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Phylogenetic of sago palm (*Metroxylon sagu*) and others monocotyledon based on mitochondrial *nad2* gene markers

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Abstract. *Abbas B, Tjolli I, Dailami M, Munarti. 2019. Phylogenetic of sago palm* (Metroxylon sagu) *and others monocotyledon based on mitochondrial* nad2 *gene markers. Biodiversitas 20: 2249-2256.* Sago palm forest and sago palm semi cultivated are found in the Papua islands as well as Ambon and Seram islands. The diversity center of sago palm is found in the Papua Islands. The objectives of this study are revealed sequence DNA mitochondrial associated with *nad2* genes in sago palm accessions and molecular phylogenetic of sago palm and other monocotyledon plants. Plant materials used in the studies were derived from Sago Research Center (SRC) and sequencing and other monocotyledon were retrieved from the GenBank, NCBI accessions. Young fresh leaflets were derived from the experimental field of SRC and DNA extraction by following the procedure of Plant Genomic DNA Mini Kit and then PCR performed by using *nad2* primer sets. Thereafter, DNA PCR product was sequenced by Macrogen Inc., Seoul, Korea. Sequences of *nad2* genes in sago palm accessions from Papua, Indonesia were registered by GenBank NCBI for further used in the future as biological authenticity from the certain location. Mitochondrial DNA sequences associated with *nad2* genes in the genome of sago palm were shown no differences among sago palm accessions. Molecular phylogenetic of sago palm and others monocotyledon based on *nad2* gene markers showed the sago palm and others monocotyledon incorporated into two major clades and five subclades. Sago palm, coconut, and date palm were described as close related and being in the same subclades.

Keywords: Mitochondrial, monocotyledon, *nad2* gene, phylogenetic, sago palm

INTRODUCTION

Sago palm (*Metroxylon sagu* Rottb.) is found growing in the countries of Southeast Asia, Oceania, and the Pacific Islands at latitude 10º South and 10º North (Ishizuka et al. 1996), longitude 90º to 180º East, and altitude up to1000 meters above sea level (Bintoro 2011). Sago palm forest and sago palm semi cultivated is found in the Papua islands as well as Ambon and Seram islands (Flach 1997; Abbas et al. 2014). Schuiling (1995) reveals the diversity center of sago palm found in the Moluccas and New Guinea. Flach (1997) reported that New Guinea (Papua-Indonesia and Papua New Guinea) as a center of diversity of sago palm. McClatchey et al. (2005) believe that sago palm endemic in Papua-Indonesia, New Guinea, New Britain, and the islands of the Moluccas. Sago palm found widespread in Southeast Asia, Melanesia, and some islands in Micronesia and Polynesia (McClatchey et al. 2005). Estimation 1.25 million hectares of sago forest and 148,000 hectares of sago palm plantations located in Indonesia. Papua Province and West Papua province, Indonesia territorial is estimated 1.2 million hectares of sago palm forest and 14,000 hectares of sago palm plantations (Flach 1997). Recently reported the sago palm areal widely distributed in Papua Province Indonesia territorial with it has 4.749.325 hectares and 510.213 hectares in the West Papua Province, Indonesia (Bintoro 2015) and mostly sago palm growth in the inundated areas and semi inundated areas (Yater et al. 2019). Distribution of sago palm area in Indonesia is uneven. Papua Island, Indonesia territorial has the largest sago palm areas compared with other islands in Indonesia. Abbas et al. (2014) reported that 92% of sago palm area is located on the island of Papua and 8% of sago palm area is in the other islands in Indonesia.

Morphological characters of sago palm in Indonesia were reported high variation. Riyanto et al. (2018) revealed that sago palm seedling showed in high variations based on morphology and genetic using RAPD markers. Abbas (2018) reported that sago palm high variation based on molecular markers, such as RAPD markers, Waxy gene markers, and rpl1671, NTCP21, and NTCP22 of cpDNA markers. Furthermore, sago palm reported also variation in the ability to result carbohydrates. The ability of sago palm to produce carbohydrates is higher than other starch-producing plants. Karim et al. (2008) reported that sago starch production was 3 to 4 times higher than rice, maize or wheat production and 16 times higher than cassava production. Bujang (2008) reported that the yield potential of sago palm in Malaysia is reached 25 tons of starch ha^{-1} $year^{-1}$. The dried starch produced by sago palm was reported between 200-400 kg $tree^{-1}$ (Dewi et al. 2016). Sago palm encountered in Sentani, Papua, Indonesia with local names Para, Panne, Yebha, Wanny sequentially have average production capability of 674 kg, 576 kg, 512 kg, 491 kg $tree^{-1}$ (Yamamoto 2011). Starch production of

others sago palm varieties reaches 49 tons of starch ha^{-1} in Indonesia (Abbas 2015; Abbas 2018).

