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ORIGINAL ARTICLE

Morphological Variations of *Amorphophallus* spp. Blume ex Decne. in Peninsular Malaysia

S. Phornvillay[1], S. H. Ahmad[1]*, N. A. P. Abdullah[2], A. B. Rosenani[3], N.K. Yusof[1] and N.A.M. Rashid[1]
[1]Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2] Department of Crop Science, Faculty of Agriculture and Food Science, UPM Bintulu Sarawak Campus, 97800 Bintulu, Sarawak, Malaysia
[3]Department of Soil Management, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
*Corresponding email: sitihajarahmad@gmail.com

ABSTRACT
Amorphophallus has attracted much attention as it contains glucomannan and also possess other medicinal properties. Prior to the collection of propagating materials and cultivation, identification and diversity information of the Amorphophallus species are essential as different species perform differently under cultivation. Sixty accessions of Amorphophallus spp., with 10 accessions representing six locations, were used to assess morphological of vegetative characters variations. Thirty-four morphological characters of each accession were observed and recorded. Cluster and principal coordinate analysis using Gower's similarity coefficient classified the accessions into two groups. The first group included all 10 accessions. The second group consisted of 50 accessions. The component analysis (PCA) results revealed the diversity among 60 Amorphophallus spp. accessions with the first three principal components contributed 66.34% of the total variability. The PCA show that there were variations in morphological characteristics among accessions of Amorphophallus spp. based on corm size, corm shape, cormel number per corm and petiole nature. The morphological analysis results suggest that two different species, A. paeoniifolius and A. prainii, were identified.
***Keywords**: genetic diversity, classification, principal component analysis, Gower's coefficient, elephant foot yam*

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INTRODUCTION

The *Amorphophallus* plant possesses medicinal properties and has long been used in traditional medicine [1,2]. Also, it has a significant ornamental value for horticultural materials and ecotourism [3,4]. Among the 200 *Amorphophallus* species of the Araceae family, *A. konjac* K. Koch, *A. albus* Liu & Wei and *A. muelleri* Bl. are planted commercially due to the high glucomannan content of their corms [5]. Glucomannan, a water-soluble polysaccharide, fermentable dietary fibre, has been used for obesity, diabetes, hypertension and high cholesterol problems [6,7]. In Peninsular Malaysia, *A. paeoniifolius* (Dennst.) Nicolson and *A. prainii* Hook f. are common while *A. muelleri* and *A. elegans* Ridl. are scarce [8].
A. praiini, *A.aphyllus* (Hook.) Hutch, *A. paeoniifolius* and *A. sylvaticus* (Roxb.) Kunth are traditionally used for snake bite, arrow poison and as an analgesic [9]. *A. paeoniifolius* is commercially grown in India. The corm, young shoot and flower are eaten and used in ayurvedic medicine. The corm's ash is prescribed to treat piles, haemorrhoids, gout, asthma, bronchitis and stomach indigestion while the petiole juice is used to cure diarrhoea [2]. The corm extract possess anti-tumour, antioxidant and cytotoxic properties and has synergistic depressant effect when used with diazepam [10]. Thus, to be introduced as a new crop, the genetic diversity of the *Amorphophallus* spp. needs to be studied.
Morphological variations study is crucial as it could provide the information for the plant genetic diversity in order to increase the efficiency of germplasm collection management, conservation and

improvement of breeding programs [11]. Morphological characterization forms the basic in describing and taxonomic classification of the plant. Despite numerous studies reported on the *Amorphophallus* genetic diversity, there is lack of information on the variations of morphology characteristics of the Malaysian *Amorphophallus* spp. Such knowledge is needed in cultivating the *Amorphophallus* spp. as a new crop. Corm collection is carried out during the vegetative stage of the plant as flowering is uncommon. Thus, the *Amorphophallus* spp. cannot be identified by using flower morphology. Therefore, the objectives of the study were to characterize vegetative morphological variations of *Amorphophallus* spp. collected in Peninsular Malaysia.

MATERIAL AND METHODS

Plant Materials

Corms of 60 accessions of *Amorphophallus* spp. from six populations in Peninsular Malaysia were collected (Table 1). The samples were planted under about 50% shade provided by Brazil nut trees (*Bertholletia excels*) at the herbal garden, Universiti Putra Malaysia, in the year 2012-2013. The location coordinates were N02° 59.293’ E101° 42.544'. Cultural practices, such as watering and organic fertilizer application, were conducted as required.

