut (one of the important parts of inflorescence) in plant identification, sixteen different sedge species were collected from various parts of the country. They were analyzed under stereo and scanning electron microscope to understand the differences among nut characters which help to differentiate among species of the same family. The three most important characteristics considered were Nut shape, size and color. All the species showed variation enabling identification easily. The surface of nut was also observed under the scanning electron microscope and showed great variation. Principal Component Analysis (PCA) was performed to check the impact of characters on the grouping of the species. Based on the results of this study, it can be said that nuts play a very important role in the identification and classification of the plant on a morphological and micromorphological basis.

Keywords: Sedges, Nut, Scanning Electron Microscopy, Stereomicroscopy, Micromorphology, PCA, UPGMA

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Introduction

Cyperaceae (commonly known as the sedge family) the third-largest monocot and seventh-largest angiosperm family having a cosmopolitan distribution. The family has around 5500 species distributed among 106 genera (Govaerts et al., 2021). They may be perennial or annual herbaceous plants having rhizomes and some may be stoloniferous. Sedges have significant importance both ecologically and ethnobotanically. The cultural development is initiated by plant domestication (Gul et al., 2020). They grow in specific pH and salinity conditions therefore indicating the specific habitat. The plants help to control the mountain overflows due to their strong root system (Shah et al., 2024). Some species are used in making baskets, hats and mats constituting the cottage industry of some countries and some are used in making wine (Tande and Lipkin, 2003). Some of the species act as weeds for the paddy fields but they can be managed by ploughing the infested field in winters. The occurrence of the weed in the field can be alleviated by a paddy land rotation cropping system. There are certain species which have thick culms, and they compete with the plants growing in the fields for sunlight, water and nutrition. Species like *Schoenoplectus tabernaemontani* (C.C.Gmel.) Palla serves to provide food for the birds inhabiting the wetland, especially ducks. Its dense colonies provide shelter and nest to some bird species (Xu et al., 2017). About 10% of the sedges are used by humans for ethnobotanical purposes (Simpson, 2008; Simpson and Inglis, 2001). They are an important contributor to the local and regional economics. Cyperaceae finds great importance in different bioactivities of life. Due to the ability of Cyperaceae, it is used worldwide against different pathogens and other microbes. Some species are used as anti-lice while others are used against scorpions, and snake stings and to obtain the essential oils. Species like *Cyperus scariosus* have sweet-smelling rhizomes used in perfumery and cosmetics. They are useful in the treatment of chest disorders and nasal discharge, blood enrichers, digestive system disorders, genito-urinary system disorders, metabolic system disorders, and infection / infestation. *Eleocharis dulcis* is edible (raw and cooked) and is palatable and nutritious. *Cyperus cyperoides* is used as vermifuge, plant ash is also used to apply on wounds (Clarke and ex Kunth, 2016). Flour is made from corms and is widely eaten in China, Japan, India, the U.S.A., Philippines Leaf protein concentrate extraction is used as cattle fodder (Simpson and Inglis, 2001).

The taxonomic identification of Cyperaceae is quite challenging because most of its species resemble a lot with each other and have exceedingly small and reduced flowers. They are herbs like linear leaves having parallel venation and they are arranged spirally around the stem. The leaf blade is normally flat with a prominent mid-rib. The edges and underside of the leaf are mostly rough. The plants have triangular stems that differentiate them from grass and rushes and have small anemophilous flowers. The shape of the inflorescence is determined by the branching pattern. It may be either branched or unbranched. The flower can be either unisexual or bisexual (Goetghebeur, 1998). Nuts are one of the important parts of the inflorescence that differs in varied species. The microscopic cellular structure plays a significant role in the classification of the family at generic and species levels. It differs widely at the cellular level and is visible only under light and scanning electron microscope. SEM plays a vital role in studying the micromorphological parts that cannot be observed under the regular light microscope (Shah et al., 2024). According to the literature, most of the research done on the nut is on the genus *Carex* but this article focuses also on the other genera along with the *Carex*. The distinguishing micromorphological features of the nut can play a vital role in the taxonomic identification of the family Cyperaceae (Lamiaa and Gazer, 2015).

