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RESEARCH **P**APER

Morphology of flower, pollen and orbicules of Meghalayan *Dioscorea* L. (Dioscoreaceae), North-East India: A pivotal taxon in the evolution of monocot

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Flowers, pollen and orbicules morphology is an important source of information for systematic and evolutionary studies among different species, genera and families level. In the present study the floral, pollen and orbicules morphology of 80 samples of the genus *Dioscorea* L. representing 8 species native to Meghalaya were studied. *Trichopus* and *Tacca*, the allied genera with *Burmannia* of Burmanniaceae, nearest family of Dioscoreaceae were selected as an outgroup for the present study. Variation in floral, pollen and orbicules characters were observed and 62 descriptors or traits were selected for morphometric analysis. Pollen of *Dioscorea* is monosulcate or bisulcate with perporate or microrecticulate sexine sculptures. Orbicules were mostly spherical with smooth surface.

Key words : Floral morphology, Pollen aperature, Sexine ornamentation, Orbicule morphology, Morphometric analysis

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INTRODUCTION

Dioscorea L., one of the core genus of the family Dioscoreaceae are mainly distributed in the tropics and subtropical regions of the world, of which India accounts for 50 species of *Dioscorea* (Anonymous, 1952) in distribution. Meghalaya, one of the eight states of North-East India is rich in biodiversity and has luxuriant growth of *Dioscorea* species in wild habitat. Nearly 16 species of *Dioscorea* has been reported from Meghalaya (Sheikh *et al.*,2009). This genus of the family Dioscoreaceae occupies a basal position among all extant monocotyledonous plants (Dahlgren, 1989 and Chase *et al.*, 2006). Despite of its large morphological diversity, this genus lacks in proper taxonomic treatment. Several workers like Knuth (1924); Burkill (1960); Coursey (1967) and Ayensu (1972) have classified the genus based on seed morphology, floral characters, underground organs and anatomical characters, yet the systematic of the genus is confusing and not completely resolved. Floral and pollen morphology plays an important role in taxonomic identification at species, generic or even higher level of classification. The arrangement, size, nature and numbers of different floral parts are included in taxonomic identification. Few workers who suggested the taxonomic significance of pollen morphology within Dioscoreaceae were Su (1987); Caddick *et al*. (1998) and Xifreda (2000). Others who contributed to the palynological data of *Dioscorea* species using light microscopy (LM) were Selling (1947); Kuprianova (1948); Sharma (1967); Erdtman (1969); Heusser (1971); Huang (1972) and Chavez *et al*. (1991). Microphological data remained

scarce untill Schols *et al*. (2001 and 2003) examined pollen of 96 *Dioscorea* species with light microscopy (LM), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) and concluded that pollen of *Dioscorea* are monosulcate or disulculate with perporate, perporate to microreticulate or striate exine and suggested that aperature number and sexine ornamentation to be frequently consistent within samples from each section. Schols *et al*. (2005) with correspond to the combine work of Wilkin *et al.* (2005) and Caddick *et al.* (2002) presented the pollen characters evolution in the genus *Dioscorea*. Though pollen characters assessment for phylogenetic study are comparatively rare at generic level but are mostly within family or at higher level. Such study made an approach for using micromorphological characters in systematic study of large genera.

With support to the work of Schol *et al*.(2001, 2003 and 2005), the present paper considers the importance of detailed floral, pollen and orbicules morphological study and aims at not only considering micromorphological characters but also incorporating the neglected floral characters and implicating the use of these characters for the proper systematics and evolutionary studies of large genera like *Dioscorea*.

RESEARCH METHODOLOGY

Fresh flowers were collected from wild habitat of Meghalaya. A total of eight species (*Dioscorea alata* L., *Dioscorea belophylla* (Prain) Haines, *Dioscorea bulbifera* L., *Dioscorea glabra* Roxb, *Dioscorea pentaphylla* L., *Dioscorea pubera* Bl., *Dioscorea oppositifolia* L., *Dioscorea lepcharum* Prain *et* Burk) were collected (appendix for voucher specimen number).

LM and SEM :

Approximately 10 pollen grains belonging to each species were acetolyzed following the protocol of Shivanna and Rangaswamy (1993) for LM and then acetolyzed pollen were dehydrated through an acetone series before critical point drying (CPD). The dried pollen was mounted on specimen stubs and micrographs were taken using digital imaging on JSM- 6360, JEOL scanning electron microscope at Sophisticated Analytical Instrument Facility, North Eastern Hill University, Shillong. Micrographs were also taken for whole male flower, anther lobes, style and staminode. For each species, the longest axis (LA) and the shortest equatorial axis (SEA) were measured using LM slides of acetolyzed pollen and additional measurement was taken by SEM.

Morphometric analysis :

A total of 62 descriptors (qualitative and quantitative) traits were selected from floral, pollen and orbicule morphology (Table A). Species means for 32 quantitative characters were calculated and the differences among the means were statistically significant at $p<0.0001$. All tests were performed using STATGRAPHICS centurion XVI Version 16.0.09. Qualitative characters were scored as binary state or unordered multistate characters whereas quantitative characters were scored as multistate characters. A maximum parsimony based phylogenetic tree was constructed using NONA ver 1.6 (Goloboff, 1993). Morphological data matrix were incorporated into Winclada ver. 1.00.08 (Nixon, 2002) and submitted into NONA using "spawn". A heuristic search was conducted employing the multiple TBR+TBR strategy, with 1000 replication and 999 random seeds.

