

FOLIAR EPIDERMAL, STEM AND PETIOLE ANATOMY OF MEGHALAYAN *DIOSCOREA* L. (DIOSCOREACEAE) AND ITS SYSTEMATIC IMPLICATION

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Abstract

A comparative anatomical study of eight species of *Dioscorea* L. from Meghalaya, North East India was carried out in order to evaluate the taxonomic significance of anatomical characters to differentiate the species. Characters were coded and analyzed by PCA and cluster analysis. The combination of selected qualitative and quantitative anatomical characters of foliar epidermis, stem and petiole were significant for identification of species. The characters that contributed most to the separation of the species were type of stomata, length of stomata, stomatal index, leaf epidermal hairs, stem epidermal hairs, layer of stem sclerenchyma, number of vascular bundle in outer ring and inner ring of stem, paired or unpaired metaxylem, presence of phloem at both ends or at one end, presence or absence of starch grain in stem, petiole epidermal hair and presence or absence starch grain and crystal in petiole. An indented dichotomous key based on anatomical characters was constructed to distinguish and identify the species.

Introduction

Dioscorea L., with about 602 species (Coursey, 1967) in the tropical and subtropical regions, regarded as the core genus of the family Dioscoreaceae is a pivotal taxon in the evolution of Liliopsida and occupies a basal position among all extant monocotyledonous plants (Chase *et al.*, 2006; Dahlgren, 1989). About 50 species of *Dioscorea* in India (Anonymous, 1952) and approximately 28 species are distributed in North East India (Sharma and Hore, 1995). Meghalaya, one of the eight hot spots of North East India has wide range of distribution of *Dioscorea* species in wild habitat. Only a few species of *Dioscorea* are consumed as food and also used as medicines by the indigenous people of this area in spite of its wide range of distribution. Identification of *Dioscorea* species has always presented a challenge to taxonomists due to its morphological diversity, dioecy and small flowers. Kunth (1924) divided *Dioscorea* into four subgenera based on seed morphology. Burkill (1960) divided the genus into 23 sections based on seed characters, tuber characters and male inflorescence morphology. Coursey (1967) divided the genus into 70 sections based on underground tuber. Several authors studied the floristics of the genus including Miede (1968), Milne-Redhead (1975), N’Koukou (1993), Tellez and Schubert (1994), Miede and Sebsebe (1998) and Ding and Gilbert (2000).

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., provide evidences for the accurate delimitation of the species. Anatomical parameters play an important role in plant taxonomy (Metcalf and Chalk, 1957). Anatomical characters have proved to be more useful for delimitation of higher taxonomic ranks, such as genera and families. Few researchers studied foliar epidermal anatomy of different species of *Dioscorea* (Abdulrahman *et al.*, 2009;

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Aina and Atumeyi, 2011; Shah and Gopal, 1972), however, no concrete data of anatomical analysis used in taxonomic delimitation of this genus have been conducted earlier. Therefore, in the present study an attempt has been made to investigate the anatomical features of foliar epidermis, stem and petiole of different species of Meghalayan *Dioscorea* and to single out distinctive anatomical 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. (NEHU-11937), *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.

Foliar epidermal anatomy

For foliar epidermal study, fresh leaf epidermal peelings from both the surfaces (adaxial and abaxial) and transverse section of the leaves for epidermal study were made by hand with the help of sharp razor and forceps. Ten individual per species were studied. The epidermal peelings and the transverse section of the leaves were cut into suitable size, taken on a clean slide, stained with 5% aqueous safranin, mounted in 50% glycerin and sealed the margins of cover slips with DPX. The prepared slides were observed under a light microscope at x40 magnification and photographs were taken. To calculate the stomatal index, 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 by Metcalfe and Chalk (1950) and Metcalfe (1961) was followed to describe the stomatal types.