Material and Methods

Sample collection and tagging

Different Cyperaceae samples were collected from all over Pakistan and were tagged by the GPS for their location. The plants were then brought to the Plant systematics and evolution lab in ASAB, NUST and were mounted on the herbarium sheets for preservation. The inflorescence of the same plants was separated for micromorphological studies and microscopy. The herbarium sheets were submitted to the Pakistan Museum of Natural History to get the accession numbers for future reference. The plants were identified morphologically using The Flora of Pakistan. A list of the collected samples along with the GPS tags and location is mentioned in Table 1.

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Table 1: GPS reading and collection information of the Cyperaceae species.

Sr. No.	Species	Location	GPS reading	Collection Data
1.	*Schoenoplectus litoralis* (Schrad.) Palla	Kallar kahar	32°46′17"N-72°42′19"E	I Shah & MQ Hayat, Kallar Kahar, 2017
2.	*Fimbristylis bisumbellata* (Forssk.) Bubani	Khanpur	33°48′16"N-72°55′37"E	M Khan & MQ Hayat, Haripur near Khanpur, 2017
3.	*Cyperus flavidus / Pycreus flavidus* Retz.	Wah gardens	33°48′09"N-72°41′55"E	M Khan & MQ Hayat, Wah Gardens, 2017
4.	*Cyperus alopecuroides* Rottb.	Kallar kahar	32°46′17"N-72°42′19"E	I Shah & MQ Hayat, Kallar Kahar, 2017
5.	*Cyperus iria* L.	Khanpur	33°48′16"N-72°55′37"E	I Shah & MQ Hayat, Dera Ghazi Khan-Haripur, 2017
6.	*Cyperus difformis* L.	Islamabad	33°40′06"N-73°02′42"E	K Ullah & MQ Hayat, Islamabad, 2018
7.	*Bolboschoenus maritimus* (L.) Palla	Parachinar (Kurram)	33°54′29"N-70°05′12"E	U Laila & MQ Hayat, Parachinar, 2019
8.	*Carex viridula* (Michx.) L.H.Bailey	Parachinar	33°54′08"N-70°02′59"E	U Laila & MQ Hayat, Parachinar, 2019
9.	*Carex canescens* L.	Mansehra	34°19′54"N-73°11′52"E	HI Fakhar & MQ Hayat, Lulusar Lake Mansehra, 2018
10.	*Carex nubigena* D.Don	Ayubia National Park, Abbottabad	34°10′10"N-73°13′17"E	B Anjum & MQ Hayat, Ayubia National Park Abbottabad, 2018
11.	*Carex remota* L.	Ayubia National Park, Abbottabad	34°10′10"N-73°13′17"E	B Anjum & MQ Hayat, Ayubia National Park Abbottabad, 2018
12.	*Carex stenocarpa* Turcz. ex V.I.Krecz.	Jaffarabad, dist., Naggar	36°14'07"N-74°24'00"E	S Haider & MQ Hayat, Naggar Gilgit, 2020
13.	*Carex pseudobicolor* Boeckeler	Jaffarabad, dist., Naggar	36°14'07"N-74°24'00"E	S Haider & MQ Hayat, Naggar Gilgit, 2020
14.	*Fimbristylis littoralis var. littoralis*	Mangla, Hamlet	33°02′08"N-73°38′52"E	K Ullah & MQ Hayat, Mangla Hamlet, 2018
15.	*Carex distans* L.	Jaffarabad, dist., Naggar	36°14'07"N-74°24'00"E	S Haider & MQ Hayat, Naggar Gilgit, 2020
16.	*Carex shaanxiensis* F.T.Wang & Tang ex P.C.Li	Jahaz banda	35°23'49"N-72°18'18"E	S Malik & MQ Hayat, Jahaz banda, 2019

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Light and Scanning Electron Microscopy (SEM)