Morphological characterization

Quantitative and qualitative characteristics were recorded following the descriptions reported by Hetterscheid and Ittenbach [12] and Abraham et al. [13]. The quantitative characters identified include corm fresh weight, corm diameter, corm circumference, corm thickness, petiole length, petiole diameter, petiole circumference. The North-south canopy spread, East-west canopy spread, leaflet length and width were also determined. The qualitative characteristics assessed were plant habit, corm (shape and colour), cormel number grouping, rachis nature, foliar phenophase, petiole (nature, partition, cluster and surface pattern), leaflet (apex, margin, shape, colour and appearance), canopy type and phenology-vegetative phase. The qualitative characters were scored as binary and multi-state scores.

Data analysis

The data were subjected to principal coordinate analysis (PCoA) to identify the grouping of the accessions based on the Gower’s coefficient of similarity. Principal component analysis (PCA) was carried in order to determine the pattern of variation in the characters of the collected accessions, and to find out which characters are the main contributor in distinguishing the taxa [14]. The correlation matrix of PCA was used as it is invariant under scale changes. PCA and PCoA were performed using the PAST software [15]. The classification of the accessions was carried out by generating the Gower’s similarity matrices. The Gower coefficient of similarity was chosen as it is applicable to binary, alternative, quantitative and qualitative characters, and widely used in numeric analysis [16, 17, 18]. Both the quantitative and qualitative characters were transformed into 0 (absence) and 1 (present). Then, the similarity matrix generated was used to perform cluster analysis using Unweigthed Pair Group Method Based on Arithmetic Averages (UPGMA) [14] with multivariate statistical package (MVSP) software version 3.01 [19].

RESULTS AND DISCUSSION

Principal Coordinate Analysis (PCoA)

The results of the two-dimensional PCoA based on 34 vegetative characters revealed the diversity among the 60 *Amorphophallus* spp. accessions of the six populations collected in Peninsular Malaysia. Both of the first and the second coordinate axis explained 49.7% of the total variation in the standardized data set of 60 vegetative characters. The first coordinate axis demonstrated 37.93% of the variation and 11.77% for the second coordinate axis. Based on this morphological data, the PCoA separated the accessions into two main groups (Figure 1). Kota Bahru, Kelantan (KKB) accessions were clustered into one group indicating that these accessions have distinct vegetative morphological variations. The other accessions from Kubur Panjang, Kedah (KKP) Bukit Jambul, Penang, (PBJ), Ulu Kenas, Perak (PUK), Taiping, Perak (PT) and Hulu Langat, Selangor (SHL) were clustered into another group. However, the PT1, PT2, PT36 and PT37 accessions were slightly separated from the second group, thus, forming a subgroup of the second group.

Table 1. Accession identification, number and location of collection.

Identification	Number	Location of collection	State
KKP	3	Kubur Panjang	Kedah
KKP	4	Kubur Panjang	Kedah
KKP	5	Kubur Panjang	Kedah
KKP	6	Kubur Panjang	Kedah