Stem and petiole anatomy

For the anatomical study of stem and petiole, transverse section of stem and petiole of 10 individual plants per species were made using sharp blade from the fresh material. Temporary slides were prepared following O'Brien *et al.* (1964) using Toluidine blue. The stained sections were observed under a light microscope at x10 magnification and Photographed. Presence and absence of starch grain in stem and petiole was separately analyzed by Lugol's solution (Jensen, 1962). The anatomical characters observed under microscopes were recorded.

Anatomical data recording and statistical analysis

A total of 27 characters including both qualitative and quantitative were observed (Table 1). The characters or traits that appeared in more than one state and differed among the examined species were coded and assessed through principal component analysis (PCA). Cluster analysis (CA) was also performed in order to group the species based on the similarity of anatomical characters or traits. Both PCA and CA were performed using XLSTAT ver. 2015.4.01.22283 statistical software.

Table 1. List of anatomical characters of stomata, stem and petiole of different *Dioscorea* species.

Serial No.	Traits acronym	Characters/ descriptors	Score code-descriptor code
Stomata			
1	PAR	Absence/ presence of paracytic	Absent-0; present-1
2	ANS	Absence/ presence of anisocytic	Absent-0; present-1
3	TER	Absence/ presence of tetracytic	Absent-0; present-1
4	ANA	Absence/presence of anomocytic	Absent-0; present-1
5	Nosto	No of stomata per mm ²	1-(95-124); 2-(125-154);3-(155-184)
6	Noepi	No of epidermal cell per mm ²	1-(36-45);2-(46-55);3-(56-65);0->65
7	EpiH	Epidermal hairs	0-absent; 1-present
8	Stol	Stomatal index (%)	1-(10-15.5); 2-(16-20.5);3-(21-25.5)
9	Lsto	Length of stomata(μm)	1-(21-30μm);2-(31-40μm);3->40μm
10	Bsto	Breadth of stomata(μm)	1-(1-1.4μm);2-(1.5-1.9μm);3-(2-2.4μm)
Stem			
11	STO	Stem outline	1-Wavy;2-angular; 3- round
12	SEpiH	Epidermal hair	1-present; 0-absent
13	OR	V.B in outer ring	1-(6-7 rings);2-(8-9rings);3-(10-11rings)
14	IR	V.B. in inner ring	1-(4-5 rings);2-(6-7rings);3-(8-9rings)
15	LC	Layer of cortex	1:-6 layers; 2:-7 layers
16	LScl	Layer of sclerenchyma	1:-4 layers; 2:-5 layers;3:-6 layers
17	StG	Stem starch grain	0-absent; 1-present
18	Mxl	Metaxylem	1-paired; 2-unpaired
19	Ph	Phloem	1-both ends; 2-one ends
20	Scr	Stem crystals	1-present; 0-absent
Petiole			
21	PO	Petiole outline	1-pentagonal; 2-round; 3-others
22	PepiH	Petiole epidermal hair	0-absent; 1-present
23	Pcol	Petiole cortical layer	1:-3 layers; 2:-4 layers; 3:-5 layers
24	PSL	Petiole scherenchyma layer	1:-3 layers; 2:-4 layers; 3:-5 layers
25	V.B.	vascular bundle	1-6V.B.; 2-8V.B.; 3- 9 V.B.
26	Pcr	Petiole crystals	0-absent; 1-present
27	Pst	Petiole starch grain	0-absent; 1-present

* μm= micrometer.

Results

Foliar epidermal anatomy

The upper (adaxial) and lower (abaxial) epidermis has one layer of cells and each is covered by a cuticle. The outer epidermis is also characterized by the presence of hairs in some species. Stomata are restricted only to the lower surface (hypostomatic). Four types of stomata complex were identified namely anamocytic, tetracytic, paracytic and anisocytic (Fig. 1). *D. alata* possessed anisocytic, anamocytic and tetracytic stomata; *D. pubera* possessed paracytic and tetracytic stomata; *D. belophylla*, *D. pentaphylla* and *D. bulbifera* had paracytic, anisocytic and anamocytic types of stomata; *D. glabra* with anamocytic and tetracytic stomata; *D. lepcharum* and

D. oppositifolia possessed all the four types of stomata. In the present analysis, the stomatal index ranges from 15.3 in *D. alata* to 24.6 in *D. bulbifera*.