The morphological and micromorphological parts of the inflorescence were studied under the stereomicroscope of IRMECO (Model: IM-900, 21943 Schwarzenbek / Germany) after being dissected using the needle and forceps. The nut was separated and observed under the light microscope and the colored images of the nut were captured which are shown in Fig. 1. It was done in the Plant Systematics and Evolution Lab, ASAB, NUST. SEM was performed to observe the structure of the Nut surface. It was done by placing the sample on the copper stub using double-sided carbon tape. The sample was sputter coated using 10nm gold by using a JEOL JSM 6490A scanning electron microscope. SEM was performed partly in NUST and the rest in the University of California, Davis lab. The images are shown in Fig. 2

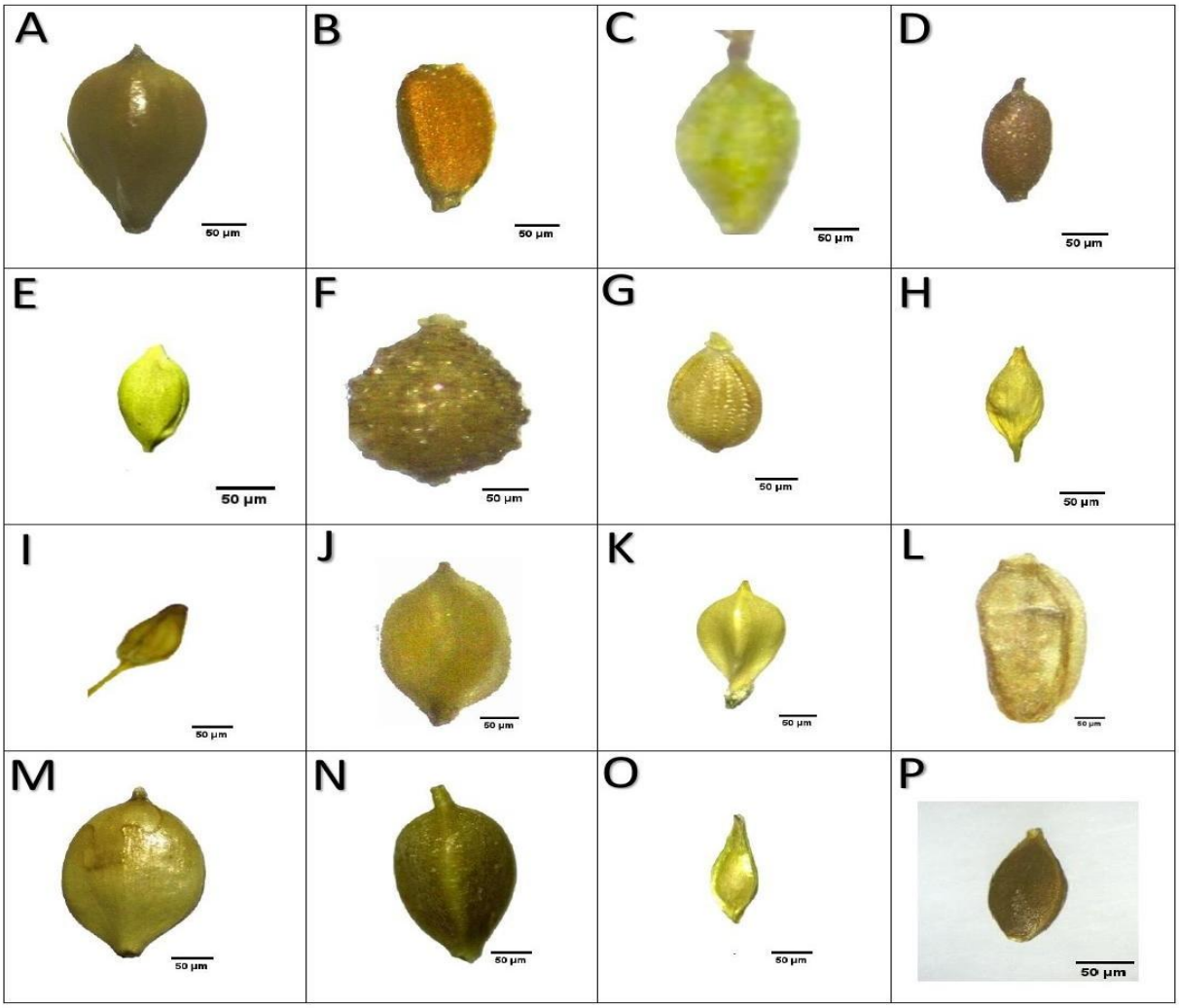

Figure-1: Light micrograph of Cyperaceae nuts.
A: *Bolboschoenus maritimus,* B*: Cyperus alopecuroides,* C: *Cyperus iria,* D: *Pycreus flavidus,* E: *Carex distans,* F*: Fimbristylis littoralis,* G*: fimbristylis bisumbelleta,* H: *Carex canescens,* I*: Schoenoplectus litorelis,* J: *Cyperus difformis,* K*: Carex viridula,* L: *C.stenocarpa,* M*: Carex nubigena,* N: *Carex pseudobicolor,* O*: Carex remota,* P*: Carex shaanxiensis*

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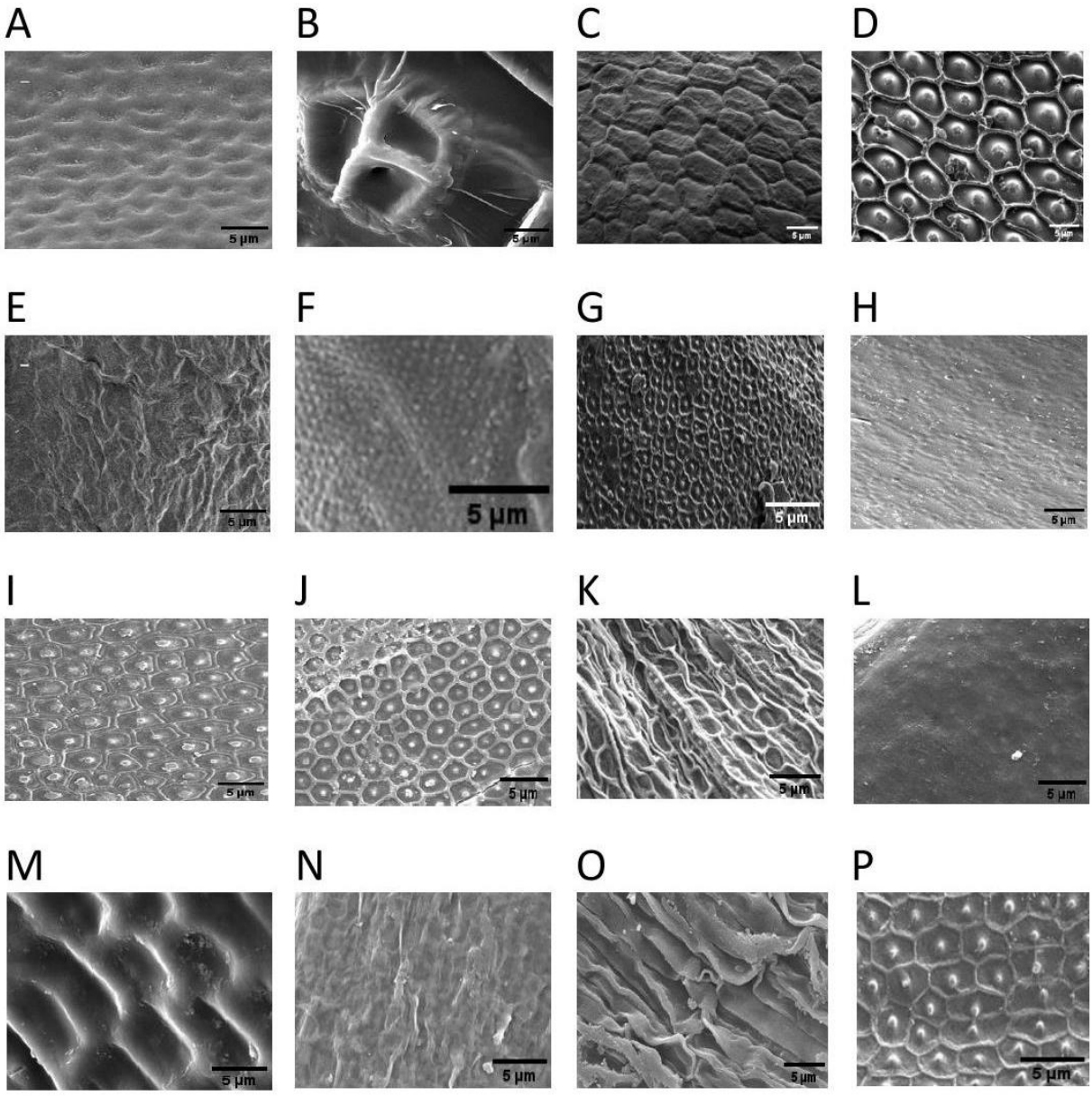

