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ASSESSING THE PHYLOGENY OF GENUS *Dioscorea* FROM MEGHALAYA (INDIA) BASED ON PRINCIPAL COMPONENT AND CLUSTER ANALYSIS

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ABSTRACT

The present study examined the morphology, anatomy and palynological characters of eight species of *Dioscorea* growing in Meghalaya (India). A total of 128 characters of *Dioscorea* species were assessed using principal component analysis (PCA) and cluster analysis methods. The first four principal components indicated 79.42% of the total observed variability. Characters with discriminating values were stem twinning direction, position of leaves, leaf apex shape, petiole colour, male flower colour, male floral bracteoles shape, inner tepal shape of male flower, style shape, fruit shape, seed shape, seed wing position, vascular bundle in outer ring, number of paracytic stomata, breath of stomata, width of pollen aperture and orbicule diameter. The cluster analysis clearly separated the species based on their close association.

Keywords: Cluster analysis, *Dioscorea*, principal component analysis, traits

INTRODUCTION

Dioscorea L. is the largest genus of family Dioscoreaceae which includes 300-400 species (Caddick *et al.* 2002) with about 70 sections mainly distributed in Southeast Asia, Africa, Central America, South America and other tropical or subtropical regions. Various studies have been conducted on the systematic and diversity of the genus (Ayensu, 1972; Saikia *et al.*, 2011; Ngo Ngwe *et al.*, 2015), yet the systematic of this genus is still confusing and not completely resolved. Meghalaya, one of the eight states of North-East India, is considered one of the biodiversity hotspot areas in the world. Out of the 50 species of *Dioscorea* distributed in India (Anonymous, 1952), 16 species have been documented from this State alone (Sheikh *et al.*, 2009).

The taxonomy of genus *Dioscorea* is considered problematic due to the continuous variability in morphological characters of aerial parts such as leaves, bulbils, flowers, tubers, etc. (Veluyadhan *et al.*, 1998). Sheikh and Kumar (2017) have studied the morphological characters of a few Meghalayan *Dioscorea* species but their study was confined only to male *Dioscorea* plants. There was no enumeration of female *Dioscorea* plants which are morphologically diverse. Hence, in the present study an attempt was made to incorporate detail morphological, anatomical and palynological characteristic features of *Dioscorea* plants and to single out the discriminating traits in species identification as well as to consider these variable characters for proper systematic approach.

MATERIALS AND METHODS

Plant materials

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) with 10 accessions of each species were collected from the wild habitats of Meghalaya states (India) lying between 25º 5´ N and 26º 10´ N latitude and 89º 47´ E and 92º 47´ E longitude with an area of 22,429 km^2. Voucher specimens were deposited in the Herbarium of the Department of Botany, North Eastern Hill University, Shillong (India).

Morphological characterization

Detailed morphological study of *Dioscorea* species was carried out on mature male and female plants. For each species at least 10 samples (individual plants) were analyzed. The morphological investigations were done with the help of magnifying glass and binocular stereomicroscope. The morphological characters (at least ninety) selected for the present study were as under:

