

RESEARCH ARTICLE

# Variation in Leaf Morphology of Sago Trees (*Metroxylon sagu*) in South Borneo Province, Indonesia.

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## Abstract

Natural populations of sago (*Metroxylon sagu*) in Indonesia show morphological variations in its organs, particularly the leaves. The existing variations resulted in sago type names provided by the locals. The sago plant types include Mahang, Buntal, Salak, Madang and Gandut. Sago leaves are economically important in Indonesia, mainly because they provide a potentially profitable and environment-friendly source of house roofing materials. Determining leaf variation among sago plants would provide information for a better way of choosing which among the current accessions/varieties of sago has the best leaf quality and production. Leaf variations in sago were recorded in shape, size, and color. The aim of this study are a) to compare differences in leaf morphology among sago types obtained from different geographic areas, and b) to determine whether these different sago types correspond to certain sago plant grouping. The qualitative and quantitative analysis showed variations in leaf characteristics of sago trees. Additionally, this study shows that based on leaf variations, the sago of South Borneo Province can be separated into two groups. The first group is Mahang and Gandut, which have long rachis, leaflets and leaves, and largest leaf area. The second group composed of Buntal, Salak and Madang accessions, which have short rachis and leaves, and smallest leaf area. Other results showed that there were characters that contributed significantly to the diversity of sago plants in the study area including spines, white stripe or banding on the rachis and petiole length. In line with our results, Mahang and Gandut accessions are the best accessions to be cultivated in the area, because they have wider leaves which support higher rates of photosynthesis. Further study should be conducted on phylogenetic using DNA sequencing to confirm

divisions of the two sago groups.

Keywords: leaf characteristics, diversity, sago accessions, leaf area, Borneo

## Introduction

Sago (*Metroxylon sagu*) is one of the most efficient carbohydrate-producing crops in Indonesia. The common and popular local name of sago is "rumbia"; it contains a large quantity of starch on its trunk. Sago has large trunk diameter about 35-60 cm and trunk length about 6-16 m (Flach, 1997), and sometimes it has leaf sheath on its trunk and spine on the leaves, leaves or crown have horizontal growth and almost founded as a cluster (Figure 1). The distribution of sago in Indonesia is in Papua (Irian Jaya), West Papua (Irian Jaya Barat), Maluku, West Sumatra Province, Aceh, Riau Islands, Borneo, Sulawesi, and other provinces (Bintoro et al., 2013).

Sago leaves in Indonesia are used as materials for making house roof. Sago leaves are the preferred roof materials because they are available in the area, easy to collect, and inexpensive. Craftsmen of sago roofs in Jambu Hulu Village, Hulu Sungai Selatan Regency, could produce 2-7 roofs per day with net profit of IDR 16,500 - 55,000 per day (Fatriani, 2010). Sago leaves could also be used as the roof of *kumbung* houses, i.e. a structure for growing mushroom, at a relatively low cost (Masdjudi et al., 2019). Sago roofs are environment-friendly, energy efficient, simple, inexpensive and easy to repair (Lestari and Alhamdani, 2013). When installed in houses, sago roofs can create a reasonably comfortable room temperature of 23°C to 29.2°C and a humidity of around 88% - 99%.

The advantages of sago roof can be attributed to the morphological characteristics of sago leaves; sago leaves have unique structures and hardy. Sago leaves consist of petiole, rachis and leaflets (Figure 2). During trunk formation stage, sago generally have leaves with length of 10 m, between 140-180 leaflets, and 10-20 leaves. Sago leaves usually grow vertical at the crown. The spines are on the abaxial position of petiole. The leaflets sometimes have dry part on the tips. Sago plants produce a large number of leaves during growth but becoming constant thereafter (Yamamoto et al., 2020). Sago leaves displays anatomical characteristics of a  $C_3$  plant, having palisade and spongy tissues without Kranz anatomy, as opposed to  $C_4$  plants (Nakamura et al., 2005). Sago leaves have several unique mechanisms of resistance to external stress. Sago plants cope with salinity stress by regulating low  $Na_+$  concentrations in their leaflets (Ehara et al., 2006). Sago can also adapt to temporary waterlogging stress by regulating the photosynthetic capacity in leaves (Azhar et al., 2020).