Outgroup selection:

Trichopus and *Tacca*, the allied genera of *Dioscorea* from Dioscoreaceae and *Burmannia* of Burmanniaceae, nearest family of Dioscoreaceae was selected as an outgroups for the present study. Data for morphometric study of these outgroups was taken from available literature *viz*., Caddick *et al.* (1998, 2000, 2002a and b); Huber (1998) and Kunth (1924).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Floral morphology :

Plant is usually dioecious in the genus *Dioscorea* but other allied genera such as *Tacca*, *Trichopus* are hermaphrodite belonging to same family Dioscoreaceae. The female flowers are borne in small numbers in axillary spikes and male in larger numbers in panicles. Both male and female flower bear one large floral bract and small bracteoles. The sepal and petal are usually similar in size and appearance and are arrange in two whorls (Fig.1). Floral colour also shows variation between the species generally being white, purplish, creamish or greenish

Table A: List of qualitative and quantitative characters used in morphometric analysis

Sr. No.	Traits acronym	Characters/descriptors	Score code-descriptor code
		Qualitative / Quantitative traits	
1.	FT	Flower type	1-dioecious; 2- hermaphodite
		Male inflorescence	
2.	InfS	Inflorescence smell	0-absent;1-present
3.	NoInf/INT	Number of inflorescence per internode	1-One or two;2-many
4.	InfG/P	Inflorescence glabrous/pubescent	1-glabrous;2-pubescent
5.	InfPo	Inflorescence position	1-upward;2-downward
6.	MFC	Male flower colour	1-whitish purple;2-pale green;3-yellow
7.	FLBS	Floral bract shape	1-ovate acuminate;2-orbicular;3-ovate
8.	FLBrS	Floral bracteoles shape	1-broadly lanceolate;2-ovate acuminate; 0-others
9.	OTS	Outer tepal shape	1-ovate;2-obovate;3-lanceolate;0-others
10.	ITS	Inner tepal shape	1-linear oblong;2-oblong obovate;3-ovate
11.	TG/P	Tepal glabrous/pubescent	1-glabrous;2-pubescent
12.	STA	No. of stamen	1-3stamens;2-6stamens
13.	STAMA/P	Staminode absent/present	0-absent;1-present
14.	LMspk	Average length of spike	1-(1-10cm); 2-(11-20cm);3->20cm
15.	MFL	Male flower length	1-(2mm); 2->2mm
16.	MFP	Male flower peduncle length	1-(1.1-2cm);2-(2.1-3cm);3-(3.1-4cm); 4-(4.1-5cm)
17.	MFPe	Male flower pedicle length	1-(0.6-1.5mm); 2-(1.6-2.5mm); 3-(2.6-3.5mm)
18.	FLBL	Floral bract length	1-(1mm); 2->1mm
19.	FLBW	Foral bract width	1-(1mm); 2->1mm
20.	FLBrL	Floral bracteoles length	1-(0.1-1.0mm); 2->1mm
21.	FLBrW	Foral bracteoles width	1-(0.1-1.0mm); 2->1mm
22.	OTL	Outer tepal length	1-(1.5-3mm); 2->3mm
23.	OTW	Outer tepal width	1-(0.1-1.0mm); 2->1mm
24.	ITL	Inner tepal length	1-(0.1-3.1mm); 2->3.1mm
25.	ITW	Inner tepal width	1-(0.1-1.0mm); 2->1mm
		Female inflorescence	
26.	FNoInf/INT	Number of inflorescence per internode	1-One or two;2-many
27.	FInfG/P	Inflorescence glabrous/pubescent	1-glabrous;2-pubescent
28.	FInfPo	Inflorescence position	1-upward;2-downward
29.	FFC	Female flower colour	1-greenish brown;2-white;3-yellowish
30.	FFLBS	Floral bract shape	1-ovate acuminate;2-orbicular;3-ovate
31.	FFLBrS	Floral bracteoles shape	1-broadly lanceolate;2-ovate acuminate
32.	FOTS	Outer tepal shape	1-ovate;2-obovate;3-lanceolate
33.	FITS	Inner tepal shape	1-linear oblong;2-oblong obovate;3-ovate
34.	FSTAM	No. of staminode	1-3nos;2-6nos
35.	STYS	Style shape	1-fanshaped;2-hookshaped
36.	LFspk	Average length female spike	1-(1-20cm); 2-(21-40cm)
37.	feFL	Fower length	1-(5.5-6.5mm); 2->6.5mm
38.	feFBL	Floral bract length	1-(1.6-2.5mm); 2-(2.6-3.5mm)
39.	feFBW	Foral bract width	1-(1mm); 2->1mm
40.	feFBrL	Foral bracteoles length	1-(0.6-1.5mm);2-(1.6-2.5mm)

Table A: Contd.……..