Figure-2: Exine surface SEM micrograph of Cyperaceae nuts.
A: *Schoenoplectus litoralis*, B: *Fimbristylis litoralis*, C: *Fimbristylis bisumbelleta*, D: *Pycreus flavidus*, E: *Cyperus alopecuroides*, F: *Cyperus iria*, G: *Cyperus difformis*, H: *Bolboschoenus maritimus*, I: *Carex viridula*, J: *Carex distans*, K: *Carex canescens*, L: *Carex nubigena*, M: *Carex remota*, N: *Carex stenocarpa*, O: *Carex pseudobicolor*, P*: Carex shaanxiensis*

Qualitative and Quantitative Analysis

The quantitative and qualitative characteristics of the Nut include nut size, nut color and nut shape. These characters were observed and recorded in Table: 2, 3, 4. The readings were taken in the lab and then compared with those available in the Flora (Kukkonen, 2001). ImageJ software was utilized to record measurements and standard deviations, with terminologies adapted by (Denton, 1983).

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Table-2: Nut size, shape, color and the surface under the stereo and Scanning electron microscope of collected taxa.

Serial No.	Species	Voucher No.	Shape	Nut Surface Cell Type	Size (mm)	Color
1.	*Schoenoplectus litoralis*	PMNH 042233	Ellipsoid finely reticulate	Smooth surface with papillae	1.2	Dark brown
2.	*Fimbristylis bisumbellata*	PMNH 042319	Obovoid with small stipe	Large rectangular cells	0.7	Yellowish white
3.	*Cyperus flavidus/ Pycreus flavidus*	PMNH 042312	Obovoid with a small stipe	Circular to oval connected with hooks	0.9	Dark brown
4.	*Cyperus alopecuroides*	PMNH 042232	Slightly flattened	Irregular shaped interlocked	0.5	Yellowish brown, shiny
5.	*Cyperus iria*	PMNH 042315	Sharply trigonous, ellipsoid	Smooth surface with small papillae	0.7	Brown to dark brown
6.	*Cyperus difformis*	PMNH 043869	Obovoid, sharply trigonous	Circular to oval connected with hooks	0.5	Yellowish brown
7.	*Bolboschoenus maritimus*	PMNH046228	Broadly obovoid, biconvex compressed trigonous	Rectangular cells	2.5	Dark brown
8.	*Carex viridula*	PMNH046229	Obovoid having beak at the apex	Circular to oval connected with hooks	0.8	Yellow to dark olive
9.	*Carex distans*	PMNH046448	Ellipsoid	Circular to oval connected with hooks	1.1	Yellowish

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10.	*Carex canescens*	PMNH043883	Ovoid, lenticular	Rectangular-shaped interlocked cells	0.9	Greenish
11.	*Carex nubigena*	PMNH043873	Ellipsoid, obscurely reticulate	Smooth surface	0.8	Greyish brown, glossy
12.	*Carex remota*	PMNH043866	Ellipsoid, trigonous	Smooth surface with papillae	0.8	Greenish
13.	*Carex stenocarpa*	PMNH046450	Ellipsoid, trigonous	Irregular surface with trichomes	2.5	Dark grey to brownish
14.	*Carex pseudobicolor*	PMNH046447	Obconical, triangular	Rectangular interlocked cells	1.1	Light brown
15.	*Fimbristylis littoralis*	PMNH044051	obovoid to largely obovoid	Surface with papillae	0.7	Yellowish brown
16.	*Carex shaanxiensis*	PMNH046217	Elliptic	Hexagonal interconnected cells with hooks	2.3	Greenish brown

Table-3: Character and character states in the collected Cyperaceae species.

