

TAXONOMIC SIGNIFICANCE OF ACHENE MORPHOLOGY IN THE GENUS *CYPERUS* L. (CYPERACEAE) IN GOA, INDIA

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ABSTRACT

The genus *Cyperus* is cosmopolitan with 700 species, distributed in temperate, subtropical and tropical regions of the whole world. In India there are about 85 species and is the largest genus of Cyperaceae in Goa having 29 species, 2 subspecies and 3 varieties. It shows lot of variations in the shape, size and the epidermal microstructures of the achenes. Achene morphology of all the 29 species and the infraspecific taxa were studied utilizing the light microscope and 21 species were studied for their micromorphology using SEM images of the achenes. Achene shape and epidermal patterns were found distinctive and consistent within the species or infraspecific taxon. Variation in the epidermal cells is evident with respect to size of the cell, nature of periclinal wall, the number, thickness and sinuosity of anticlinal walls per cell, and presence or absence of silica bodies. In the present study characteristics of epidermal cells are correlated with other morphological characters as well. The micromorphological characters of achene surface were found to be different in dissimilar taxa. However, there is close similarity of these characters in closely related taxa. Interpretation of the SEM images was found to be useful in determining the taxonomic relationships, identification and delimitation of different taxa of *Cyperus* at species level and infra specific level.

Keywords: SEM Images, Achene Morphology, Silica Bodies, Anticlinal Wall, *Cyperus*

INTRODUCTION

Cyperaceae (Sedge family) is one of the ten largest families of flowering plants and is the third largest of monocotyledons after Orchidaceae and Poaceae. Bruhl (1995) estimated approximately 5,000 species of Cyperaceae in about 80 genera and Goetghebeur (1998) included same number of species under 104 genera. Mabberley (2009) reported 92 genera and 4450 species, and Govaerts *et al.*, (2015) included 97 genera and 5486 species. Karthikeyan *et al.*, (1989) listed 537 species and 92 varieties of Cyperaceae under 38 genera and this number was later updated as 580 species under 38 genera (Karthikeyan, 1999). Singh and Prasad (2001) estimated about 570 species of 39 genera in India and the present number is estimated to be about 580 species belonging to 32 genera (Patil and Prasad, 2016). In Goa it is represented by 94 species, 2 subspecies and 9 varieties belonging to 16 genera. The genus *Cyperus* L. of family Cyperaceae is annual or perennial sedges, often rhizomatous or stoloniferous. Culms tufted or solitary, triquetrous, trigonous or sometimes subterete, without nodes above the leafy base. Leaves 3-ranked with narrowly linear, grass-like blade, sometimes reduced to subaphyllous sheaths; lower leaves often scale-like, covering base of the culm. Inflorescence a terminal anthela, simple to decomposed, sometimes becomes capitate by contraction. Involucral bracts leaf-like, without sheath. Spikelets sessile, prophyllate at base, usually compressed, bearing few to many glumes; rachilla continuous and persistent or jointed at base and deciduous, often winged. Glumes distichous, bearing an axillary flower, occasionally one or two glumes above the prophyll empty. Flowers bisexual, uppermost often staminate or barren. Hypogynous bristles or scales absent. Stamens 3, 2 or 1; connective of the anthers often produced into an apical appendage. Style continuous with the ovary, not jointed at base, 3-fid or 2-fid, rarely almost undivided. Achene trigonous, triquetrous or lenticular with one face towards the rachilla. The first basic study on epidermal silica bodies of the achenes was accomplished by Schuyler (1971) on two species of *Scirpus* L. and *Eriophorum* L. that lead to development of a new set of characters that could reevaluate the

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systematics of Cyperaceae. Verma *et al.*, (1989) studied the epidermal surface patterns of the achenes in *Eleocharis*, Govindrajalu (1990) studied SEM images of *Pycneus* sect. *Muricati*. and Wujek *et al.*, (1992) did the achene micromorphology of some Indian species of *Cyperus*, *Fimbristylis*, *Pycneus*, *Scirpus* and *Scleria*. Also, Menapace *et al.*, (2003) did the achene micromorphology of some Indian species as a possible systematic aid to the taxonomic recognition of different sections in *Fimbristylis*. Patil and Prasad (2016, 2016a) did the micromorphology of the achenes of *Fimbristylis* and *Eleocharis* species found in Goa. In the present study achenes of 21 taxa belonging to the genus *Cyperus* in Goa have been studied and interpreted for their similarities and dissimilarities. The study includes macro-morphology such as achene size, shape, etc. and the micro-epidermal cell structures, which includes nature of periclinal wall, the number, thickness and sinuosity of the anticlinal wall, and presence or absence of silica bodies. The shape and number of silica bodies per cell were also studied.