KKP	7	Kubur Panjang	Kedah
KKP	8	Kubur Panjang	Kedah
KKP	10	Kubur Panjang	Kedah
KKP	11	Kubur Panjang	Kedah
KKP	12	Kubur Panjang	Kedah
KKP	13	Kubur Panjang	Kedah
PBJ	4	Bukit Jambul	Penang
PBJ	5	Bukit Jambul	Penang
PBJ	7	Bukit Jambul	Penang
PBJ	8	Bukit Jambul	Penang
PBJ	12	Bukit Jambul	Penang
PBJ	21	Bukit Jambul	Penang
PBJ	22	Bukit Jambul	Penang
PBJ	23	Bukit Jambul	Penang
PBJ	24	Bukit Jambul	Penang
PBJ	25	Bukit Jambul	Penang
PT	1	Taiping	Perak
PT	2	Taiping	Perak
PT	8	Taiping	Perak
PT	13	Taiping	Perak
PT	16	Taiping	Perak
PT	29	Taiping	Perak
PT	30	Taiping	Perak
PT	32	Taiping	Perak
PT	36	Taiping	Perak
PT	37	Taiping	Perak
PUK	30	Ulu Kenas	Perak
PUK	40	Ulu Kenas	Perak
PUK	41	Ulu Kenas	Perak
PUK	42	Ulu Kenas	Perak
PUK	45	Ulu Kenas	Perak
PUK	46	Ulu Kenas	Perak
PUK	47	Ulu Kenas	Perak
PUK	48	Ulu Kenas	Perak
PUK	50	Ulu Kenas	Perak
PUK	53	Ulu Kenas	Perak
SHL	2	Hulu Langat	Selangor
SHL	5	Hulu Langat	Selangor
SHL	7	Hulu Langat	Selangor
SHL	10	Hulu Langat	Selangor
SHL	11	Hulu Langat	Selangor
SHL	12	Hulu Langat	Selangor
SHL	20	Hulu Langat	Selangor
SHL	22	Hulu Langat	Selangor
SHL	24	Hulu Langat	Selangor
SHL	25	Hulu Langat	Selangor
KKB	1	Kota Bharu	Kelantan
KKB	3	Kota Bharu	Kelantan
KKB	4	Kota Bharu	Kelantan
KKB	5	Kota Bharu	Kelantan
KKB	7	Kota Bharu	Kelantan
KKB	10	Kota Bharu	Kelantan
KKB	11	Kota Bharu	Kelantan
KKB	12	Kota Bharu	Kelantan
KKB	15	Kota Bharu	Kelantan
KKB	17	Kota Bharu	Kelantan

Table 2. Loadings of the 34 vegetative characters on the first three components from principal component analysis (PCA) of *Amorphophallus* spp. Eigen values, percentage of variance and total are given for each component (the boldface indicates highly loaded variables).

Character	Component		
	1	**2**	**3**
Corm fresh weight	0.687	0.390	-0.325
Corm diameter	**0.718**	0.434	-0.250
Corm circumference	**0.743**	0.472	-0.259
Corm thickness	**0.714**	0.501	-0.176
Petiole length	0.622	**0.700**	-0.092
Petiole diameter	**0.730**	0.426	0.001
Petiole circumference	**0.748**	0.482	0.006
NS spread	0.679	0.661	-0.015
EW spread	0.687	0.653	0.035
Leaflet length	-0.022	0.684	0.646
Biggest leaflet length	0.053	**0.727**	0.580
Smallest leaflet length	-0.078	0.349	0.510
Leaflet width	0.359	0.031	**0.797**
Biggest leaflet width	0.342	0.182	0.690
Smallest leaflet width	0.181	0.051	**0.837**
Habit	0.527	-0.446	0.229
Corm shape	-0.141	-0.010	0.004
Corm colour upper surface	**0.791**	-0.567	0.090
Corm colour lower surface	**0.713**	-0.615	0.045
Cormel number grouping	**0.811**	-0.547	0.090
Foliar phenophase	**-0.715**	0.142	0.312
Petiole nature	**0.853**	-0.183	0.077
Petiole surface pattern	-0.593	0.513	-0.270
Petiole number grouping	0.684	-0.542	0.055
Rachis nature	-0.107	0.059	0.454
Leaflet shape	0.562	-0.033	-0.280
Leaflet apex	-0.659	0.528	-0.037
Leaflet margin	-0.249	-0.087	-0.110
Leaflet appearance (abaxial)	**-0.769**	0.453	-0.169
Leaflet appearance (adaxial)	-0.512	0.247	-0.283
Leaflet colour	0.610	-0.629	0.083
Petiole partition	0.593	0.250	-0.357
Canopy spread	**0.723**	0.254	-0.251
Phenology vegetative state	0.013	0.127	-0.375
Eigen value	11.82	6.64	4.10
Variation (%)	34.76	19.53	12.05
Total variation (%)	34.76	54.29	66.34

Principal Component Analysis (PCA)

The grouping pattern in the scatter plot of PCoA is further explained by using PCA scores or loadings. In the PCA, the first three components showed significant differences based on the scree plot for PC scores that explained 66.34% of (Table 2). The first principal component (PC1) accounted for the largest variation of 34.76% of the total variation. The vegetative characters which showed high loading scores for PC1 were as follows: a) corm characteristics: diameter, circumference, thickness and both upper and lower surface corm colour ; b) petiole characteristics: diameter, circumference and nature; c) cormel grouping number; d) foliar phenophase; e) leaflet abaxial appearance and f) canopy spread. The second component that was used to account for the 19.53% variation comprised petiole length and biggest leaflet length as dominant characters in distinguishing the accessions. On the other hand, the third component had the highest positive (12.05%) loading scores for leaflet width and the smallest leaflet width.

