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Morphology, growth and genetic variations of sago palm (*Metroxylon sagu*) seedlings derived from seeds

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Abstract. *Riyanto R, Widodo I, Abbas B. 2018. Morphology, growth and genetic variations of sago palm (*Metroxylon sagu*) seedlings derived from seeds. Biodiversitas 19: 682-688.* Propagations of Sago palm using seeds will result in large variations of seedlings due to segregation resulting in the genetic diversity. The objectives of this study are to observe the morphology, growth and genetic variations of sago palm seedlings derived from seeds resulted from natural pollination. Morphological diversities of sago palm seedlings showed in high variations with the similarity coefficient ranging from 10% to 69%. Growth patterns of sago palm seedlings were divided into three categories, i.e., slow, medium, and fast growth. Genetic characters of sago palm seedlings showed that sago palm seedlings were varied with coefficients ranging from 23.6-74.5% and seedlings samples were separated into three groups of 49% the differentiation level. Based on the morphological, growth, and genetic characteristics of sago palm seedlings derived from seeds, it is predicted that sago palms in the natural condition may occur because of cross-pollination.

Keywords: Genetic, morphology, RAPD, sago palm, seedlings

INTRODUCTION

Sago palms were reported as a plant accumulating large amount of carbohydrate in the trunk. Sago palm belongs to the family Palmae and the genus of *Metroxylon*. The quantity of starch production of sago palm is highest compared to other plant producing carbohydrates. Karim et al. (2008) also reported that sago starch production was 3 to 4 times higher than rice, corn or wheat production and 17 times higher than cassava production. Bujang (2008) reported that the average productivity of sago year^{-1} reaches 25 tons ha^{-1}. This makes sago palm is an important commodity and needs to be developed to fulfill carbohydrates of world needs.

Sago palm can be propagated using their suckers and seeds. Propagation using seeds were rarely done because sago palm is generally harvested before it produces fruits and not all of sago palm cultivars enable to produce seeds (Flach 1997). Seeds are produced from pollination processes. Pollination of plants can occur in autogamy (self-pollinated) and allogamy (cross-pollinated).Cross-pollination causes the expansion of genetic diversity due to recombination or segregation (Sofiari and Kirana 2009). Flach (1997) reported that the pollination type of sago palm is cross-pollination, so that the fruits and seeds will be various traits or characters.

Information on genetic diversity is required in the preparation of conservation strategies and breeding programs as well as the utilization of genetic resources (Rao Hodgkin,2001). Genetic diversities of plants in the level of both individual and population needs to be evaluated. The level of genetic diversities of plants can be determined by morphological and molecular character. The assessment of sago palm variation using molecular markers has been successfully reported (Abbas et al. 2008; Abbas et al. 2009; Abbas et al. 2010; Abbas and Ehara 2012).Sago palm is known as a hapaxanthic plant which can only produce flowers and fruits at one time in the one life cycle. This also occurs in sago palm. However, the seed production is limited because this plant is generally cut down to obtain the starch before it produces fruits, so that the information about morphology, growth, and genetic diversity of sago palm seedlings is very limited. In order to ensure that sago palm in natural condition is self-pollinated or cross-pollinated and also to determine appropriate breeding strategies for improving traits of the sago palm varieties, therefore, the objective of this study was to determine the morphology, growth and genetic diversity of sago palm seedlings originated from seeds derived from natural pollination.

MATERIALS AND METHODS

The study was conducted at the Center of Tuber Crops and Sago Palm Research and Biotechnology Laboratory, University of Papua (UNIPA), Manokwari, West Papua, Indonesia. Several parameters including morphology, growth, and molecular character in the nursery without shading were observed. Data were recorded every month until seedlings at the age of eight-month-olds.

Source of seedlings

Sago palm seeds used in this work were derived from one parent tree with the spine characteristics. These seeds resulted from natural pollination occurrence. As much as

100 number of seeds were germinated which 73 of them were successfully sprouted. Seeds germination were done by soaking them in the water which was then coated, and put in the dark place. After 28 days, the sprouted seeds were then transferred into polybags with the size of 10 cm x 15 cm. After seedlings produced2-3 leaves, they were transferred into polybags with the sizes of 30 cm x 40 cm. Each polybag was filled with sandy topsoil as much as 15 kg polybag^{-1} with on the surface of the planting medium was applied with organic mulch of sago waste, which has been decomposed by sago mushroom (*Volvariella* sp.) as much as 150 g polybag^{-1}. The daily maintenance was done by watering, fertilizing, and weeding.