MATERIALS AND METHODS

Achene samples were collected from the plant specimens collected from different localities in Goa. The specimens collected were identified utilizing the available facilities in Botanical Survey of India, Pune and the herbarium in Goa University. The herbarium specimens from which achene samples were taken are deposited in BSI. For better result, fully matured specimens were selected to study the morphology of achenes by conventional method using stereo microscope and by the advanced method of interpreting the Scanning Electron Microscope (SEM) images. The shape and size of the achenes of each species were recorded and the micro-structure of the achene surface was studied using SEM images. For this, achenes were extracted from the spikelets and mounted on glass slides with sticky tape, mounted on SEM stubs and then sputter coated with platinum and examined under JOEL JSM6360 Scanning Electron Microscope. The images were then photographed at different magnifications. The SEM images of achenes of different species thus, obtained were then interpreted with the help of relevant literature. Achene shape, size, its ornamentations and micro-epidermal structures such as nature of periclinal walls, anticlinal walls and silica bodies were studied to find out the similarities or dissimilarities.

RESULTS AND DISCUSSION

The genus *Cyperus* in Goa shows a lot of differences in achene shape, size as well in the epidermal microstructures. Most of the species have trigonous achenes while in a few achenes are triquetrous (e.g. *C. alulatus*, *C. difformis*, *C. iria*, *C. pilosus*, and *C. procerus*). However, in a few like *C. arenarius*, *C. michelianus* subsp. *pygmaeus*, and *C. stoloniferus* achenes are biconvex. Based on length of the achene *C. paniceus* var. *roxburghianus* may be considered as the largest, being 2 to 2.31 mm long and achenes of *C. tenuispica* the smallest, being 0.3 to 0.42 mm long. However, when breadth is considered, the broadest achenes were found in *C. arenarius* being ≤ 1.13 mm and the narrowest in *C. squarrosus* being ≤ 0.22 mm. Closely related taxa of *Cyperus* can be easily separated based on achene size together with other morphological characters. For example *C. haspan* subsp. *haspan* with $\leq 0.45 \times 0.33$ mm achene can be differentiated from *C. haspan* subsp. *juncooides* having $\leq 0.62 \times 0.44$ mm achene. Similarly, closely related *C. alulatus* ($\leq 1.59 \times 0.82$ mm) and *C. iria* (1.26×0.64 mm) can be separated based on the achene size.

Out of the 29 taxa of the genus 21 were studied for their achene morphology using SEM images. Silica bodies are absent in the epidermal cells of *C. arenarius*, *C. digitatus*, *C. javanicus*, *C. procerus* and *C. rotundus*, while pointed or blunt ended silica bodies were found in the remaining species. The closely related species *C. squarrosus* and *C. maderaspatanus* can be easily distinguished based on microstructures on the achene surface. In *C. maderaspatanus* achenes are with isodiametric, polygonal epidermal cells having convex periclinal wall and with central spherical silica bodies. But in *C. squarrosus* achenes are with irregular epidermal cells, having pointed silica bodies on the periclinal wall. In typical *Cyperus paniceus* anticlinal walls are inconspicuous in the epidermal cells of the achenes, but conspicuous in its variety *roxburghianus*.

A brief account of the important findings is depicted in Table 1 below and the SEM images are shown on plates 1 to 5.

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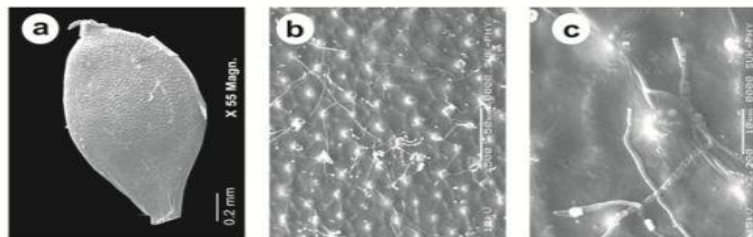
Nature of the anticlinal walls was found to be quite different in different taxa of *Cyperus*. It may be slender or thick, straight or sinuous, raised or depressed and based on these characters different taxa can be identified and delimited from each other. Based on the nature of anticlinal walls the 21 *Cyperus* taxa studied can be broadly grouped into three groups as given below:

Group I: Taxa with straight anticlinal walls. *C. alulatus*, *C. compressus*, *C. difformis*, *C. distans*, *C. exaltatus*, *C. iria*, *C. malaccensis*, *C. pilosus*, *C. rotundus* and *C. tenuispica* comes under this group.