Higher plants including sago plants have three genetic information centers nam[25] the nuclear genomes, chloroplast genomes, and mitochondrial genomes. The function of mitochondria in higher plants is as an energy-generating organelle in the cell. Mitochondrial (mt) genomes are organelles that have DNA in a circular shape and maternally inherited (Castro et al. 1998) with a size around 222 to 773 kb for angiosperm (Kitazaki and Kubo 2010). The composition of mt genome was not influenced by the presence of crosses pollination. Changes mitochondrial DNAs (mtDNA) in the plant genome were reported that caused by the evolution in a [49]g time, approximately 10,000 to100,000 years (Mower et al. 2007). Pervaiz et al. (2015) reported that the mt genome among *Prunus* species has a high conservative level. Genetic differentiation occurs in very small amounts in the maternally inher[48] marker such as mtDNA and cpDNA markers (Petit et al. 2005). The size of the plant mitochondrial genome varies from 200 kbp to 2,000 kbp (Morley and Nielsen 2017). Mitochondrial genomes undergo very low mutation processes (Christensen 2013) so it is good to use as a molecular marker for reveling phylogenetic of sago palm and others monocotyledon. The objectives of this study were to explore nucleotide sequence of *nad2* gene associated in the genome of sago palm and reveled phylogenetic of sago accessions and others monocotyledon.

[41]

MATERIALS AND METHODS

Plant materials

Plant materials used in the studies were derived from several regions in Papua that has been collected by the Sago Palm Research Center (SRC) University of Papua (UNIPA) and others monocotyledon plant were retrieved from the NCBI GenBank accessions. Leaf samples were taken from accessions of sago palm in a growth russet stage. Accession name is not based on its loca[22]me because it is confusing. The Accession names are SP001, SP002, SP003, SP004, SP005, SP006, SP007, SP008, SP009, SP010, and SP011. Young leaf samples of sago palm accessions were preserved using silica gel in a zip lock plastic. The sago palm sample was used this study the same as were used Abbas et al. (2015) and Abbas et al. (2017).

DNA extraction

DNA extraction was done by following the procedure of Plant Genomic DNA Mini Kit (Geneaid 2012). The outlines of DNA extraction using Geneaid protocols are tissue dissociation, lysis, DNA binding, wash, and DNA elution. Tissue dissociation was done by grind the sample to a fine powder. As much as 20 mg fine powder of the sample wa[37]ansfer to a 1.5 ml microcentrifuge tube, then following step 2, step 3, step 4, and step 5 in the protocols. The genomic DNAs were extracted it is stored at-20 °C freezer until ready used.

PCR and sequencing

Design of *nad2* primer sequences was used in this study it is adopted from Duminil et al. (2002) and synthesize by Genetica Science Company. The Primer sequences used for amplified mitochondrial which association with *nad2* gene in the sago palm genome as follows: forward 5' TTC ATA TAG AAT CCA TGT CC 3'and reverse 5' CTA TTT GTT CTT CGC CGC TT 3'. PCR mixtures and cycles condition were followed by 25 ml total volume that contains: 1 x PCR buffe[36]ntained 1.5 mM MgCl 2 (KAPA 2G Robust HotStart), 10 mM dNTP mix, 10 µM of forward and reverse primer, 1 x KAPA Enhancer, 0.5 U KAPA 2G Robust Hotstar polymerases, and 10 ng genomic DNA. PCR cyc[47] condition as follows: initial denaturation for 15 seconds at 9[35], followed by 30 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 50°C, for 45 seconds extension at 72°C. PCR amplification fragments were separated on 1% agarose gels by electrophoresis, staining was done using Ethidium Bromide and visualization by using UV illumination apparatus. Sequencing and purification of DNA PCR product were performed by Macrogen Inc., Seoul, Korea.