From the 1st to the 3rd months of sago seedling maintenance, watering was done twice a week but after four to eight month old, the seedlings were watered every day. Fertilization was done using Yara Mila^{TM} compound fertilizer. The dose of fertilizer was 50 g polybag^{-1}, which was applied after seedlings were at 3-month-old. The composition of Yara Mila^{TM} fertilizer consisted of16% N in the form of 9.5% NH_4 and 6.5% NO_3, 16% P_2O_5, 16% K_2O, 1.5% MgO, and 5.0% CaO. Weeding was done during seedlings grown in polybags.

Morphological character and growth

Morphological performances and growth measurements were done in each of individual seedlings. The morphological characteristics of sago palm were observed including leaf spear, leaf shape, young leaflet color, old leaflet color, spines on the leaf, leaf position, spear color, the shape of the canopy, the number of spines, the length of the spines, the longest spines, the shortest spines, the length of the petiole, the width of the petiole, the height of the plant, the number of midrib, and the presence of spear during observations I, II, III, IV, and V. The determination of color in certain plant organs is determined based on the color chart (RHS 1986).

The growth characteristics including height of seedlings, number of midribs, length of midrib, length of leaf, and width of leaflets was also observed. The number of seedlings used in morphological characters was 73 seeds. The classification of the growth criteria was based on the normal distribution curve of seedling growth. Individuals in the left quadrant were classified into slow growth group, individuals in the middle quadrant were classified into moderate growth group, and individuals in the right quadrant were classified into rapid growth group.

Genetic characters

The genetic characteristics were determined based on the RAPD markers by selecting 15 individual seedlings which representing each class of morphological and growth characters. DNA was extracted from young leaves of sago palm seedlings. The extraction protocol used in this study was based on the method of GENEAID's Genomic DNA kit (plant) following its manufacture protocols. The quality of the extracted DNA was visualized by horizontal electrophoresis using agarose 1% at a voltage of 110 V for 25 min.

Five RAPD primers were used for amplification of genomic DNA of sago palm seedlings, i.e., OPA 04 (AAT CGG GCT G), P01 (GCG GCT GGA G), OPA 01 (CAG GCC CTT C), OPD 08 (GTG TGC CCC A) and OPAW 05 (CTG CTT CGA G). The volume of amplification reaction used was 10 µL that consisting of 9 µL Master Mix and 1 µL genomic DNA of sago palm seedlings. Master mixed consists of 3 µL ddH2O, 1 µL primer and 5 µL GO Taq Green (Promega). Amplification was performed using PCR Bio-RAD Gene Cycler PCR machine programmed with the PCR condition as follows: predenaturation cycle for 1 minute at 94^{o}C, 35 cycles with the denaturation temperature at 94^{o}C for 30 second, annealing at 40^{o}C for 1 minute, extension at 72°C for 1 minute 30 seconds; 1 post-extension cycle at 72°C for 5 minutes and 37°C for 1 minute. Electrophoresis was performed at a voltage of 95 V for 1 hour. The staining was done by immersion into ethidium bromide solution for 15 minutes, which were then rinsed with water. Visualization was done using UV transilluminator.

Data analysis

Data of growth observation was analyzed statistically using Minitab 17 application for morphological variables. Morphological character data and molecular character based on RAPD marker were analyzed using multivariate analysis. Unweighted Pair Group Method with Arithmetic (UPGMA) of sago palm seedlings was analyzed using NTSys software version 2.02.

RESULTS AND DISCUSSION

Morphology character of sago seedlings

The morphological characters, i.e., color expression of leaf spear, young leaf, and other organ colors of the sago seedlings were presented in Figure1.Results showed that variations occurred in the morphological characters indicating that segregation of propagation sago seedlings through seeds. The results of similarity analysis using 29 morphological characters showed that sago seedlings propagated using seeds had phenotypic similarity ranges from 10 to 69%. Seeds having the highest similarity coefficient (69%) were $F1_{51}$ seedlings with $F1_{61}$, while the lowest similarity coefficient (10%) was$F1_{49}$ seedlings with $F1_{12}$ and $F1_{44}$ with $F1_{31}$.The wide range of phenotypic similarity coefficients indicated that seedlings derived from seeds resulted from natural pollinated were varied. This phenomenon was in line with Dewi et al. (2016) who reported that the morphological characters of 12 sago palm accession grown naturally in Sayal Village, South Sorong, West Papua, Indonesia was diverse.

