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VARIABILITY AND RELATIONSHIP ANALYSIS OF SAGO ACCESSIONS FROM NATURAL POPULATION OF PAPUA BASED ON MORPHOLOGICAL CHARACTERS

A.J. PRATAMA[1], M.H. BINTORO[2] and TRIKOESOEMANINGTYAS[2*]

[1]Graduate student of Agronomy and Horticulture Major, Bogor Agricultural University, Indonesia
[2]Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia
*Corresponding author's email: trikadytia@gmail.com
Email addresses of coauthors: agiefjuliopratama@gmail.com, hmh_bintoro@yahoo.com

SUMMARY

Sago (*Metroxylon sagu* Rottb.) is a potential starch-producing plant in the world, since sago can be used as raw material of food, bioethanol, environmentally friendly plastic, pharmaceutical and sweetener. Indonesia has a high diversity of sago, especially in Papua. The morphological characteristics of sago is important to elucidate as the distinguishing feature of each sago accession and assess the diversity of the natural population for further optimum utilization. This study aimed to determine the variability and relationship of sago accession in the natural population based on morphological characters. The study was conducted in Mioko Village, Central Mimika Sub-district, Mimika Regency, Papua Province with height of ± 3 m above sea level. The material used was eight accessions of sago from the natural population of Mioko Village selected through purposive sampling approach. Observations were conducted on 17 morphological characters. Principal component analysis result showed that the 76.61% variability of sago accessions in the natural population can be explained by three principal components (PC). Further analysis indicated that morphological characters of rachis length, plant height and petiole width are important characters to distinguish genetic variability of sago. The sago accessions that has the highest similarity level is Taina and Omoroko with similarity on rachis length, number of right-side leaflets, leaflets length ¼ leaf and petiole width.

Key words: Heat map, traditional knowledge, plant height, principal component analysis

Key findings: The study of sago variability based on morphological characters revealed that morphological characters of rachis length, plant height and petiole width can be used to distinguish sago accessions in natural populations.

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INTRODUCTION

Sago (*Metroxylon sagu* Rottb.) is one of the most potential palm trees not widely known to the public. Sago can be used as food, bioethanol, environmentally friendly plastic, pharmaceutical and sweetener and as a source of carbohydrate with low glycemic index suitable for diabetics (Bantacut 2014; Ehara 2009; Yetti *et al*., 2007; Bukhari *et al*., 2017; Utami *et al*., 2014; Oladzadabbasabadi *et al*., 2013; Ozturk *et al*., 2011; Vatanasuchart *et al*., 2012). Sago can grow in an environment where other crops cannot grow and produce well, such as peat soil swamps. Sago accumulates carbohydrates in the form of starch in the trunk with high productivity compared to other food crops. The potential of dry starch production reaches 20-40 ton / ha / year (Bintoro *et al*., 2010) with the production of dry starch in one trunk reaching 116.69 - 372.89 kg (Dewi *et al*., 2016), even as high as 835 kg as reported by Saitoh *et al*. (2008).

Indonesia is one of the countries with the largest sago area in the world with 5.5 million hectares (Bintoro *et al*., 2016). Flach (1983) considered Papua as center of diversity of *M. sagu* due to the vast natural stands and the high genetic variation of sago palm trees that have been found in Papua. The distribution of genotypes of sago palm in Indonesia based on Wx gene markers showed that the populations possessing a specific genotype are found in Serui of Papua and Palopo of Sulawesi. This indicates that both Papua and Sulawesi are the origin of sago palm variation because of the existence of specific genotypes in their populations; both locations are, therefore, considered to be the original sources of sago palm germplasm in Indonesia. Papua exhibited the largest genotype numbers existing in their population based on Wx gene markers; therefore, it is considered to be the center of sago palm genetic diversity in Indonesia (Abbas 2018).

Mioko a village in Mimika Regency of the Papua Province has a wide sago area, which is a natural population. The natural population allows for a very high diversity of sago, since sago is a cross-pollinated plant. The local community of Mioko Village has classified sago diversity based on plant performance and starch production. Sago classification based on morphological characters in the natural population needs to be done in order to manage and conserve sago genetic resources. Information on morphological characters of the natural variability can be used as a reference for researchers in a sago to determinate elite sago trees for further development and plant-breeding program, as well. The high variability of sago present in the natural population can be utilized to analyze the relationship between the morphological characters of sago plants. Mimika Regency with a large natural population is an ideal location to study the variability of sago.

