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ORIGINAL ARTICLE

Characterization of Selected *Tacca* species and Their Relationship Based on Morphology

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ABSTRACT
Tacca (Dioscoreaceae) is a native under storey plant with a great potential to be developed as an ornamental medicinal plant and it was considered rare in Malaysia. Their populations become reduced due to over collecting and habitat disturbance. Thus, ex situ conservation was carried out at Field 2, University Putra Malaysia where the species were collected from Terengganu, Penang, Selangor and Sarawak. The conserved Tacca species has been characterized for important vegetative and morphological characters for utilization as an ornamental medicinal plant. The most significant variations among Tacca species were identified in their seed shape, bract and bracteoles colour and apices of innermost bracts. Three different groups were determined from group cluster and UPGMA dendrogram based on 24 qualitative and 15 quantitative characteristics among Tacca species using Multivariate Statistical Package (MVSP) programme. The coefficient of similarities matrix was calculated based on the Gower distance.
***Keywords**: Dioscoreaceae, Morphological characterization, Tacca, MVSP*

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INTRODUCTION

The morphology description of plants has been fundamental in the history of botany and provided the keys for taxonomy. The accurate morphological description of plant structures based on a mathematical approach may be a fundamental aspect in the identification of such process and their role for the integration of plant development. The description of biological shapes constitutes an important field of study that is being recently recognized in books and articles. New magnitudes are utilized in the description of forms, such as curvature [1] and methods for data analysis are optimized in morphometrics [2]. The morphological description study paradoxically has remained in the shadow, because most studies in development have covered molecular approaches. It is true that molecular data provide good information in relation with morphology, but it is also true that the role played by genes and proteins in the maintenance or stability of shapes will be better understood once a sufficient description of plant forms has been done. The precise description of plant structures is still lacking in many case. In addition to statistics and geometry, such a description provides the basis for discussion on conceptual aspects.

Now a day, although morphological characterization provides only indirect method to measures genetic variability, there are many documented studies that have used it successfully as a toll for classification of plants. Morphological still remain useful because germplasm regeneration curators and plant breeders usually routinely record morphological data during germplasm regeneration or evaluation [3]. The objectives of the present study were describing the morphological characteristics among selected *Tacca*

species for the purpose of identification and *ex situ* conservation and determine relationships among the species.

MATERIALS AND METHODS

Morphological characterization

Four species of *Tacca* recognized by Drenth [4] that was recorded in Malaysia and also the World Checklist of Monocotyledon from Kew [5] were examined. In contrast, *T. nivea* [6], also known as *T. integrifolia* variety Tibet [7] is a new species and not been listed in World Checklist of Monocotyledon. In this study, 15 samples per species were selected to represent almost all morphological types that suggested by Wieclaw and Podlasinki [8]. The taxonomic system defined by Drenth were used and voucher specimens were prepared and deposited in the herbarium of Institute of BioScience, Universiti Putra Malaysia (Table 1) and the collections were planted in the nursery at Field 2, Universiti Putra Malaysia. Vegetative and reproductive morphological characters for each species were measured and recorded at selected location in Malaysia. Qualitative and quantitative characteristics were categorized and transformed into scores to represent each category (Table 2).

Table 1 : List of voucher specimens used in morphological characterization study.

Voucher no.	Family name	Scientific name	Local name
SK 2496/14	Discoreaceae	*Tacca integrifolia* Ker Gawl	Janggut Adam
SK 2497/15	Discoreaceae	*Tacca chantrieri* Andre	Bat plant
SK 2498/16	Discoreaceae	*Tacca nivea* Alba	Janggut Adam Putih
SK 2499/17	Discoreaceae	*Tacca leontopetaloides* (L) Kuntze	Arrowroot