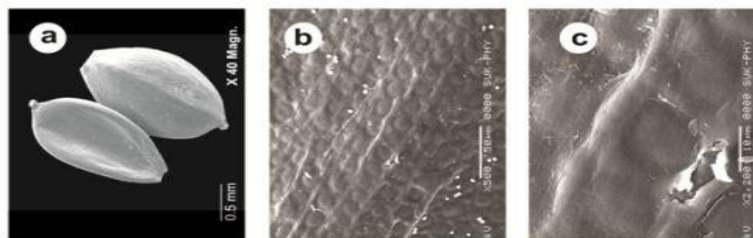
Group II: Taxa with sinuous anticlinal walls. These are *C. arenarius*, *C. digitatus*, *C. haspan* subsp. *haspan*, *C. haspan* subsp. *juncooides*, *C. javanicus*, *C. pangorei*, *C. paniceus* var. *roxburghianus* and *C. procerus*.

Group III: Taxa with inconspicuous anticlinal walls. *C. maderaspatanus*, *C. paniceus* var. *paniceus* and *C. squarrosus* comes under this group.

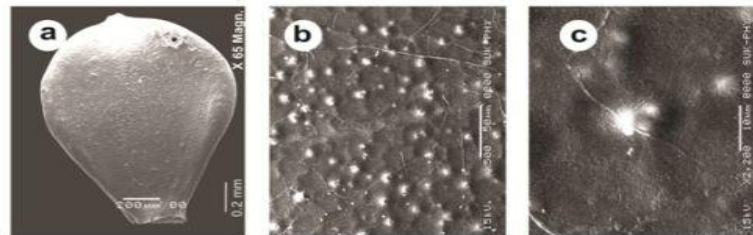
**SEM MICROGRAPHS OF CYPERUS L. ACHENES
 PLATE 1**



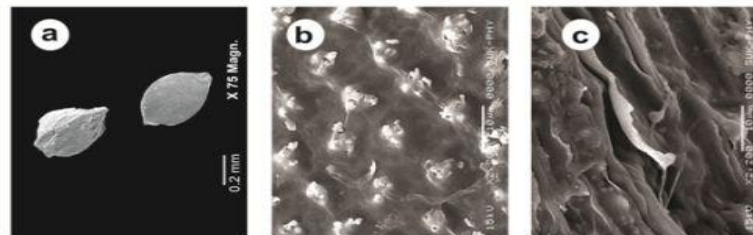
Cyperus alulatus J. Kern - a. Achene, b & c. Epidermal cells



Cyperus arenarius Retz. - a. Achene - dorsal & ventral view, b & c. Epidermal cells



Cyperus compressus L. - a. Achene, b & c. Epidermal cells



Cyperus difformis L. - a. Achene - ventral & dorsal view, b. Epidermal cells - ventral surface
 c. Epidermal cells - dorsal surface

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Table 1: Macro- and Micro-Morphology of Achenes in the Genus *Cyperus*

Sr. No.	Plant Name and Specimen	Voucher	Macromorphology	Micromorphology (SEM)
1.	<i>Cyperus alulatus</i> J. Kern <i>R.T. Patil</i> 192606 (BSI). PLATE 1		Triquetrous, obovoid with concave sides, mucronate at apex, 1.59 x 0.82 mm.	Epidermal cells inconspicuous, formed of somewhat hexagonal cells; anticlinal walls 6-7, unequal, straight, with minute projections at the junction of two walls; periclinal wall with centrally placed small apical nodular silica bodies; buttresses distinct.
2.	<i>Cyperus arenarius</i> Retz. <i>R.T. Patil</i> 3023 (BSI). PLATE 1		Planoconvex with angulate dorsal surface and concave ventral surface, broadly obovate, apiculate at the obtuse apex, not stipitate but notched on posterior side, 1.82 x 1.13 mm.	Epidermal cells shows distinct pattern on angles and margins of achene, otherwise without particular pattern. Rows of vertical ridges contain superimposed rectangular cells. Anticlinal walls slightly sinuous; periclinal wall concave towards periphery and convex at the centre; silica bodies absent.
3.	<i>Cyperus compressus</i> L. <i>R.T. Patil</i> 192579 (BSI). PLATE 1		Conspicuously trigonous, broadly obvoid, minutely apiculate at the obtuse apex, 1.46 x 1.04 mm.	Epidermal cells smaller in size, longitudinally elongated pentagonal or hexagonal; anticlinal wall straight, thin, sometimes with reduced silica bodies at their corners; periclinal walls smooth and flat, with 1 or 2 nodular silica bodies at the centre; buttresses present.
4.	<i>Cyperus difformis</i> L. <i>R.T. Patil</i> 192569 (BSI). PLATE 1		Triquetrous, obovate-elliptic, minutely apiculate at apex, shortly stipitate, 0.52 x 0.33 mm.	Epidermal cells circular polygonal, at times not clearly visible; anticlinal wall inconspicuous but straight; periclinal wall concave near the silica body; silica bodies with 4-5 nodular projections; buttresses prominent. Silica bodies of nearby cells unite through the buttresses and appear in vertical rows on the achene surface.
5.	<i>Cyperus digitatus</i> Roxb. <i>R.T. Patil</i> 192558 (BSI). PLATE 2		Trigonous, elliptic-oblong, apiculate at apex, 0.8 x 0.41 mm.	Epidermal cells isodiametric quadrangular; anticlinal walls 4, thick, raised, weakly sinuous showing beaded appearance; periclinal wall smooth and slightly concave, otherwise flat; silica bodies absent.
6.	<i>Cyperus distans</i> L. <i>R.T. Patil</i> 3014 (BSI). PLATE 2		Trigonous, elliptic-oblong to oblong, apiculate at apex, 1.5 x 0.38 mm, with a dorsal groove.	Epidermal cells hexagonal, distinct on margins of the achene; anticlinal wall thin, straight, raised; periclinal wall smooth, with central silica bodies. Epidermal pattern is indistinct in most part of the achene.
7.	<i>Cyperus exaltatus</i> Retz. var. <i>exaltatus</i>		Trigonous, elliptic to elliptic-obovate, minutely apiculate at apex, 0.77 x 0.40	Epidermal cells polygonal; anticlinal walls raised; periclinal wall with inconspicuous spherical silica bodies;