Results from dendrogram construction based on morphological characters showed that the seedling samples were clustered into 4 clusters at a coefficient of 30% similarities (Figure 2). Cluster 1 consisted of 61 seedlings, cluster 2 consisted of 6 seedlings, and both cluster 3 and 4 consisted of 3 seedlings. The grouping patterns were presented on the dendrogram (Figure 2) showed that individuals belonging to one group had similar

morphological characters. Matta et al. (2015) reported that the pattern of phenotype groups on dendrograms was influenced by genetics and environment.The morphological diversity in a uniform environment might be influenced by genetic character. Pandin and Matana (2015) reported that oil palms of Dura and Tenera types grown under the same environmental conditions had different characters in both vegetative and generative characters. If environment values of plant grown at the uniform environment conditions are equal to zero, it will cause the variant of phenotype value is the same as variant of genotypes, so that the heritability will obtain 100%. This value reinforces the notion that the morphological diversity that appears in sago seedlings is an expression of the genetic diversity of seedlings. In addition, based on the criteria of maturity time of male and female flowers, sago palms are included in the protandrous (Jong 1995).The male sago flower is mature before the female flower is mature, making it possible for cross-pollination (Flach 1997). The mature female flowers will be pollinated by male flowers from other plants.These conditions also lead to the variation of morphological characters of seedlings derived from sago seeds.

Character of sago seedling growth

The growth of sago palm seedlings individuals observed at 6-month-olds or at 8-month-olds of seedlings was varied. (Table 1). According to Flach (1997), sago seedlings produced approximately one leaf per month. The increasing number of leaf will increase the photosynthesis product. In such conditions, it will produce a lot of photosynthate results to trigger the growth and to increase the biomass production. This is in line with the results from Özköse and Tamkoç (2014) which revealed that the character of the length and width of the leaf correlated positively to the character of plant growth and production.

Based on the growth responses analysis of seedlings with the normal distribution curve, it showed that the growing power of seedlings in the nursery can be divided into three criteria, i.e., slow growth, moderate, and fast growth (Table 2). The growth variations in sago seedlings might occur due to the genetic factor of each seed. Flach (1997) states that by type of pollination, sago plants are included in allogamy plant types, so that the seed variation might arise due to the segregation of properties through cross-pollination of two or more different parents.

Other factors which may influence the increase growth of sago seedlings are the availability of nutrients and water for seedlings. The type of media used in this study was sandy-top soil layers with the addition of mulch of decomposed sago waste. The nutrient elements contained in the decomposed sago waste are sufficient to meet the needs of seedlings, so that the growth process will not be inhibited. Elton et al. (2014) show that compost of sago waste plus cow dung contains 3.14% N, 1.31% P, 0.85% K, 1.10% Ca, and 0.32% Mg. Moreover, the addition of sago waste in cacao and spinach cultivation has been shown to increase the growth response and plant productivity (Bintoro 2011). Water availability can ensure plant survival because water has an important function, which it cannot be replaced by other compounds.The frequency of watering determines the amount of water available, which can be utilized by the plant. The results of study conducted by Maryani (2012) showed that the different volume quantity of water supply gave different responses to all observed growth variables of oil palm seedlings.

Figure 1. Performance of phenotypic variation of sago palm seedlings. Color of spear show that A1 is *Strong red* (46A), A2 is *Light yellowish pink* (159A), and A3 is *Strong yellowish green* (142A); color of newly expaded leaf show that B1 is *Brilliant yellowish green* (150B), B2 is *Strong yellow* (153D), and B3 is*Pale yellow* (161C); color of young midrib show that C1 is*Light yellowish pink* (19A), C2 is *Moderate red* (179A), C3 is*Mosaik*, and C4 is*Pale yellow* (158A); and color of young spineshw that D1 is*Strong purplish red* (59D) + *pale purplish pink* (65C), and D2 is *Strong purplish red* (59D) + *brilliant yellowish green* (154C)