Table 2: Qualitative measurement of morphological characters

Traits	Technique of measurement
Inflorescence shape	simple umbel (present=1, absence=0)
Leaf shape	1-3 scale (oblong-elliptic=1, lanceolate=2, lobed=3)
Leaf base	1-2 scale (attenuate=1, cuneate=2)
Leaf venation	arcuate (present=1, absence=0)
Leaf apices	1-3 scale (entire=1, undulate=2, lobate=3)
Petiole colour	1-3 scale (purple=1, green=2, light green=3)
Petiole structure	1-2 (chambered=1, hollow=2)
Bract colour	1-4 scale (purple=1, dark purple=2, white=3, green=4)
Shape of outermost bract	1-3 scale (aristate=1, cordate=2, rhomboid=3)
Bases of outermost bract	1-2 scale (attenuate=1, cordate=2)
Apices of outermost bract	broadly acute (present=1, absence=0)
Shape of innermost bract	1-2 scale (aristate=1, falcate=2)
Bases of innermost bract	truncate (present=1, absence=0)
Apices of innermost bract	1-4 scale (caudate=1, narrowly acute=2, broadly acute=3, double apiculate=4)
Bracteoles colour	1-4 scale (purple=1, dark purple=2, white=3, green=4)
Fruit shape	1-3 scale (obovate=1, ovate=2, obicular=3)
Colour of immature fruit	1-2 scale (dark purple=1, green=2)
Colour of mature fruit	1-3 scale (darker purple=1,black=2, yellow=3)
Placentation	1-3 scale (parietal=1, free central=2, basal=3)
Ovary structure	1-2 scale (inferior epigenous=1, inferior hypogenous=2)
Seed shape	1-4 scale (kidney=1,broad kidney=2, narrow kidney=3, broad ovate=4)
Seed colour	1-3 scale (dark brown=1, black=2, light brown=3)
Type of root	1-2 scale (rhizome=1, tuber=2)

Multivariate statistical algorithms were used to quantify the genetic similarity where it permitted standardization of multiple types information of a set characteristics [9]. The most widely used algorithms principal component and canonical variable analysis, as well as clustering methods were used [10,11]. The accessions were clustered by the unweighted pair group method using arithmetic average (UPGMA) method which it was indicated as a good cophenetic correlation of the original distance matrices.

In this study, data were subjected to analysis of variance, mean comparison being conducted through Least Significantly Different (LSD) test using SAS statistical package version 9.4. Multivariate analysis was done by transforming the scores for the presence and absence from each species and analyzed using Gower's Similarity Index. Dendrogram and coefficient of similarities were produced using MVSP (Multivariate Statistical Programme) to estimate the variability among the *Tacca* species.

RESULTS AND DISCUSSION

The overall size of inflorescence showed distinct characteristics since *T. nivea* had large inflorescence*, T. leontopetaloides* had longer inflorescence stalk with lobed leaves while *T. integrifolia* and *T. chantrieri* were moderately in sized (Fig. 1). The average for each morphological character and results of least significant different tested for four selected *Tacca* species were listed (Tables 3, 4). The fruits of *T. leontopetaloides* were easily observed from based on the mesocarp colour compared to other three selected species which was green to yellowish in colour (Fig. 2). The largest seeds were observed in *T. leontopetaloides* with light brown colour and the smallest seeds were found in *T. nivea* with black in colour (Fig. 2, Table 5). The shape of seeds were relatively distinct among selected *Tacca* species which *T. integrifolia* in kidney shape, *T. chantrieri* in broad kidney shape, *T. nivea* in narrow kidney shape and *T. leontopetaloides* in broad ovate shape. Rhizome and tuber are the common storage organs for *Tacca* species. Among the selected *Tacca* species, only *T. leontopetaloides* produced a tuber that can be easily distinguished from other 3 species.

Evidently, the differences were supported by the relationships showed in the dendrogram and coefficient of similarity index (Fig. 3, Table 6). The UPGMA dendrogram was obtained based on Gower general similarities coefficient calculated from the morphological data (Fig. 3). Based on cluster analysis, our investigation revealed that four selected *Tacca* species were divided into three cluster group where *T. integrifolia* and *T. chantrieri* were grouped together and separated from *T. leontopetaloides* and *T. nivea*. The result was supported by Zhang *et al.* [7] where they used molecular markers such as ITS and cytoplasmatic region to obtain phylogenetic tree for eleven *Tacca* species.

Based on qualitative and quantitative measurement (Tables 3,4,5), a similarity index was worked out between the four species by considering both positive and negative similarities in features of *Tacca* species (Table 6). Evidently, the figure showed that the *T. integrifolia* and *T. chantrieri*, *T. chantrieri* and *T. nivea* shared similarities up to 63 %.