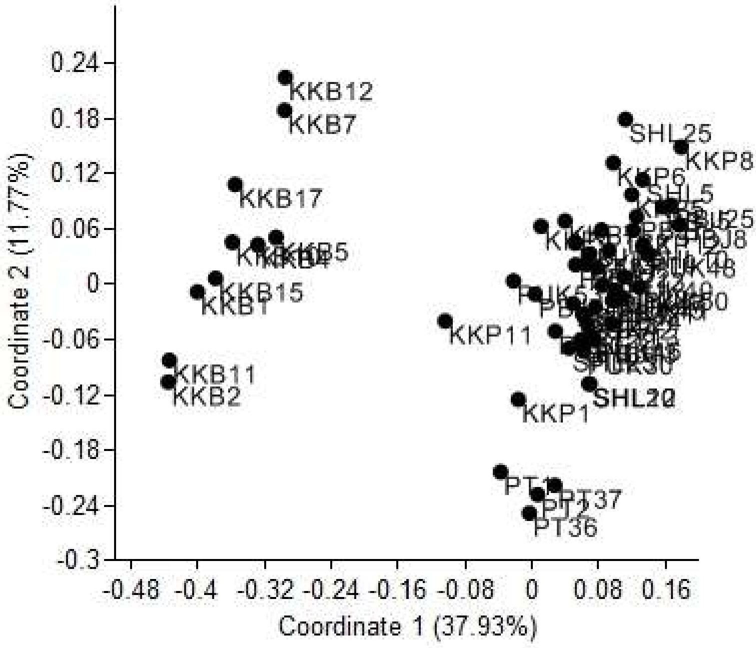

Figure 1. Scatter plot of principal coordinate analysis based on vegetative characters of the 60 *Amorphophallus* spp. accessions by using Gower's similarity coefficient.

Figure 2. (A) The scabrous and muricate epidermal excrescence of KKB accessions; (B) Cormels of KKB8 (42 cormels/corm).

The PCA results on the qualitative characters evaluated in the 60 *Amorphophallus* spp. accessions showed that petiole nature contributed the most variation with a loading of 0.853 (Table 2), indicating the high grouping distinction based on the petiole nature. KKB accessions obviously had scabrous and muricate epidermal excrescence on the petiole surface as compared to KKP, PBJ, PUK, PT and SHL accessions (Figure 2A). The later accessions of the five populations mostly had smooth petiole, and a few had minutely rounded protuberances (verrucate) on the lower part of petiole surface. It was also reported that muricate petioles are a crucial vegetative character used to differentiate wild and cultivated *Amorphophallus* spp. [20].

Cormel number grouping also was another character that separated KKBs from other accessions. The KKB accessions had a larger number of cormels per corm with six to 40 cormels/corm (Figure 2B). On the contrary, the other accessions had no cormel, except for KKP11, which had only one cormel/corm. In addition, the corm surface colour (upper and lower) was another discriminator, whereby KKB accessions had a distinctly different corm surface colour than other accessions. The colour was brown and light brown on the upper and lower corm surface, respectively. The other accessions had dark brown upper and brown lower corm surface colour. Also, the KKB accessions had distinct foliar phenophase and leaflet appearance. It was observed that all the KKB accessions had a petiole which lasted for one growing season and had shiny leaflet appearance (abaxial).

About 66.34% of the total variability explained by the first three components showed a high degree of positive correlation between the quantitative characters of corm, petiole and leaflet sizes. In India, a study on the morphological variation of wild and cultivated *A. peaoniifolius*, showed that there was a significant correlation between petiole circumference and corm diameter, corm thickness, corm weight and canopy spread [21]. Therefore, petiole and canopy size could be used to indicate the corm size. In addition, the corm/plant age could be determined through the corm and petiole size and canopy spread. It was previously reported that the corm age is proportional to the corm size, particularly corm weight [22]. Also, the branching of the leaf partition and pattern was reported to indicate the corm age of *Amorphophallus* spp. A thick canopy spread usually has a larger number of the leaf petiole branching [5, 23].