Data analysis

DNA sequences in the form of electropherogram were edited and checked to obtain correct DNA sequence. Editing and proofreading sequences were performed by matching the peak color of the electropherogr[46] to the sequence of nucleotides produced using Molecular Evolutionary Genetics Analysis (MEGA) 5.0.5 software (Tamura et al. 2011). Each sequence in this study was obtained from the forward and reverse sequences of each sample. The editing result of a nucleotide sequence is stored in the Fasta file format. Cluster alignment was performed based on Clustal W with MEGA 5.0.5 software. Comparis[26]f sample sequences with GenBank database (NCBI) is done using Basic Local Alignment Search Tools (BLAST) available on the National Center for Biotechnology Information (NCBI) web (Zhang et al. 2000; Morgulis et al. 2008). Phylogenetic construction of sago palm accession an[40]her related plant-based on *nad2* gene was calculated by using Mega 6 s[12]ware (Tamura et al. 2013). Outgroup was performed by using the UPGMA method (Sneath and Sokal (1973) and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). The certain species were choose based on the potential of resulting large amount carbohydrate of monocots plant.

RESULTS AND DISCUSSION

Nucleotide sequence of *nad2* genes

Forward and reverse sequences *nad2* genes of sago palm were performed by Macrogen Inc., Seoul, Korea. Alignments of the mitochondrial sequences associated with *nad2* genes of sago palm accessions were presented in Figure 1. All of accessions has the number of sequences as much as 1304 base and just showed 61 bases in Figure 1. The complete sequences of sago palm accessions used in

this study were prepared and accessible by retrieved in the GenBank, NCBI. Mitochondrial sequences associated with *nad2* genes in the sago palm accessions showed that no differences event through morphological differences (Table 1). Blast analyses of sago palm and other monocotyledon were presented in Table 2. The nucleotide sequences *nad2* genes of sago palm were registered in the GenBank, NCBI with the accession sequence number KY849955.1, KY849956.1, KY849957.1, KY849958.1, KY849959.1, KY849960.1, KY849961.1, and KY849962.1. Actually, we performed eleven accessions for sequencing of sago palm that representative of morphological differentiation, but other three accessions were figured out that their sequence contaminated. The peaks of electropherograms of those three accessions ware not clear separation. The *nad2* gene sequences of eight sago palm accessions showed no differences event the morphological different (Abbas et al. 2017). Based on investigation of mitochondrial genome in the previous studied such as beet showed that plant mitochondrial genome possesses a low mutation rate, a little compactness, large size, and high rearrange structure (Darracq et al. 2011). Furthermore, it was reported that mt genome of plants have a mechanism of base excision repair pathway (Boesch et al. 2009) so that the nucleotide structure is very conservative, even though morphologically different. Morphological differences were probably controlled by multigenic function which associated in the nucleus and mitochondrial, or chloroplast genomes, such as young petiole color, spine types, and spear color. Genes associated with mitochondrial genome such as nad2 gene were generally known their function as energy regulation in biological metabolism. Chen et al. (2017) reported that the mitochondria are responsible as primary source of cellular energy for growth, development, and reproduction of organism. The registration number of sago palm sequences associated with mitochondrial *nad2 gene* as follows: (i) KY849955.1Metroxylon sagu accession 1 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (ii) KY849956.1Metroxylon sagu accession 2 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (iii) KY849957.1Metroxylon sagu accession 3 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (iv) KY849958.1Metroxylon sagu accession 4 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (v) KY849959.1Metroxylon sagu accession 5 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (vi) KY849960.1Metroxylon sagu accession 6 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (vii) KY849961.1Metroxylon sagu accession 7 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial; (viii) KY849962.1Metroxylon sagu accession 8 NADH dehydrogenase subunit 2 (*nad2*) gene, intron; mitochondrial.

Basic local Alignment Search Tool (BLAST)

Comparison the DNA sequence homology of sago palm accessions and other others monocotyledon in the NCBI GenBank DNA database were performed by BLAST analysis (Table 2). The results of BLAST analysis showed that sago palm accessions and other related plant have high similarity. The *Maximum score* range from 1274 to 2374 indicated that the largest value of the plant genus is the highest similarity of sago palm. The *Query cover* for all species used has value 100%, this indicated high degree of alignment to BLAST sequences. The *E-value* of 0.0 indicates the number of alignments with scores equivalent to the database and better quality of the alignment BLAST search. The DNA sequences have high similarity if the query cover is closed to 100% and the *E-value* is closed 0.0 (Claverie and Notredame 2003). The identity for 51 plant samples have value in the range of 85% to 99%. The smallest of the identity value is *Butomus umbelatus* and the highest of the identity value is *Cocos nucifera* and *Phoenix dactilifera*. These indicated that *C. nucifera* and *P. dactilifera* is the highest similarity with sago palm based on mitochondrial *nad2 gene*.