Traits acronym	Characters/descriptors	Score code-descriptor code
	Qualitative	
	Stem	
STD	Twinning direction	1-clockwise (left); 2-anticlockwise (right)
STR/A	Stem ridged/angled	1-ridged; 2-angled
STC	Stem colour	1-green; 2-purplish green; 3-brownish green
STA/U	Stem armed/unarmed	1-armed; 2-unarmed
STG/P	Stem glabrous/pubescent	1-glabrous; 2-pubescent
	Leaves	
POL	Position of leaves	1-alternate; 2-opposite; 3-mixed
LT	Leaf type	1-simple; 2-compound
NoL	No. of leaflets in compound leaf	1-mainly 3; 2-mainly 5
LC	Leaf colour	1-yellowish; 2-pale green; 3-dark green
LD	Leaf lobation	1-shallowly lobed; 2-deeply lobed; 0-no lobe
LS	Leaf shape	1-ovate; 2-cordate; 3-ellliptic oblong
DL	Distance between lobes	0-no measurable distance; 1-intermediate; 2-very distant
LAN	Leaf nerves	1- 5n; 2- 7n; 3- 9n; 0- others
LAS	Leaf apex shape	1-obtuse; 2-acute; 3-emarginate; 0-others
LG/P	Leaf glabrous/pubescent	1-glabrous; 2-pubescent
PC	Petiole colour	1-brownish green; 2-purplish green; 3-green
PL/LF	Petiole length in relation to leaf length	1-short; 2-median; 3-long
PG/P	Petiole glabrous/pubescent	1-glabrous; 2-pubescent
	Male Inflorescence	
InfS	Inflorescence smell	0-absent; 1-present
NoInf/INT	No. of inflorescence per internode	1-One or two; 2-many
InfG/P	Inflorescence glabrous/pubescent	1-glabrous; 2-pubescent
InfPo	Inflorescence position	1-upward; 2-downward
MFC	Male flower colour	1-whitish purple; 2-pale green; 3-yellow
FLBS	Floral bract shape	1-ovate acuminate; 2-orbicular; 3-ovate
FLBrS	Floral bracteoles shape	1-broadly lanceolate; 2-ovate acuminate; 0-others
OTS	Outer tepal shape	1-ovate; 2-obovate; 3-lanceolate; 0-others
ITS	Inner tepal shape	1-linear oblong; 2-oblong obovate; 3-ovate
TG/P	Tepal glabrous/pubescent	1-glabrous; 2-pubescent
STA	No. of stamen	1-3stamens; 2-6stamens
STAMA/P	Staminode absent/present	0-absent; 1-present

	Female inflorescence	
FNoInf/INT	No. of inflorescence per internode	1-One or two; 2-many
FInfG/P	Inflorescence glabrous/pubescent	1-glabrous; 2-pubescent
FInfPo	Inflorescence position	1-upward; 2-downward
FFC	Female flower colour	1-greenish brown; 2-white; 3-yellowish
FFLBS	Female floral bract shape	1-ovate acuminate; 2-orbicular; 3-ovate
FFLBrS	Female floral bracteoles shape	1-broadly lanceolate; 2-ovate acuminate
FOTS	Female outer tepal shape	1-ovate; 2-obovate; 3-lanceolate
FITS	Female inner tepal shape	1-linear oblong; 2-oblong obovate; 3-ovate
FSTAM	No of staminode	1-3nos; 2-6nos
STYLS	Style shape	1-fanshaped; 2-hookshaped
	Fruiting	
FF	Fruit formation	0-no; 1-yes
FP	Fruit position	1-upward; 2-downward
FS	Fruit shape	1-<3cm; 2-<3cm
SS	Seed shape	1-elongated; 2-circular
SWP	Seed wing position	1-circular; 2-apical; 3-basal
	Aerial tubers	
BA/P	Absence/presence of bulbil	0-absent; 1-present
BS	Aerial tuber shape	0-round; 1-oval; 2-irregular; 3-ellongated
BST	Surface texture	1-smooth; 2-wrinkled; 3-rough
B ab/P	Bulbil abundant/less	1-abundant; 2-less
BSC	Bulbil skin colour	1-greyish; 2-light brown; 3-dark brown
	Underground tuber	
TUS	Tuber shape	0-round; 1-cylindrical; 2-oval-oblong; 3 -other
RTTU	No. of roots on the tuber surface	1-few; 2-many
TUSC	Skin colour of tuber	1-off-white; 2-black; 3-dark brown
TUFC	Tuber flesh colour	1-off-white; 2-orange; 3-light purple; 0- others
	<u>Qualitative</u>	
	Stem	
STH	Stem height	1-(1-5 cm); 2-(6-10 cm); 3>10 cm
	Leaves	
LML	Length of a mature leaf	1-(1-10 cm); 2-(11-20 cm); 3->20 cm
WML	Width of mature leaf	1-(1-10 cm); 2-(11-20 cm); 3->20 cm
PL	Petiole length	1-(1-5 cm); 2-(6-10 cm); 3->10 cm
	Male infloresences	
LMspk	Average length of spike	1-(1-10 cm); 2-(11-20 cm); 3->20 cm
MFL	Male flower length	1-(2 mm); 2->2 mm
MFP	Male flower peduncle length	1-(1.1-2 cm); 2-(2.1-3 cm); 3-(3.1-4 cm); 4-(4.1-5 cm)
MFPe	Male flower pedicle length	1-(0.6-1.5 mm); 2-(1.6-2.5 mm); 3-(2.6-3.5 mm)
FLB L	Floral bract length	1-(1 mm); 2->1 mm
FLBW	Floral bract width	1-(1 mm); 2->1 mm
FLBrL	Floral bracteoles length	1-(0.1-1.0 mm); 2->1 mm
FLBrW	Floral bracteoles width	1-(0.1-1.0 mm); 2->1 mm
OTL	Outer tepal length	1-(1.5-3 mm); 2->3 mm
OTW	Outer tepal width	1-(0.1-1.0 mm); 2->1 mm
ITL	Inner tepal length	1-(0.1-3.1 mm); 2->3.1 mm
ITW	Inner tepal width	1-(0.1-1.0 mm); 2->1 mm
	Female inflorescence	
LFspk	Average length female spike	1-(1-20 cm); 2-(21-40 cm)
feFL	Flower length	1-(5.5-6.5 mm); 2->6.5 mm
feFBL	Floral bract length	1-(1.6-2.5 mm); 2-(2.6-3.5 mm)
feFBW	Floral bract width	1-(1 mm); 2->1 mm