Morphological character consists of quantitative and qualitative characters observed in all parts of the plant. Qualitative morphological characters are controlled by major genes and are not influenced by environmental factors, so it can be a characteristic in studying the relation of sago accession kinship.

Identification of morphological characters is one way to determine the relationship of a species (Purwantoro *et al*., 2005). The analysis of relationships based on morphological characters with distinctive characteristics has been demonstrated in some plants (Susantidiana *et al*., 2009; Suketi *et al*., 2010; Fatimah, 2013; Aryanti *et al*., 2015).

Morphological characters that have the greatest contribution to variability need to be known for sago plant improvement. Key Component Analysis can be used to integrate characters that can ultimately be selected as criteria for plant improvement (Mohajer *et al*., 2013), in addition, key component analysis can also identify genotypes or accessions variability (Tar'an *et al*., 2005). Each major component explains the magnitude of the variability distribution of observed characters, so there is a strong relationship between individuals (Denton & Nwangburuka, 2012). Different characters in key component analysis results contribute differently to total variability (Nwangburuka *et al*., 2011). This study aims to determine the relationship of sago accessions in a natural population based on the morphological characters.

MATERIALS AND METHODS

Sampling method of genetics materials

Morphological characterization was conducted in the natural population of Mioko Village, Central Mimika Sub-district, Mimika Regency, Papua Province. The study was conducted from July to September 2016. The materials used were 8 accessions of local sago resulted from sampling in natural populations. The trees were selected using is purposive sampling method based on result of Focus Group Discussion (FGD) and the sampling of sago trees was conducted together with community elders. Each accession was observed in one sample tree and the sample tree taken has to fulfill the criteria of maturity at flower initiation phase, which was then logged.

Morphological characters observation

Observations were made on morphological characters and agronomic traits. The quantitative morphological characters include plant height, stem diameter, stem circumference, leaf length, leaflet length, leaflet width, bark thickness, number of leaves, right-sided leaflets, number of left leaflets, rachis length, length of the petiole, the width of the petiole, spine of mature trees, the spacing of the seedling and the length of ¼ of the leaf. The qualitative morphological characters that include the shape of the stem, the spine pattern and the color of the leaflets. The agronomic traits observation include starch production per tree and starch yield (Dewi *et al*., 2016; Ahmad *et al*., 2016).

Data analysis

Data were analyzed using STAR application version 2.01 and R version 3.1. Correlation analysis of some morphological characters on starch production was conducted using application R version 3.1 with package Agricolae. Characters that can be differentiated between accessions can be identified through the Principle Component Analysis that is then displayed in the biplot graph using STAR application version 2.01. To identify the relationship the sago accessions were analyzed using application of R version 3.1 with Agricolae package.

RESULTS AND DISCUSSION

Analysis of variability and relationship of sago accessions

The indigenous people of Mioko Village, in Papua recognize and are able to differentiate the variability of sago from traditional knowledge passed down from generations to generation orally. Based on their knowledge, the local people generally distinguish sago based on the presence or absences of spines, however, in Mioko Village sago with spines were further distinguished into different types. Sago that has no spines is called Ikimina. Sago trees that have spines on petiole and rachis are divided into 7 accessions that have a variety of spines and stems. The accession of sago that has dense spines is called Taina. The accession of sago with rough spines is called Oko. The accession of sago which has spines and straight and short stems and the rest of the midrib is still attached to the surface of the stem is called Mbapare. The accession of sago that has spines and trunked straight and high is called Omoroko. The accession of sago having spines and funnel-like trunk (center and the middle part is bigger than base) is called Omiya 1. The accession of sago having spine and bottle-like trunk (middle part smaller than base and tip) is called Omiya 2. Accession of sago that has spine and trunk that secrete water when cut down is called Durumu.