Genetic distance of sago palm and others monocotyledon

Genetic distance of sago palm accessions and related plant from several families based on *nad2* gene markers showed range from 0.000 to 0.171 (Table 3). Sago palm accessions have calculated no differences between the others based on *nad2* gene markers with molecular distances 0.000. In the reverse, the largest genetic distances were calculated between species *Triticum aestivum* and *Butomus umbellatus* with genetic distance value 0.171. Reverse of that overall sago palm accessions used being in one species of is a *Metroxylon sagu.* The species closer to sago palm is *Cocos nucifera* and following *Phoenix dactylifera* with genetic distances value of 0.003 and 0.006 respectively (Table 2).

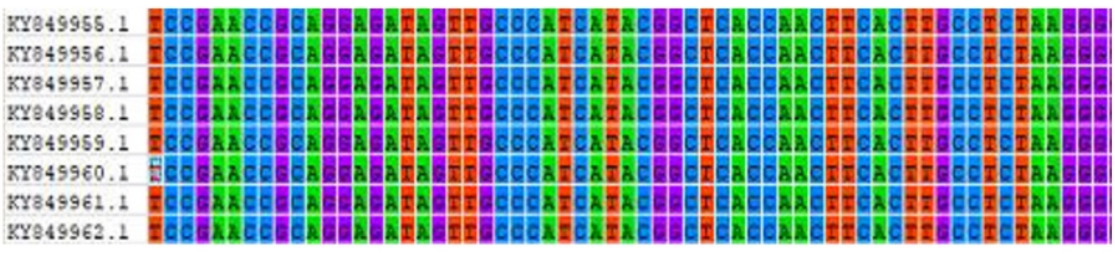

Figure 1. Alignment of nucleotide sequence of eight sago palm accessions

Table 1. Morphological characteristic in the russet stages of sago palm accessions from Papua, Indonesia

Accession	Morphological characteristic					
	Spine type	Spear color	Young leaf color	Leaf color	Young petiole color	Petiole
SP001	Spiny	RHS67A	RHS67A	RHS142A	RHS67A	RHS154A
SP002	Spineless	RHS67A	RHS67A	RHS142A	RHS67A	RHS142A
SP003	Spineless	RHS142A	RHS150C	RHS142A	Strips 154B & 153D	Strips RHS150C & RHS33D
SP004	Spineless	RHS142A	RHS150C	RHS142A	150C	142A
SP005	Spineless	RHS142A	RHS150C	RHS142A	150C	142A
SP006	Spineless	RHS142A	RHS150C	RHS142A	Strips 154B & 153D	Strips RHS150C & RHS33D
SP007	Spineless	RHS142A	RHS150C	RHS142A	RHS67A	154A
SP008	Spineless	RHS67A	RHS67A	RHS142A	RHS67A	154A

Notes: RHS67A is strong purplish-red, RHS142A is strong yellowish-green, RHS154A is vivid yellowish-green, RHS150C is brilliant yellowish-green, RHS33D is Moderate yellowish pink, RHS154B is brilliant yellowish-green, RHS153D is strong yellow

Table 2. Blast analysis of sago palm and others monocotyledon based on *nad2* genes