feFBrL	Floral bracteoles length	1-(0.6-1.5 mm); 2-(1.6-2.5 mm)
feFBrW	Floral bracteoles width	1-(1 mm); 2->1 mm
feOTL	Outer tepal length	1-(1.5 mm); 2->1.5 mm
feOTW	Outer tepal width	1-(1 mm); 2->1 mm
feITL	Inner tepal length	1-(1.5 mm); 2->1.5 mm
feITW	Inner tepal width	1-(1 mm); 2->1 mm
STL	Style length	1-(1-5 mm); 2-(6-10 mm)
feSTAL	Staminode length	1-(0.8 mm); 2-(0.9 mm)
fePL	Peduncle length	1-(1-5 cm); 2-(6-10 cm)
fePeL	Pedicel length	1-(1.1-2 mm); 2-(2.1-3 mm)
	Fruiting	
FrL	Fruit length	1-(1.1-2 cm); 2-(2.1-3 cm)
FrW	Fruit width	1-(0.6-1.5 cm); 2-(1.6-2.5 cm)
SL	Seed length	1-(0.1-1.2 cm); 2->1.2 cm
SW	Seed width	1-(1-10 mm); 2->10 mm
SWL	Seed wing length	1-(2.5-3.5 mm); 2->3.5 mm
SWW	Seed wing width	1-(1-1.5 mm); 2->1.5 mm

Anatomical investigations

Foliar epidermis: Fresh mature leaves were collected and epidermal peelings were made by hand with the help of sharp razor and forceps. The peelings were cut to suitable size, taken on a clean slide stained with 5% aqueous safranine, mounted in 50% glycerin and the margins of cover slips sealed with DPX. At least five slides were made for each sample. The prepared slides were observed under microscope and photographs were taken. Twenty-six anatomical characters recorded were as under:

Traits acronym	Characters/descriptors	Score code-descriptor code
	Anatomy stomata	
PAR	No. of paracytic	1-(11-20); 2-(21-30); 3-(31-40)
ANS	No. of anisocytic	1-(21-30); 2-(31-40); 3-(41-50); 0->50
TER	No. of tetracyctic	1-(21-30); 2-(31-40); 3-(41-50); 0->50
Nosto	No. of stomata mm^{-2}	1-(95-124); 2-(125-154); 3-(155-184)
Noepi	No. of epidermal cell mm^{-2}	1-(36-45); 2-(46-55); 3-(56-65); 0-.65
EpiH	Epidermal hairs	0-absent; 1-present
StoI	Stomatal index (%)	1-(10-15.5); 2-(16-20.5); 3-(21-25.5)
Lsto	Length of stomata (µm)	1-(21-30 µm); 2-(31-40 µm); 3->40 µm
Bsto	Breadth of stomata (µm)	1-(1-1.4 µm); 2-(1.5-1.9 µm); 3-(2-2.4 µm)
	Stem anatomy	
STO	Stem outline	1-wavy; 2-angular; 3- round
SEpiH	Epidermal hair	1-present; 0-absent
Scoc	Shape of cortical cell	1-oval; 2-others
OR	V.B in outer ring	1-(6-7 rings); 2-(8-9 rings); 3-(10-11 rings)
IR	V.B. in inner ring	1-(4-5 rings); 2-(6-7 rings); 3-(8-9 rings)
NC	Nature of cuticle	1- thin; 2-others
LC	Layer of cuticle	1 -6 layers; 2 -7 layers
LScl	Layer of sclerenchyma	1-4 layers; 2-5 layers; 3 -6 layers
PiStG	Pith starch grain	1- wide; 2- narrow
		0-absent; 1-present
	Petiole anatomy	
PO	Petiole outline	1-pentagonal; 2-crescent; 3-round
PepiH	Epidermal hair	0-absent; 1-present
CCS	Chollenchyma cell shape	1-oval; 2-hexagonal
PSL	Scherenchyma layer	1-3 layers; 2-4 layers; 3-5 layers
V.B.	Vascular bundle	1-6V.B.; 2-8V.B.; 3- 9 V.B.
PCCS	Cortical cell shape	1-hexagonal; 2-oval
Pst	Starch grain	0-absent; 1-present

The quantitative data such as number of stomata, size of stomata, stomatal index, etc. were recorded. The qualitative characters such as type of stomata and presence or absence of hairs were noted. The finding was based on 10 observations, two each from five slides for each leaf section. To calculate stomatal index, the following formula was used:

$$I = \frac{S}{E + S} \times 100$$

Where 'I' = stomatal index, S = number of stomata per unit area and E = number of epidermal cells per unit area. The terminology adopted for the type of stomata was described by Metcalf and Chalk (1950) and Metcalf (1961).

Stem and petiole anatomy: Transverse section of stem and petiole were made using sharp blade from the fresh material. Temporary slides were prepared following O'Brien *et al*. (1964) technique using toluidine blue. The stained sections were observed under microscope at 10X and 4X magnifications. Photographs were also taken.

Palynological investigation

Pollen was acetolyzed as per protocol of Shivanna and Rangaswamy (1993) for light microscopy (LM) and then acetolyzed pollen were dehydrated through an acetone series before critical point drying. The dried pollen was mounted on specimen stubs and micrographs taken using digital imaging on JSM-6360, JEOL scanning electron microscope at Sophisticated Analytical Instrument Facility, North Eastern Hill University, Shillong (India). Micrographs were also taken for whole male flower, anther lobes, style and staminode. For each species, the longest axis and the shortest equatorial axis were measured using LM slides of acetolyzed pollen and additional measurement was taken by SEM. Twelve palynological characters recorded in the present study were under:

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

Numerical taxonomic analysis

Combining morphological, anatomical and palynological data, a total of 128 traits comprising of 82 qualitative and 46 quantitative characters were examined in all *Dioscorea* species. A standard descriptor list for yam was adopted for morphological study with a few modification (IPGRI, 1997), whereas for palynological and anatomical study a detailed experimental analysis was done and codes were given on the basis of observations. The data were recorded either directly from the measurement, using a 1-9 scale or as a binary recording (1= present and 0 = absent). All the data were standardized and subjected to PCA and cluster analysis. Principal component analysis (PCA) and cluster analysis were performed using XLSTAT ver. 2014 statistical software.