Serial No.	**Characters**	**Character States**
1.	Nut shape	Obovoid (0), Obconical (1), Ellipsoid (2), Flattened (3), Lenticular (4), Elliptic (5)
2.	Nut size	>3mm (0), >2.5-2.0mm (1), >1-1.5mm (2), 0.9-0.8mm (3), 0.7-0.6mm (4), 0.5-0.4mm (5)
3.	Nut color	Blackish brown (0), Dark brown (1), Light brown (2), Yellowish brown (3), Greenish (4), Yellowish white (5), Yellow to dark olive (6), Greyish brown (7), Greenish brown (8)

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Table 4: Species and their character states explained in Table 3.

Sr. No.	**Species**	**Abbreviations**	**Character states**		
			1	2	3
1.	*Schoenoplectus litoralis*	LIT	2	2	1
2.	*Fimbristylis bisumbellata*	BIS	0	4	5
3.	*Cyperus flavidus/Pycreus flavidus*	FLA	0	3	1
4.	*Cyperus alopecuroides*	ALO	3	5	3
5.	*Cyperus iria*	IRI	2	4	1
6.	*Cyperus difformis*	DIF	0	5	3
7.	*Bolboschoenus maritimus*	MAR	0	1	1
8.	*Carex viridula*	VIR	0	3	6
9.	*Carex canescens*	CAN	4	3	4
10.	*Carex nubigena*	NUB	2	3	7
11.	*Carex remota*	REM	2	3	4
12.	*Carex stenocarpa*	STE	2	1	4
13.	*Carex pseudobicolor*	PSE	1	2	2
14.	*Fimbristylis littoralis*	FLI	0	4	3
15.	*Carex distans*	DIS	2	2	5
16.	*Carex shaanxiensis*	SHA	5	1	8

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Statistical analysis

Principal Component Analysis (PCA) analysis was performed using MVSP software to check the impact of nut characters like shape, size and color on the grouping of species. Fig 3. PCA spread the species on the base of variables and 4 groups have been recognized. CLUSTER analysis was performed to compare with the observations obtained from the PCA scatter plotting. (Fig 4).

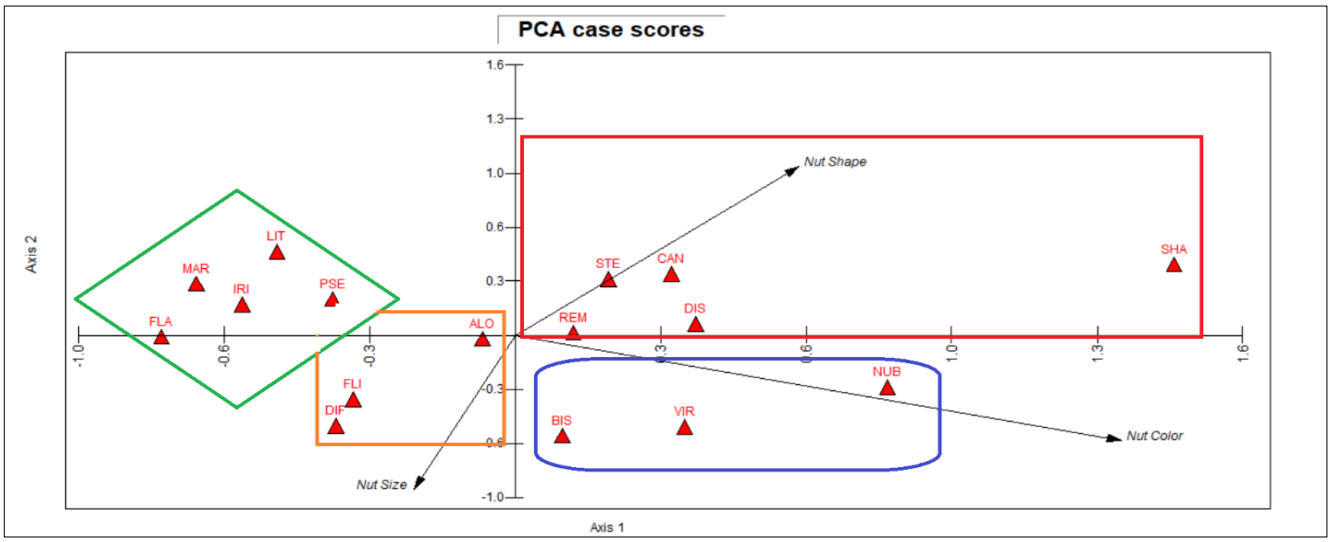

Figure-3: PCA analysis of the selected species based on nut characters.