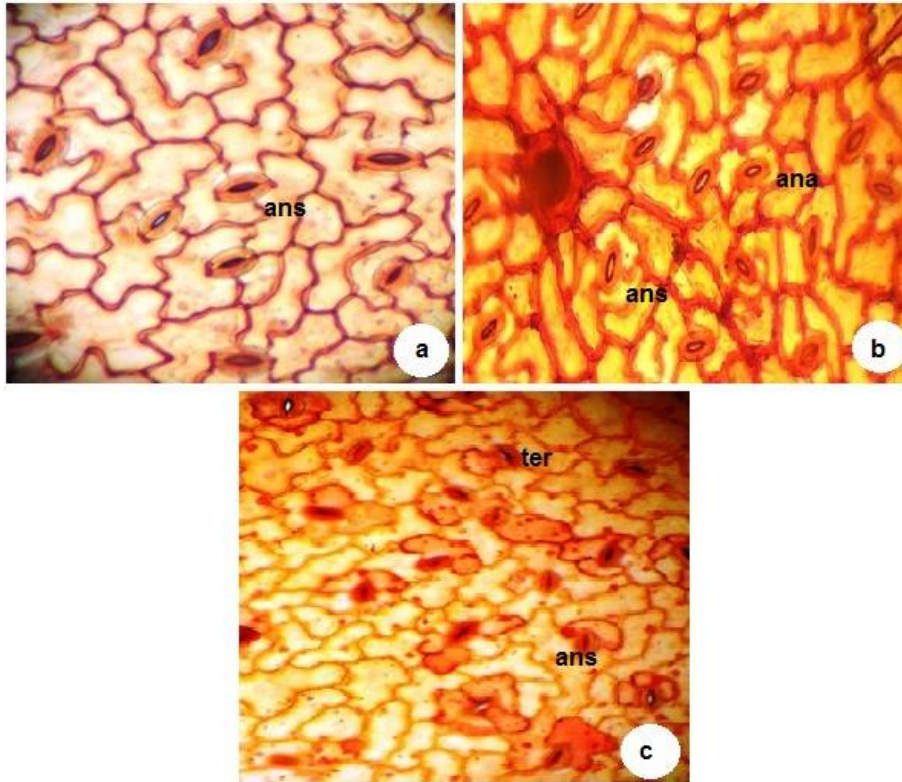


Fig.1. Foliar epidermal structures of *Dioscorea* species. **a)** *D.bulbifera*, **b)** *D. pubera*, **c)** *D. alata*. (*ana* - anomocytic stomata, *ans* - anisocytic stomata, *ter* - tetracytic stomata).

Stem anatomy

Transverse sections of stems are generally circular with longitudinal ridges or wings (Fig. 2). The epidermis consists of thin-walled rectangular, cuboidal or rounded cells. The epidermal cells are 1-cell layer thick. The cuticle is generally thin. In *D. pubera* some epidermal cells contained numerous hairs. The cortex lying just beneath the epidermis is composed of 3-7 layers of cells. The cortical cells are of various size and shapes. The inner boundary of cortex is located at a zone of sclerenchyma with 2-many cells. The pith occupies the central position and composed of thin-walled hexagonal parenchyma cells. The vascular bundles of the stem are arranged in two concentric circles. The vascular bundles of the outer circle are smaller than the inner, with 2 metaxylem vessels together with 1 phloem unit at the middle in the bundles. The bundles of inner circle have mostly paired metaxylem vessels with phloem unit present at both ends in all species except but in *D. bulbifera*, where the bundles of inner circle have unpaired metaxylem with phloem unit at one end. The number of vascular bundles in transverse section varies within the species.