In addition to the qualitative characters, the PCA loading also showed that the sizes of corm, petiole and leaflet contributed to the grouping of the accessions. These explain the forming of subgroup that consisted of PT1, PT2, PT36 and PT37 (Figure 1). This was because the four PT accessions had larger sizes of corm, petiole and leaflet as compared to the other accessions in the second group (KKP, PBJ, PUK, PT, and SHL) despite having similar characters to the other accessions in the same group.

Cluster Analysis

The standardisation of morphological data was used, and UPGMA phenogram was constructed using cluster analysis based on Gower's similarity coefficient of the 34 vegetative characters shown in Figure 3. The phenogram showed similar grouping with the scatter plot of PCoA. The 60 *Amorphophallus* spp. accessions were clustered into two distinct groups. All of the KKB accessions were clustered as one group (Cluster I) and the other accessions of KKP, PBJ, PUK, PT and SHL were clustered into another group (Cluster II).

In a cluster I and II, each small subgroup indicated high morphological variations in vegetative characters among the collected *Amorphophallus* spp. This is in agreement with the findings of a previous study, whereby *Amorphophallus* spp. displayed variations in corm and petiole characters, such as corm shape, size and colour; and petiole nature, pattern and size [12]. Based on the UPGMA phenogram, Cluster II showed that the accessions from different populations of KKP, PBJ, PUK, PT and SHL were not classify accordingly. This may suggest the possibility of human interference in transferring the corm or seed source across the state. Also, the seeds could be dispersed by birds that feed on the berries. Mayo et al. [24] also reported that birds are the main seed dispersal of the *Amorphophallus* spp.

The collected *Amorphophallus* spp. accessions exhibited variations in the petiole surface pattern. It was observed that petiole surface pattern of all KKB accessions was different from the other accessions of the five populations. The petioles were light green with white blotches on the surface, while the petioles of the other accessions were dark green, olive green or brown to dark brown, with small black dots and white blotches. In India, a study to classify wild and cultivated *A. peaoniifolius* also showed that petiole surface pattern played an important key role [21]. Therefore, some characters of corm and petiole could be useful to identification of *Amorphophallus* spp. in Peninsular Malaysia. Nonetheless, the vegetative characters showed a continuous variation and had a high degree of plasticity and instability. Therefore, the taxon identification is ambiguous or insufficient if only the vegetative plant parts are evaluated [25].

During the field maintenance of the collections, eight plant samples flowered. Accessions from Kelantan (KKB1 and KKB3) produced pale green to maroon spathe (11-35 cm long) with campanulate shape, spreading limb and strong wavy margin (Figure 4A). Length of spadix is 11.0-19.2 cm. The appendix is conical and never narrowly elongated (12.8-31.5 cm long). The peduncles are short (2.2-4.5 cm), smooth and pale. The inflorescence characteristics fit the description of *A. paeoniifolius* by Hetterscheid and Ittenbach [12]. Figure 4B shows inflorescence characteristics similar to *A. prainii* as described by Hetterscheid and Ittenbach [12]. The spathe is creamy white, campanulated with less wavy margin and either ovate or circular spreading. The spadix (3.5-9.5 cm) is shorter than the spathe (13.5-25.0 cm long).

