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Full Length Research Paper

Morpho-playnological and foliar epidermal anatomy of genus *Cenchrus* L.

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This study confined to four species of genus Cenchrus using multiple parameters that is morphology, epidermal leaf anatomy (LM and SEM) and palynology (LM and SEM). The species *Cenchrus biflorus* Roxb, *Cenchrus ciliaris* Linn*., Cenchrus pennisetiformis* Hochst. and Steud. ex Steud and *Cenchrus setigerus* Vahl. Playno-anatomical characterization based on SEM techniques provides useful information for differentiation of species in Genus Censhrus. The study demonstrated that all the four species may differ from each other on the basis of morphology, anatomy and palynology.

Key words: Morphology, palynology, foliar epidermal anatomy, Cenchrus.

INTRODUCTION

Morphological features can be used in identification and delimitation of taxa and genera into tribes and subfamilies generally. The leaf epidermal characters have proved to be very important in providing information of taxonomic importance (Ogie-Odia et al., 2010). Leaf epidermal characters have significance in grass systematics and characterization of broad groups, particularly subfamilies and tribes (Brown, 1958; Watson and Dallwitz, 1988). Epidermal characters are very useful for the differentiation of the different taxa at higher level (Tateoka et al., 1959).

Anatomical studies have been used successfully to clarify taxonomic status and help in the identification of different species (Gilani et al., 2002). Leaf epidermal characters such as the arrangement of long and short cells, distribution and morphology of the macrohairs, microhairs, prickles, silica bodies and stomata are important taxonomically (Holm 1896; Metcalfe, 1960).

In the past anatomical studies incorporation with morphological and palynological studies for the resolution of taxonomic problems of monocots has been used. Cenchrus, the type genus of Paniceae, is a large and taxonomically difficult group and its leaf epidermis is of prime importance in solving taxonomic problemsand the epidermal cells have a higher degree of specialization (Prat, 1936).

The aim of the present study was to find out the solution of existing taxonomic problems of species, which overlap in most of their morphological characters and to elucidate relationship of the critical taxa by utilization of leaf epidermal and palynological characters. One of the objectives of the study was to correlate these multiple parameters for taxonomic position and decision of these species in the particular genus and then into a particular tribe.

MATERIALS AND METHODS

Morphology

For morphological study herbarium and preserved as well as fresh specimens were used. 1 to 5 specimens per species were studied under the binocular light microscope.

Measurement of various parts of a specimen was taken 3 to 5 times to ensure the reading and calculated mean. Morphological characteristics were reconfirmed by using various Floras (Nasir and Ali, 1982; Townsend and Guest, 1968; Hooker, 1875; Tutin and

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Heywood, 1972; Hooker and K.C.S.I, 1885a, 1894; Saldanha and Nicolson, 1976).

Leaf epidermal anatomy

Foliar leaves were collected from field and dried specimens. Leaves samples were prepared according to the method of Cotton (1974) who followed Clark's (1960) technique, but with a little modification (Ahmad et al., 2009, 2010). Both qualitative and quantitative micromorphological foliar characteristics were observed using light microscope (LM) and scanning electron microscope (SEM). Microhistological photographs of both surfaces were taken by Camera equipped light microscope (Mejii- MX 5200H, Japan) and by SEM.

Scanning electron microscope (SEM) study of pollen

Pollen samples were obtained from herbarium specimens housed in Quaid-e-Azam University, Islamabad. Pollen slides were prepared by adopting the method of Zafar et al. (2011). The measurements are based on 15 to 20 readings from each specimen, shape, type, structure, diameter, exine thickness; P/E ratio and sculpturing were measured. SEM images were taken on a jeol microscope JSM-T200.

RESULTS AND DISCUSSION

Morphological studies are important in order to know the genetic diversity and patterns of geographic variation in grasses. Ambrose et al. (2000) and Kyozuka et al., (2000) discussed that the ancestry and origin of the lodicules, palea, lemma, and glumes in genus Cenchrus have been the subject of a vast and largely inconclusive literature.