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	<i>R.T. Patil</i> 3020 (BSI). PLATE 2	mm.	buttresses present.
8.	<i>Cyperus haspan</i> L. subsp. <i>haspan</i> <i>R.T. Patil</i> 192561 (BSI). PLATE 2	Trigonous, broadly obovate, prominently stipitate, minutely apiculate at apex, 0.45 x 0.33 mm.	Epidermal cells elongated hexagonal; anticlinal wall 5-6, sinuous, raised; periclinal wall smooth, flat but convex at base of the nodular silica bodies.
9.	<i>Cyperus haspan</i> subsp. <i>juncooides</i> (Lam.) Kuk. <i>R.T. Patil</i> 3008 (BSI). PLATE 3	Trigonous, broadly obovate, minutely apiculate at apex, prominently stipitate, 0.62 x 0.44 mm.	Epidermal cells elongated hexagonal; anticlinal walls 4-5, weakly sinuous, raised; periclinal wall smooth, flat but convex at the base of nodular silica bodies; buttresses 1 or 2.
10.	<i>Cyperus iria</i> L. <i>R.T. Patil</i> 192581 (BSI). PLATE 3	Triquetrous, obovate, minutely apiculate at apex, 1.26 x 0.64 mm.	Epidermal cells with 6-8 anticlinal walls; anticlinal wall thick, straight, raised, with knob like structures at the junction of two walls; silica bodies nodular; buttresses 3-4, prominent between silica bodies and anticlinal walls.
11.	<i>Cyperus javanicus</i> Houtt. <i>R.T. Patil</i> 192687 (BSI). PLATE 3	Trigonous, subovoid-ellipsoid, cunneate and apiculate at apex, 1.22 x 0.62 mm.	Epidermal cells elongated hexagonal; anticlinal walls slender, weakly sinuous, depressed; periclinal wall smooth, convex, without silica bodies.
12.	<i>Cyperus maderaspatanus</i> Willd. <i>R.T. Patil</i> 192611 (BSI). PLATE 3	Trigonous, oblong, slightly curved, minutely apiculate at apex, shortly stipitate, 1.36 x 0.29 mm.	Epidermal cells isodiametric polygonal; anticlinal walls inconspicuous, weakly depressed; periclinal wall convex, with central spherical silica bodies arranged in a row; buttresses 1-3 for each cell.
13.	<i>Cyperus malaccensis</i> Lam. <i>R.T. Patil</i> 3017 (BSI). PLATE 4	Trigonous, narrowly oblong, minutely apiculate at apex, 1.22 x 0.33 mm.	Epidermal cells isodiametric pentagonal-hexagonal; anticlinal walls thick, straight, beaded, prominently raised; periclinal wall undulate, with flat, circular silica bodies; buttresses 4- 5, conspicuous in few cells only.
14.	<i>Cyperus pangorei</i> Rottb.	Trigonous, oblong-obovate, minutely apiculate at apex, shortly stipitate, 1.3	Epidermal cells isodiametric hexagonal; anticlinal walls thick, very slightly sinuous, raised; periclinal wall smooth,