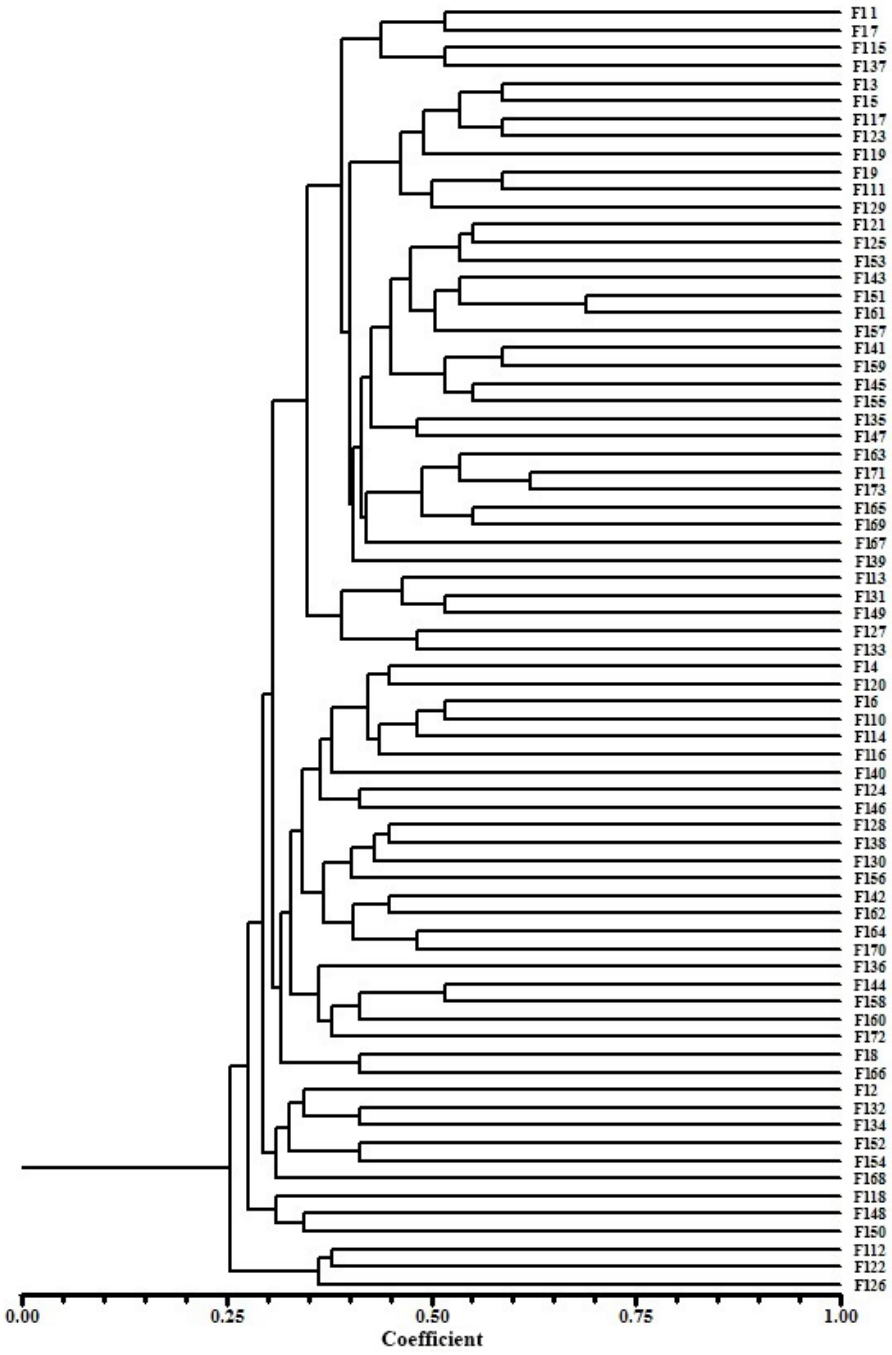

Figure 2. Dendrogram construction of sago palm seedlings based on morphological characteristics

Based on the response of seedling growth, it is suspected that watering has been able to meet the needs of seedlings. This was supported by the absence of symptoms of water shortage in sago seedlings.Cha-Um et al. (2012) reported that the seedlings were subjected to drought stress will be growth hampered, leaf chlorosis, leaf burn, and green leaf area reduction. Karuwalet al. (2017) and Jafari et al. (2018) added that plants grown under low water shortages had a decrease of biomass, chlorophyll content, physiological response, and relative water content.