Vegetative and floral morphology of the taxa studied revealed that the inflorescence type was helpful in identification of the species generally. The character of inflorescence types have been mainly utilized by Ali and Qaiser (1986). Morphologically, a number of spikelets were observed in different species. The number of spikelets observed in *C. pennisetiformis* were 2 to 4 per burr while *C. bifroris* and *C. setigerus* comprises 1 to 3 and *C. ciliaris* 1 to 4 (Table 1). These results incorporate with the findings of Shaukat (1989). The length of lemma varied from 0.2 to 0.6 mm and the length of palea from 0.3 to 0.5 mm in different species. Tzvelev (1984) (Table 1) mentioned that in the genus *Cenchrus* lemma is similar to the upper glume, but often three veined and palea of the sterile flower are membranous.

The length, width and colour of spikes are also variable characters (Table 1). Skerman and Riveros (1990) discussed that in *Cenchrus. Bifloris*, false spikes were surrounded by a rigid involucre and outer bristles were short and spiny where as *C. pennisetiformis* had paler involucres and wide spreading spacing of the spikelets on the rachis.

Morphologically, culm and leaf blades length is also variable among taxa. Hooker (1897) reported variation exist in the length and width of the leaf blades of *Cenchrus* species. It is also found that morphological diversity may exist in different taxa of Cenchrus at inter and intra specific level.

Foliar epidermal anatomy

Epidermal characters have potential taxonomic significance and are helpful as an additional taxonomic character (Stace, 1965). The differences among species of Cenchrus were not clear by considering their ranges of length and breadth of the organelles, but when their average length and breadth were calculated, remarkable differentiaton were observed. Long cells are present mostly on both surfaces and possess rectangular shape and thin sinuous walls in majority of the taxa except in *Cenchrus setigerus*, which possess thick sinuous walls almost cubical to rectangular, shaped (Plate 3a). Short cells in the Costal regions are predominantly arranged in rows of more than five cells, in majority of the taxa, except *C. ciliaris* in which short cells possess a row of more than four cells (Table 2).

Three types of silica bodies, that is, dumb bell, dumb bell cross-shaped and cross-shaped were observed in *C.* species. All the three types of silica bodies were found in *C. bifloris*, *C. ciliaris and C. Pennisetiformis* (Plate 3b) whereas *C. setigerus* possess only dumb-bell shaped silica bodies. (Table 2) Sharma and Kaplia (1985) in their studies showed that the leaf epidermal characters of various species of genus Digitaria have revealed the presence of dumb-bell shaped, tall and narrow, cross-shape or nodular silica bodies, characters corresponding to “Panicoid type” of leaf epidermis of Prat (1936).

Microhairs were observed in the three species that is *C. bifloris, C. pennisetiformis* and *C. setigerus*. No microhairs were found in *C. ciliaris*. In *C. bifloris* and *C. setigerus* macrohairs were observed on both abaxial and adaxial surfaces whereas in *C. ciliaris* and *C. pennisetiformis* macrohairs were present only on adaxial surface (Plate 1b and 2a). Sharma and Kaplia (1985) that microhairs are present in the costal and intercostal regions of both surfaces of epidermis in majority of the taxa of the genus Digitaria. The shapes of prickles play an important role in the identification of the *Cenchrus.* In *C. bifloris* prickles were narrowly oblong not much broader at the tip. In *C. ciliaris* prickles were of two types, with narrow beak and elongated distil portion or broad beak and short and blunt distil portion. (Plate 1a) In *C. pennisetiformis* prickles were found to be short, broad and elongated whereas in *C. setigerus* the prickles were distil and narrowly elongated, beak-like portion with tapering (Plate 2b).

Stomata arranged in well-defined rows in the intercostal regions, are characteristic of the majority of the species. The subsidiary cells, two in number, are mostly low dome shaped or triangular shaped. Shouliang et al. (1996)

Table 1. Morphological distinctions among the species of genus cenchrus.