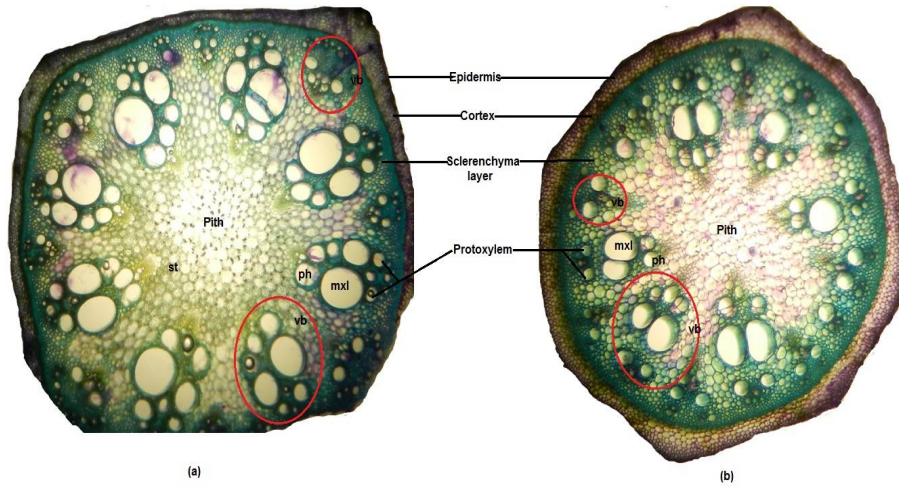


Fig. 2. Anatomy of the stem of *Dioscorea* species at 4X magnification. **a)** *D.alata*, **b)** *D.belophylla*. (*st* - starch grains, *ph* - phloem, *mxl* - metaxylem, *vb*- vascular bundle).

Petioles anatomy

The cortex is composed of collenchymatous tissues. The vascular bundles are arranged in a ring and are basically collateral with the presences of 2-3 phloem units in each bundle. The number of vascular bundles in each petiole is also variable but constant in each species (Fig. 3).

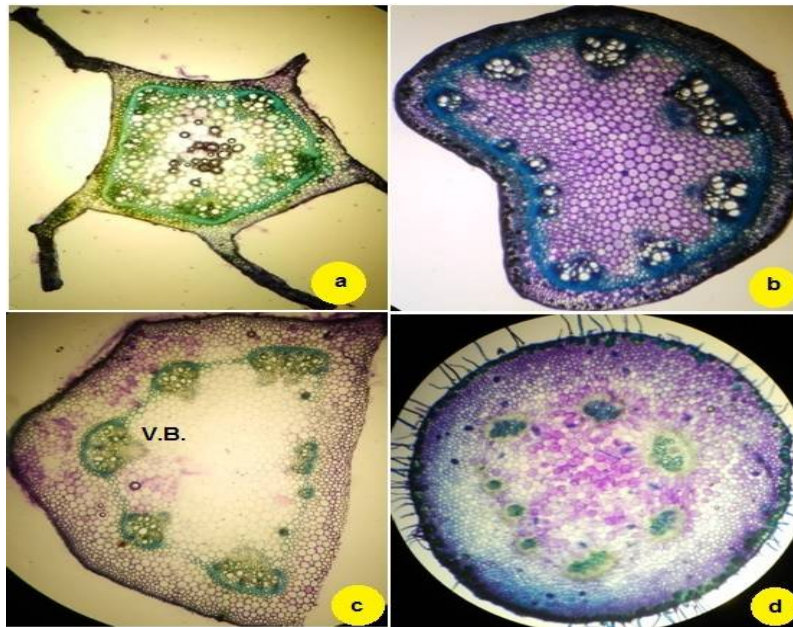


Fig.3. Anatomy of the petiole of *Dioscorea* species at 4X magnification. **a)** *D.alata*, **b)** *D. lepcharum*, **c)** *D. belophylla*, **d)** *D. pubera*.(V.B. vascular bundle).

