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Palynological Implication to the Systematic of the Genus *Dioscorea* in Meghalaya, North East India

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ABSTRACT

A comparative palynological study of eight species of Dioscorea L. from Meghalaya, North East India was carried out in order to evaluate the taxonomic significance of palynological characters to differentiate the species. Characters were coded and analyzed by Principal components analysis. An indented dichotomous key based on palynological characters was constructed to distinguish and identify the species.

Key words: *Dioscorea; Palynology; Principal Component Analysis; Systematics*

INTRODUCTION

Dioscorea L., with about 602 species[5] in the tropical and subtropical regions, regarded as the core genus of the family Dioscoreaceae. Identification of *Dioscorea* species has always presented a challenge to taxonomists due to its morphological diversity, dioecy and small flowers. Kunth[12] divided *Dioscorea* into four subgenera based on seed morphology. Burkill[1] divided the genus into 23 sections based on seed characters, tuber characters and male inflorescence morphology. Coursey[5] divided the genus into 70 sections based on underground tuber. Though morphological approaches provide a workable system of taxonomy, yet it cannot be denied that data from other field like anatomy, cytology, palynology, molecular studies etc. also provide evidences for the accurate delimitation of the species. Palynological data has also proved to accurately delimit between species and help in taxonomic classification.Pioneer workers to suggest the possible taxonomic significance of pollen morphology within Dioscoreaceae were Su[20], Caddick *et al.*[2] and Xifreda[22]. Su[20] considered that the pollen grains of *Dioscorea* species are mostly bisulcate except for the section *Stenophora* which are monosulcate. She also suggested correlation between pollen size and tuber type. Few others who also contributed to palynological data of few *Dioscorea* species using light microscopy (LM) were Selling[17], Kuprianova[13], Sharma[18], Erdtman[7], Heusser[10], Huang[11], Chavez, Ludlow-Wiechers and Villanueva, 1991.

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Microphological data remained scarce untill Schols *et al.*[14,15] who 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* as monosulcate or disulculate with a mainly 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*.[16] with correspond to the combine work of Wilkin *et.al.*[21] and Caddick *et al.*[2] presented the pollen character evolution in the genus *Dioscorea*. But no detailed description regarding the use of palynological characters for taxonomic classification and identification was evaluated. Hence in the present study an attempt has been made to investigate the palynological features of different species of Meghalayan *Dioscorea* and to single out distinctive palynological characters potentially useful for infrageneric classification.

MATERIALS AND METHODS

Plant material

Fresh material of eight species of Dioscorea viz., D. pentaphylla L. (NEHU-11946), D. alata L. (NEHU-11944), D. belophylla (Prain) Haines (NEHU-11950), D. glabra Roxb. (NEHU11937), D. pubera Bl. (NEHU-11949), D. oppositifolia L. (NEHU-11941), D. lepcharum Prain et Burk. (NEHU-11942) and D. bulbifera L. (NEHU-11935) were collected from wild habitats of Meghalaya. Voucher specimens were deposited in the Herbarium of Botany Department, North Eastern Hill University, Shillong. Palynological investigationPollen was acetolyzed following the protocol of Shivanna and Rangaswamy[19] for light microscope (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. Twelve palynological characters were recorded for the present study (Table 1).Numerical analysis Pollen and orbicules morphology varies from species to species. A total of twelve characters were taken for the present analysis. The character traits were subjected to PCA by XLSTAT ver.2015.

RESULT

Pollen morphology of taxonomic significance

Palynology deals with the detailed study of pollen grains. Pollen morphology forms an important role in the confirmation of relationship and affinities between the related taxa. Pollen morphology deals with the size of pollen grain, aperature number, sexine sculpture, presence or absence of orbicule on the locule wall etc which are important micromorphological characters of taxonomic importance.Size of Pollen and Aperature numberThe size of pollen varies from species to species. The size of pollen is measured in longest axis (LA) and also along its shortest equatorial axis (SEA). Number of aperature in pollen also marks the palynological importance in taxonomy. In *Dioscorea* species number of aperature were found to be monosulcate or disulcate (Fig 1)Sexine is the outermost layer of pollen wall which are variously sculptured such as perporate, striate or microrecticulate etc. The sculpturing of sexine can be also considered as important character for palynological study. Sexine sculpturing in *Dioscorea* species were found to be perporate to microrecticulate (Fig 2).