Characteristics	*C. biflorus*	*C. ciliaris*	*C. pennisetiformis*	*C. setigerus*
Habit	Annual	Perennial with a rootstock	Annual perennial	Perennial
Clums	Erect	Tufted, erect or geniculately ascending	Tufted, usually branched near the base	Tufted, geniculately ascending
Spikelets	Ovate, 1 to 3 per burr	Lanceolate, 1 to 4 per burr	Lanceolate or ovate, 2-4 per burr	Ovate, 1-3 per burr
Inner bristles	Longer than the spikelet	Much longer than the spikelet	Longer than the spikelet	Shorter than the spikelet
Involucre	Elongate	Elongate	Elongate	Cupulate
Lower glume	Lanceolate	Ovate	Lanceolate or ovate	Ovate
Lower glume Apex	Acute or obtuse	Acute	Acute or obtuse	Acute or obtuse
Upper glume	Ovate	Ovate	Lanceolate or ovate	Ovate
Upper glume Apex	Obtuse or acute	Acute	Obtuse or acute	Obtuse or acute
Glumes nerves	1-5 nerved	1-3 nerved	1 to 3 nerved	1-nerved
Lemma	Ovate	Ovate	Elliptic	Ovate
Lemma apex	Acute	Acute	Acute	Truncate
Lemma surface	Coriaceous	Chartaceous	Asperulous	Chartaceous or coriaceous
Lemma nerves	5-nerved	5-nerved	5-nerved	5-7 nerved

Table 2. Comparative foliar epidermal characterization among *Cenchrus* Species.

Characteristics	*C. biflorus*	*C. ciliaris*	*C. pennisetiformis*	*C. setigerus*
Short cells	In rows of 5 or more cells	In rows of 4 or more cells	In rows of 5 or more cells	In rows of 5 or more cells
Silica bodies	Cross-shaped, less dumb bell shaped and in between cross shaped and dumb-bell shaped	Cross shaped and in between cross-shaped and dumb-bell shaped	Dumb-bell shaped, cross-shaped and in between dumb-bell shaped and cross shaped	Dumb-bell shaped
Macro-hairs	Numerous, between the veins, 40-50 μm	Over the veins, 90 to 110 μm	Occasionally present between the veins, 190 to 220 μm	Occasionally present between the veins, 210 to 230 μm
Micro-hairs	Mostly at the side of small veins, knife like, 10 to15 μm	None seen	Occasionally present between the veins, 30 to 60 μm	Occasionally present between the veins, 20 to 75 μm
Prickles	A few, over the veins, narrowly oblong, not much broader at the tip, 7 to 10 μm	Over the veins, two types, with narrow beak and elongated distil portion or broad beak and short and blunt distil portion, 5 to 7 μm	Over the veins, short, broad and elongated, 4 to7 μm	Over the veins, distil and narrowly elongated, triangular shaped, broader beak shaped, 7 to 12 μm

Table 2. Contd.

Hooks	Present, 2 to 3 µm	Present, 2 to3 µm	Present, 2 to 4 µm	Present, 1 to 3 µm
Stomata	With 2 to 7 rows, triangular shaped, subsidiary cells, some are dome shaped, 4 to 6 µm	With 2-3 rows, triangular shaped, subsidiary cells, some are dome shaped, 4 to 7 µm	With 1-3 rows, triangular shaped, subsidiary cells, 4 to 5 µm	With 2 to 5 rows, triangular shaped, subsidiary cells, 5 to 6 µm
Long cells	Thin sinuous walls, rectangular shaped, 8 to 25 µm	Thin sinuous walls, rectangular shaped, 10 to 30 µm	Thin sinuous walls, rectangular shaped, 27 to 37 µm	Thick sinuous walls, almost cubical to rectangular shaped, 10 to 30 µm