Species name	Max. score	Query cover %	E-value	Identity %	Accession ID
Cocos nucifera	2374	100	0.0	99	KX028885.1
Phoenix dactylifera	2370	100	0.0	99	JN375330.1
Ferrocalamus rimosivaginus	2170	100	0.0	97	JN120789.1
Ferrocalamus rimosivaginus	2170	100	0.0	97	JQ235171.1
Bambusa oldhamii	2156	100	0.0	97	EU365401.1
Triticum aestivum	2159	100	0.0	97	GU985444.1
Hordeum vulgare	2154	100	0.0	96	AP017301.1
Hordeum vulgare	2154	100	0.0	96	AP017300.1
Lolium perenne	2146	100	0.0	96	JX999996.1
Saccharum officinarum	2134	100	0.0	96	LC107875.1
Aegilops speltoides	2126	100	0.0	96	AP013107.1
Triticum timopheevii	2126	100	0.0	96	AP013106.1
Aegilops speltoides	2126	100	0.0	96	AP013107.1
Triticum timopheevii	2126	100	0.0	96	AP013106.1
Triticum aestivum	2126	100	0.0	96	EU534409.1
Triticum aestivum	2126	100	0.0	96	AP008982.1
Sorgum bicolor	2128	100	0.0	96	DQ984518.1
Oryza minuta	2109	100	0.0	96	KU176938.1
Oryza sativa Indica Group	2098	100	0.0	96	AP017386.1
Oryza rufipogon	2098	100	0.0	96	AP012528.1
Oryza sativa Indica Group	2098	100	0.0	96	JN861112.1
Oryza sativa Indica Group	2098	100	0.0	96	JN861111.1
Oryza sativa Indica Group	2098	100	0.0	96	JF281154.1
Oryza sativa Indica Group	2098	100	0.0	96	JF281153.1
Oryza sativa Indica Group	2098	100	0.0	96	AP011077.1
Oryza rufipogon	2098	100	0.0	96	AP011076.1
Oryza sativa Japonlca	2098	100	0.0	96	BA000029.3
Oryza sativa	2098	100	0.0	96	DQ167400.1
Oryza sativa	2098	100	0.0	96	DQ 167807.1
Orvza sativa	2098	100	0.0	96	DQ 167399.1
orvza sativa Japonica Group	2093	100	0.0	96	AP014957.1
Oryza sativa Jaoonica Group	2093	100	0.0	96	AP003280.2
Oryza rufipogon	2091	100	0.0	96	AP012527.1
Triticum aestivum	2076	100	0.0	96	Y14434.1
Tripsacum dactyloides	2073	100	0.0	96	DQ984517.1
perennis	2073	100	0.0	95	DQ645538.1
Zea luxurians	1993	100	0.0	95	DQ645537.1
Zea mays subsp mays genotype CMS-S	1989	100	0.0	94	DQ490951.2
Zea mays parviglumis	1989	100	0.0	94	DQ645539.1
Zea mays subsp mays genotype CMS-C	1989	100	0.0	94	DQ645536.1
Zea mays subsp mays genotype CMS-T	1989	100	0.0	94	DQ490952.1
Zea mays subsp mays genotype male-fertile	1989	100	0.0	94	NC_007982.1
Zea mavs isolate SM10	1986	100	0.0	94	KY018916.1
Scheuchzeria sp.	1421	100	0.0	87	KX363631.1
Butomus umbellatus	1274	100	0.0	85	KC208619.1