RESULTS AND DISCUSSION

The principal component analysis revealed the correlation between the original variables and the

Table 1: Eigen values and cumulative variance for seven major factors obtained from PCA and significant parameters within each component for *Dioscorea* species based on combined analysis for selected traits

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
STD	0.968	-0.244	-0.036	-0.030	0.012	0.017	-0.006
STC	-0.417	0.553	0.131	-0.541	-0.029	0.180	-0.421
STG/P	-0.166	0.692	-0.385	0.434	-0.396	0.013	0.015
POL	0.830	-0.155	-0.307	0.269	-0.330	0.085	-0.065
LT	-0.454	0.868	0.006	0.042	0.095	-0.115	0.129
NoL	-0.454	0.868	0.006	0.042	0.095	-0.115	0.129
LC	-0.301	0.024	0.886	0.009	0.150	-0.317	0.035
LS	-0.144	0.254	0.079	0.578	0.530	0.017	0.542
LAS	0.814	0.548	-0.041	0.003	0.110	-0.093	0.122
LG/P	-0.166	0.692	-0.385	0.434	-0.396	0.013	0.015
PC	0.620	-0.595	0.064	0.431	-0.101	-0.142	0.203
MFC	0.679	0.338	-0.125	-0.543	0.134	0.189	-0.244
FLBS	0.415	0.462	0.666	0.215	0.000	-0.341	0.087
FLBrS	0.968	-0.244	-0.036	-0.030	0.012	0.017	-0.006
ITS	0.734	0.326	-0.369	-0.229	-0.185	0.237	-0.276
STAMA/P	-0.454	0.868	0.006	0.042	0.095	-0.115	0.129
FInfG/P	-0.166	0.692	-0.385	0.434	-0.396	0.013	0.015
STYS	0.814	0.548	-0.041	0.003	0.110	-0.093	0.122
FS	0.968	-0.244	-0.036	-0.030	0.012	0.017	-0.006
SS	0.968	-0.244	-0.036	-0.030	0.012	0.017	-0.006
SWP	0.968	0.244	0.036	0.030	-0.012	-0.017	0.006
SEpiH	-0.166	0.692	-0.385	0.434	-0.396	0.013	0.015
OR	0.596	-0.566	-0.314	-0.085	-0.224	0.086	0.401
PAR	0.521	-0.048	-0.366	0.462	0.103	0.122	-0.594
TER	0.055	-0.523	0.431	0.165	0.151	0.600	-0.357
Bsto	0.619	-0.244	0.304	-0.009	0.652	-0.153	-0.125
Nosto	-0.069	0.017	-0.090	0.976	0.049	0.146	-0.101
Noepi	-0.246	-0.413	0.350	0.561	0.101	-0.354	0.442
StoI	-0.283	-0.386	0.598	0.206	-0.354	-0.270	0.417
EpiH	-0.166	0.692	-0.385	0.434	-0.396	0.013	0.015
Eigenvalue	44.231	23.076	15.540	14.850	10.920	8.394	5.989
Variability (%)	35.960	18.761	12.634	12.074	8.878	6.824	4.869
Cumulative %	35.960	54.721	67.355	79.429	88.307	95.131	100.000

Value in bold signify eigen value>0.52

first seven principal components (Table 1). The first four components explained about 79.42% of the total observed variability. PC1 is accounted for 35.96% of the variance. PC2 is accounted for 18.76% of variance. PC3 and PC4 accounted for 12.6 and 12.0% variances. The remaining component explained less variability. Out of 128 morphological traits which were analyzed, only a few traits showed high variability such as STD, POL, LAS, PC, MFC, FLBrS, ITS, STYLS, FS, SS, SWP, OR, PAR and Bsto. Various classical approaches have been illustrated by various workers (Sastrapradja, 1982; Sayed *et al.*, 2006; Mwirigi *et al.*, 2009) on the systematic study of genus *Dioscorea* which included characters such as stem twining direction (STD), position of leaves (POL) and petiole colour (PC), Seed wing position (SWP). Therefore, through PCA the traits which have high degree of positive variability could somewhat be helpful for taxonomic study of the genus by implementing these characters for identification.