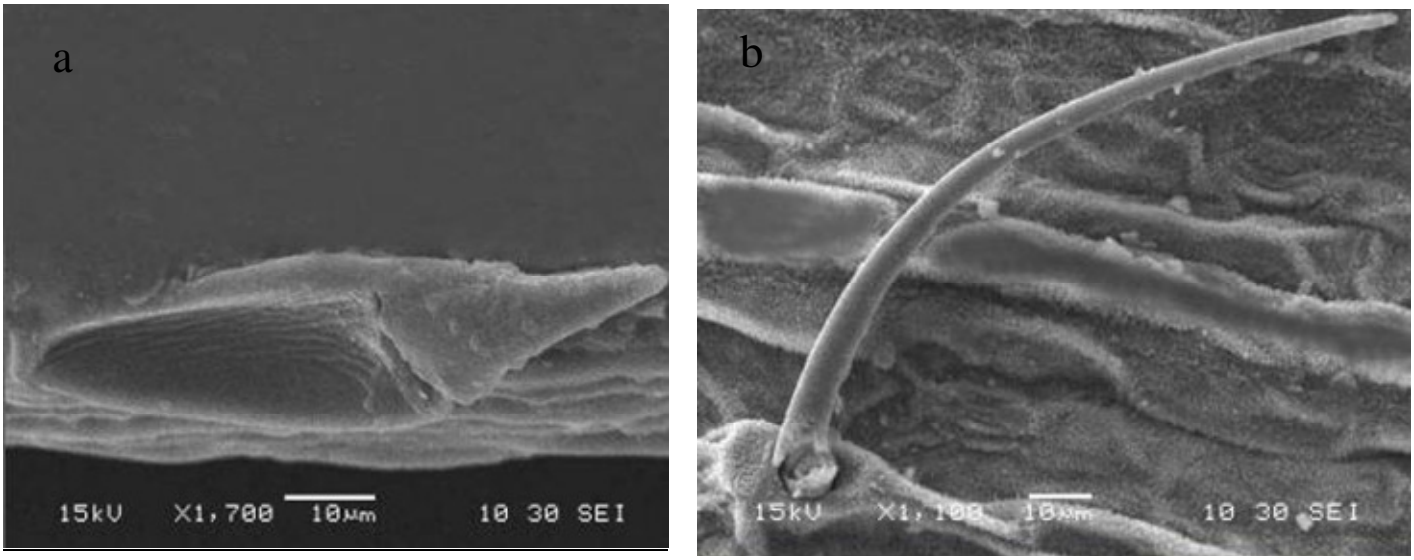

Plate 1. (a) *Cenchrus ciliaris* Beaked shaped Prickle, (b) Macrohair (SEM).