Orbicules

Orbicules are small sporopollenin bodies in the anther locule. Orbicules characters such as presence or absence, shape, diameter and density of orbicules can be also included as important taxonomic characters. The orbicules

of *Dioscorea* species appears to be mostly circular in shape (Fig 3)

Principal component analysis

The result of PCA indicates that 100% of the observed variability was explained by the first seven components (Table 2). The first two components explained about 52.3% of the total observed variability. PC1 represented mainly from LA, SEA and LeA accounted for 31% of the variance. PC2 represented ANB, OrbT and OrbD accounted for 21.17% of the variance. PC3 represented ANL and EOX accounted for 19.15% of the variance. The remaining component explained less variability.

Discussion

The character trait, LA (longest axis) and SEA (Shortest equatorial axis) are important trait for palynological study. Principal Component Analysis confirms that these two traits show high variability among the species which could be used for delimiting of the taxa. The size of longest axis (LA) of Pollen grain of *Dioscorea* species collected from Meghalaya ranges from 15.48µm in *D.bulbifera* to 20.48µm in *D.glabra*. Schols *et al*.[14] suggested that pollen grains of *D.bulbifera* (*sect. Opsophyton*) is smallest in size having an average LA of 16.2µm. He also suggested that almost all species which were examined from section *Opsophyton, Enantiophyllum* have pollen grains that are smaller than 32µm. The result of the present study confirms this statement. 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. D.pentaphylla* (sect.*Botryosicyos*) is bisulcate as suggested by Schols *et al*.[14] but our observation showed that it is monosulcate. Monosulcate pollens are generally accepted to be the plesiomorphic character state within the monocot[6], Furness and Rudall[8,9].

Table 1: Palynological characters of taxonomic significance

No.	Traits acronym	Characters/descriptors	Score code-descriptor code
		Palynology	
1			
2	NAL	no.of anther lobe	1: -3 lobes; 2: - 4 lobes
3	EOX	exine orientation	1-perporate; 2-microrecticulate
4	NPoA	no. of pollen aperature	1-1 aperature; 2- 2 aperature
5	OrbT	orbicule type	1-spherical; 2- elliptical
	ANL	anther length	1-(151-200µm); 2-(201-250µm);3-(251-300µm); 0->300µm
6	ANB	anther breadth	1-(60-88µm); 2-(89-109µm); 3-(110-130µm); 0->130µm
7	LA	longest axis	1-(10-16µm); 2-(17-23µm); 3->23µm
8	SEA	shortest equatorial axis	1-(5-11µm);2-(12-18µm); 3->18µm
9	LeA	length of aperature	1-(1-10µm); 2-(11-20µm);3->20µm
10	WiA	width of aperature	1-(0.51-0.80µm); 2-(0.81-1.00µm); 3->1µm
11	FiL	Filament length	1-(101-150µm);2-(151-200µm);3-(201-250µm)
12	OrbD	Orbicule diameter	1-(0.1-.50µm); 2-(0.6-1.00µm); 3->1µm