Sago leaves have fairly high diversity, especially in the leaf area. The Para and Rondo sago accessions around Lake Sentani, Jayapura, and Papua have a maximum leaf area of 29 m<sup>2</sup> and 17 m<sup>2</sup> (Yamamoto et al., 2020a). Sago leaves in Mioko Village, Mimika Regency, and Papua Province vary between 4.88 m<sup>2</sup> - 18.81 m<sup>2</sup> (Pratama, 2018). Sago leaves in Aimas District, Sorong Regency, West Papua vary from 5.39 m<sup>2</sup> to 24.18 m<sup>2</sup> (Fathnoer et al., 2020). Sago accessions in several villages in Meranti Islands Regency, Riau had a leaf area of 5.24 m<sup>2</sup> - 20.27 m<sup>2</sup> (Ayulia, 2019). The Molat and Rattan sago accessions in Kendari, Southeast Sulawesi had leaf area with a variation of 13 to 22 m<sup>2</sup> and 10 to 13 m<sup>2</sup> (Yamamoto et al., 2014).

Qualitative and quantitative measurements on leaf are common approaches used in the study of sago leaf morphology (Ahmad et al., 2016; Pratama., 2018), which has been used for this study. Morphological data can be used to obtain dendrogram that shows placement and grouping of variable plants within a

certain taxon, such as different accessions with the same species. The result of dendrogram base on morphological data showed that sago were grouped into some groups and individuals belonging to one group had similar morphology (Riyanto et al., 2018).

The aims of this study are a) to compare differences in leaf morphology among sago plant types obtained from different geographic areas in South Borneo and b) to determine whether these different sago plant types correspond to certain sago plant grouping. The results from this study provided important information related to the identity and morphological characteristics of the types of sago trees that we found in South Borneo, Indonesia.

## Material and Methods

### Location of Sample Collection

A total of 15 samples of representing three replicates from each of the five accessions (group of related plant material from a single species which is collected at one time from a specific location) of sago (*Metroxylon sago*) were collected from three regencies in South Borneo (Kalimantan) Province, Indonesia. The regencies (sampling areas) were selected based on endemic sites and they include Tapin (Candi Laras Selatan District), Banjar (Sungai Tabuk), and Barito Kuala (Anjir Muara). The accessions were identified and differentiated from each other based on information provided by local land owner and harvester. The accessions included Mahang, Buntal, Salak from Tapin Regency, Madang from Banjar Regency, and Gandut from Barito Kuala Regency.

### Data Collection and Analysis

Measurements and data collection were made where trunk formation was apparent and by selecting the second mature and completely open leaf from the top of the plant. Both qualitative and quantitative data were collected.