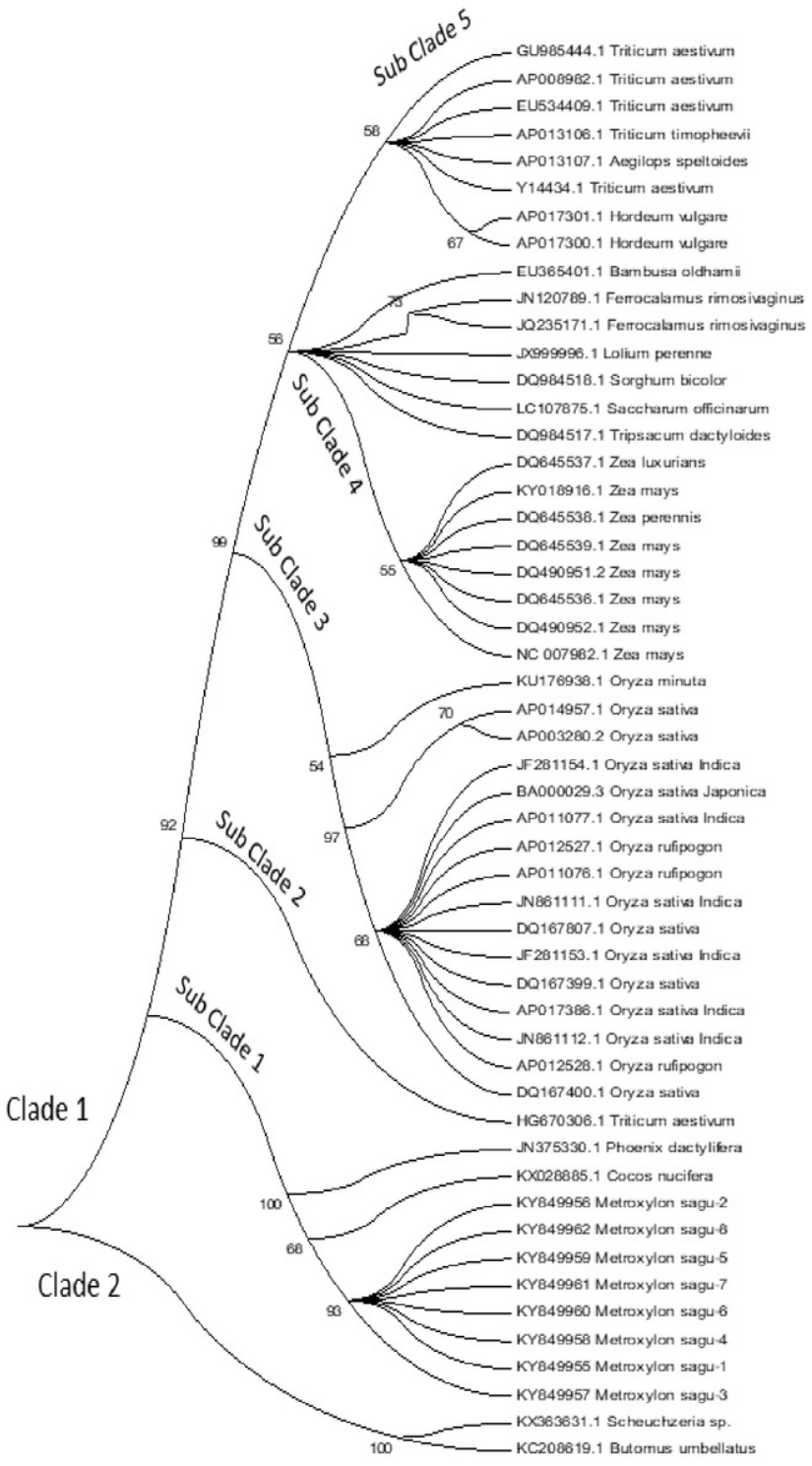

Figure 2. Phylogenetic construction of sago palm accession and related plant species based on *nad2* gene markers

Table 3. Genetic distance of sago palm accessions and related other plant producing carbohydrate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
KY849955 Metroxylon sagu-1																																																			
KY849955 Metroxylon sagu-2	0.00																																																		
KY849957 Metroxylon sagu-3	0.00	0.00																																																	
KY849958 Metroxylon sagu-4	0.00	0.00	0.00																																																
KY849959 Metroxylona sagu-5	0.00	0.00	0.00	0.00																																															
KY849960 Metroxylon sagu-6	0.00	0.00	0.00	0.00	0.00																																														
KY849961 Metroxylon sagu-7	0.00	0.00	0.00	0.00	0.00	0.00																																													
KY849956 Metroxylon sagu-8	0.00	0.00	0.00	0.00	0.00	0.00	0.00																																												
KX028885.1 Cocos nucifera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																																											
JN375330.1_Phoenix_dactylifera	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00																																										
JN120789.1 Ferrocalamus rimosivaginu	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03																																									
JQ235171.1_Ferrocalamus_rimosivaginu	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.00																																								
GU985444.1 Triticum aestivum	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00																																							
EU365401.1_Bambusa_oldhamii	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.00	0.00	0.00																																						
AP017301.1 Hordeum vulgare	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00																																					
AP017300.1_Hordeum_vulgare	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00																																				
JX999996.1 Lolium perenne	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00																																			
LC107875.1_Saccharum_officinarum	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00																																		
AP013107.1 Aegilops speltoides	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																																	
AP013106.1_Triticum_timopheevii	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																																
EU534409.1 Triticum aestivum	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																															
AP008982.1_Triticum_aestivum	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																														
DQ984518.1 Sorghum bicolor	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00																													
KU176938.1_Oryza_minuta	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01																												
AP017386.1 Oryza sativa Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00																											
AP012528.1_Oryza_rufipogon	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00																										
JN861112.1 Oryza sativa Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00																									
JN861111.1_Oryza_sativa_Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00																								
JF281154.1 Oryza sativa Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00																							
JF281153.1_Oryza_sativa_Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00																						
AP011077.1 Oryza sativa Indica	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00																					
AP011076.1_Oryza_rufipogon	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																				
BA000029.3 Oryza sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																			
DQ167400.1_Oryza_sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																		
DQ167807.1 Oryza sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																	
DQ167399.1_Oryza_sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00																
AP014957.1 Oryza sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00															
AP003280.2_Oryza_sativa	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00														
AP012527.1 Oryza	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00													
HG670306.1_Triticum_aestivum	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02												
Y14434.1 Triticum aestivum	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02											
DQ984517.1_Tripsacum_dactyloides	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.00										
DQ645538.1 Zea perennis	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00									
DQ645537.1_Zea_luxurians	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00								
DQ490951.2 Zea mays	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00							
DQ645539.1_Zea_mays_subsp	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00						
DQ645536.1 Zea mays	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00					
DQ490952.1_Zea_mays_subsp	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00				
NC 007982.1 Zea mays subsp.	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
KY018916.1_Zea_mays	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
KX363631.1 Scheuchzeria sp.	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.14	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	
KC208619.1 Butomus umbellatus	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.15	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.06