Genetic characters of sago seedlings

The results of DNA amplification of sago seedlings using RAPD primers showed that there were 13 polymorphic and 5 monomorphic bands ranging in size from 250 bp to 1900 bp. An example of an amplified DNA banding pattern was presented in Figure 3. The OPA4 primer produced four polymorphic DNA band patterns, while both P01 and OPA1 primers produced 3 polymorphic DNA band patterns, the OPAW5 primer produced two polymorphic DNA band patterns, and the OPD8 primer

Table 1. Growth rate average of seedlings at the eight months old

Growth category	HS (cm)	LM (cm)	NM (sheets)	NL (sheets)	LL (cm)	WL (cm)	Number of Seedlings
Slow	66.9	45.5	8	14	23.7	2.4	18
Moderate	82.1	61.3	10	18	25.8	2.8	37
Fast	96.2	80.8	12	23	34.5	3.3	18

Note: height of seedlings (HS), length of midrib (LM) which closer to the spear leaf, number of midrib (NM), and number of leaflet (NL) per midrib which closer to the spear leaf, length of leaflet (LL), width of leaflets (WL) in the middle position

Table 2. Seedlings label of slow, moderate, and fast growth

Growth category	Number
Slow	$F1_1$, $F1_6$, $F1_8$, $F1_9$, $F1_{10}$, $F1_{12}$, $F1_{13}$, $F1_{18}$, $F1_{19}$, $F1_{21}$, $F1_{22}$, $F1_{27}$, $F1_{35}$, $F1_{47}$, $F1_{50}$, $F1_{52}$, $F1_{53}$, $F1_{54}$
Moderate	$F1_2$, $F1_3$, $F1_4$, $F1_5$, $F1_7$, $F1_{11}$, $F1_{14}$, $F1_{15}$, $F1_{16}$, $F1_{17}$, $F1_{20}$, $F1_{23}$, $F1_{24}$, $F1_{25}$, $F1_{26}$, $F1_{28}$, $F1_{30}$, $F1_{31}$, $F1_{32}$, $F1_{39}$, $F1_{40}$, $F1_{41}$, $F1_{42}$, $F1_{44}$, $F1_{45}$, $F1_{51}$, $F1_{59}$, $F1_{60}$, $F1_{61}$, $F1_{62}$, $F1_{64}$, $F1_{65}$, $F1_{66}$, $F1_{67}$, $F1_{68}$, $F1_{69}$, $F1_{71}$
Fast	$F1_{29}$, $F1_{33}$, $F1_{34}$, $F1_{36}$, $F1_{37}$, $F1_{38}$, $F1_{43}$, $F1_{46}$, $F1_{48}$, $F1_{49}$, $F1_{55}$, $F1_{56}$, $F1_{57}$, $F1_{58}$, $F1_{63}$, $F1_{70}$, $F1_{72}$, $F1_{73}$

Note: F1 is first inheritance, individualseedlings ($F1_1$-$F1_{73}$)

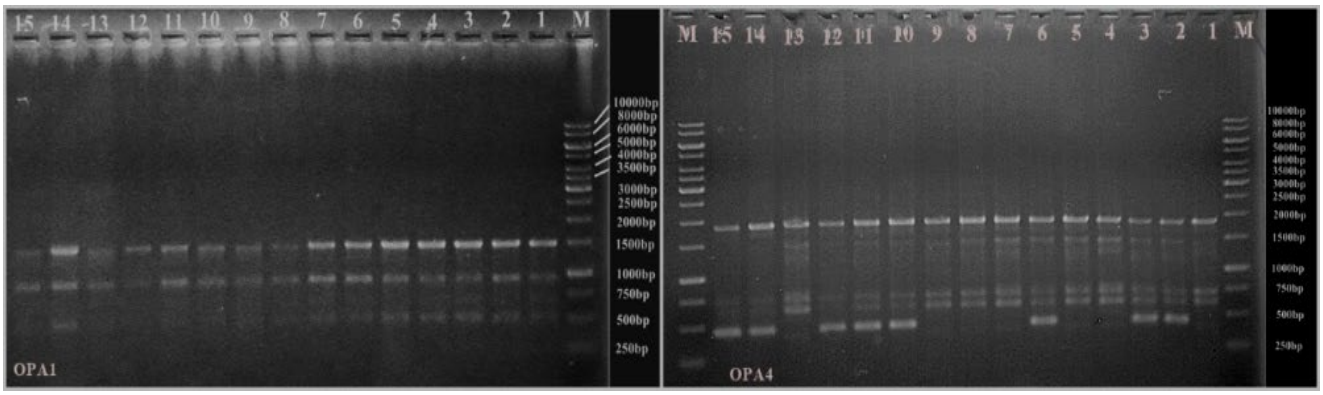

Figure 3. Performance of RAPD amplification fragments on 1% agarose gel by using primer OPA1 and OPA4. The well number represents the sample number, M: marker

