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Research Paper

SEM studies support the narrow generic limit of genus *Cyperus* L.

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Abstract

The nut morphology of 08 species of *Cyperus* L. was examined under LM and SEM. The nuts display considerable diversity in shape, size, dimensions, colour and nuts surface in different species. SEM studies at high magnification show different types of surface patterns like papillate, reticulate, with different anticlinal and periclinal walls. The shape of all studied nut was elliptic, obovoid, oblong or ovate. Nut size ranges from 0.5 to 3 mm length and from 0.3 to 1 mm width. Controversy on narrow and broad generic limit of genus *Cyperus* L. The nut exomorphic characters from LM and SEM investigations support the narrow generic limits of genus *Cyperus* L.

Keywords: Cyperus L., LM, Narrow generic limit, Nut surface, SEM.

Introduction

Cyperus L., one of the most important genera of the family Cyperaceae, has worldwide distribution and it consist of annual and perennial herbs. Cyperus L., is a taxonomically difficult genus because of small inconspicuous flowers with complicated morphology and difficulties in their collection. Of all characters of the Cyperus L., morphology of the mature nut provides the most reliable diagnostic characters. It includes size, shape, sides, interfacial angles, apex, colour, texture and particularly the surface pattern. We aim in this study, with the aid of scanning electron microscope (SEM), to provide detailed data on nut morphology of Cyperus L., to determine which characteristics of their nuts may be used for taxonomic purpose. Heywood, Brisson and Pterson drew attention to the importance and impact of Scanning Electron Microscope in the study of systematic problems ^{[1] [2]}. Value of the nut as a practical guide in the study of Cyperaceae is indisputable. Blatter and McCann regarded nut as a "very reliable means of determination"^[3]. Morphology of the nuts and associated parts, viz. utricle, style base, bristles and scales, have been utilized by Clarke in discerning genera like Pycreus P. Beauv., Juncellus Clarke., Carex Linn., Kobresia Willd., Fimbristylis Vahl, Bulbostylis Kunth. and Eleocharis R. Br.^[4]. According to Clarke "The form, size and surface appearance of the nut are characters of primary importance" in the identification of the Indian species of Cyperus Linn.^[5]. Blatter to the North America species of the genus Scirpus Linn. is based on nut characters ^[6]. The outline and form of the nut have been fully utilized by Kern in his key to the Malaysian species of Fimbristylis Vahl.^[7]. Recently, the application of LM and SEM in the study of seed coat has become widespread ^[8] ^[9]. Hence, to examine the characters of nut surface and help to the taxonomic disposition of certain taxa the present mark has been undertaken.

Materials and Methods

Nuts of 08 species of *Cyperus* L. were collected from the field of Khandesh region of Maharashtra. The collected specimens were identified with the help of Dr. M. A Wadood Khan and by the matching with herbarium specimens of Botanical Survey of India, Pune-Maharashtra, India. Only mature and dry nuts were taken for investigation. Dry nuts were cleaned, and examined under light microscope to

observe the different exomorphic parameters viz., shape, dimensions, colour and nut surface texture. Five nuts of each species were taken to cover the range of variations.