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

Table 2: Eigen values and cumulative variance for seven major factors obtained from PCA and significant parameters within each component for *Dioscorea* species based on palynological traits

traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
NAL	0.339	-0.067	0.150	**0.856**	0.304	0.160	-0.082
ANL	-0.117	0.458	**0.727**	-0.411	0.146	-0.144	-0.194
ANB	-0.431	**0.819**	-0.026	-0.036	0.126	0.354	0.016
LA	**0.938**	0.015	0.241	-0.041	0.134	0.017	-0.203
SEA	**0.834**	-0.184	0.337	-0.006	-0.122	0.116	0.357
EOX	0.213	-0.261	**0.735**	-0.489	-0.253	0.201	0.045
NPoA	-0.713	-0.515	-0.386	-0.258	0.030	-0.022	0.097
LeA	**0.863**	0.177	-0.404	0.055	0.122	-0.185	0.094
WiA	0.372	0.102	-0.657	-0.514	0.252	0.302	-0.029
FiL	-0.397	-0.191	0.456	-0.074	**0.736**	-0.065	0.217
OrbT	-0.375	**0.732**	0.201	0.440	-0.248	0.007	0.165
OrbD	0.324	**0.821**	-0.209	-0.340	0.061	-0.203	0.131
Eigenvalue	3.731	2.541	2.299	1.794	0.912	0.397	0.326
Variability (%)	31.090	21.178	19.158	14.946	7.602	3.311	2.716
Cumulative %	31.090	52.268	71.425	86.371	93.973	97.284	100.000