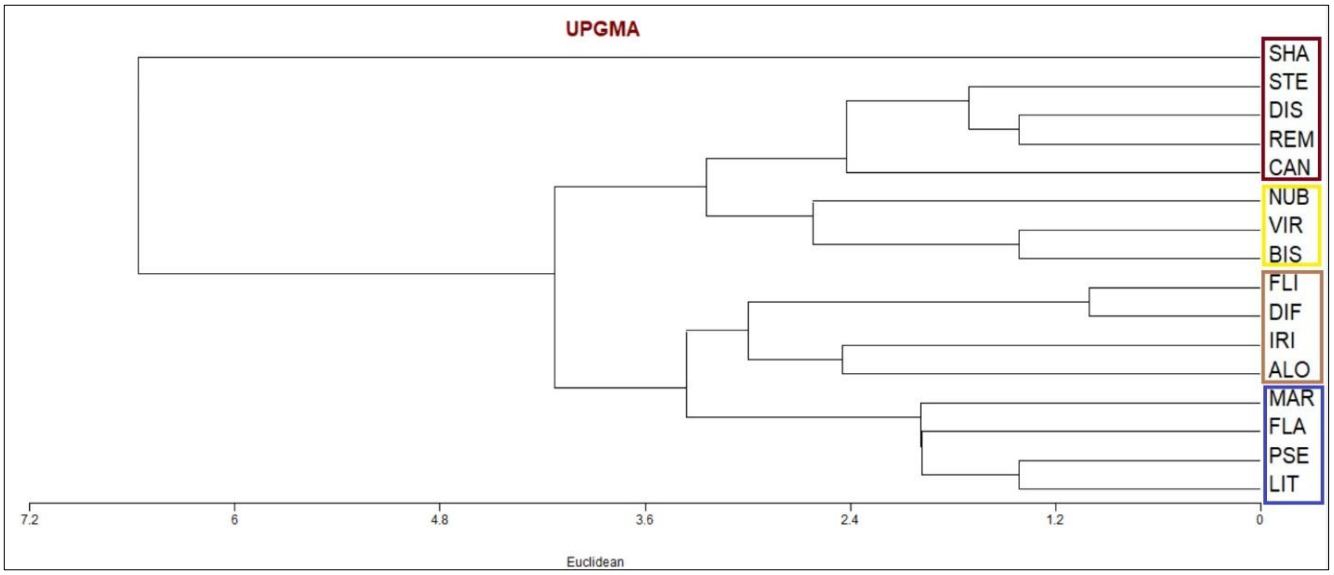

Figure-4: UPGMA phenogram based on Nut micromorphology of 16 collected species.
See Table 4 for taxa abbreviation.

Results and Discussion

For the correct morphological identification of the sedges, the nut is considered a very important part. It is considered important for the assessment of taxonomic value. Fruit and seed diversity is important character to classify species in family Cyperaceae (Reutemann et al., 2024). For this reason, we collected around 16 species of Cyperaceae from different regions of Pakistan and the micromorphological investigation of their nut was performed to analyze the

differences among their structure. Nuts showed great diversity among all the species considering the size, color and shape of the nut. Fig 1 shows the micrographs of the nut taken from the stereomicroscope. Nuts were also observed under the SEM to analyze the surface of nuts and the differences among them. The illustration is shown in Fig 2.