The morphological characters of the eight sago accessions in Mioko Village showed different for all the observed characters in trunks, leaves and spines. The Principal Component Analysis results show that the contribution of sago accessions variability of 76.61% can be explained by three principal components (PC) (Table 1). The morphological character of the principal component I (PC1) describe 45.93% of the variability between sago accessions consisting of the length of leaf, the length of rachis, the length of leaflets, the width of the leaflets and the number of leaflets on the right hand side with values of 0.3358, 0.3363, 0.3061, 0.3112 and 0.3307. The principle component II (PC2) describes 19.43% of the diversity between sago accessions consisting of plant height, stem circumference, petiole width and the number of leaves with consecutive values of 0.5016, 0.3880, 0.4283 and 0.3326. The principal component III (PC3) describes 11.25% of the variability between sago accessions consisting of the length of the petiole and the spines of tillers with values of 0.3448 and 0.4251.

The characters that have the largest and positive values in PC1, PC2 and PC3 are rachis length, plant height and petiole width with values of 0.3363, 0.5016 and 0.4251 (Table 1), respectively. These characters are assumed to have a large contribution to the variability among sago accessions in Mioko Village. A total of 11 morphological characters were observed to explain the variability and the characteristic of sago accessions in Mioko Village. According to Haydar *et*

Table1. Result of the principal component analysis based on morphological characters.

Characters	PC1	PC2	PC3
T	-0.1198	0.5016	-0.0340
D	0.2476	-0.2841	0.2530
DTK	0.2425	-0.2879	0.2431
LB	0.1966	-0.3880	0.2429
TK	0.1676	0.0454	0.2872
PD	0.3358	0.1596	-0.0385
PR	0.3363	0.1506	-0.0991
PP	0.1419	0.1216	0.3448
LP	0.0079	0.4283	0.4233
PAD	0.3061	0.1056	-0.1383
LAD	0.3112	0.1568	-0.0554
PAD4	0.2841	0.1826	-0.1856
JDD	-0.2200	0.0695	-0.1313
JDA	-0.1748	-0.0337	0.4251
JD	-0.0804	-0.3326	-0.3471
ADR	0.3307	-0.0106	-0.1434
ADL	0.2942	-0.0095	-0.1709
Eigen Values	7.8087	3.3029	1.9125
Proportion of variance	0.4593	0.1943	0.1125
Cumulative proportion	0.4593	0.6536	0.7661
Standard deviation	2.7944	1.8174	1.3829

Note: T = trunk length; D = trunk diameter; DTK = trunk diameter without bark; LB = circumference; TK = thickness; PD = leaf length; PR = rachis length; PP = petiole length; LP = petiole width; PAD = leaflet length; LAD = leaflet width; PAD4 = one fourth leaflet length; JDD = spine distance ofmature tree; JDA = spine distance of seedling; ADR = total of leaflet on right side; ADL = total of leaflet on left side.

al. (2007) characters that contribute the most to the variability of genetic materials are those that have the largest and positive vector values. According to Afuape *et al*. (2011), the principal component analysis is a technique used to find out how large a character contributes to the variability so that the results can be used to identify characters that characterize a variety. The results were in accordance with the information provided by the indigenous people of Mioko that some sago accessions are distinguishable by the variation of the stem, i.e. tall and short trunks.

The PC1 and PC2 are able to explain the variability of sago accessions present in the natural population illustrated in the biplot graph (Figure 1). Based on the biplot graph, PC1 and PC2 shows the overall accessions spread over four quadrants. The sago accession Omiya 2 is in quadrant I, the accessions Durumu and Omiya 1 are in quadrant II, sago Oko and Ikimina accessions are in quadrant III and in the quadrant IV located the accessions Omoroko, Taina and Mbapare. The position of sago accessions Taina and Mbapare are closer together than other sago accessions. It shows that both accessions have a strong similarity in morphological characters. The similarity can be seen in the high number of leaves on both accessions. The sago accession Omoroko has a high value of spines in mature trees and seedlings. The sago accession Omiya 2 has a high value on plant height. The sago accession Oko is an accession that has the highest trunk circumference value compared to