Zhang *et al.* [7] suggested using Drenth's system where *Tacca* species were divided into four sections based on fruit and leaf morphology, presence or absence of filiform bracteoles and geographically distribution. But in this study, only two sections were established as shown in the dendrogram (Fig. 3).

The first section of Drenth's system includes exclusively entire-leaved species with filiform bracteoles and a vertical elongate rhizome with apical growth. However, in this study, the three species; *T. nivea, T. chantrieri* and *T. integrifolia* occur in different clades. In phylogenetic tree from Zhang *et al.* [7] reported that *T. integrifolia* from Malaysia was not monophyletic and separated from those *T. nivea* (also known as *T. integrifolia* Tibet) with very high bootstrap support. There were large morphological differences among them indicated that they should be accepted as separated species. *T. nivea* is a rare white form of the ever popular "Bat Flower" offers magnificent white, purple veined blooms with long bracteoles. This finding was supported by Boo *et al.* [6] that claimed *T. nivea* or *T. integrifolia* Tibet is a seperate species with *T. integrifolia.* In this study, we can concluded that *T. nivea* considered as new angiosperm of *Tacca* genus in Malaysia. The second section of Drenth's system contain a single species, *T. leontopetaloides* (Fig. 2D) is widely distributed in coastal area and commonly used as a food because the tubers are rich in starch.

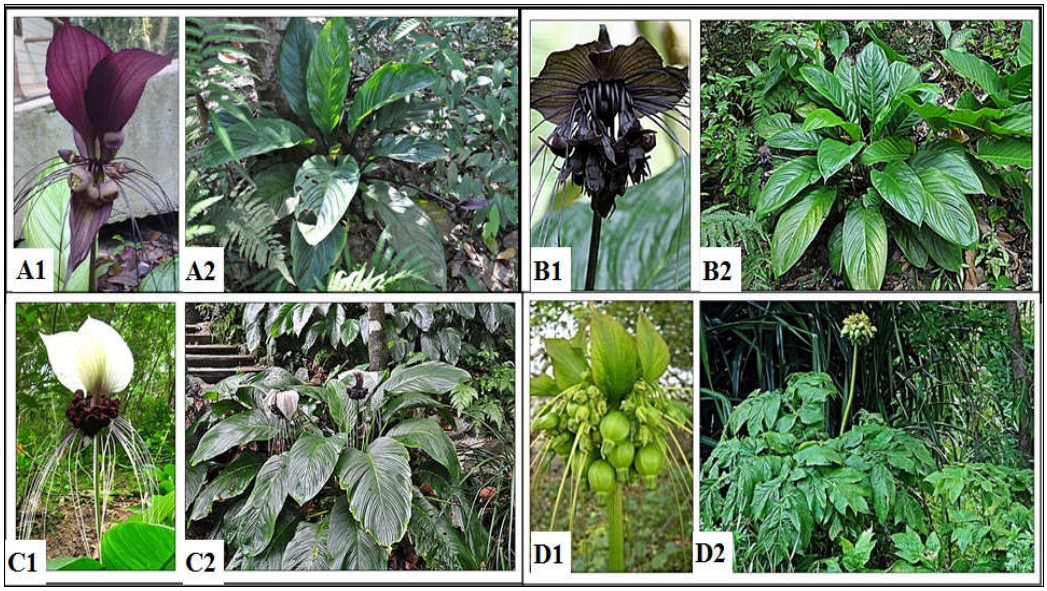

Figure 1: Distinct morphological character inflorescence among selected *Tacca* species. The figure showed inflorescences (**1**) and foliage (**2**) of **A**; *Tacca integrifolia*, **B**; *Tacca chantrieri*, **C**; *Tacca nivea* and **D**; *Tacca leontopetaloides*