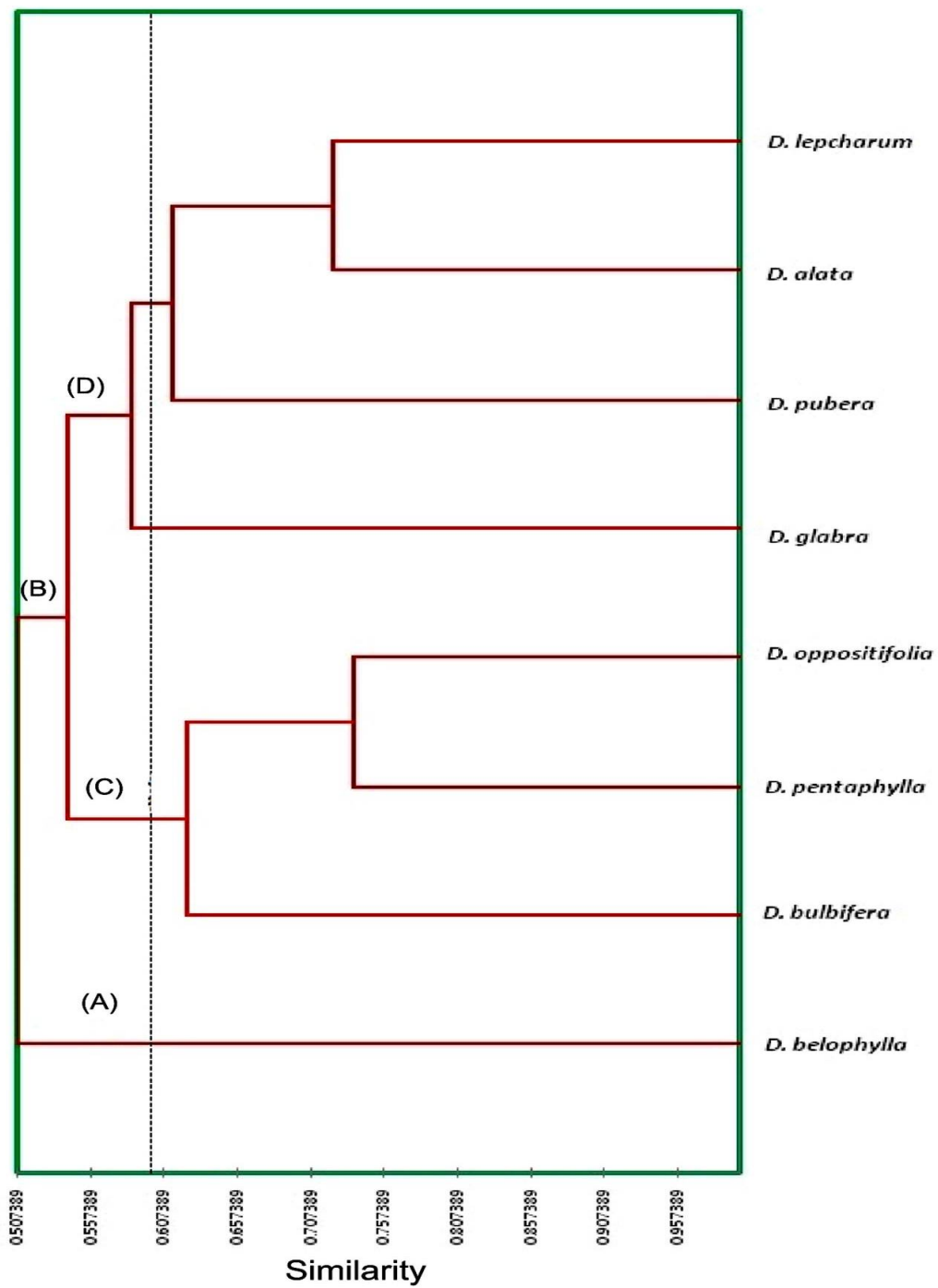


Fig.4. Dendrogram showing relationships among eight *Dioscorea* species based on anatomical characters.