Table A : Contd……

41.	feFBrW	Floral bracteoles width	1-(1mm); 2->1mm
42.	feOTL	Outer tepal length	1-(1.5mm); 2->1.5mm
43.	feOTW	Outer tepal width	1-(1mm); 2->1mm
44.	feITL	Inner tepal length	1-(1.5mm); 2->1.5mm
45.	feITW	Inner tepal width	1-(1mm); 2->1mm
46.	STL	Style length	1-(1-5mm); 2-(6-10mm)
47.	feSTAL	Staminode length	1-(0.8mm); 2-(0.9mm)
48.	fePL	Peduncle length	1-(1-5cm); 2-(6-10cm)
49.	fePeL	Pedicel length	1-(1.1-2mm); 2-(2.1-3mm)
		Palynology	
50.	NAL	No.of anther lobe	1:-4 lobes; 2:- more than 4 lobes
51.	EOX	Exine stratification	1-perporate; 2-microrecticulate; 3-striate
52.	NPoA	No. of pollen aperature	aperature; 2- 2 aperature
53.	OrbP/A	Presence or absence of Orbicule	1-presence; 2- absence
54.	OrbT	Orbicule type	1-spherical; 2- elliptical
55.	ANL	Anther length	1-(151-200µm); 2-(201-250µm);3-(251-300µm); 0->300µm
56.	ANB	Anther breadth	1-(60-88µm); 2-(89-109µm); 3-(110-130µm); 0->130µm
57.	LA	Longest axis	1-(10-16µm); 2-(17-23µm); 3->23µm
58.	SEA	Shortest equatorial axis	1-(5-11µm);2-(12-18µm); 3->18µm
59.	LeA	Length of aperature	1-(1-10µm); 2-(11-20µm);3->20µm
60.	WiA	Width of aperature	1-(0.51-0.80µm); 2-(0.81-1.00µm); 3->1µm
61.	FiL	Filament length	1-(101-150µm);2-(151-200µm);3-(201-250µm)
62.	OrbD	Orbicule diameter	1-(0.1-.50µm); 2-(0.6-1.00µm); 3->1µm

*cm= Centimeter; mm= Millimeter; µm= Micrometer

APPENDIX

Section	Species	Specimen vouchers[A]
Botriyosicyos (Hochst.) Uline	*Dioscorea pentaphylla* L.	NEHU-11946 (M) NEHU-11947 (F)
Enantiophyllum Uline	*Dioscorea alata* L.	NEHU-11944 (M) NEHU-11945 (F)
	Dioscorea belophylla (Prain) Haines	NEHU-11950 (F) NEHU-12034 (M)
	Dioscorea glabra Roxb,	NEHU-11937 (M) NEHU-11938 (F)
	Dioscorea pubera Bl.	NEHU-11948 (M) NEHU-11949 (F)
	Dioscorea oppositifolia L.	NEHU-11940 (M) NEHU-11941 (F)
	Dioscorea lepcharum Prain *et* Burk.	NEHU-11942 (M) NEHU-11943 (F)
Opsophyton Uline	*Dioscorea bulbifera* L.	NEHU-11935 (M) NEHU-11936 (F)

[A] specimen vouchers deposited at the herbarium, Department of Botany, North Eastern Hill University, Shillong;
M- Male plant, F- Female plant

Fig. 1: **Male and female flowers of *Dioscorea species:* A- a single male spike of *D.pentaphylla;* B- a portion of male spike of *D.oppositifolia;* C-C1- a single male flower and spike of *D.pubera;* D-D1- a portion of male inflorescences with single flower of *D. bulbifera;* E- female spike of *D.bulbifera;* F- a single female flower of *D.bulbifera;* G- a single female flower of *D.glabra;* H- a single female flower of *D.oppositifolia;* I-J -upper view of the female flowers of *D.oppositifolia* and *D.belophylla;* K- a single female flower of *D.pentaphylla***

shades.

Out of the eight species collected, male flower of *D. bulbifera* was larger in size compared to other species ranging (3.5-4) mm in length, whereas others ranges (1.5-2) mm in length. A peculiar characteristic features of male flower inflorescences of *D. bulbifera, D.pubera* and *D. pentaphylla* show the presence of sweet scented smell which was absent in other species. Presence of pubescent in the outer tepal of *D.pubera* and *D.pentaphylla* is another important characteristic feature for morphological study. Stamens are usually 6 in number but in *D.pentaphylla* it was observed to be 3 in number along with 3 staminodes. The staminodes of *D. pentaphylla* are longer in length then the stamen (Fig. 2). Female flower of *Dioscorea* species also shows variation in its morphological features such as floral length of *D. bulbifera* and *D. pentaphylla* was larger in size ranging (8-9) mm in length. Style length, shape in *Dioscorea* varies from species to species. Number of staminodes was observed to be usually 6.

Pollen and orbicules morphology :

Pollen and orbicule morphology varies from species to species (Fig. 3). The size of longest axis (LA) of pollen of *Dioscorea* species collected from Meghalaya ranges from 15.48µm in *D. bulbifera* to 20.48µm in *D. glabra*. pollen aperature was found to be bisulcate in species of *D. bulbifera, D. alata, D. oppositifolia, D. belophylla* and *D. glabra* whereas monosulcate in *D. lepcharum, D. pubera* and *D. pentaphylla*. Sexine sculpturing was found to be perporate to microrecticulate. Orbicules type ranges from elliptical to spherical with smooth surface.