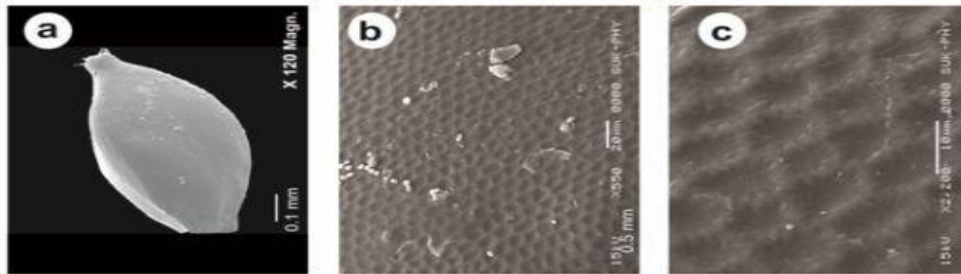
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		x 0.53 mm.	flat, with granular silica bodies dispersed in whole cells.
	<i>R.T. Patil</i> 192686 (BSI). PLATE 4		
15.	<i>Cyperus paniceus</i> (Rottb.) Boeckeler var. <i>paniceus</i>	Trigonous, elliptic-oblong, slightly curved, 2.27 mm x 0.60 mm.	Epidermal cell shape not clearly visible; anticlinal walls inconspicuous; periclinal wall smooth, with spherical silica bodies arranged in rows.
	<i>R.T. Patil</i> 192658 (BSI). PLATE 4		
16.	<i>Cyperus paniceus</i> var. <i>roxburghianus</i> (C.B. Clarke) Kuk. <i>R.T. Patil</i> 192677 (BSI). PLATE 4	Trigonous, elliptic-oblong, apiculate at apex, 2.31 x 0.64 mm.	Epidermal cells hexagonal; anticlinal walls conspicuous, weakly sinuous, slightly raised; periclinal wall smooth, with central spherical silica bodies in each cell; buttresses sometimes 1 or 2.
17.	<i>Cyperus pilosus</i> Vahl <i>R.T. Patil</i> 3019 (BSI). PLATE 5	Triquetrous, broadly elliptic-obovate, apiculate at apex, 1.05 x 0.55 mm.	Epidermal cells irregular in shape; anticlinal walls slender, straight, raised; periclinal wall smooth, flat, with central spherical silica bodies; buttresses 4 to 6.
18.	<i>Cyperus procerus</i> Rottb. <i>R.T. Patil</i> 192528, (BSI). PLATE 5	Triquetrous, obovoid-ellipsoid, minutely apiculate at apex, 1.37 x 0.57 mm.	Epidermal cells subisodiametric pentagonal to hexagonal; anticlinal wall thick, slightly sinuous with beaded appearance, raised; periclinal wall smooth, concave, without silica bodies.
19.	<i>Cyperus rotundus</i> L. <i>R.T. Patil</i> 192583 (BSI). PLATE 5	Trigonous, oblong-obovate, apiculate at apex, 1.16 x 0.35 mm.	Epidermal cell shape not clearly visible; anticlinal walls thick, straight, slightly raised; periclinal wall smooth, flat, without silica bodies.
20.	<i>Cyperus squarrosus</i> L. <i>R.T. Patil</i> 192575 (BSI). PLATE 5	Trigonous, oblong-obovate, minutely apiculate at the obtuse apex, 0.71 x 0.22 mm.	Epidermal cells irregular, shape not clearly visible; anticlinal walls inconspicuous; periclinal wall with pointed silica bodies arranged in longitudinal rows; buttresses occasionally present.
21.	<i>Cyperus tenuispica</i> Steud. <i>R.T. Patil</i> 192639 (BSI). PLATE 5	Obtusely trigonous, ovate-obovate, minutely apiculate at apex, 0.42 x 0.43 mm.	Epidermal cells elongated hexagonal; anticlinal walls slender, straight, depressed; periclinal wall convex, with blunt spherical silica bodies at centre of the cell.

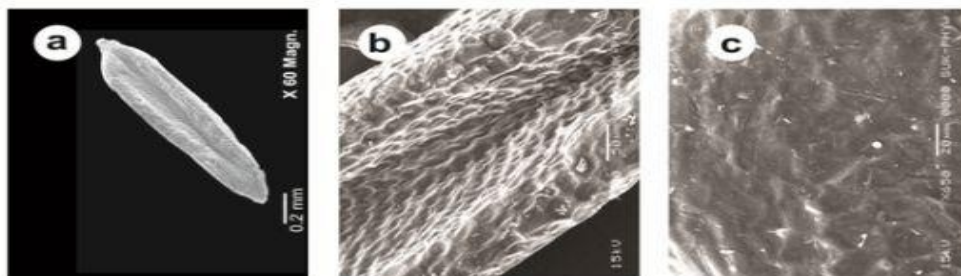
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Thus, SEM study of the achenes in the genus *Cyperus* was found to be useful in determining the taxonomic relationship at species level or infraspecific level. However, the conventional method of achene morphology using the ordinary stereomicroscope with light arrangement was found to be more convenient, less time consuming and inexpensive. But whenever there is confusion about identity of closely related taxa SEM images of the achenes can surely be helpful in their correct identification.