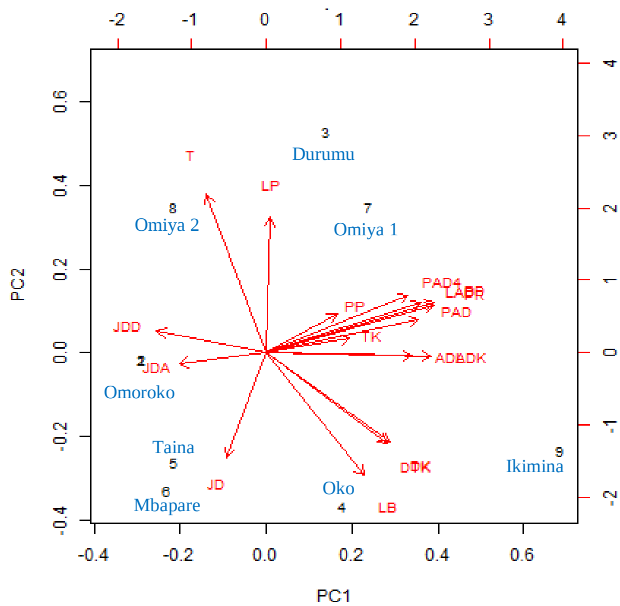

Figure 1. Biplot diagram of principal component analysis based on morphological characters.

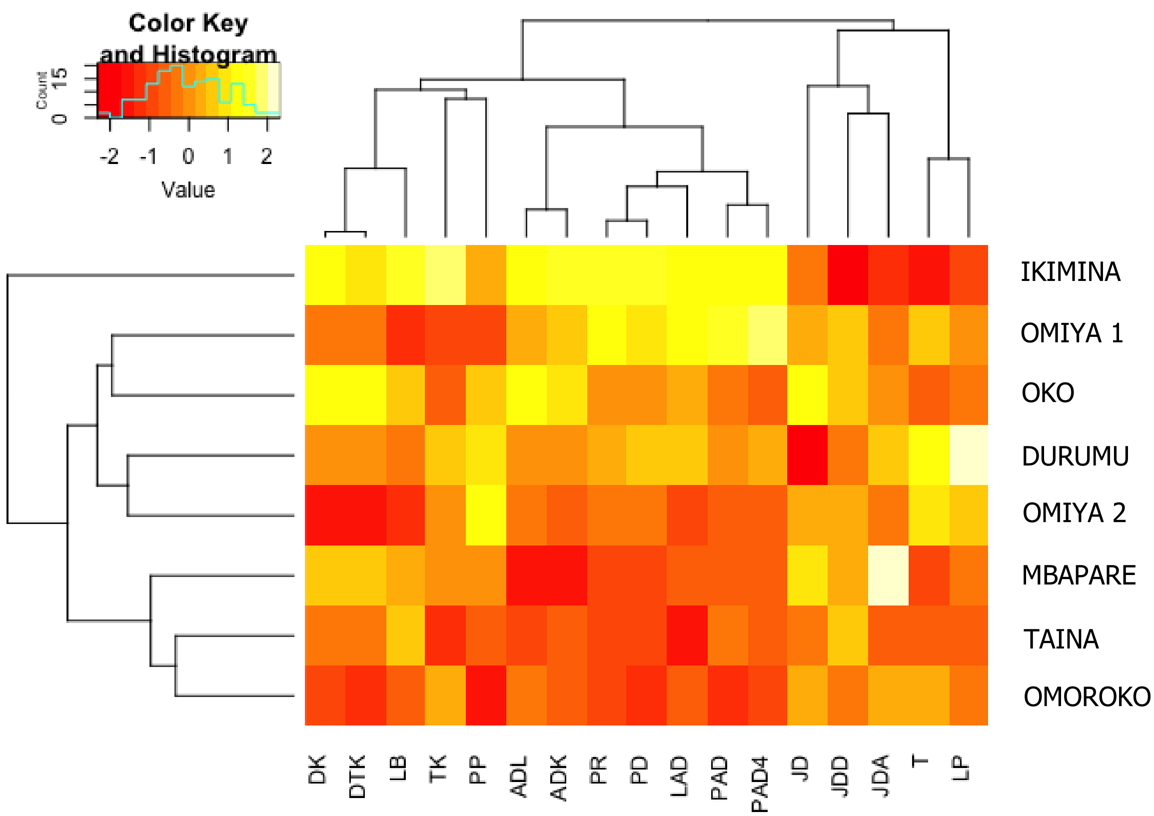

Figure 2. Heatmap and dendrogram based on morphological characters.

other accessions. The results of the analysis proved that the accession that is considered different by the indigenous community does have distinguishing morphological characteristics.