Cluster analysis

The result of cluster analysis based on unweighted pair group average method (UPGMA) initially produced two main clusters, cluster A with a single species *D. belophylla* and the other cluster B at 0.507 level of similarity (Fig. 4). Within the cluster B, two subcluster C and D are separated at similarity level of 0.54. Subcluster C with *D. bulbifera* is separated from *D. pentaphylla* and *D. oppositifolia*. *D. pentaphylla* and *D. oppositifolia* forms a clade at 0.74 level of similarity. Similarly, subcluster D with *D. glabra* and *D. pubera* are separated from *D. alata* and *D. lepcharum* which forms a clade at 0.72 level of similarity.

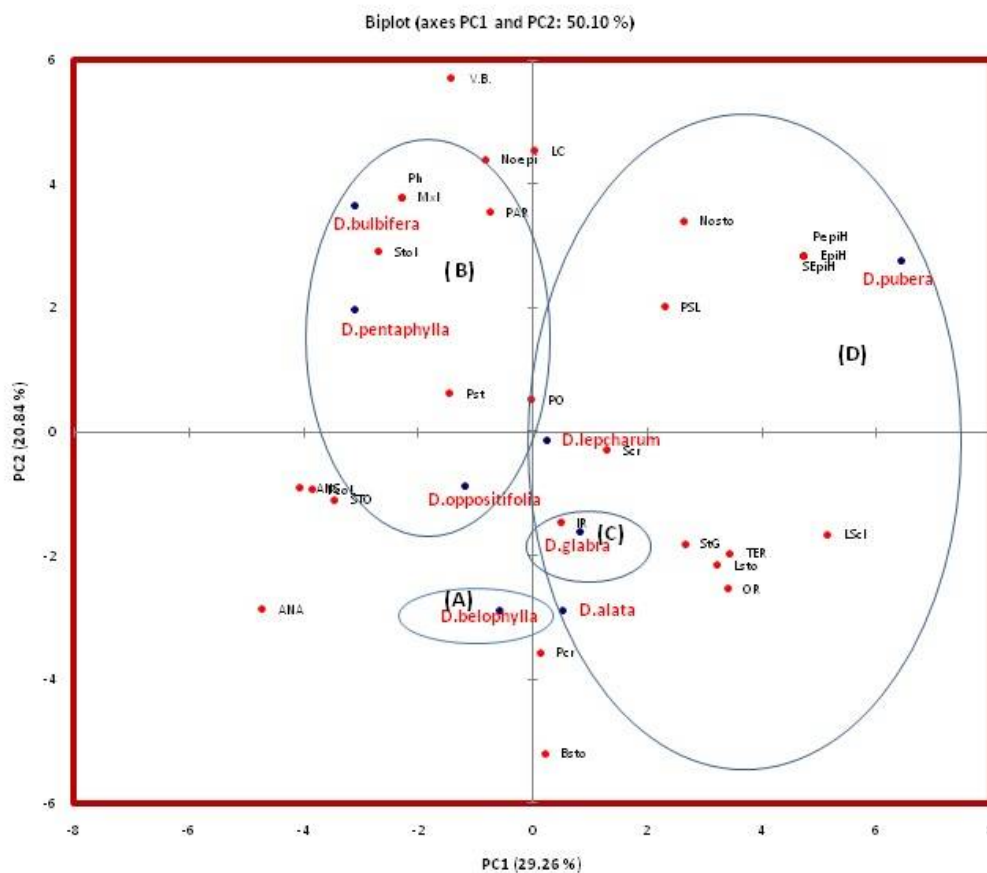


Fig. 5. A biplot based on first and second principal components of anatomical characters for eight *Dioscorea* species.