Table 3: Quantitative measurement of vegetative features

Traits	Species			
	TI*	TC	TN	TL
Plant height (cm)	68.70^{d**}	93.70^{c}	105.60^{b}	117.10^{a}
Number of leaves per plant	12.00^{b}	20.00^{a}	11.00^{b}	2.00^{c}
Petiole length (cm)	28.70^{b}	23.70^{c}	28.30^{b}	75.20^{a}
Leaf length (cm)	54.60^{d}	57.20^{c}	58.40^{b}	60.30^{a}
Leaf width (cm)	17.70^{d}	21.50^{c}	23.60^{b}	28.60^{a}
Number of outermost bract	2.00^{b}	2.00^{b}	2.00^{b}	6.00^{a}
Number of innermost bract	2.00^{a}	2.00^{a}	2.00^{a}	2.00^{a}
Bracteoles length (cm)	25.30^{c}	28.40^{b}	38.40^{a}	23.60^{d}
Number of bracteoles	16.00^{d}	21.00^{c}	22.00^{b}	30.00^{a}

*TI = *T. integrifolia*, TC = *T. chantrieri*, TN = *T. nivea* and TL = *T. leontopetaloides*
**Means with the same letter are not significantly different according to Least Significant Different (LSD) test at P=0.05%

Table 4: Quantitative measurement of reproductive features

Traits	Species			
	TI	TC	TN	TL
Length of inflorescence stalk (cm)	52.20^{b}	54.80^{b}	57.70^{b}	117.10^{a}
Number of flower per inflorescence	7.00^{c}	6.00^{c}	12.00^{b}	21.00^{a}
Fruit length (cm)	5.60^{b}	4.70^{c}	8.30^{a}	3.90^{d}
Fruit width (cm)	4.10^{a}	3.10^{c}	3.80^{b}	4.40^{a}
Seed length (cm)	0.60^{b}	0.30^{c}	0.40^{c}	0.90^{a}
Seed width (cm)	0.25^{c}	0.36^{b}	0.26^{c}	0.44^{a}

*TI = *T. integrifolia*, TC = *T. chantrieri*, TN = *T. nivea* and TL = *T. leontopetaloides*
**Means with the same letter are not significantly different according to Least Significant Different (LSD) test at P=0.05%

Table 5: Qualitative estimation of morphological characters

Traits	Species			
	TI*	TC	TN	TL
Inflorescence shape	simple umbel	simple umbel	simple umbel	simple umbel
Leaf shape	oblong-elliptic	oblong-elliptic	lanceolate	lobed
Leaf base	attenuate	attenuate	attenuate	cuneate
Leaf venation	arcuate	arcuate	arcuate	arcuate
Leaf margin	entire	entire	undulate	lobed
Leaf apices	acuminate	caudate	broadly acute	acute
Petiole colour	purple	green	light green	light green
Petiole structure	chambered	chambered	chambered	hollow
Bract colour	purple	dark purple	white	green
Shape of outermost bract	aristate	cordate	cordate	rhomboid
Bases of outermost bract	attenuate	cordate	attenuate	attenuate
Apices of outermost bract	broadly acute	broadly acute	broadly acute	broadly acute
Shape of innermost bract	aristate	aristate	aristate	falcate
Bases of innermost bract	truncate	truncate	truncate	truncate
Apices of innermost bract	caudate	narrowly acute	broadly acute	double apiculate
Bracteoles colour	purple	dark purple	white	green
Fruit shape	obovate	ovate	obovate	obicular
Colour of immature fruit	dark purple	dark purple	dark purple	green
Colour of mature fruit	darker purple	black	black	yellow
Placentation	parietal	parietal	free central	basal
Ovary structure	inferior epigenous	inferior epigenous	inferior epigenous	inferior epigenous
Seed shape	kidney	broad kidney	narrow kidney	broad ovate
Seed colour	dark brown	black	black	light brown
Type of root	rhizome	rhizome	rhizome	tuber

*TI = *T. integrifolia*, TC = *T. chantrieri*, TN = *T. nivea* and TL = *T. leontopetaloides*

Table 6: Coefficient of similarity matrix

Species	*T. integrifolia*	*T. chantrieri*	*T. nivea*	*T. leontopetaloides*
T. integrifolia	1.00			
T. chantrieri	0.63	1.00		
T. nivea	0.63	0.55	1.00	
T. leontopetaloides	0.30	0.28	0.38	1.00