The longest variable length vector in the biplot graph is shown in the plant height variable, while the shortest variable vector is shown in the variable of petiole length. This shows that the character plant height has high variability, while the character of petiole length has a low variability.

There is a similarity of information between results of principal component analysis with the results of interviews with the community about the variability of sago present in the natural population. The similarity is shown in the variable height of the plant. Accession sago Omiya 2 is the accession that has the highest plant height compared to other accessions of 13.09 m.

There are two types of sago accession found in the village of Mioko, spine and spine-less sago. The spine sago has a spine on petiole and rachis. The Ikimina sago accessions are spineless sago while other accessions are spine sago. The Ikimina sago accession is not in the same cluster with other accessions (Figure 1). The appearance of a spine in sago plants is thought to be due to an epigenetic event influenced by the growing environmental factors of a wet swamp (Novero *et al*., 2012). According to Lira-Medeiros *et al*. (2010) epigenetic diversity in natural populations in addition to generating morphological diversity, also has an important role in helping plant individuals to cope with different environments.

Based on the indigenous people of Mioko village, Ikimina sago can be found mainly in the middle of the sago forest. One of the distinguishing features of Ikimina, sago is the presence of ribbon/tape at the bottom of the petiole and rachis. According to Ehara *et al*. (2000), the existence of the tape on the back of the petiole and rachis is used by the community in distinguishing sago accession where the difference is due to the growing culture in every region in Indonesia. However, the indigenous community of Mioko does not use this character to distinguish sago accessions.

The results of dendrogram showed that sago accessions are grouped into two large groups namely (Figure 2). The Ikimina accession is in one separate group, while the sago accession Omiya 1, Oko, Durumu, Omiya 2, Mbapare, Taina and Omoroko are grouped into one group of spiny sago. The Taina sago has higher similarity with the accession Omoroko than with other sago accessions. The similarity is shown in the length of rachis, the number of right-sided leaflets, leaflets length in ¼ leaf and petiole width. Most of the similarities in accessions Taina and Omoroko are indicated by the intensity of morphological values between the two accessions that tend to be low (color key value -2).

A heatmap is used in this study to where the morphological values are represented as colors ranging from blue to dark red. The heatmap shows morphological characters that have high or low intensity in each accession (Figure 2). The high value of morphological characters is shown by the color shown in the heatmap. The high or low value of morphological character becomes the characteristic of the diversity of each accession of sago. Based on the information given by the indigenous community of Mioko, the accession of sago Omiya 1 and Omiya 2 show variability in the trunk. The trunk of accession Omiya 1 is the upper part of the trunk has a larger diameter than the middle and the base so that people identify it as the funnel. The trunk of Omiya 2 sago is like a bottle with the center of the trunk is smaller in diameter than the base and the upper part. The distance of spines (JDA) of the Mbapare sago accession has a high value, while the lowest value was found in sago Taina accession. The low spine distance indicates a tightly located spine. This is in accordance with the information provided by the local people that the Taina sago accession has high spine density compared to other sago accessions.

Based on the intensity of the color key values of morphological characters, accessions of sago present in the natural population was divided into 3 groups namely those with low, medium and high color key value (Figure 2). Accessions of sago with low color key value consist of accession sago Omoroko, Taina and Mbapare. The accession of sago with medium color key values composed of sago Omiya 2, Durumu, Oko and Omiya 1. Accession Ikimina sago has the color key values that tend to be high on almost all morphological characters.

The accession of sago with medium color key values is sago accession group that has high value for plant height. Based on information from the local community, tall sago trees produce high starch yield. Of the four accessions only accession sago Omiya 2 which has a low starch production of only 143.87 kg per tree, while accession sago Omiya 1, Oko and Durumu produce 402.09, 384.10 and 312.14 kg per tree, respectively. The people of Mioko Village believe all tall accessions have high of starch production, but the study found sago accession which has low starch production. The plant height was unable to predict high starch production in the natural population. The previous study showed that starch accumulation and flowering time of sago varies among accessions and starch production in sago plant correlates with leaf area per plant not with plant height (Yamamoto *et al*., 2010; Yamamoto *et al*., 2014).