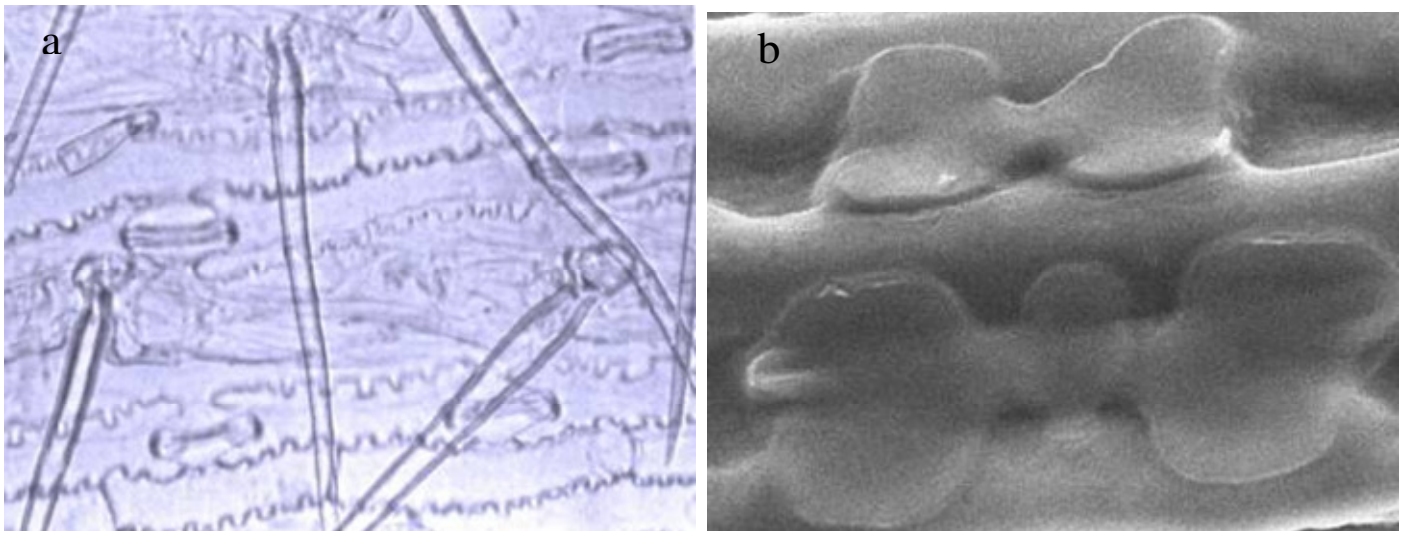

Plate 2. (a) *Cenchrus pennisetiformis* (macrohairs in the intercostal region), (b) Dumb-bell shaped silica bodies.

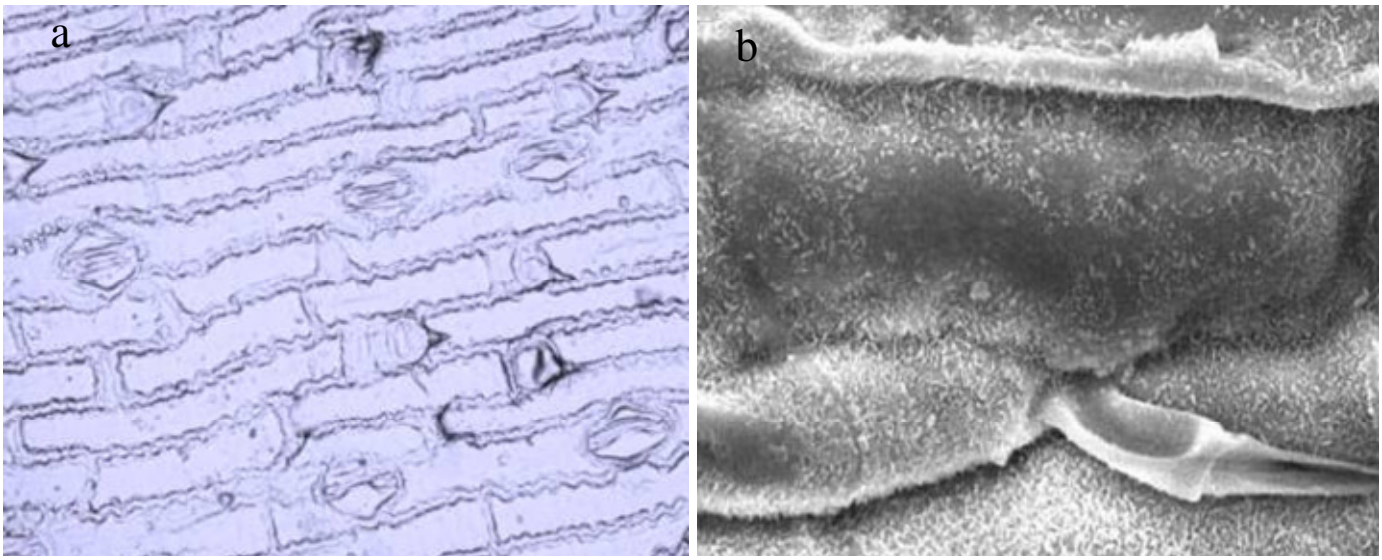

Plate 3. (a) *Cenchrus setigerous* Thick sinuous walls, (b) Prickle (SEM).

Table 3. Palynological comparison among the *cenchrus* species.