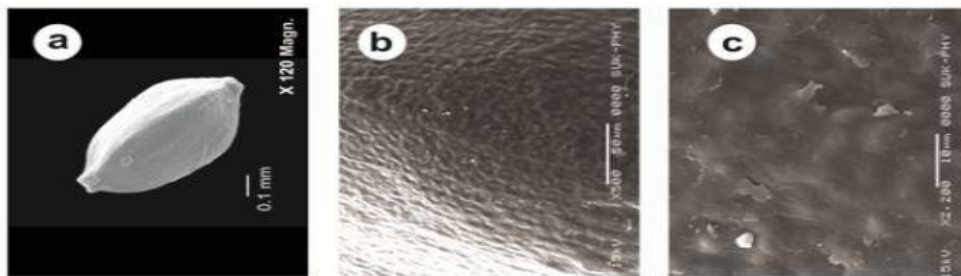
PLATE 2



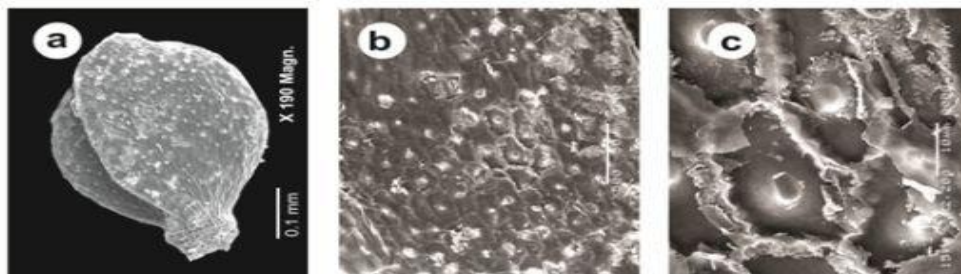
Cyperus digitatus Roxb. - a. Achene, b & c. Epidermal cells



Cyperus distans L. var. *distans* - a. Achene, b & c. Epidermal cells

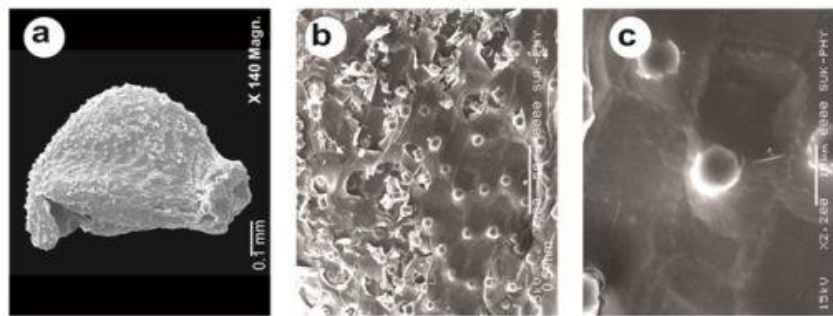


Cyperus exaltatus Retz. var. *dives* (Delile) C. B. Clarke - a. Achene, b & c. Epidermal cells