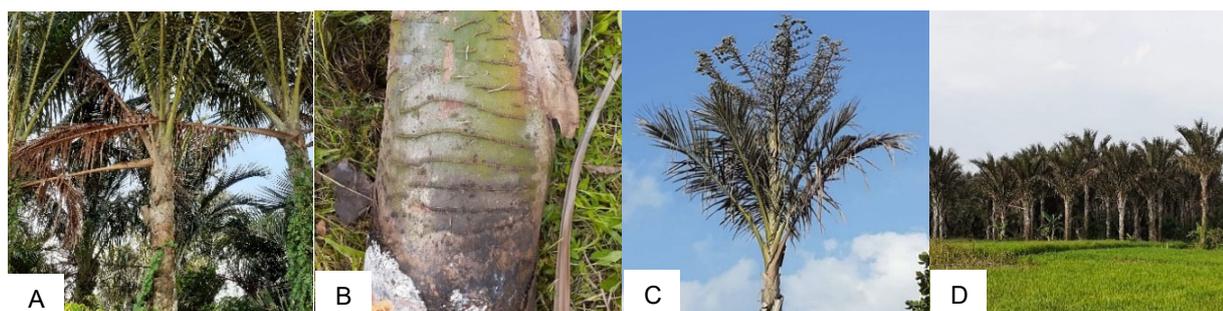


Figure 1. (A) trunk surface, (B) spine on leaf, (C) crown, (D) sago cluster

Morphological characteristics of the leaves including leaf shape, the presence of spine, color, and other unique characteristics were recorded and formed part of the qualitative data. Other morphological characteristics including the number of leaflets, rachis length (m), petiole length (m) and width (cm), rachis length (m), leaf length (cm) and width (cm), and leaf area (m<sup>2</sup>) were measured and comprised the qualitative data of the study (Figure 2).

Leaf color was determined by comparing the leaf color using the Royal Horticulture Society (RHS) Colour Chart (RHS., 2015). RHS colour chart has been used to determine color expression of leaf and other organ colors of the sago seedlings (Riyanto et al., 2018). The number of leaflets was counted on both sides of

## Results and Discussion

Qualitative and quantitative data on sago morphology are provided in Tables 1-4. The relationship of the different types of sago based on morphological variation are shown in Figures 6-7.

### Qualitative Data

Our study demonstrated that the shape of the sago leaf samples are generally similar for all accessions. The leaves in the rosette stage have fan-like shape when opening, while in the trunk formation stage, the sago leaves are tighter or narrower at the leaflets (Figure 3).

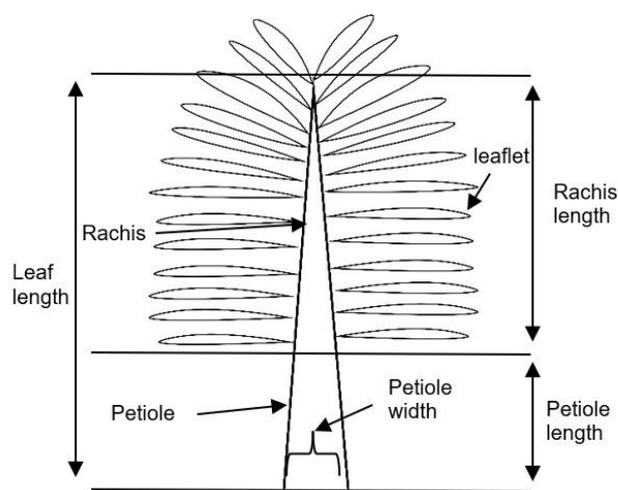


Figure 2. Illustration of a sago leaf

the midrib. The length of the petiole was measured from the base petiole to the leaflets. The width of the petiole was measured at the base of the petiole. The length of the rachis was measured from the leaf base to the tip of the leaf midrib. The length and width of leaflets were observed at maximum growth or maturity using the longest leaflets on leaves. Leaf area was calculated using the method of Nakamura et al. (2009).

Data were analyzed using IBM SPSS Statistics (IBM., 2020). Identification of sago characters were analyzed using STAR IRRI application version 2.01 and displayed in the biplot graph (Pratama et al., 2018). The accessions were also be classified based on their similarity using NTSYS-pc version 2.02 through UPGMA method (Unweighted Pair Group Method of Arithmetic Average) with SimQual function and are displayed in the dendrogram (Rohlf., 1998).

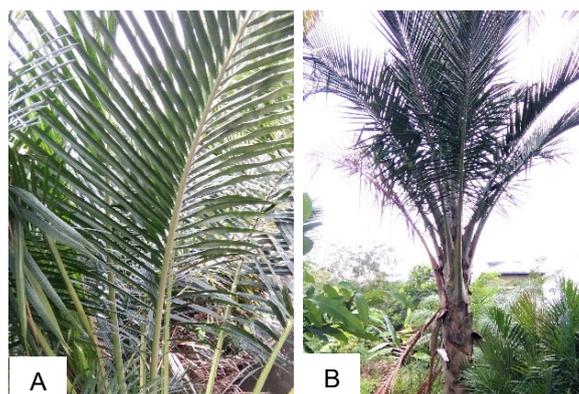


Figure 3. Typical morphology of sago leaves at rosette stage of 3.5 to 6 year-old sago trees (A); sago leaves in the trunk stage of 6 to 14 year-old sago trees (B).