produced a polymorphic DNA band pattern. The success of a primer for amplifying DNA of the genome was determined by the homologous primary nucleotide sequence with the DNA sequence of the genome.Tidke et al. (2017) stated that the difference in the number of bands amplified in the 12 soybean genotypes using 10 primers was due to differences in the primary sequence and the DNA arrangement of each soybean genotype. The frequency of polymorphic bands in sago palm seedlings might be caused by genetic changes because of deletion, insertion or recombination of DNA through cross-pollination causing segregation amongst seedlings. In the previous studies, it was reported that genetic variations of sago palm in Indonesian were high based on RAPD markers (Abbas et al. 2009; Abbas 2018). The genetic differences of sago palm in sago forests were thought to be caused by cross-pollination. Abbas et al. (2014) andAbbas et al. (2015)reported that genetic diversities of sago palm forests based on molecular markers showed high diversities.Singh et al (2014) detected genetic changes in oil palm and found a changing in DNA sequences among clones of 400 accessions from sub-Saharan Africa that account for the dominant-negative virescens phenotype.

The result of genetic distance calculation showed that sago seedlings differentiated from 0.236 to 0.745 (Table 3). The values of the genetic distance extrapolated to the percentage values, so the differences level among sample of seedlings ranged from 23.6% to 74.5%. Seedlings that had smaller value of genetic distance were $F1_4$and $F1_7$, $F1_7$and $F1_{13}$. Seedlings that had large genetic distance were $F1_{13}$and $F1_{56}$, $F1_{35}$and $F1_{56}$, $F1_{35}$and $F1_{68}$ (Table 3). Smaller genetic distance mean that small variation among seedlings and large genetic distance mean that large variation among seedling.Dendrogram construction showed that sago palm seedlings grouped into three groups at the coefficient of dissimilarity 0.49 (dush line) or in the level of

differentiation 49%. Group one is $F1_2$, $F1_{19}$, $F1_{26}$ and $F1_{35}$, group two is $F1_4$, $F1_7$, $F1_{13}$, $F1_{18}$, $F1_{36}$, $F1_{61}$, $F1_{39}$ and $F1_{40}$, and group three is $F1_{56}$, $F1_{68}$, and $F1_{72}$ (Figure 4). Seedlings in the same group were identified lowe differences of characteristic and seedlings among group were determined high differences of characteristic. Genetic diversity among seedlings showed a high value; it might be caused by seeds derived from cross-pollination. This opinion was consistent with Lengkong and Runtunuwu (2005) who reported that the genetic diversity among West African Tall coconut seedlings was quite high, the diversity was estimated due to seeds derived from open pollination so that individual coconut has a different genotype from one to another. In the related study, it was reported that the seedlings of sago palm derived from seeds were differentiated (Abbas et al. 2017). This phenomenon shows that generally, sago palm in the natural condition was the result of cross-pollination.

Tabel 3. The genetic distance of 15 seedlings sample performed by dissimilarities calculation of 13 loci from 5 RAPD primers