Characteristics	*C. biflorus*	*C. ciliaris*	*C. pennisetiformis*	*C. setigerus*
Polar diameter	32.5 (30 to 35)	32.5 (30 to 35)	22.5 (20 to 25)	45 (40 to 45)
Equatorial diameter	30 (20 to 40)	27.5 (20 to 35)	27.5 (25 to 30)	35 (30 to 40)
P/E ratio	1.08	1.18	0.81	1.28
Exine thickness	0.75 (0.4 to 1.1)	0.75 (0.4 to 1.1)	0.75 (0.3 to 1.2)	0.9 (0.6 to 1.2)
Pollen fertility	87.69%	98.76%	98.72%	96.25%
Shape in equatorial view	Prolate-spheroidal	Spheroidal	Circular	Circular
Position of pore	Ectoporus	Endoporus	Endoporus	Endoporus or ectoporus

considered the tall dome shaped subsidiary cells as primitive while parallel and triangular subsidiary cells as advanced characters.

Pollen characteristics

The size of pollen grains in polar view ranged from 22.5 (20 to 25) µm to 45 (40 to 45) µm. *C. pennisetiformis* have smallest in size whereas *C. setigerus* is the largest. In equatorial view, the size ranged from 27.5 (20 to 35) µm to 35 (30 to 40) µm. *C. ciliaris* and *C. pennisetiformis* appears to be the smallest and *C. setigerus* is the largest in pollen size (Table 3). P/E ratio ranged from 0.81 to 1.28 µm. *C. pennisetiformis* appears to be the lowest while *C. setigerus* comprises the highest value of P/E ratio (Figure 1). Exine thickness ranged from 0.75 to 0.9 µm among the species. *C. biflorus*, *C. ciliaris* and *C. pennisetiformis* show the lowest value in exine thickness whereas *C. setigerus* shows the highest value. Meo et al. (1989) recorded that in *C. setigerus* exine is granulate and in *C. pennisetiformis* exine is psilate to punctate.

A little variation in the shapes of pollen grains is observed. In polar view, all pollen grains were circular or semi-circular (Plate 4a) whereas in equatorial view, the pollen grains in *C. biflorus* are prolate-spheroidal and in *C. ciliaris* pollen grains are spheroidal (Plate 4b). According to Meo et al. (1989) the shape of pollen grains observed in *C. pennisetiformis* is spherical-perbolate and in *C. setigerus* is spheroidal-subulate. The pollen grains are monoporate and psilate (Plate 4c). The pore position ranged from ectoporus to endoporus. Three species *C. biflorus*, *C. ciliaris* and *C. pennisetiformis* appear to be endoporus (Plates 4d) while *C. setigerus* is endoporus or ectoporus. In the genus Cenchrus, the pollen size and shape are of taxonomically significant characters.

In the genus Cenchrus, the pollen fertility for the taxa showed that all the taxa almost have high range of fertility. *C. ciliaris* shows the highest value of pollen fertility as 98.76 whereas C. biflorus shows the lowest value as 87.69 (Figure 2). It is indicated that pollen fertility is generally high and the species are fertile, stable and distributed well in the area.