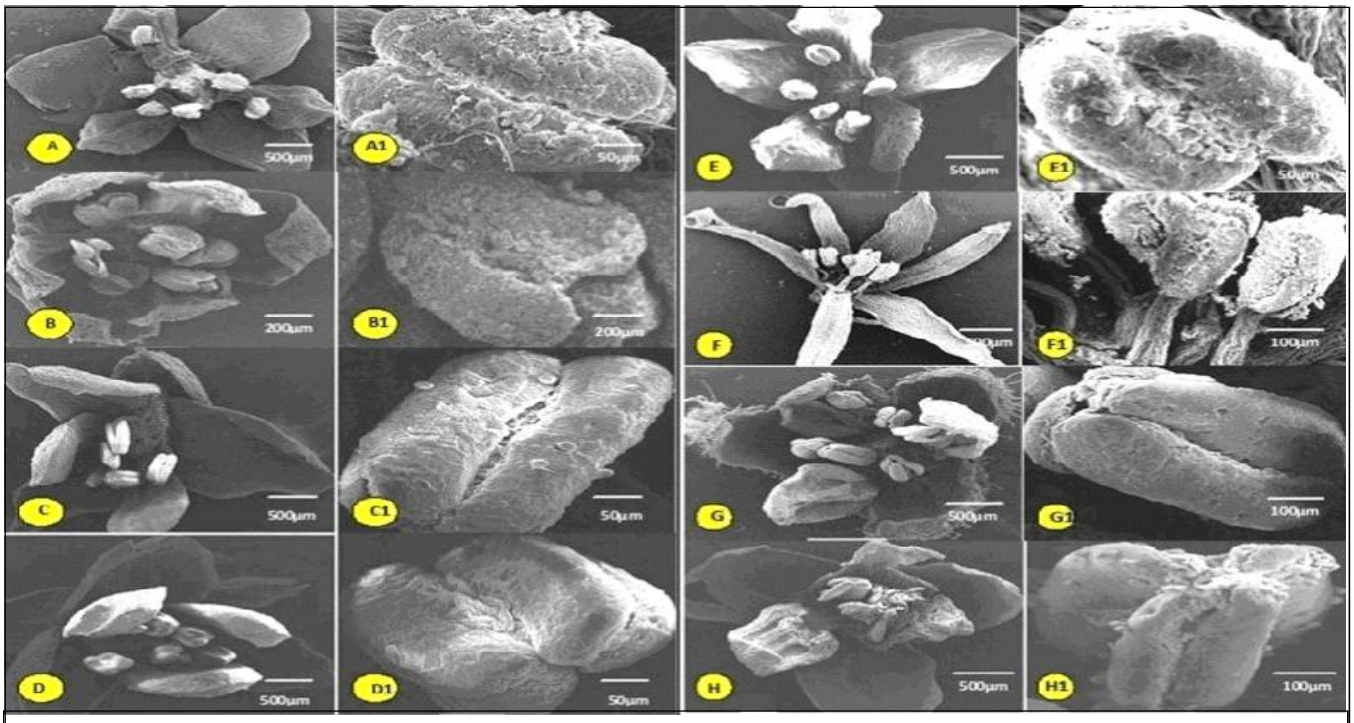

Fig. 2: **Mature male flowers and anther lobes of *Dioscorea* species viewed under SEM. A-A1-*D.pentaphylla;* B-B1- *D.alata;* C-C1-D. *glabra;* D-D1-*D.belophylla;* E-E1-*D.lepcharum;*F-F1-*D.bulbifera;*G-G1-*D.pubera;*H-H1-*D.oppositifolia***