Metroxylon sagu, *C. nucifera*, and *P. dactylifera* are being in the group of palm tree family (Arecaceae), so hierarchical closed each other. Otherwise, it was reported that among sago palm accessions were calculated differently by using cpDNA gene marker (Abbas et al. 2010). The use of different molecular markers might result in different result, even though in the same plant genome object. Touzet and Delph (2009) reported that mitochondrial cytochrome b (cob) and cytochrome oxidase (cox1) was very little until no difference in mitochondrial nucleotide sequences in the hermaphroditic such as *Silene scouleri*, *S. virginica*, *S. noctiflora* and dioecious suc as *S. latifolia*, S. dioica, and *S. diclinis*, whereas gynodioecious such as *S. vulgaris, S. acaulis*, and *S. nutans* found many haplotypes based on mitochondrial DNA which means there are many differences in the arrangement of mitochondrial DNA. Sago palm is a hermaphroditic plant that it might cause their mitochondrial DNA sequence no differences among accessions based on *nad2* gene marker. Barr et al. (2007) were reported that the mitochondrial plant is possessing low substitution rates and no recombination.

Phylogenetic of sago palm and others monocotyledon

Phylogenetic of sago palm and others monocotyledon that potentially produce large amount carbohydrate based on *nad2* gene markers showed that the individual sample incorporated into two major clades. Clade 1 consist of five subclades and no subclade at clade 2. In cladistics or phylogenetics of clade 2 were determined an outgroup plant. Both plants of clade 2 are being monocotyledon plants but do not correlate produced large amount carbohydrate. All of sago palm accessions are being in the same subclade in the clade 1, not separated to the other brunch of phylogenetic. Sago palm, coconut, and date palm are being in the same subclade at clade 1. Cereal plants that were known produce a lot of carbohydrates such as wheat, rice, and corn are being in the position of cluster 2 and flowering plants are being in the cluster 3 (Figure 2). In the previous studies of sago palm were reported that sago palm in the forest and in the semi cultivated have high diversity and divided into several clusters based on molecular marker of Waxy gene marker (Abbas et al. 2007; Abbas and Ehara 2012), RAPD marker (Abbas et al. 2009; Abbas et al. 2017; Abbas 2017). Sago palm accessions were in the same clusters of palm family that producing large amount of carbohydrates and other clusters are cereal plant and flowering plant. *nad2* gene might be related to energy resources for accumulating starch in the stained part of plant. The NAD and NADP pyridine nucleotide pools were known to play critical roles for regulating energy-producing in the catabolic process (Blacker and Duchen 2016).

In conclusions, Sequences of *nad2* genes in sago palm accessions from Papua, Indonesia were registered into GenBank NCBI for further used in the future as biological authenticity from the certain location. the study indicated that mitochondrial DNA sequences associated with *nad2* genes in the genome of sago palm were shown no differences among accessions. Molecular phylogenetic of sago palm and others monocotyledon based on *nad2* gene markers showed the sago palm and related plant incorporated into two major clades and five subclades. Sago palm, coconut, and date palm were described as closely related and being in the same subclades.

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