Taina and Omoroko sago accessions in the natural population show a noticeable similarity in agronomical characters as shown by plant height. Both accessions have a straight rod shape and are included in tall sago accession. The local community distinguishes these two accessions by looking at variations of spine and trunk shapes. The Taina accession is an accession that has the characteristic of dense spines on petiole and rachis while accession Omoroko has the characteristic of straight and high trunk shape. Information obtained from the local community needs to be incorporated into the results of this study in order to maintain and preserve sago germplasm. According to Zuraida and Sumarno (2003), the participation of farmers or local communities needs to be incorporated in order to preserve the genetic variability of plant species cultivated. According Widiarti (2013) community participation in managing a forest area as one effort to fulfill social, economic and ecological functions.

Relationship between morphological characters

Sago observed morphological characters are correlated. The character of leaf length correlated positively and highly significant with length and width of leaflets with a value of 0.92 and 0.90. The long rachis character is also positive and significantly correlated (Table 2). The leaf characters of sago plant observed consists of petiole, rachis and leaflets. The character of a long leaf is closely correlated with long rachis leaf with correlation coefficient value of r = 0.99. The rachis is part of a leaf covered by the leaflets. According Nakamura and Gotto (2015) sago leaves will increase in size especially in the stem-forming phase with leaflets stick on the rachis. This study showed that in natural sago plants observed, there are no leaf characters that are correlated with sago production.

The height character of the plant has a non-directional relationship with the circumference of the stem with a correlation coefficient value of r = -0.86. It shows the higher the plant, the smaller the circumference of the trunk. There is a correlation of agronomic character with plant growth. Plant height shows a highly significant correlation but negatively correlated with plat circumference. Accession sago that has a characteristic of short plants, but has a high starch production is known by the local community with the name of Mbapare. The accession has plant height and a trunk circumference of 7.13 m and 1.67 m, while accession Omiya 1 with a plant height and trunk circumference of 13.09 m and 1.23 m showed low starch production.

Starch production is the character that became the main goal of sago plant improvement. Selection for starch yield in sago is difficult because it takes a long time for sago to mature. Therefore, a morphological character associated with starch production is needed as a secondary selection character. Characters that have a strong relationship can be used as a secondary selection character for crop improvement if you want to obtain a plant that has high production.

In this study, there are no agronomic and morphological characters that have a strong correlation with starch production. In contrast to what Ahmad *et al*. (2016) and Dewi *et al*. (2016) found that sago starch production is correlated with the diameter and length of sago tree trunk. According to Ehara *et al*. (2000) the diameter of the trunk is not affected by the length of the growing period, but appears to be more related to genetic background and growing environment.

According to Tenda and Miftahorrachman (2014), there are six vegetative characters, namely plant height, rachis length, petiole length, number of leaflets, trunk weights and pith weight which affect the production of Baruq sago in the Sangihe regency of North Celebes. According to Ehara *et al*. (2000), the production of sago starch present in the eastern archipelago of Indonesia can be estimated using the character of trunk diameter at chest height. The increasing number of leaflets has an impact on the extent of the leaf surface resulting in higher photosynthesis and accumulation of storage of starch in the sago tree trunk. According to Mendez *et al*. (2011) during initial growth and

Table 2. Correlation based on morphological characters of sago accessions.