The sago leaf samples studied vary in petiole and rachis morphology. In terms of petiole, the Salak accessions were found to have spine on the abaxial side of petiole (Figure 1). These spines are absent in

the other accessions. The rachis of sago plants has the stripes or banding pattern (pattern of bands at the abaxial of the rachis). These bandings vary in color among the different accessions. White bandings were observed in Mahang, Buntal and Madang accessions, while brown banding were seen in the Gandut sago accessions. There were no bandings pattern observed in Salak accessions (Figure 4). Leaf color (including petiole, rachis and leaflets) varies among the different accessions observed, with yellowish green as the dominant one (Table 1). This color is similar to what was found by Abbas et al. (2019) in leaves and petioles of sago accessions at the Sago Palm Research Center (SRC), University of Papua (UNIPA). The usual petiole colors were yellowish-green (Mahang), dark yellowish-green (Buntal), moderate yellow-green (Salak, Madang and Gandut). The color of the rachis observed in all accessions was moderate yellowish-green. The dominant color of the leaflets was dark yellowish-green (Buntal, Salak and Madang). In contrast, the other accessions did not appear to have dominant colors: Mahang (dark green, deep yellowish-green and dark yellowish-green) and Gandut (moderate olive green, dark green, and dark yellowish-green). Genotypic factors can influence the color of the petiole, rachis and leaflets. Leaf color can be influenced by certain genes, such as NAD2. This gene found in the mitochondria has a role in regulating energy in biological metabolism (Abbas et al., 2019).

#### Quantitative Data

Results from measurements and analysis show that

all accessions included in this study vary significantly in their leaflet characteristics. Gandut and Mahang have significantly greater leaf area (1023.38 cm<sup>2</sup>) compared to Buntal, Salak and Madang (1019.19 cm<sup>2</sup>) (Table 2). Additionally, Gandut and Mahang have significantly longer rachis and leaves and bigger leaf area than the other accessions. Finally, Gandut samples showed significant difference in petiole width from the rest of the samples, but did not vary significantly from Mahang in terms of petiole length (Table 3). The morphological characteristics of sago leaves can be influenced by environmental factors (phenotype), although genetic factors (genotype) also play a role. According to Azhar et al. (2018), the temperature range (25-29°C) can lower photosynthetic ability resulting in less green leaf color and narrower leaf area. Azhar et al. (2020) also added that normal soil conditions and adequate water amounts could help plants produce higher photosynthesis and more stable sago production.

Principal component analysis showed that leaf characters, including the presence of spines, white banding, and length of petioles, contributed significantly to the diversity of sago accessions in South Borneo with a value of 0.1431, 0.4592 and 0.2401. These three leaf characters were the variables with the most extensive and positive values for PCI, PCII and PCIII (Table 4). Haydar et al. (2007) stated that the variable with the most extensive and positive vector value maximizes genetic material diversity. This variable can also be a characteristic of the sago variety in South Borneo. According to Afuape et al.

Table 1. Color\* variations found on petiole, rachis and leaflets of various sago accessions

Accessions	Petiole	Rachis	Leaflets
Mahang 1	moderate yellow green	moderate yellow green	dark green
Mahang 2	strong yellowish green	strong yellow green	deep yellowish green
Mahang 3	strong yellowish green	strong yellowish green	dark yellowish green
Buntal 1	moderate yellow green	moderate yellow green	dark yellowish green
Buntal 2	dark yellowish green	dark yellowish green	dark yellowish green
Buntal 3	strong yellowish green	strong yellowish green	deep yellowish green
Salak 1	moderate yellowish green	moderate yellowish green	dark yellowish green
Salak 2	moderate yellow green	moderate yellow green	moderate olive green
Salak 3	moderate yellow green	moderate yellow green	dark yellowish green
Madang 1	moderate yellow green	moderate yellow green	dark yellowish green
Madang 2	moderate yellow green	moderate yellow green	dark yellowish green
Madang 3	moderate yellow green	moderate yellow green	dark yellowish green
Gandut 1	moderate yellow green	moderate yellow green	moderate olive green
Gandut 2	moderate yellow green	moderate yellow green	dark green
Gandut 3	moderate olive green	moderate olive green	dark yellowish green