| S. | | | | | Dimensions in mm |
|-----|--|-----------------------------------|-------------------------------|---|--------------------------|
| No. | Species | Shape | Colour | Seed surface | |
| 1 | <i>Cyperus alulatus</i> Kern | Obovoid | Shining dark brown | Reticulate-minutely papillate | 1.5-1.8 x <i>ca</i> 1 mm |
| 2 | Cyperus corymbosus Rottb. | Narrowly elliptic- oblong | Brown or grayish- brown | Reticulate with regularly rectangular radial walls, minutely elevated central papillae | 1-1.2 x 0.2-0.4 mm |
| 3 | <i>Juncellus alopecuroides</i> (Rott b.) Clarke | Broadly ellipsoid- obovoid | Yellow to golden yellow | Reticulate with similar wall thicking | 0.6-1 x 0.5-0.6 mm |
| 4 | <i>Juncellus laevigatus</i> (L.) Clarke | Ellipsoid | Grey to brown | Reticulate with broad anticlinal walls (irregular reticulum) | 1-1.5 x 0.8-1 mm |
| 5 | <i>Mariscus clarkei</i> (Cooke) Koyama | Narrowly ellipsoid- oblong | Pale yellow to brown | Reticulate, anticlinal and periclinal wall not prominent | 2-3 x 0.7-1 mm |
| 6 | <i>Mariscus cyperinus</i> (Retz.) Vahl | Oblong to narrowly elliptic | Brown | Reticulate, with prominent radial walls forming regular polygonal cells. Papillae smooth, raised. | 2-2.5 x 0.6-0.9 mm |
| 7 | <i>Pycreus pumilus</i> (L.) Nees | Narrowly obovoid | Brownish | Reticulate with distinct polygonal cells, smooth prominent papillae which are more or less interconnected. | 0.5-0.6 x 0.3-0.4 mm |
| 8 | <i>Pycreus punctatus</i> (Roxb.) Govind. | Oblong- ovate, or obovoid | Brownish- black | Reticulate with distinct polygonal cells. Each cell is distantly arranged from its adjacent cell, anticlinal and periclinal wall prominent with smooth prominent papillae which are interconnected by narrow lines | 0.8-0.9 x 0.4-0.5 mm |

Table 1: Morphological characters of nut surface studied as revealed by LM and SEM

For SEM investigation, dry nuts were mounted on metal stubs and examined using Philips XL 30 ESEM with EDAX at the Sophesticated Instrumentation Center for Applied Research and Testing (SICART), Vallabh Vidyanagar, Gujarat, India. The whole surface of each nut was examined under the SEM and photographs were taken at two magnifications: lower magnification to show whole nut and higher magnification to show fine surface details. The terms used for describing the nut coat patterns have been adopted according to Stearn^[10].

Results and Discussion

The nut morphological characters of the studied species of *Cyperus* L. as shown by LM and SEM are reviewed in Table I. There have been often controversial opinions among the Cyperologists as regards the generic & intrageneric delimitation of several genera. Conflict opinions and inconsistent treatment of several species which have been recorded here, are difficult to accommodate in narrow generic limit. However, there are certain genera such as *Cyperus* L., *Scirpus* L., *Fimbristylis* Vahl, *Scleria* P.Berg. and *Lipocarpha* R. Br. are problematic. Growing tendency appears to be in favour of splitting the genera and accepting narrow generic limit. Even after the close morphological similarities together with uniform embryography *Cyperus* L. has been split into smaller genera like *Kyllinga* Rottb., *Pycreus* P. Beauv., *Juncellus* Clarke, *Mariscus* Vahl., *Courtoisina* Nees, *Quinslandiella* Dom. and considered to be distinct from *Cyperus* L. Taxonomists supporting narrow generic limit include Palla and Clarke took *Scirpus* L. in broad sense and *Cyperus* L. in narrow sense^[11], recently Hooper, Koyama, Simpson & Koyama, Goetghebeur & Simpson, J. Bruhl, Prasad and Singh, and a few others ^{[12][13[[14][15]]}. Some Cyperologists treat the genera such as *Cyperus* L., *Scirpus* L., *Scleria* P.Berg. in broad sense with different species groups under Sections.

In present investigation an attempt is made to solve the complex of narrow generic limit and broad generic limit of the genus *Cyperus* L. which has been split into many smaller genera like *Kyllinga* Rottb., *Pycreus* P. Beauv., *Juncellus* Clarke, *Mariscus* Vahl., *Quinslandiella* Dom. by the taxonomist like Palla, Hooper, Koyama, Simpson, Bruhl etc, who followed narrow generic limit. While others like Bentham, Clarke, Kern etc. are in favor of taking genera in broad sense and here they have merged all these in one common genus *Cyperus* L. and thus a complex is formed in Cyperaceae^{[5][7]}. This complex can be solved by the thorough study of all species of this genus with respect to their anatomy, embryology, palynology and nut morphology. Nut morphology is one of the recent tool. A keen investigation based on extensive and intensive field and laboratory exploration is the need of present taxonomy of family.