Principal component analysis

The first seven principal components explained about 100% of the variation, while the first component (PC1) alone counted for 29.26% of the variability (Table 2). Characters such as tetracytic type of stomata, presence or absence of leaf epidermal hair, length of stomata, presence of stem epidermal hair, number of vascular bundle present in outer ring of stem, number of schlerenchyma of in stem, presence of epidermal hair in petioles has the highest loading on PC1. The second component (PC2) explained 20.84% of the total variation was highly correlated with paracytic type of stomata, number of stomata, layers of cortex in stem, presence of paired or

unpaired metaxylem in stem, presence of phloem at one end or at both ends and number of vascular bundles in petioles. The remaining component explained less variability. To assess the scores of the individual species with its characters or traits, PC1 and PC2 were plotted (Fig. 5). The group of species on the left side of the biplot is separated from the species on the right side of biplot due to certain combination of anatomical characters, viz., anamocytic and paracytic types of stomata, presence of starch in petiole, number of vascular bundle in petiole, presence of metaxylem paired or unpaired in stem, presence of phloem at one end or at both ends in stem,

Table 2. Eigenvalues, variance, cumulative variance and component scores (eigenvectors) of the first 7 principal components (PC) for anatomical characters of *Dioscorea* species.

	Component scores						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	7.900	5.626	4.836	3.554	2.746	1.372	0.965
Variance (%)	29.260	20.836	17.912	13.162	10.171	5.083	3.576
Cumulative %	29.260	50.096	68.008	81.170	91.341	96.424	100.000
PAR	-0.138	0.548	-0.090	-0.734	0.078	0.270	-0.236
ANS	-0.742	-0.138	-0.471	-0.360	-0.275	0.043	-0.042
ANA	-0.863	-0.439	0.043	0.175	-0.109	0.056	-0.124
TER	0.625	-0.302	0.169	0.274	-0.522	0.349	0.143
Nosto	0.483	0.525	0.500	0.400	0.094	-0.138	-0.231
Noepi	-0.150	0.678	0.437	0.518	-0.131	-0.187	-0.081
EpiH	0.863	0.439	-0.043	-0.175	0.109	-0.056	0.124
StoI	-0.493	0.450	0.147	-0.357	0.559	-0.079	-0.294
Lsto	0.586	-0.331	-0.530	-0.259	-0.142	-0.197	-0.373
Bsto	0.041	-0.799	0.371	0.379	0.013	0.237	-0.147
STO	-0.634	-0.169	-0.091	-0.583	0.217	-0.062	0.413
SEpiH	0.863	0.439	-0.043	-0.175	0.109	-0.056	0.124
OR	0.619	-0.389	-0.566	-0.027	-0.017	0.161	-0.343
IR	0.089	-0.225	-0.351	0.848	0.187	0.249	0.047
LC	0.006	0.701	0.571	0.116	-0.224	0.341	-0.052
LScl	0.937	-0.255	-0.002	-0.132	-0.005	0.200	0.013
StG	0.484	-0.278	0.392	-0.207	0.564	0.374	0.183
Mxl	-0.418	0.584	-0.430	0.400	0.297	0.217	-0.057
Ph	-0.418	0.584	-0.430	0.400	0.297	0.217	-0.057
Scr	0.236	-0.044	-0.802	-0.238	-0.055	0.486	-0.068
PO	-0.003	0.081	0.856	-0.114	-0.459	0.184	-0.052
PepiH	0.863	0.439	-0.043	-0.175	0.109	-0.056	0.124
PcoL	-0.704	-0.143	0.303	-0.272	-0.529	0.171	-0.095
PSL	0.419	0.312	-0.470	0.081	-0.613	-0.316	-0.155
V.B.	-0.259	0.880	-0.171	-0.047	-0.124	0.332	-0.028
Pcr	0.022	-0.548	0.346	0.138	0.686	-0.071	-0.290
Pst	-0.265	0.096	-0.742	0.545	-0.044	-0.104	0.246

Coefficient in bold indicate descriptors that are highly correlated with the corresponding principal component.

whereas species on the right side of the plot is represented with characters such as presence of foliar epidermal, stem epidermal and petiole epidermal hairs, number of petiole schlerenchyma layers, number of stomata per unit area etc. The finding was consistent with the separation of species into four major groups by UPGMA clustering (Fig. 4).