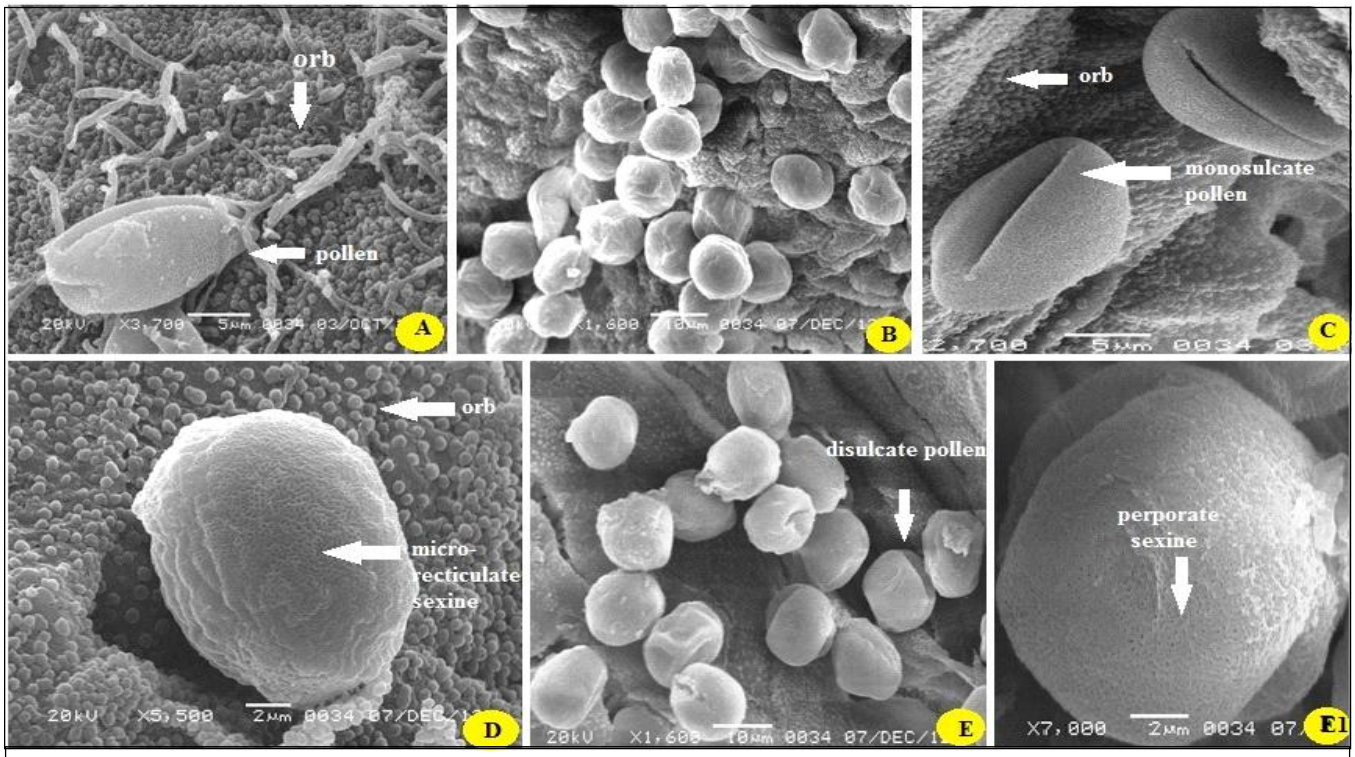

Fig. 3 : **Pollen, orbicules and sexine sculptures viewed under SEM. A. *D.pentaphylla;* B. *D. Bulbifera;* C. *D. Pubera;* D. *D.glabra;* E. *D. oppositifolia;* E1.*D.oppositifolia (enlarged view of pollen).Orb=orbicules***

Elliptical orbicules was found in *D. lepcharum* whereas rest species represent spherical orbicules.

Phylogeny based on morphometric analysis :

Result of morphometric analysis based on floral characters (Fig. 4) is different from analysis based on micromorphological (pollen and orbicules) characters. The dendrogram from floral characters shows three seperate clade *D. bulbifera* (sect.*Opsophyton*) and *D. pentaphylla*; *D. pubera* and *D. belophylla*; *D. glabra* and *D. lepcharum* forming a separate clade whereas the dendogram from morphometric analysis based on micromorphological characters shows aperature number shift from monosulcate pollen at the basal position to disulcate pollen in the rest species (Fig.5). *D.lepcharum* that forms the basal position of the genus in the

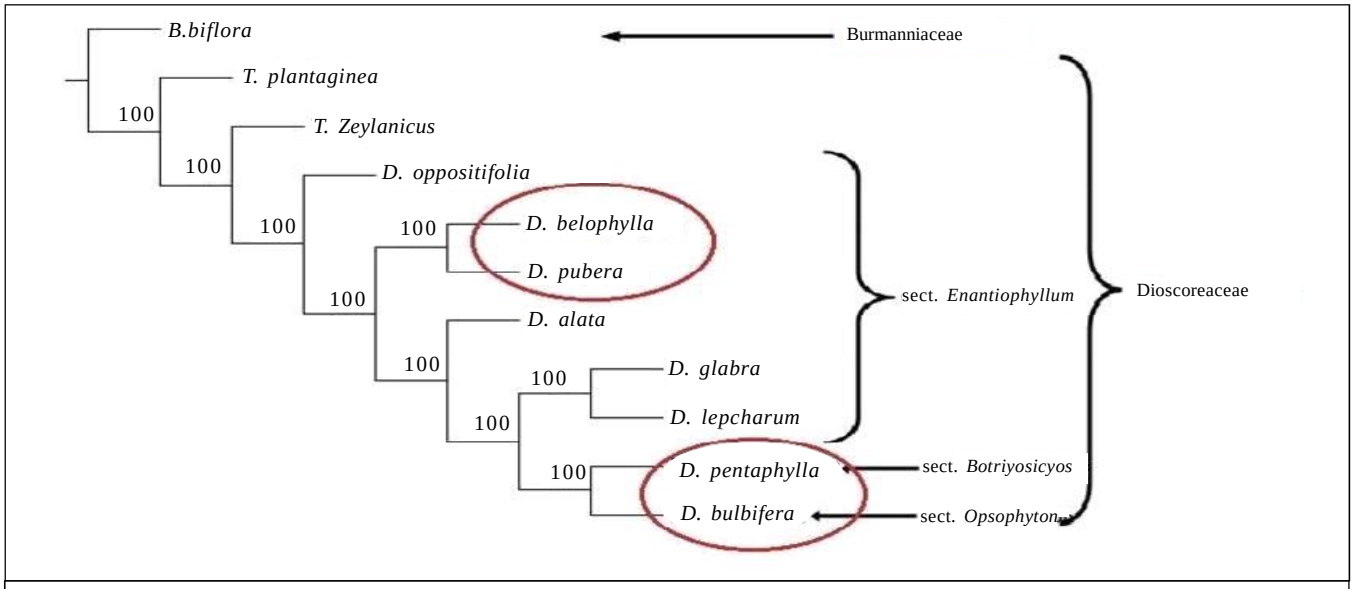

Fig. 4 : Relationship among eight species of the genus Dioscorea based on maximum parsimony analysis of 52 morphological characters of flower using NONA. Values at nodes indicate bootstrap values