All the investigations of previous workers like Blatter and McCan, Clarke, Koyama and Kern including the present one reveal that nut surface are usually species specific. Light and scanning electron microscopy reveals that shape, size, colour and nut surface pattern varies considerably in *Mariscus* Vahl., *Juncellus* Clarke and *Cyperus* L. If the shape is considered then it significantly varies in all genera and species. In *Mariscus clarkei* (Cooke) Koyama nut shape is narrowly ellipsoid-oblong and in *Mariscus cyperinus* (Retz.) Vahl., oblong to narrowly elliptic. however in *Juncellus alopecuroides* (Rottb.) Clarke broadly ellipsoid-obovoid and *Juncellus laevigatus* (L.) Clarke ellipsoid where as in *Pycreus pumilus* (L.) Nees narrowly obovoid and *Pycreus punctatus* (Roxb.) Govind. oblong-ovate or obovoid. Hence at generic level in genus *Mariscus* Vahl., the shape is ellipsoid-oblong, however it is broadly obovoid in *Juncellus* Clarke, where as in *Pycreus* P. Beauv. it is ellipsoid-obovoid. While *Cyperus* L. shows diversity in nut shape like obovoid, elliptic, oblong, pear shape, obovoid etc. Hence on the basis of nut shape all genus are distinct. Nut size ranged from 0.5-3 mm in length and from 0.3-1 mm in width. The smallest nut was found in *Pycreus pumilus* (L.) Nees and the biggest nut was found in *Mariscus clarkei* (Cooke) Koyama (Table1).

Similarly, SEM studies of nut surface also show outstanding differences within genus and species. In *Mariscus clarkei* (Cooke) Koyama nut surface is reticulate, anticlinal and periclinal wall not prominent and *Mariscus cyperinus* (Retz.) Vahl., surface is reticulate, with prominent radial walls forming regular polygonal cells, papillae smooth, raised. however in *Juncellus alopecuroides* (Rottb.) Clarke surface is reticulate with similar wall thicking and *Juncellus laevigatus* (L.) Clarke reticulate with broad anticlinal walls (irregular reticulum). where as in *Pycreus pumilus* (L.)

Nees reticulate with distinct polygonal cells, smooth prominent papillae which are more or less interconnected and *Pycreus punctatus* (Roxb.) Govind. nut surface is reticulate with distinct polygonal cells, each cell is distantly arranged from its adjacent cell, anticlinal and periclinal wall prominent with smooth prominent papillae which are interconnected by narrow lines. While in *Cyperus alulatus* Kern surface is reticulate-minutely papillate and in *Cyperus corymbosus* Rottb. Nut surface shows the reticulate with regularly rectangular radial walls, minutely elevated central papillae.



Cyperus alulatus Kern. Nut 75X



Cyperus corymbosus Rottb. Nut 75X



Pycreus pumilus L. Nut 75X



Pycreus punctatus Roxb. Nut 75X



C. alulatus Kern. Nut surface 300X



C. corymbosus Rottb. Nut surface 300X



P. pumilus L. Nut surface 300X



P. punctatus Roxb. Nut surface 300X

Plate-I

Under SEM all genera shows different surface sculpturing pattern in genus *Mariscus* Vahl. papillae are distinct are much raised (Plate-II). While in *Juncellus* Clarke papillae are absent (Plate-II). In *Pycreus* P. Beauv. central portion is with smooth papillae and anticlinal and periclinal walls are prominent (Plate-I). *Cyperus* L. exhibit different types of surface like smooth papillate, surface with knob like papillae, reticulate surface, much raised papillae prominent anticlinal and periclinal wall and also some with vertical ridges (Plate-I). Present investigation conforms to those of *Pycreus* P. Beauv., *Mariscus* Vahl. and *Juncellus* Clarke regarding narrow generic limits of *Cyperus* L.



Mariscus clarkei Cooke Nut 75X



Mariscus cyperinus Retz. Nut 75X



Juncellus alopecuroides Rottb. Nut 75X J. alopecuroides Rottb. Nut surface 300X



Juncellus laevigatus L. Nut 75X



M. clarkei Cooke Nut surface 300X



M. cyperinus Retz. Nut surface 300X





J. laevigatus L. Nut surface 300X

Plate-II

Conclusion

Light and scanning electron microscopy reveals that nut surface pattern varies in the Mariscus Vahl., Juncellus Clarke, Pycreus P. Beauv. and Cyperus L. hence SEM study support the narrow generic limit of genus Cyperus L.

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