Different nuts show different traits under stereo and scanning electron microscope. *Schoenoplectus litoralis, Bolboschoenus maritimus, Cyperus flavidus/Pycreus flavidus* and *Cyperus iria* have dark brown color to brown color. Some species like *Cyperus alopecuroides, Fimbristylis littoralis* and *Cyperus difformis* have yellowish brown color. *Carex remota* and *Carex canescens* have greenish color. *Carex distans, Carex viridula* and *Fimbristylis bisumbellata* have yellowish white to yellow greenish shade. *Carex stenocarpa* and *Carex nubigena* have greyish brown shade. *Carex pseudobicolor* is light brown in color. All of them have different sizes ranging from 0.5-2.5mm. *Cyperus alopecuroides* and *Cyperus difformis* have the smallest nut of 0.5mm while *Bolboschoenus maritimus* and *Carex stenocarpa* have the largest nut of 2.5mm (Table 2).

The shape of the nut varied greatly among the species. *Fimbristylis bisumbellata, Cyperus flavidus/Pycreus flavidus, Cyperus difformis, Bolboschoenus maritimus, Carex viridula* and *Fimbristylis littoralis* are large to regularly obovoid. *Schoenoplectus litoralis, Cyperus iria, Carex distans, Carex nubigena, Carex remota* and *Carex stenocarpa* are Ellipsoid. *Cyperus alopecuroides* is slightly flattened. *Carex canescens* is ovoid while *Carex pseudobicolor* is obconical.

A study carried out by Nasar et al. (2024) used multivariate approach to classify sedges on basis of taxonomic characteristics like size of stomata, shape of subsidiary cells, shape and wall sinuosity of long cells, presence/absence of intercostal short cells, presence/absence of silica bodies, presence/absence of bulliform cells and presence/absence of papillae. They concluded that multivariate approach provides clear features of relationships in family.

Previously not much information had been reported in the literature regarding the nut and its micromorphology. Pashirzad et al. (2014) explained the characteristics of the nut based on micromorphology but the number of species and even genus was limited. Due to the hard and isolating nature of fruit tissue which prevents anatomical techniques, the anatomy of Cyperaceae fruits is less known (Reutemann et al., 2024). A comprehensive study was carried out by Reutemann et al., 2024 in which structural diversity characterization of fruit and seed of 29 species using light microscopy was analyzed in Cyperaceae. They concluded that The Cyperaceae seed structural diversity is high and related to the evolutionary history of the species. This study focused on sixteen species belonging to five different genera of the family. The variation shown among these species can be implied to access the taxonomical value to nut morphology in genus and species level identification. Achenes of *Eleocharis* R. Br. were analyzed using SEM to explore the systematic value of the achene wall. The study revealed that the epidermis possesses distinct microscopic characteristics valuable for the systematic classification of *Eleocharis*. After acid treatment, which removed the cuticle and outer periclinal cell walls, micromorphological variations in epidermal features were observed across the 26 taxa examined (Menapace and Francis, 2011). Nutlet morphological and micromorphological traits were examined in 38 taxa across 13 genera of the Cyperoideae subfamily within Cyperaceae, utilizing Light Microscopy and SEM to evaluate their taxonomic relevance. Findings from this study confirm that these nutlet characteristics are effective tools for identifying and classifying different taxa within Cyperoideae, underscoring their importance in systematic studies of the family (Majumder et al., 2024). PCA showed the impact of nut shape, size and color on the grouping of species. It spread the species based on the nut characters into 4 groups. Further elaborate research is needed to completely study the nuts based on other different characters.

Conclusion

In this study nut morphological and micromorphological characteristics of sixteen Cyperaceae species were analyzed to assess their taxonomic value. Significant diversity in nut size, shape, and color was observed across species that enabled effective differentiation within family. Using stereo and scanning electron microscopy distinct surface patterns and structures were identified. It is major contribution to species classification. PCA further highlighted the role of these nut traits in species grouping, forming distinct characters. This research emphasizes the utility of nut morphology in Cyperaceae taxonomy. This also suggests that nut characteristics could serve as reliable markers for species identification and classification within family.

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Contribution of Authors

Iqra Shah: Conducted the study, compilation of data and manuscript write-up.
Muhammad Qasim Hayat: Conceptualization of study and supervision of research work.
Umme Laila, Saleem Haider, Kaleem Ullah, Beenish Anjum, Madeeha Khan & Summaya Malik: Sampling of the plants and technical assistance.
Alvina Gul: Co-supervision of research work and guidance.

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