Discussion

The use of anatomical characters or traits for taxonomic studies has proved useful for identification of fragmented plant and herbarium specimens (Metcalf and Chalk, 1957). Anatomy can provide useful information for establishing interrelations between taxa at the species and supra species levels. Sometimes it can also help in individual identifications. The internal structure of leaf is more affected by environmental factors and thus is of little value for delimiting taxonomic groups. Other characters of leaf, such as the epidermis and stomata have proved to be much more reliable for taxonomic consideration in many genera (Uphof, 1962; Dickison, 2000; Yang and Lin, 2005; Strgulc-Krajsek *et al.*, 2006). The petiole structure is of considerable taxonomic importance in many genera, since it is less affected by environmental changes (Metcalf and Chalk, 1957). The result of the present study allows the selection of some diagnostic anatomical characters for the identification of Meghalayan *Dioscorea* species. Stebbin and Khush (1961) and Ayensu (1972) reported that all stomata in *Dioscorea* species were anomocytic which was found in consistent with Shah and Gopal (1972) and Abdulrahman *et al.* (2009). Our results showing presence of different types of stomata including paracytic, tetracytic and anomocytic etc were found congruent with those of Shah and Gopal (1972) and Abdulrahman *et al.* (2009). The present study reveals an anomocytic character trait contributes much towards variability. Presence of starch grain in stem and petiole is also an important character trait for differentiating among the species (Onwuene, 1978). From the PCA analysis, the character trait i.e. presence of starch grain in petiole was another important trait which shows high rate of variability. Hence the foregoing analysis and presented illustration clearly show the importance of anatomical data as an additional tool in the taxonomy of the genus and also contributes to the identification of species. The results provided some useful features for future phylogenetic and taxonomic studies. Based on these features, an artificial indented dichotomous key is presented hereunder to delimit the species.

Dichotomous key to the Meghalayan species of *Dioscorea* based on anatomical characters.

- | | |
|---|-----------------------|
| 1. Leaf, stem and petiole pubescent | <i>D. pubera</i> |
| - Leaf, stem and petiole glabrous | 2 |
| 2. Absence of tetracytic stomata | 3 |
| - Presence of tetracytic stomata | 5 |
| 3. Layer of schlerenchyma in stem is more than 5; absence of starch grain in stem; vascular bundle in outer ring in stem is more than 9 | <i>D. belophylla</i> |
| - Layer of schlerenchyma in stem is less than 5; presence of starch grain in stem; vascular bundle in outer ring in stem is less than 9 | 4 |
| 4. Inner ring of vascular bundle in stem is less than 6; metaxylem in stem is paired; phloem on both ends of vascular bundle | <i>D. pentaphylla</i> |
| - Inner ring of vascular bundle in stem is more than 6; metaxylem unpaired; phloem at one end of vascular bundle | <i>D. bulbifera</i> |

- | | |
|--|-------------------------|
| 5. Presence of starch grain in stem; outer ring of vascular bundle more than 9 | 6 |
| - Absence of starch grain in stem; outer ring of vascular bundle less than 9 | 7 |
| 6. Inner ring of vascular bundle more than 9; petiole outline mainly pentagonal; layer of cortex in petiole less than 4 | <i>D. alata</i> |
| - Inner ring of vascular bundle less than 9, petiole outline crescent shape; layer of cortex in petiole more than 4 | <i>D. lepcharum</i> |
| 7. Inner ring of vascular bundle in stem less than 8; presence of paracytic stomata; absence of petiole crystal, layer of cortex in petiole is more than 4 | <i>D. oppositifolia</i> |
| - Inner ring of vascular bundle more than 8; absence of paracytic stomata; presence of petiole crystal, layer of cortex in petiole is less than 4 | <i>D. glabra</i> |

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