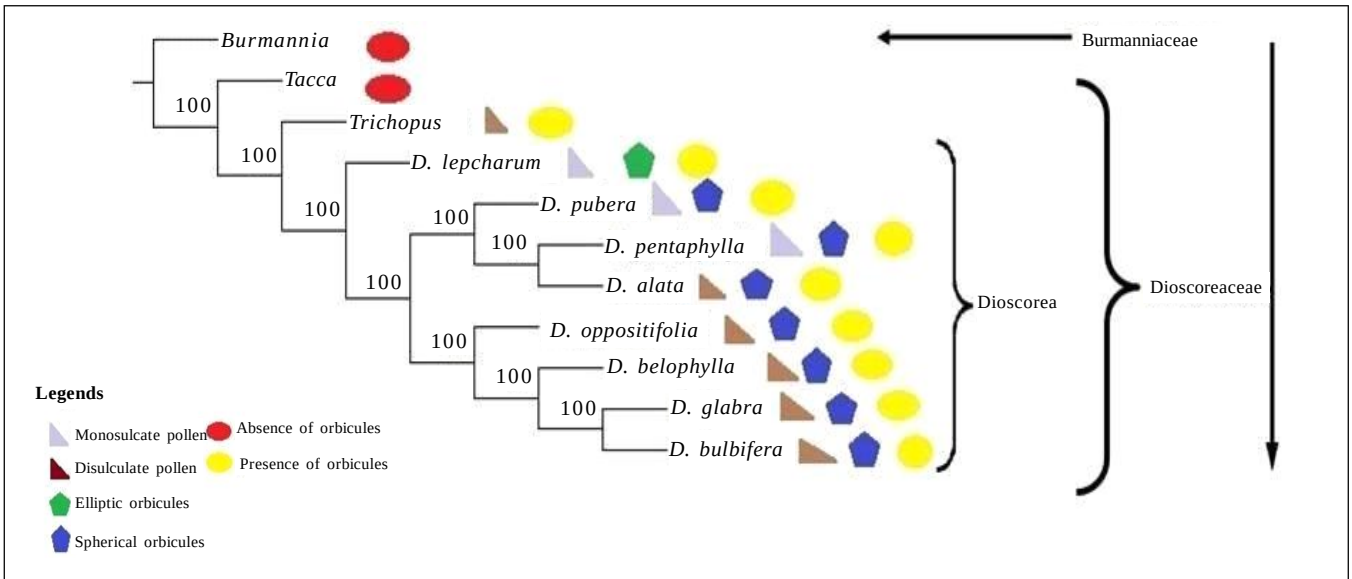

Fig. 5 : Relationship among eight species of the genus Dioscorea based on maximum parsimony analysis of 10 micromorphological characters of flower using NONA. Values at nodes indicate bootstrap values

dendrogram is characterized by the presence of elliptic orbicules with smooth surface whereas other species is characterized by the presences of spherical orbicules with smooth surface. Morphometric analysis based on combined data of floral and pollen characters (Fig. 6) illustrate that *D. pentaphylla* and *D. bulbifera* forming a separate clade whereas all the other species belong to sect. *Enantiophyllum* were grouped together in a separate clade.

The size of floral and pollen characters varies from species to species within the genus. According to Schols *et al.* (2001), the size of pollen grains of *D.bulbifera* (sect. *Opsophyton)* is smallest in size having an average LA of 16.2µm. They also suggested that almost all species which they examined from sect. *Opsophyton*, sect. *Enantiophyllum* have pollen grains that are smaller than 32µm. Su (1987) suggested that pollen of sections with annual tubers (*Enantiophyllum*, *Opsophyton*) is smaller than that of almost persistent tubers. The result from the present study seems to confirm these statements. *D.pentaphylla (*sect.*Botryosicyos)* is bisulcate as suggested by Schols *et al.* (2001) but our observation showed that it was monosulcate. Monosulcate pollen is generally accepted to be the plesiomorphic character state within the monocot (Dahlgren *et al.*, 1985); Furness and Rudall (1997 and 1999a). The transition from a single aperature to multiaperature pollen grains has occurred in numerous groups within monocot such as Alismatales (Zavada, 1983; Blackmore and Crane, 1998). In the present analysis similar transition was observed during morphometric analysis of micromorphological data. Presence of orbicules in all the species of *Dioscorea* and absence in the outgroup like *Burmannia* and *tacca* (Schols *et al.*, 2005) could be considered as an important character for evolutionary study within the genus and its allied genera.