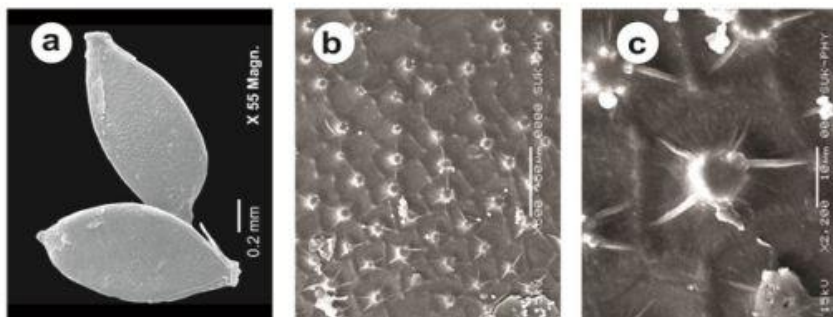


Cyperus haspan L. subsp. *haspan* - a. Achene, b & c. Epidermal cells

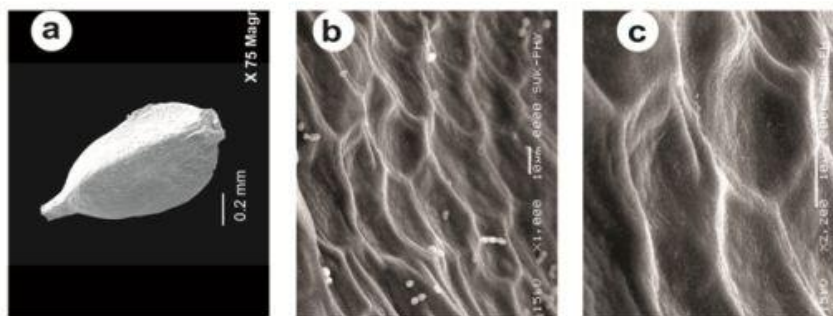
PLATE 3



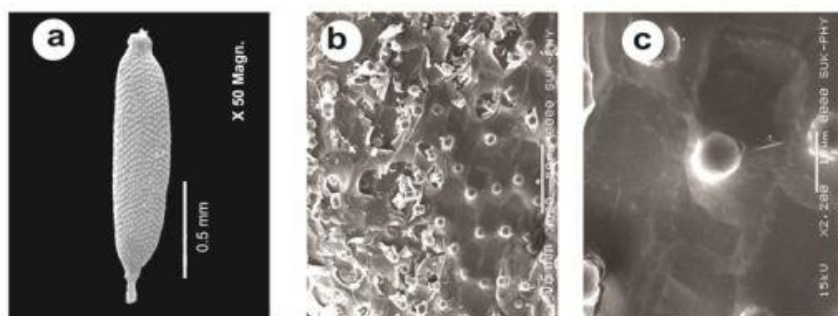
Cyperus haspan L. subsp. *juncooides* (Lam.) Kuk. - a. Achene, b & c Epidermal cells