Value in bold indicates eigen value >0.5

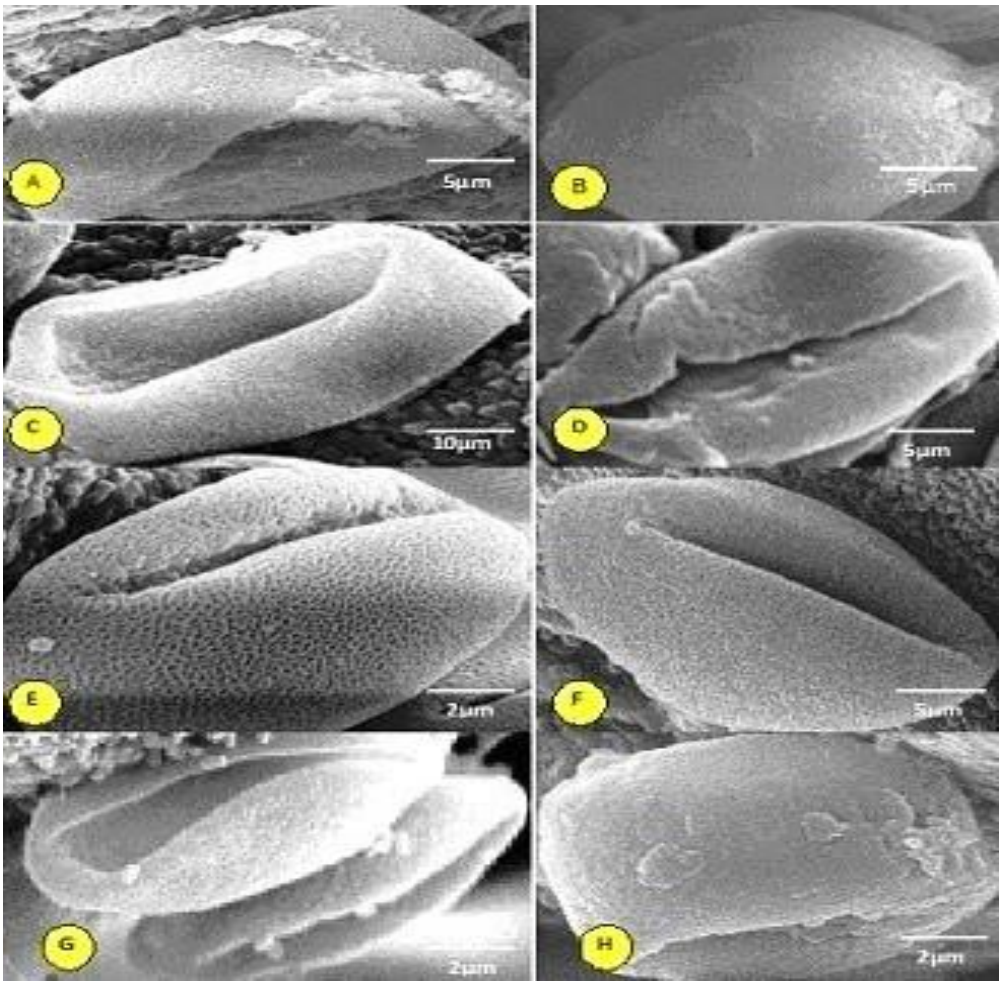

Fig. 1: Pollen and aperature (SEM). A- *D. alata*; B- *D. belophylla*; C- *D. bulbifera*; D- *D. pentaphylla*; E- *D. oppositifolia*; F- *D. pubera*; G-*D.lepcharum*; H- *D. glabra*

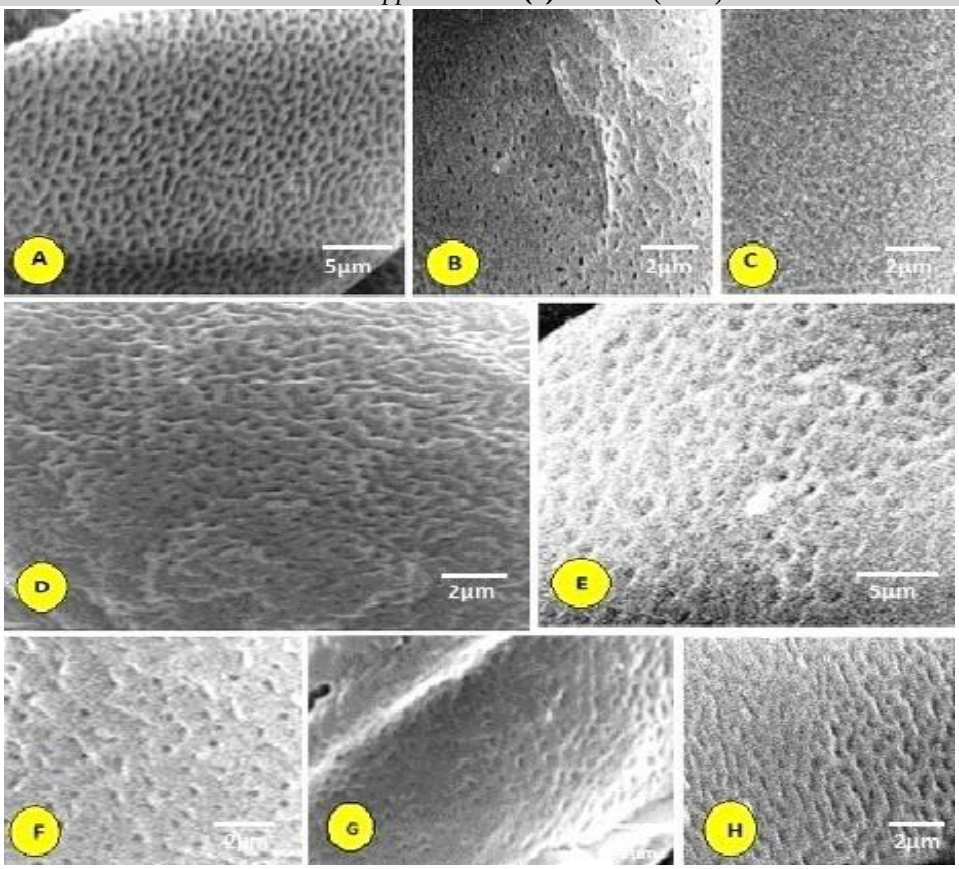

Fig. 2: Sexine sculpture (SEM). A- *D. oppositifolia*; B- *D. bulbifera*; C- *D. pubera*; D- *D. belophylla*; E- *D. alata*; F- *D. glabra*; G- *D. lepcharum*; H- *D. pentaphylla*