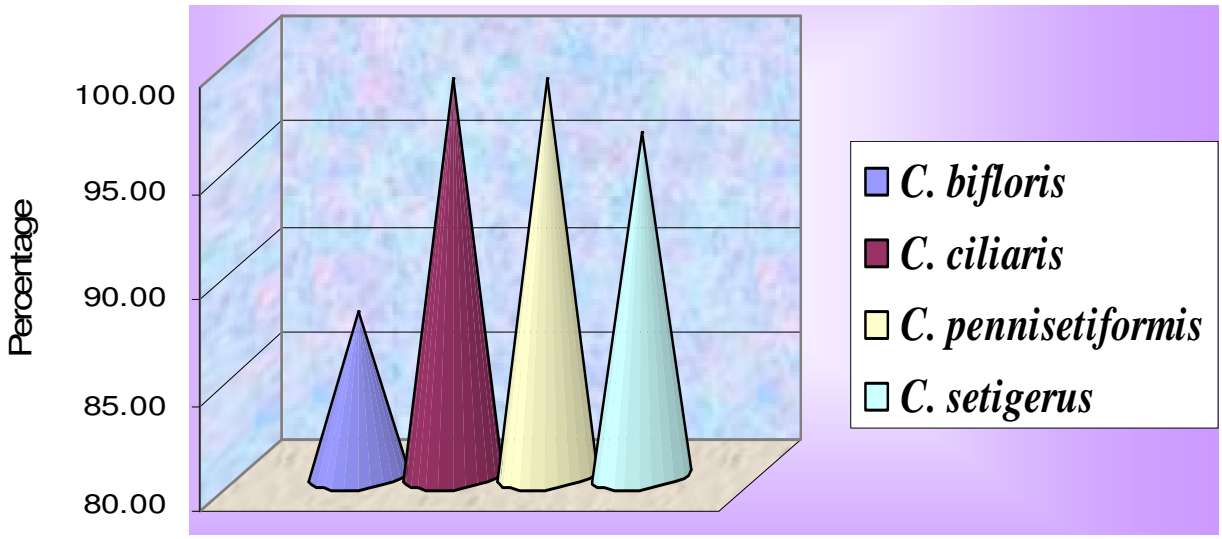

Figure 1. Summary of pollen measurements of *Cenchrus* species.