Conclusion :

Evolutionary relationship within large genus like *Dioscorea* is unclear and more new data are required for systematic study. Floral morphological characters were always being useful in systematic investigation but if we incorporate the micromorphological characters like pollen size, aperature number, presence or absence of orbicules for systematic study could be best for systematic investigation and using morphometric analysis will help to show the evolution among the species within the genus and reflect the true phylogeny and taxonomy of large genera like *Dioscorea*.

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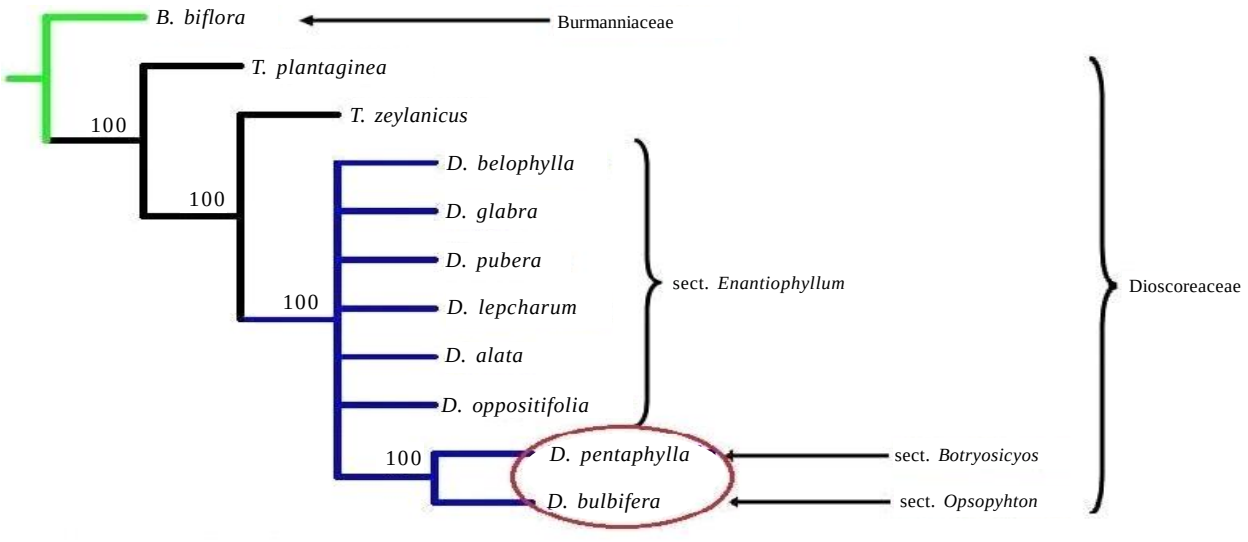

Fig. 6 : Relationship among eight species of the genus *Dioscorea* based on maximum parsimony analysis of 62 morphological characters (combined flower and pollen characters) using NONA. Values at nodes indicate bootstrap values

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LITERATURE CITED

Anonymous (1952). *The wealth of India* vol. 3.D-E, CSIR, NEW DELHI, INDIA.

Ayensu, E.S. (1972). Dioscoreales,In : Metcalfe, C. (Ed.), *Anatomy of the monocotyledons*, **6**. Oxford, Clarendon Press, pp.1-182.

Blackmore, S. and Crane, P.R. (1998). The evolution of aperatures in the spores and pollen grains of embryophytes, In: Owens, S.J., Rudall, P.J. (Ed.), *Reproductive biology in systematic, conservation and economic botany.* Kew, Royal Boyanic Gardens, pp. 159-182.

Burkill, I.H. (1960). The organography and the evolution of the Dioscoreaceae, the family of the yams. *Bot. J. Linnean Societ.,* **56** : 319-412.

Caddick, L.R., Furness, C.A., Stobart, K.L. and Rudall, P.J. (1998). Microsporogenesis and pollen morphology in Dioscoreales and allied taxa. *Grana,* **37** : 321-336.

Caddick, L.R., Rudall, P.J., Wilkin, P. and Chase, M.W. (2000). Yams and allied: systematics of Dioscoreales, In: Wilson, K.L., Morrison, D.A. (Ed.) Systematics and evolution of monocots. Proceedings of the 2nd International monocot conference Melborne, CSIRO, pp.475-487.

Caddick, L.R., Rudall, P.J., Wilkin, P., Hedderson, T.A.J. and Chase, M.W. (2002a). Phylogenetics of Dioscoreales based on combined analysis of morphological and molecular data. *Bot. J. Linnean Societ.,* **138** : 123 - 144.

Caddick, L.R., Wilkin, P., Rudall, P.J., Chase, M.W. and Hedderson, T.A.J. (2002b). A formal revised classification of Dioscoreales. *Taxon.,* **51** : 103-114.

Chase, M.W., Fay, M.F., Devey, D.S., Maurin, O., Ronsted, N., Davies, T.J., Pillon, Y., Petersen, G., Seberg O., Tamura M.N., Asmussen, C.B., Hilu, K., Borsch, T., Davis, J.I., Stvenson, D.W., Pires, J.C., Givnish, T.J., Sytsma, T.J., McPherson, M.A., Graham, S.W. and Rai, H.S. (2006). Multigene analysis of monocot relationships. a summary. *Aliso,* **22** : 63-75.