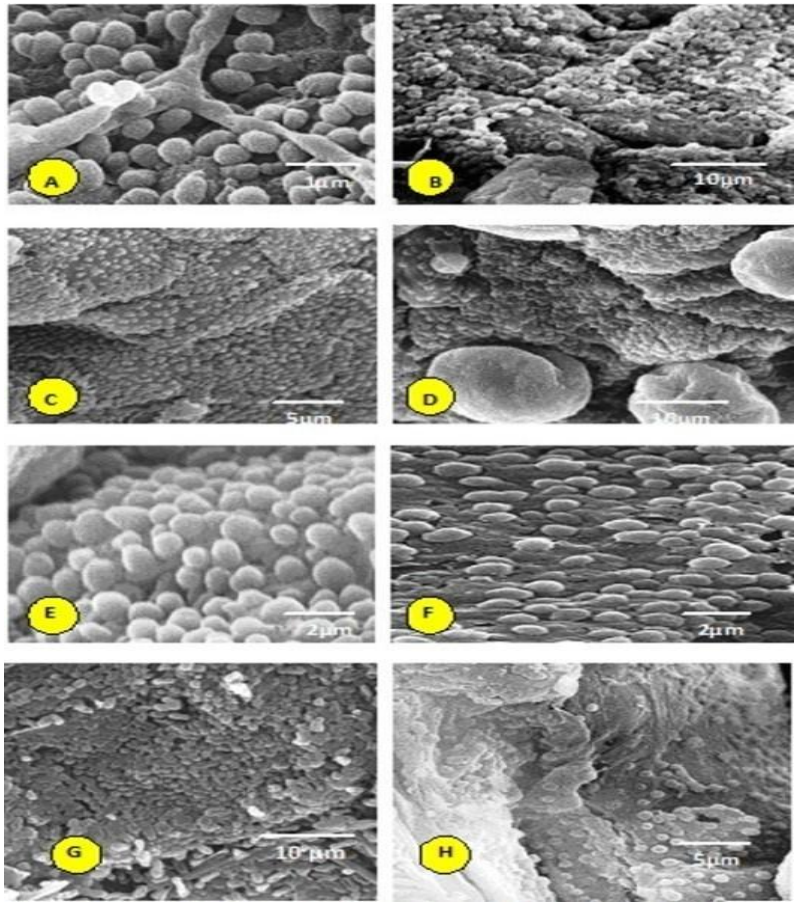

Fig. 3: Orbicules on the locule wall (SEM). A- *D. pentaphylla*; B- *D. alata*; C- *D. pubera*; D- *D. bulbifera*; E- *D. glabra*; F- *D. belophylla*; G- *D. lepcharum*; H- *D. oppositifolia*

CONCLUSION

The present study was an approach towards the systematic study of a complicated genus, Dioscorea through palynological investigation. Though morphological investigation has made an attempt to classify the genus yet problem had arisen for it wide range of morphological diversity in nature which prevent the species for proper classifying and identification. Evolutionary relationship within large genus like *Dioscorea* is unclear. So if we incorporate the micro morphological characters like pollen size, aperature number, presence or absence of orbicules for systematic study will help to show the evolution among the species within the genus and reflect the true phylogeny and taxonomy of large genera like *Dioscorea.*

Dichotomous key based on palynological characters of Meghalaya Dioscorea species

1a. Elliptical type of orbicules……..*D. lepcharum*
b. Spherical type of orbicules……2
2a. Bisulcate type of pollen grains……..3
b. Monosulcate type of pollen grains……4
3a. No. of anther lobes is 3……*D. oppositifolia*
b. No. of anther lobes is 4…… 5
4a. Perporate exine orientation……*D. pubera*
b. Microrecticulate exine orientation……...6
5a. Length of axis of pollen grain less than 16 µm……*D. bulbifera*
b. Length of axis of pollen grain more than 16 µm…..*D. glabra*
6a. Orbicule diameter less than 0.5µm……*D. belophylla*
b. Orbicule diameter more than 0.5 µm……7
7a. Length of anther greater than 200 µm……..*D. pentaphylla*
b. Length of anther less than 200µm……*D. alata*

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