Characters	T	DB	LB	PD	PAD	LAD	TK	JD	ADR	ADL	PR	PP	LP	P
DB	-0.68^{tn}													
LB	-0.86**	0.82**												
PD	-0.02^{tn}	0.41^{tn}	0.21^{tn}											
PAD	-0.10^{tn}	0.42^{tn}	0.18^{tn}	0.92**										
LAD	0.02^{tn}	0.46^{tn}	0.13^{tn}	0.90**	0.83**									
TK	-0.17^{tn}	0.25^{tn}	0.34^{tn}	0.48^{tn}	0.20^{tn}	0.51^{tn}								
JD	-0.44^{tn}	0.07^{tn}	0.01^{tn}	-0.31^{tn}	-0.17^{tn}	-0.24^{tn}	-0.37^{tn}							
ADR	-0.24^{tn}	0.55^{tn}	0.45^{tn}	0.84**	0.71*	0.78*	0.39^{tn}	-0.10^{tn}						
ADL	-0.16^{tn}	0.50^{tn}	0.36^{tn}	0.74*	0.58^{tn}	0.74*	0.34^{tn}	0^{tn}	0.97**					
PR	-0.05^{tn}	0.39^{tn}	0.21^{tn}	0.99**	0.94**	0.92**	0.47^{tn}	-0.29^{tn}	0.84**	0.73*				
PP	0.15^{tn}	0.21^{tn}	0.12^{tn}	0.37^{tn}	0.15^{tn}	0.13^{tn}	0.23^{tn}	-0.22^{tn}	0.26^{tn}	0.27^{tn}	0.24^{tn}			
LP	0.75*	-0.19^{tn}	-0.39^{tn}	0.17^{tn}	-0.01^{tn}	0.16^{tn}	0.19^{tn}	-0.73*	-0.11^{tn}	-0.09^{tn}	0.09^{tn}	0.54^{tn}		
P	0.04^{tn}	0.54^{tn}	0.08^{tn}	0.40^{tn}	0.56^{tn}	0.52^{tn}	-0.35^{tn}	-0.01^{tn}	0.42^{tn}	0.43^{tn}	0.41^{tn}	0.05^{tn}	0.12^{tn}	
RD	-0.50^{tn}	0.31^{tn}	0.30^{tn}	0.21^{tn}	0.54^{tn}	0.10^{tn}	-0.44^{tn}	0.30^{tn}	0.20^{tn}	0.06^{tn}	0.27^{tn}	-0.35^{tn}	-0.62^{tn}	0.41^{tn}

Note : T = trunk length; D = trunk diameter; LB = circumference; PD = leaf length; PAD = leaflet length; LAD = leaflet width; TK = thickness; JD = total of leaf; ADR = total of leaflet on right side; ADL = total of leaflet on left side; PR = rachis length; PP = petiole length; LP = petiole width; P = production; RD = starch content; tn, ** and *: non-significant, and significant at P<0.05 and P<0.01, respectively.

production, assimilated carbon is stored temporarily in the trunk and leaf stem as carbohydrate reserves. According to Kjaer *et al*. (2004) variation in plant height in sago plants is a variable that almost stands alone and little influenced by other nine variables. The variation is not only caused by genetics, but also influenced by edaphic or climate factors.

Land environment at the observation site are not permanent inundated land, when the rainy season will be inundated and if the dry season will become dry but surface water is still available. The level of canopy closure in the observation area is quite tight so the incoming sunlight is low. The relationship between the low received sunlight is shown in the components of leaf character that correlates with each other including leaf length, leaflet length, leaf width and rachis length. According to Ariyanto *et al*. (2015) plant density can affected plant growth and development due to water competition, absorption of sunlight, growing space and nutrient uptake. Moreover, Dwyer *et al*. (1992) and Shibles and Weber (1995) stated that light interception has a direct relationship with leaf area and the radiation received by the leaves increases.

The characters of plant height and trunk circumference are characters that can be easily observed. Both of these characters are traditionally used by the Mioko Village community to select high-yielding sago. The community checks by partially cutting the stem with axes, if sticky starch is present in the eyes of the axe, then it is concluded that the sago tree has high starch content. Such information is the local wisdom possessed by the community past down through generations.

Sago in Mioko Village has an important role in people lives. Sago has become to capable plant to producing staple food since the past generation. The lives of local people cannot be separated from sago, canoe and river. Sago as a staple food, canoe as a transportation and river as a place to play and fish finding. Sago is able to grow naturally without cultivation and only harvesting is carried out. Sago in the local community like a "mama" and sago can be prosperity and peace.

CONCLUSION

Sago accessions in natural population of Mioko Village, Central Mimika Sub-district, Mimika Regency, Papua Province showed morphological diversity with 76.61% of variability can be described by 3 principal components. The characters that have the greatest contribution to the variability of sago are rachis length, plant height and petiole width. The sago accession that has the highest similarity level is Taina and Omoroko with similarity on rachis length, number of right-side leaflets, leaflets length in ¼ leaf and petiole width. There are no agronomic and morphological characters that have a strong correlation with starch production.

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