	$F1_2$	$F1_4$	$F1_7$	$F1_{13}$	$F1_{18}$	$F1_{19}$	$F1_{26}$	$F1_{35}$	$F1_{36}$	$F1_{39}$	$F1_{40}$	$F1_{56}$	$F1_{61}$	$F1_{68}$	$F1_{72}$
$F1_2$	0.000														
$F1_4$	0.624	0.000													
$F1_7$	0.577	0.236	0.000												
$F1_{13}$	0.527	0.333	0.236	0.000											
$F1_{18}$	0.408	0.471	0.408	0.333	0.000										
$F1_{19}$	0.471	0.527	0.471	0.527	0.408	0.000									
$F1_{26}$	0.471	0.527	0.577	0.527	0.408	0.333	0.000								
$F1_{35}$	0.527	0.667	0.624	0.577	0.471	0.408	0.408	0.000							
$F1_{36}$	0.527	0.471	0.527	0.471	0.471	0.527	0.408	0.577	0.000						
$F1_{39}$	0.471	0.527	0.471	0.527	0.408	0.333	0.471	0.527	0.408	0.000					
$F1_{40}$	0.471	0.527	0.471	0.527	0.527	0.471	0.577	0.624	0.408	0.333	0.000				
$F1_{56}$	0.624	0.667	0.707	0.745	0.667	0.624	0.624	0.745	0.577	0.527	0.527	0.000			
$F1_{61}$	0.577	0.527	0.471	0.408	0.408	0.471	0.471	0.408	0.408	0.471	0.471	0.707	0.000		
$F1_{68}$	0.624	0.471	0.527	0.577	0.577	0.624	0.623	0.745	0.471	0.527	0.527	0.471	0.624	0.000	
$F1_{72}$	0.624	0.667	0.624	0.667	0.667	0.624	0.707	0.667	0.577	0.527	0.408	0.471	0.527	0.471	0.000

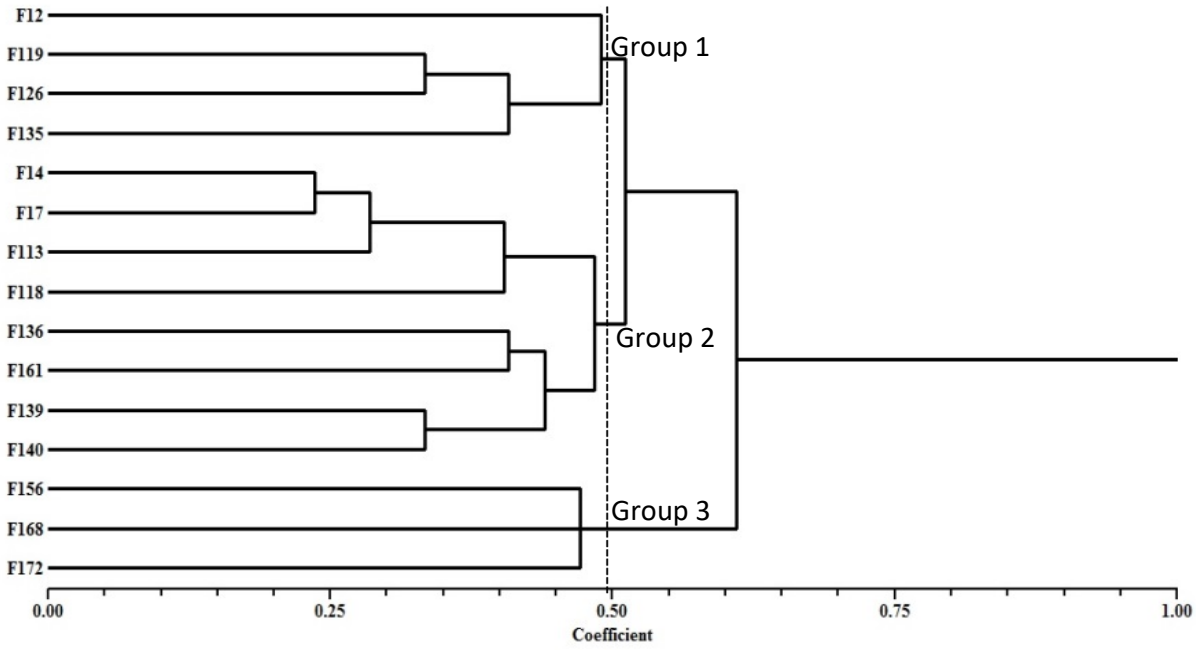

Figure 4. Dendrogram construction of sago palm seedlings based on RAPD marker. Dush line is grouping position in the coefficient of 0.49 or in the level of 49%

As a conclusion, the study indicated that morphological diversities of sago palm seedlings derived from seeds showed high variation with similarity coefficient in the range of 10% to 69%. The growth patterns of sago palm seedlings were divided into three criteria that is slow, medium, and fast growth. Genetic characters of sago palm seedlings confirmed using RAPD molecular markers showed that sago palm seedlings were varied with diversity coefficients ranging from 23.6-74.5% and the individual samples were separated into three groups of 49% the differentiation level. Based on the morphological, growth, and genetic characteristics of sago palm seedlings derived from seeds, it could be confirmed that the seed variation was the result of cross-pollination.

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