Note: \* Color criteria according to Royal Horticulture Society Colour Chart (2015)

Table 2. Variation of sago leaflet length, leaflet length at the middle leaf, leaflets length at a quarter leaf leaflets width, number of leaflets, and leaflet area

Variables	Accession					CV (%)	F-Test
	Mahang	Buntal	Salak	Madang	Gandut		
Leaf length (cm)	143.33ab	104.33c	81.67c	112.33bc	150.67a	12.40	**
Leaflet length a (m)	2.87a	12.09b	1.63b	2.36ab	3.01a	12.41	**
Leaflet length b (m)	2.23ab	1.45bc	1.52c	2.30a	2.49a	10.16	**
Leaflet width (cm)	9.00a	6.33bc	6.00c	4.67c	8.67ab	13.92	**
Number of leaflets	133.00ab	83.67c	104.00bc	92.67c	137.33a	10.96	**
Leaflet area (cm <sup>2</sup> )	1019.19a	525.43b	390.67b	411.86b	1023.38a	12.29	**

Note: The mean values followed by different letters indicates a significant difference based on Tukey's Honestly Significant Difference (HSD) test at  $\alpha = 0.05$ ; \*\*: significant according to F-test at  $\alpha = 0.01$ ; a is leaf length measurement at the middle leaf; b is leaf length measurement at  $\frac{1}{4}$  leaf; CV= Coefficient of Variance.

Table 3. Variation of sago petiole length, petiole width, rachis length, leaf length and leaf area

Variables	Accession					CV%	F-Test
	Mahang	Buntal	Salak	Madang	Gandut		
Petiole length (m)	0.69ab	0.36c	0.36c	0.52bc	0.85a	19.86	**
Petiole width (m)	0.19ab	0.13b	0.16b	0.18b	0.28a	15.43	**
Rachis length (m)	4.46a	2.16b	2.36b	1.97b	5.30a	11.31	**
Leaf length (m)	5.15a	2.52b	2.72b	2.48b	6.15a	10.79	**
Leaf area (m <sup>2</sup> )	10.09a	3.70b	3.37b	4.09b	12.86a	15.79	**

Note: The mean values followed by different letters indicates a significant difference based on Tukey's Honestly Significant Difference (HSD) test at  $\alpha = 0.05$ ; \*\*: significant according to F-test at  $\alpha = 0.01$ ; CV= Coefficient of Variance

(2011) the principal component analysis is useful to determine the level of a character's contribution to diversity. The character can become a characteristic of variety identification.

Biplot charts resulting from principal components I and II show that all accessions are spread across four quadrants (Figure 5). Mahang 1, Mahang 2, Mahang 3 and Gandut 2 are located in quadrant I. Buntal 2, Buntal 3, Madang 1, Madang 2 and Madang 3 are located in quadrant II. Buntal 1, Salak 1, Salak 2 and Salak 3 are located in quadrant III. Gandut 1 and Gandut 3 accessions are located in quadrant IV. The length of the variable vector significantly affects a variety of variables. The variable vector's length is almost the same for each variable, except for the variable color of the leaflet. The leaf color variable had the smallest variation in effect, because it had the shortest vector variable length.

Mahang 2, Buntal 2, and all Madang 1, 2 and 3 accessions are close together, showing a substantial similarity to the white banding variable. Accession Mahang 1 is close to Mahang 3 with the highest value on all leaflets. Gandut 1 and Gandut 3 accessions are also close to each other, with the highest values

on the variable brown banding and the number of leaflets. Salak 1, 2 and 3 accessions have a position close to each other with the highest value on the variable rachis color and spines' presence. Pratama et al. (2018) said that the accessions with closed position in the biplot graph have a strong similarity in morphological characters.