The agglomerative hierarchical analysis based on unweighted pair-group average method produced four main clusters at 0.58 level of similarity (Fig. 1). The 1st cluster (A) consisted of a single species i.e. *D. bulbifera* which is characterized by the presence of left twinning direction of

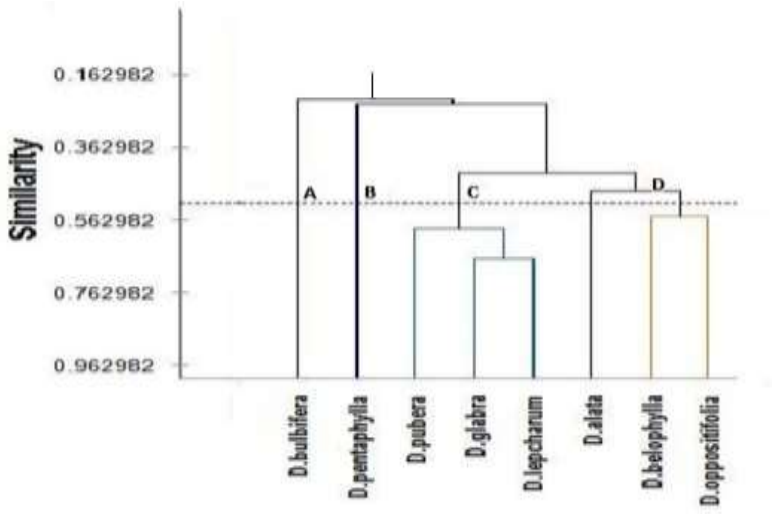

Fig. 1: Dendrogram based on Unweight pair group average method for 8 species of *Dioscorea* based on combined data

stem, alternate position of leaves, whitish purple colour of male flower, colour of petiole, elongated fruit shape and presence of wings at the proximal end of seed. The 2nd cluster (B) included *D. pentaphylla* which is characterized by left twinning direction of stem, alternate position of leaves, elongated fruit shape and presence of wings at the proximal end of the seed. The 3rd cluster (C) included *D. glabra, D. pubera* and *D. lepcharum*, whereas *D. alata, D. belophylla* and *D. oppositifolia* were included in 4th cluster (D). Among the eight *Dioscorea* species of Meghalaya, *D. pubera, D. glabra, D. lepcharum, D belophylla, D. oppositifolia* and *D. alata* are considered under section *Enantiophyllum* (Knuth, 1924; Burkill, 1960) which are characterized by right-twining stems, opposite leaves and winged all-round the margin of the seed. Wilkin *et al.* (2005) who worked on phylogenetic analysis of *Dioscorea* species based on molecular approaches suggested that the sections *Enantiophyllum* strongly support monophyly. He also suggested that *D. pentaphylla* (sect. *Botryosicyos*) with one main vein per leaflet and *D. bulbifera* (sect. *Opsophyton*) were in close association in a clade which was similar with the result of the present cluster analysis based on morphology, anatomy and palynology.

Conclusion: The present study was just a preliminary approach towards assessing the phylogeny of the genus based on morphometric characters (morphology, anatomy and palynology). These characters can be used as a marker for taxonomic identification within and between the species. Morphological, anatomical and palynological studies provides a means of evaluating the species at basic level rather than using molecular or biochemical characters for species evaluation. However, the phenotype of the plant is mostly affected by various biotic and abiotic factors because of which the study base on morphology remain restricted. Therefore, a complementary study is required for example using molecular markers, are needed for accurately identifying and classifying the species.

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