Cyperus iria L. - a. Achene, b & c. Epidermal cells

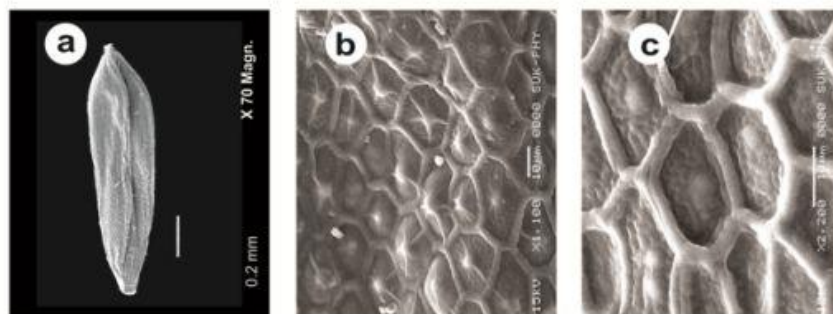


Cyperus javanicus Houtt. - a. Achene, b & c. Epidermal cells

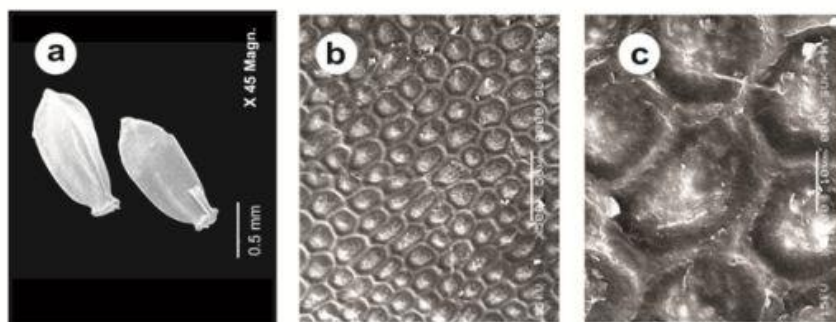


Cyperus maderaspatanus Willd. - a. Achene, b & c. Epidermal cells

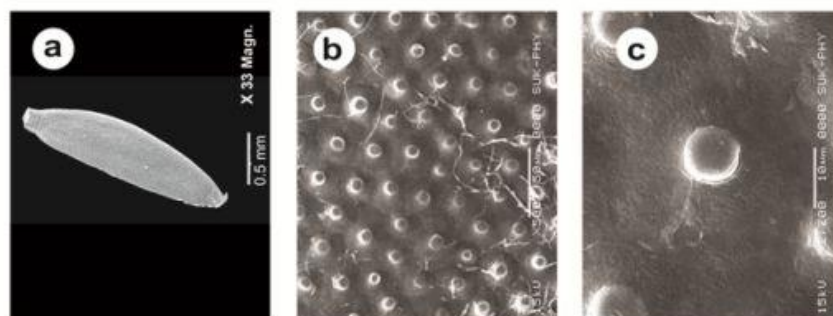
PLATE 4



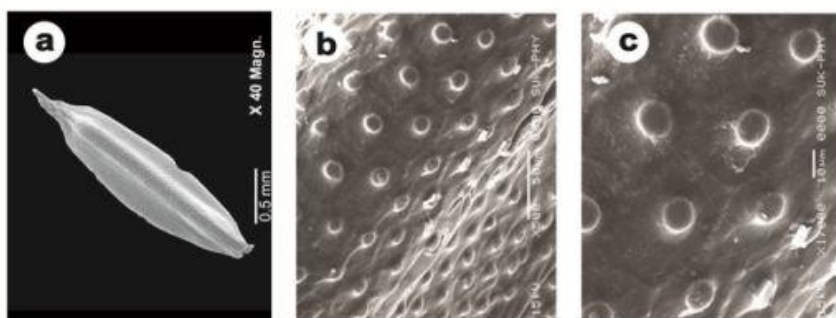
Cyperus malaccensis Lam. - a. Achene, b & c. Epidermal cells



Cyperus pangorei Rottb. - a. Achene - dorsal & ventral view, b & c. Epidermal cells

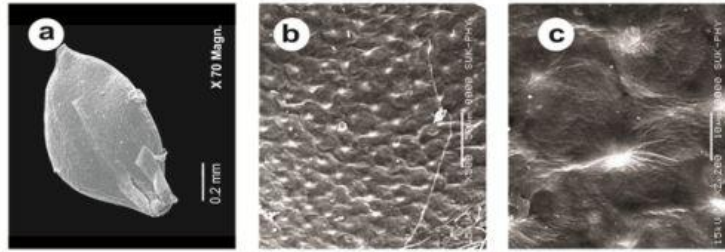


Cyperus paniceus (Rottb.) Boeckeler var. *paniceus* - a. Achene, b & c. Epidermal cells

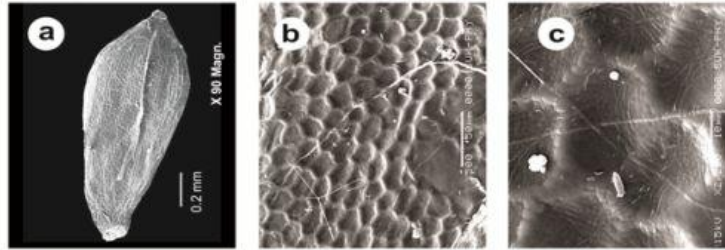


Cyperus paniceus (Rottb.) Boeckeler var. *roxburghianus* (C.B. Clarke) Kuk. - a. Achene, b & c. Epidermal cells

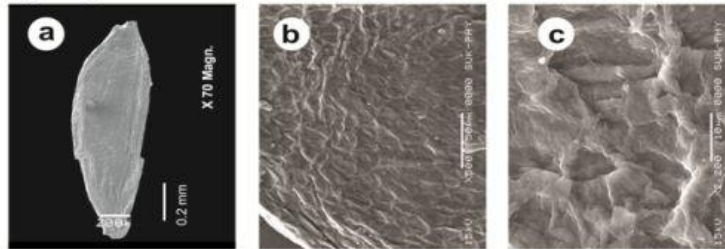
PLATE 5



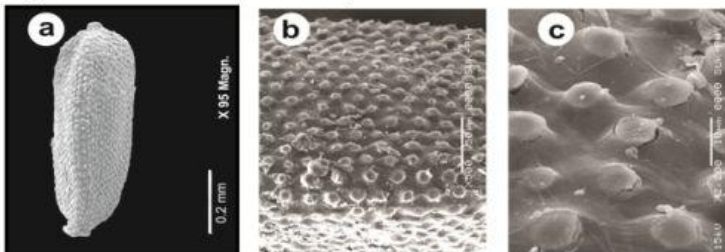
Cyperus pilosus Vahl - a. Achene, b & c. Epidermal cells



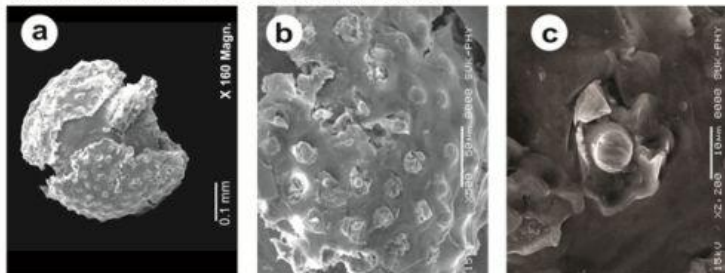
Cyperus procerus Rottb. - a. Achene, b & c. Epidermal cells



Cyperus rotundus L. - a. Achene b & c. Epidermal cells



Cyperus squarrosus L. - a. Achene, b & c. Epidermal cells



Cyperus tenuispica Steud. - a. Achene (broken), b & c. Epidermal cells

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