The results of the dendrogram generated through UPGMA method showed that all (except for Buntal) accessions were grouped together according to their type of accession (Figure 6).

The dendrogram shows that the types of sago in this study can be broadly divided into two groups. The first group consisted of Mahang and Gandut accessions, while the second group consisted of Buntal, Salak and Gandut accessions. The two accession groups have a similarity value of 26%. Gandut 1 and Gandut 3 accessions have an extensive similarity coefficient value of 100% (Figure 6).

All sago accessions showed similarities and grouping according to One-way ANOVA (Table 4), biplot charts (Figure 5) and dendrogram (Figure 6). Those similarity and grouping are likely to be affected by the epigenetic

Table 4. Principal component analysis based on sago leaf variation

Variable	PCI	PCII	PCIII
Number of leaflets	-0.2684	-0.1222	-0.0593
Petiole length	-0.2776	-0.0141	0.2401
Petiole width	-0.2586	-0.0874	0.0702
Rachis length	-0.2915	-0.0929	-0.036
Leaf length	-0.2943	-0.0845	-0.0021
Leaflet length	-0.2812	0.1575	-0.008
Leaflet width	-0.2552	-0.023	-0.3394
Leaflet area	-0.2867	0.0538	-0.1851
Midrib length	-0.2915	-0.0929	-0.036
Leaflets length a	-0.2748	0.1781	0.0174
Leaflets length b	-0.2385	0.1854	0.2186
Leaf area	-0.2999	-0.0401	0.0249
Spine	0.1431	-0.3795	-0.2943
White banding pattern at the abaxial leaf	0.066	0.4592	0.0899
Brown banding pattern at the abaxial leaf	-0.224	-0.1829	0.1843
Petiole color	0.0188	-0.4833	0.1388
Rachis color	0.0188	-0.4833	0.1388
Leaflet color	-0.0558	0.0384	-0.7522
Eigen Values	10.8698	3.7694	1.2973
Proportion of variation	0.6039	0.2094	0.0721
Cumulative proportion	0.6039	0.8133	0.8854

Note: a = measured at middle leaf; b = measured at ¼ leaf length; PC = principal component analysis



Figure 4. White banding pattern on Mahang (A), Buntal (B), and Madang (C), Brown banding pattern on Buntal, and no banding pattern on Salak (E)

events, which can be seen in Mahang and Gandut accessions. Mahang and Gandut accessions grow in different locations, but have similarities and grouping. Epigenetic variations in natural plant population has important roles in supporting individual tree to cope with different environments (Lira-Medeiros et al., 2010) such as cytosine methylation, are inherited in plant species and may occur in response to biotic or abiotic stress, affecting gene expression without changing genome sequence. *Laguncularia racemosa*, a mangrove species, occurs in naturally contrasting

habitats where it is subjected daily to salinity and nutrient variations leading to morphological differences. This work aims at unraveling how CpG-methylation variation is distributed among individuals from two nearby habitats, at a riverside (RS. We highly recommend a further study to be conducted on phylogenetic using DNA sequence to confirm the probability of epigenetic characters in the sago leaves.