Chavez, R.P., Ludlow-Wiechers, B. and Villanueva, G. (1991). Flora palinologica de la reserva de la biosfera de sian kaan, quintana roo, Mexico: Centro de Investigaciones de Quintana Roo.

Coursey, D.G. (1967). *Yams: An account of the nature, origins, cultivation and utilization of the useful members of the Dioscoreaceae: Tropical agricultural series*. Longmans, green and Co. Ltd., London, 230pp.

Dahlgren, G. (1989). An undated angiosperm classification. *Bot. J. Linnean Societ.,* **100** : 197-203.

Dahlgren, R.M.T., Clifford, H.T. and Yeo, P.F. (1985). *The families of the monocotyledons: Structure, evolution and taxonomy*. Berlin, Springer- Verlag.

Erdtman, G. (1969). *Handbook of palynology-An introduction to the study of pollen grains and spores*. Copenhagen, Munksgaard.

Furness, C.A. and Rudall, P.J. (1997). Systematics of Acorus: ovule and anther. *Internat. J. Plant Sci.,* **158** : 640-651.

Furness, C.A. and Rudall, P.J. (1999a). Inaperature pollen in monocotyledons. *Internat. J. Plant Sci.,***160** : 395-414.

Goloboff, P.A. (1993). NONAver.1.6. Published by author, Tucuman, Argentina (*www.cladistics.com*).

Heusser, C.J. (1971). *Pollen and spore of Chile*. Arizona: University of Arizona Press.

Huang, T.C. (1972). *Pollen flora of Taiwan,* Taiwan: National Taiwan University, Botany Department Press.

Huber, H. (1998). Dioscoreaceae, In: Kubitzki, K., (Ed.), The families and genera of vascular plants.Vol.**3**. *Monocotyledons, Lilianae* (except Orchidaceae). Springer Verlag, Berlin, pp. 21-235.

Kunth, R. (1924). Dioscoreaceae, In: *Das Pflanzenreich,* 87(IV. 43), Ed. H. G. A. Engler. Leipzig: H. R. Engelmann (J.Cramer), pp.1–387.

Kuprianova, L.A. (1948). Pollen morphology and phylogeny of the monocotyledons. Trudy Boranicheskogo Institute Academii Nauk SSSR series 1.*Flora Sistematika,* **7**: 279-390.

Nixon, K.C. (2002). *Winclada ver.1.0000*. Published by the author, Ithaca, NY, USA.

Schols, P., Furness, C.A., Wilkin, P., Huysman, S. and Smet, E. (2001). Morphology of pollen and orbicules in some *Dioscorea* (Dioscoreaceae) species and its systematic implications. *Bot. J. Linnean Soc.,* **136** : 295-311.

Schols, P., Furness, C.A., Wilkin, P., Huysman, S. and Smet,

E. (2003). Pollen morphology of Dioscorea (Dioscoreaceae) and its relation to systematic. *Bot. J. Linnean Societ.,* **143** : 375-390.

Schols, P., Furness, C.A., Wilkin, P., Huysman, S. and Smet, E. (2005). Pollen evolution in Yams (Dioscorea: Dioscoreaceae). *Systemat. Botany*, **30**(4) : 750-758.

Selling, O.H. (1947). *Studies in Hawaiian pollen statistics.* Part II. The pollens of the Hawaiian phanerograms. Bishop Museum Special Publication 38. Honolulu, Hawaii; Bishop Museum.

Sharma, M. (1967). Pollen morphology of Indian monocotyledons. *J. Palynology*. Special Volume. Lucknow, Palynological Society of India.

Sheikh, N., Kumar, Y., Misra, A.K. and Pinokiyo, A. (2009). Status documentation of *Dioscorea* L. (Dioscoreaceae) in Meghalaya: an approach towards food security. *Pleione*, **3**(1) : 74-82.

Shivanna, K.R. and Rangaswamy, N.S. (1993). *Pollen biology- A laboratory manual*, Narosa Publishing House, NEW DELHI, INDIA.

Su, P. (1987). Pollen morphology of *Dioscorea* in China. *Acta Phyto-taxonimica Sinesis*, **25** : 357-365.

Wilkin, P., Schols, P., Chase, M.W., Chayamarit, K., Furness, C.A., Huysman, S., Rakotonasolo, F., Smets, E. and Thapyai, C. (2005). A plastid gene phylogeny of the Yam genus, Dioscorea: roots fruits and Madagascar. *Systematic Botany*, **30** (4) : 736-749.

Xifreda, C.C. (2000). Evaluation of pollen and vegetative characters in the systematic of South American species of *Dioscorea* (Dioscoreaceae), in: Systematic and evolution of monocots. Proceedings of the 2nd International Monocot Conference,eds. K.L. Wilson and D.A. Morrison. Melbourne, CSIRO Publishing, pp. 488-496.

Zavada, M.S. (1983). Comparative morphology of monocot pollen and evolutionary trends of aperatures and wall structures. *Botanical Rev.,* **49** : 331-379.