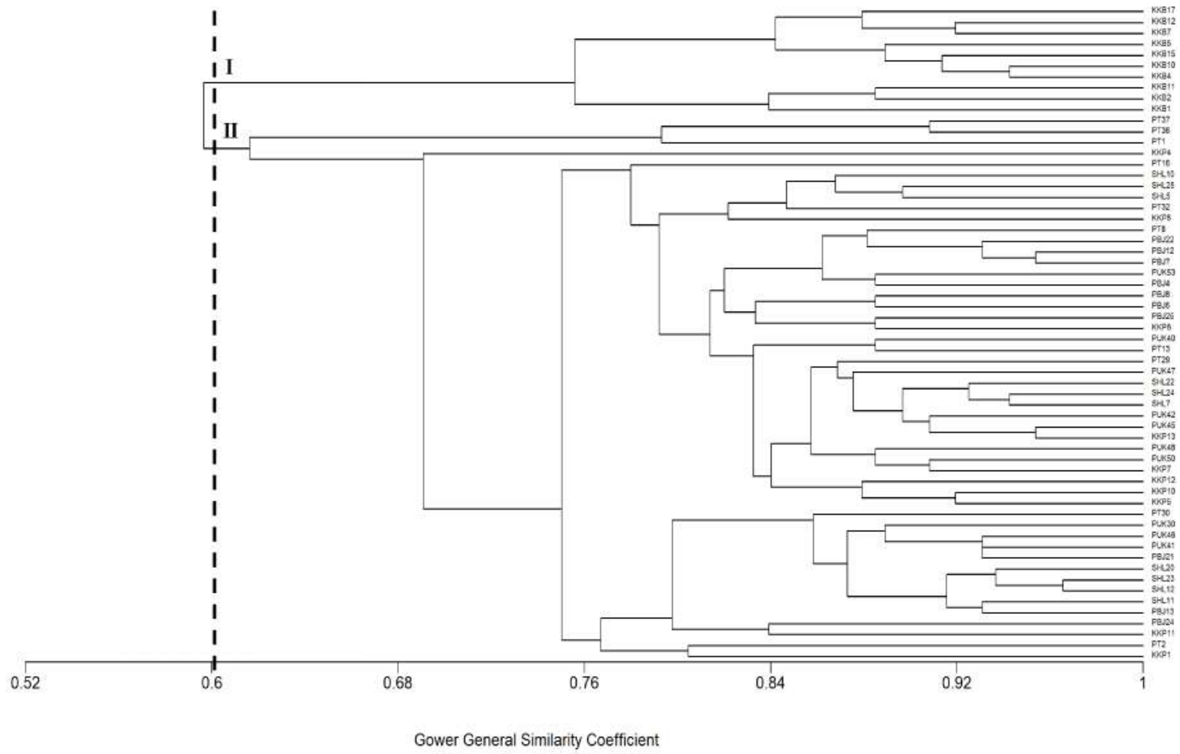

Figure 3. Phenogram of UPGMA cluster analysis showing the relationship among 60 accessions of Amorphophallus spp. collected from six locations in Peninsular Malaysia based on 34 vegetative characters by using Gower's similarity coefficient.

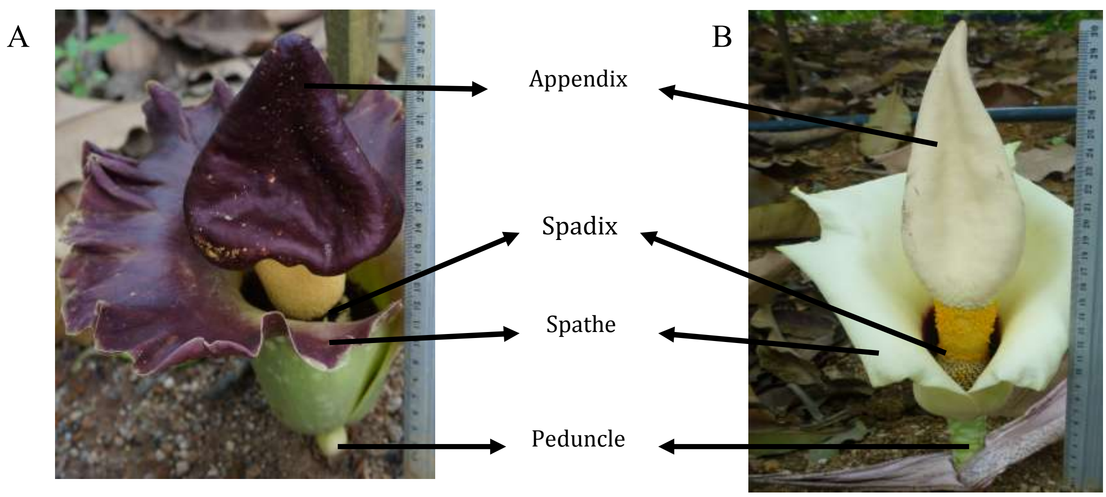

Figure 4. Inflorescence of (A) Kelantan (KKB): dark crimson to maroon spathe and (B) Perak, Taiping (PT): creamy white spathe.

The peduncle is pale and short (2.1-9.3 cm). The appendix (6-23 cm long) is creamy white and ovate-fusiform in shape. Five of the populations studied had similar inflorescence characteristics as *A. prainii*. The PCO results showed that the Kelantan accessions were clustered as one main group called *A. paeniifolius* while the remaining accessions were clustered as *A. prainii*. In conclusions, the present study suggests that morphological characters such as corm size, corm shape, cormel number per corm and petiole nature can be used to distinguish the *Amorphophallus* spp.

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