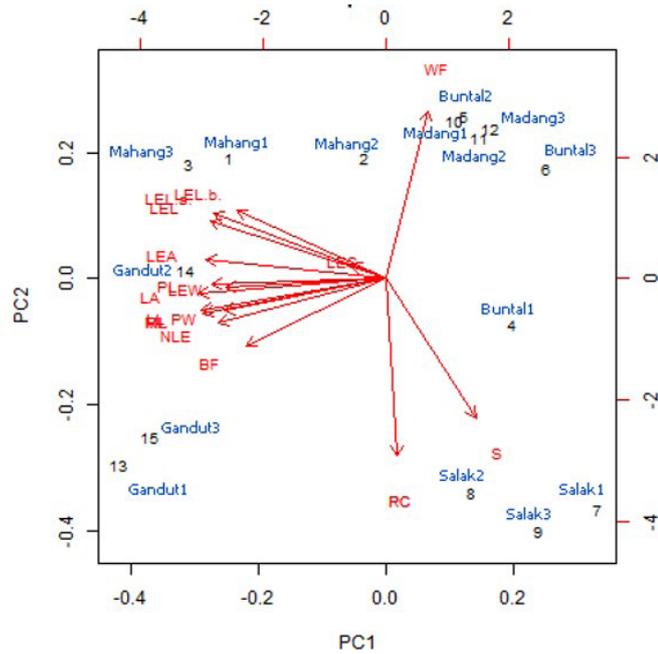


Figure 5. Biplot chart on leaf characters of sago. NLE = number of leaflets; PL = petiole length; PW = petiole width; RL = rachis length; LL = leaf length; LEL = leaflet length; LEW = leaflet width; LEA = leaflet area; ML = midrib length; LEL (a) = leaflet length at middle leaf; LEL (b) = leaflets length at ¼ leaf; LA = leaf area; S = spine; WF = white banding; BF = brown banding; PC = petiole color; RC = rachis color; LEC = leaflet color; PC= principle component analysis.

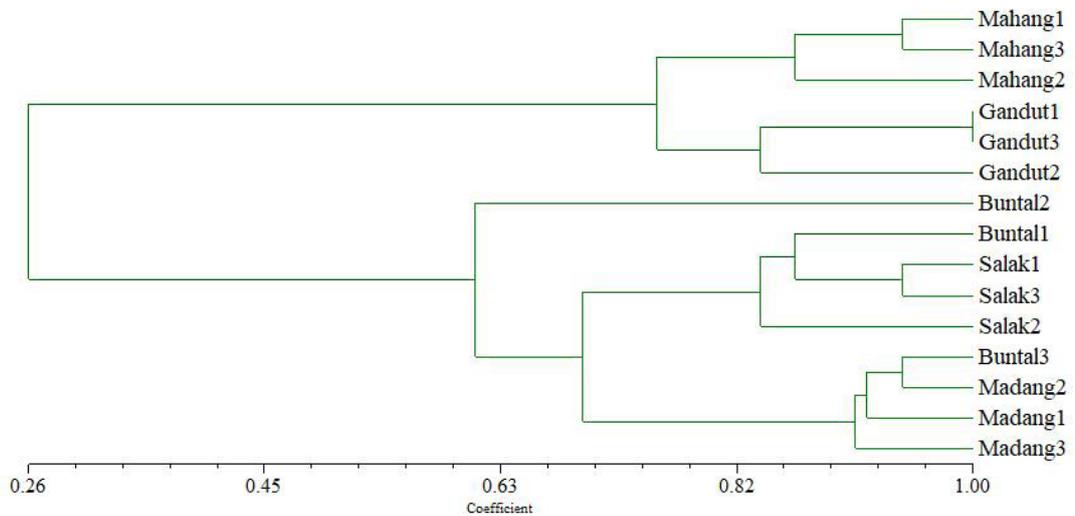


Figure 6. A dendrogram showing relationships of different types of sago based on leaf morphology variation

## Conclusion

Principal components analysis and dendrogram generated based on morphological variables of sago leaves showed that accessions of sago plants from the South Borneo Province in Indonesia fall into two groups. The first group, composed of Mahang and Gandut accessions, showed long rachis, leaflets and leaves, and greater leaf area. The second group, composed of Buntal, Salak and Madang accessions, which showed short rachis, leaflets and leaves,

and small leaf area. Morphological characteristics, such as presence of spines, banding pattern of leaf, and length of petioles can also be used as obvious criteria for determining the type of sago accession. Those similarities could be an epigenetic event because some accessions were located in different location. These results provide the difference of some accessions in the leaf morphology and are important in first identification of the sago accessions. Finally, our results have provided clues and important information that could be used for future phylogenetic

studies involving morphological and molecular data, that will in turn ascertain the species affiliation of the different sago accession types.

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