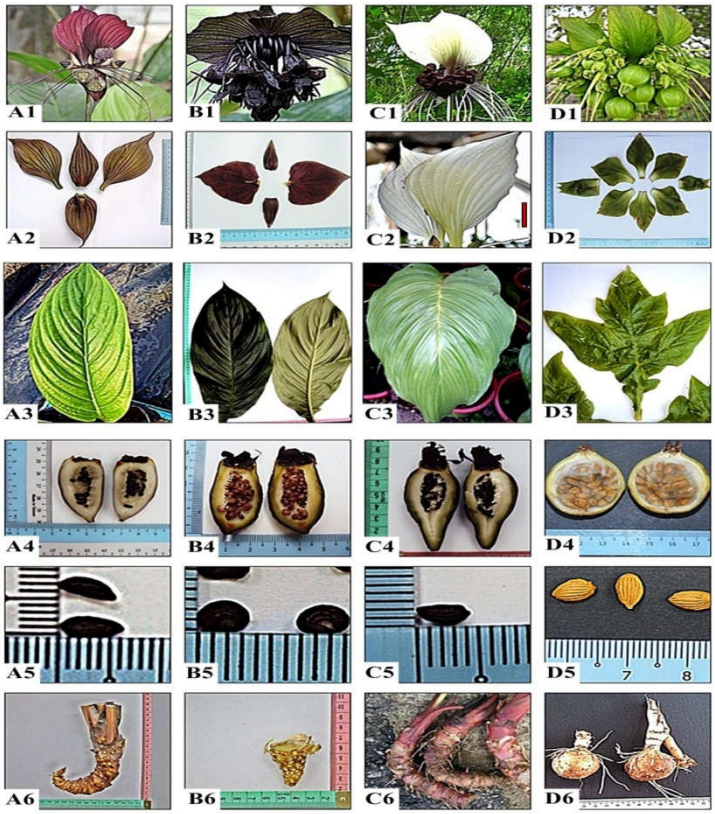

Figure 2: Vegetative and reproductive morphological characters among selected *Tacca* species. **A**: *T. integrifolia*, **B**: *T. chantrieri*, **C**: *T. nivea* and **D**: *T. leontopetaloides*. Morphological characters; **1** = inflorescences, **2** = bracts, **3** = leaves, **4** = fruits, **5** = seeds and **6** = roots. Scale bar = 2 cm.

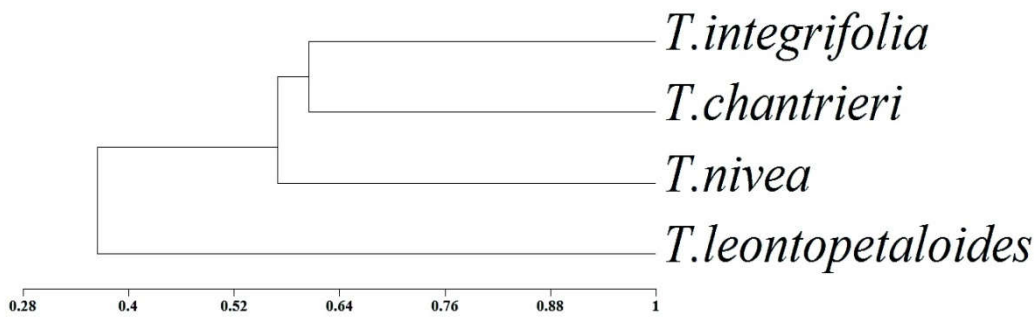

Figure 3: Dendrogram generated by UPGMA

CONCLUSION

The data obtained clearly showed the similarities and dissimilarities amongst selected *Tacca* species based on its morphological characterization. Four morphological characteristics namely, seed shape, apices of innermost bracts, bracts and bracteoles color showed the most variation in *Tacca* species especially *T. nivea,* as a new angiosperm. This study provides a morphological basis understanding to differentiate between selected *Tacca* species. The genetic basis for some of these key characters needs to be studied to ascertain their consistencies within the population. The development of a simple biochemical technique to quantify secondary metabolites will provided important complement identifications to the morphological description that can be used for subsequent selection for commercial ornamental plants.

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