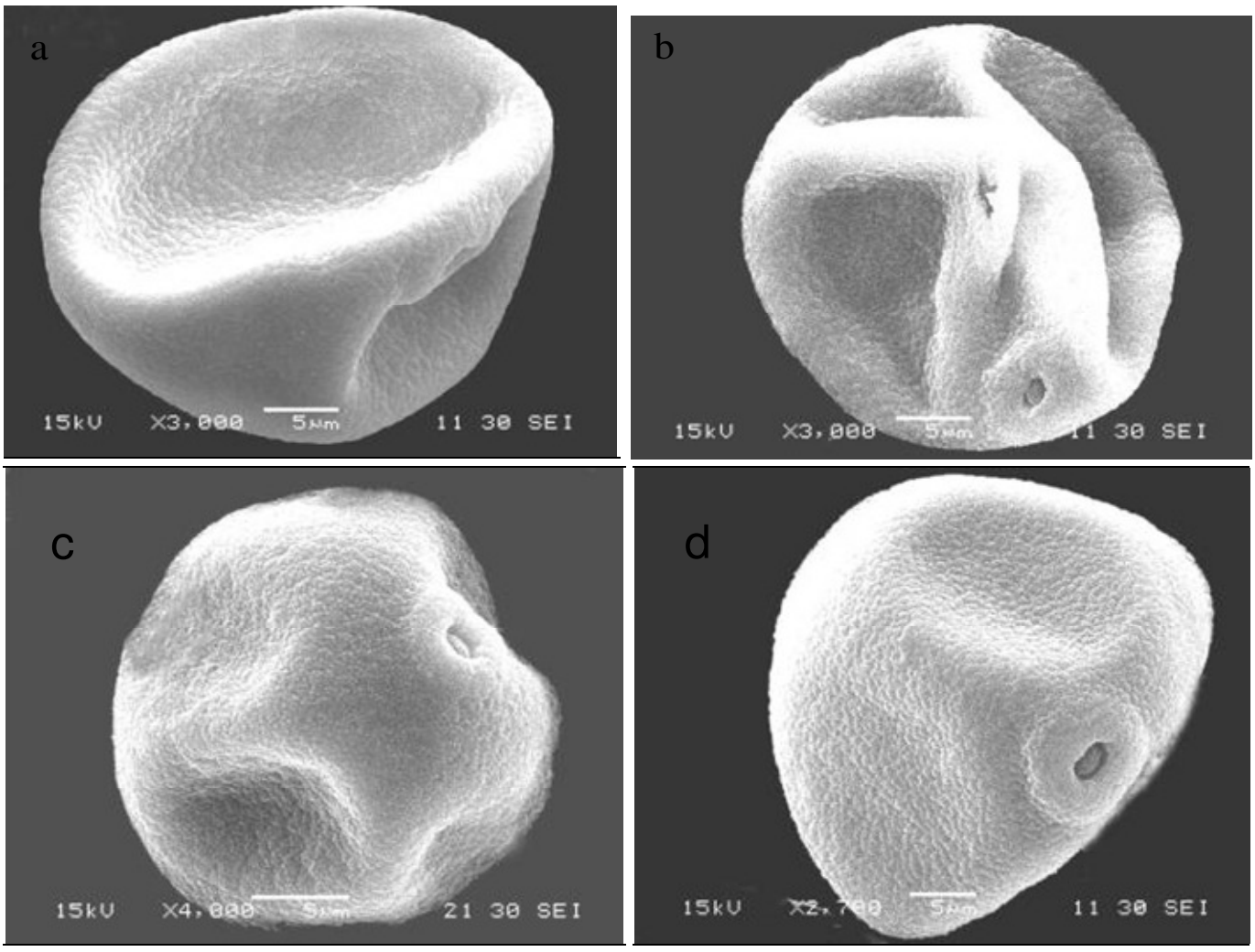

Plate 4. (a) Semi-circular shaped pollen (SEM). (b) Spheroidal pollen. (c) Monoporate and Psilate pollen. (d) Endoporus pollen (SEM).

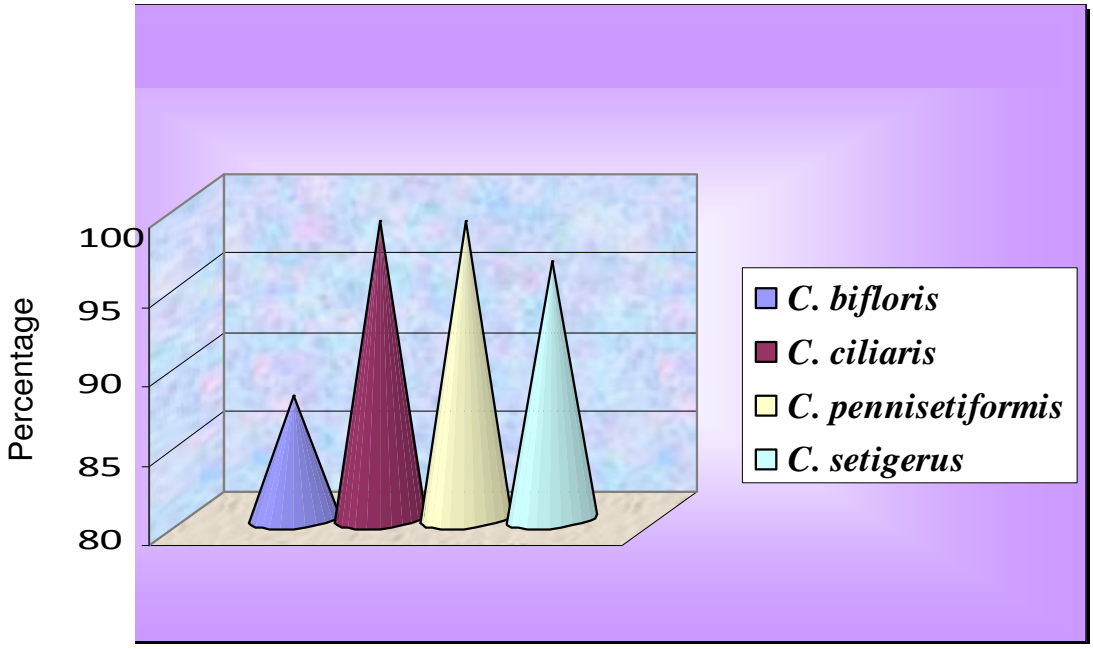

Figure 2. Pollen fertility of Cencrus.

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