

Assessment of Genetic Arrangement of Sago palm Collection Based on Mitochondrial *nad2* gene Marker

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Abstract

Mitochondrial DNA is an organelle found in plants and animals that play a role in the formation of energy. NADH and ATP are forms of chemical energy which resulting by plants and animals for their growth and development. The genes that play a role in the biosynthesis of NADH and ATP sequentially are the *nad* and *atp* genes. Gent *nad* and *atp* are inherited maternally so it is very conservative. The aims of work are evaluated genetic characteristic of sago palm accessions based on *nad-2* gene. Methods of research were DNA extraction by using Geneaid DNA plant mini Kit, PCR amplification fragments DNA by using KAPA 2G Robust Hotstar polymerases and perform by using Biorad PCR instrument, purifying and sequencing DNA by Macrogen Inc. Seoul, Korea. Data analyses were perform by using MEGA 6 software. Morphological characteristics in the russet stages of sago palm accessions were divided into four phenotypic and several accessions show phenotypically the same. Based on *nad-2* gene was shown that the sago palm accessions have the same genetic constitution among the others. Phylogenetic constructions of sago palm accession and other related plant based on *nad-2* gene markers were described that sago palm closer to the coconut and date palm and then following others plant producing large amount carbohydrates.

Keywords: Sago palm accession, molecular assessment, mitochondria, *nad-2* gene marker, phylogenetic

Introduction

Sago palm is belong to the family of Palmae, genus of *Metroxylon*, and species of *Metroxylon sagu* which has the ability to accumulate high quantities of carbohydrates in their stem. 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 17 times higher than cassava production. Bujang (2008) reported that the yield potential of sago palm in Malaysia is reached 25 tons 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, Wannu sequentially have average production capability of 674 kg, 576 kg, 512 kg, 491 kg per tree (Yamamoto, 2011). Sago palm productivity in Papua is annually reaches 49 tons starch ha⁻¹ (Abbas, 2015; Abbas, 2017). This is makes sago palm an important commodities that needs to be developed for fulfilling the food and energy needs of the world population.

Generally plants store their genetic information in the nucleus and organelle genomes, namely chloroplasts and mitochondria. Some mechanisms such as deletion, inversion, translocation, and transposition that can occur naturally or induced, can lead to the replacement or change of

nucleotide bases in DNA sequences. That mechanism does not necessarily alter the phenotype of plants so that the use of morphological markers is limited. The presence of DNA markers that directly integrate with the genetic system more reflects the true state of the genome.

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 long time, approximately 10,000 to 100,000 years (Mower et al., 2007). Pervaiz et al (2015) reported that the mt genome among *Prunus* species have a high conservative level. Genetic differentiation occurs in very small amounts in the maternal inherited marker such as mtDNA and cpDNA markers (Petit et al., 2005). Based on a high conservative level of mtDNA that is made used as a marker to determine the level of genetic diversity of organisms. Molecular marker used in this research is a molecular markers associated with *nad2* gene in the mt genome to evaluate the genetic arrangement of sago palm accessions that have been collected by sago research consortium (SRC) in the experimental field of University of Papua (UNIPA). Molecular marker associated with the expression of certain genes such as *nad2* is interesting because it is known variations of the genes that encode certain characters.

Materials and Methods

1. Plant Materials

Sago palm used in the study was derived from several regions in Papua that has been collected by the SRC UNIPA. Leaf samples were taken from eleven accessions of sago palm in a growth russet stage. Accession name is not based on its local name because it is confusing. The Accession names are SP001, SP002, SP003, SP004, SP005, SP006, SP007, SP008, SP009, SP010, and SP011. Young leaf samples of eleven sago palm accessions were preserved using silica gel in a zip lock plastic. The sago palm sample were used this study the same as were used Abbas *et al.* (2015).

2. DNA Extraction

DNA extraction is done by following the procedure of Plant Genomic DNA Mini Kit, available in the www.geneaid.com. 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 dried sample to a fine powder. As much as 20 mg fine powder of the sample were transfer to a 1.5 ml micro centrifuge tube, then following step 2, step 3, step 4, and step 5 in the protocols. The genomic DNAs were extract it is stored at -20 °C freezer until ready used.

3. PCR and Sequencing

Design of *nad-2* primer sequences was used in this study it is adopted from Duminil et al. (2002) and synthesize by Genetica Science Company. The Primer sequences were used for analyses of mitochondrial genetic arrangement which associated *nad-2* gene that were 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 buffer contained 1.5 mM MgCl₂ (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 cycles condition is as follows: initial denaturation for 15 second at 94°C, Followed by 30 cycles of denaturation for 30 second at 94°C, annealing for 30 second at 50°C, for 45 second extension at 72°C (Abbas *et al.* 2015). 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.

4. Data Analysis

Morphological data were observed visually and determined of the color based on the Color Chart Azalea Society of America. Data sequencing results were analyzed using Mega6 software (Tamura et al., 2013). Alignments of DNA sequences were performed by using ClustalW. Pairwise distance analyses were conducted using the Maximum Likelihood Composite models (Tamura et al., 2004). Phylogenetic construction of sago palm accession and other related plant producing starch based on nad-2 gene was calculated by using Mega 6 software statistical method.

Results and Discussions

1. Morphological Characteristic

The morphological characteristics of 11 samples used in this study were identified by Abbas *et al.* (2015) as follow: sago palm accessions that were used as samples are divided into four groups (Table 1 and Figure 1). Group one is the accession number SP002, SP008, SP009, and SP010 with spineless type and their spear leaf, young leaves, and young petioles have strong purplish red color (RHS67A). The morphological appearance is presented in Figure 1A. Group two is the accession number SP003, SP004, SP005, SP006, and SP007 with spineless type and their spear leaf, young leaves and petioles has strong light yellowish green color (RHS142A) the morphological appearance is presented in Figure 1B. Groups three is accession number SP001 with spiny type and their spear leaf, young leaves and petioles colored RHS67A which the morphological appearance is presented in Figure 1C. Groups four is accession number SP011 with spiny type and their spear leaf, young leaves and petioles colored RHS142A which the morphological appearance is presented in Figure 1D. Given the number of accessions that have been collected are still few and morphological observations were performed it is still limited to the russet stages so that accession is only formed four groups. People around Sentani lake, Papua, Indonesia are mention that there are 21 types of sago palm. Furthermore, reporting by scientist that sago palm encounter around Sentani lake in Jayapura, Indonesia was found 15 types (Yamamoto et al. 2005).

Table 1. Morphological characteristic in the russet stages of sago palm accessions from Papua, Indonesia were identified by Abbas *et al.* 2015

| No | Accession | Morphological characteristic | | | | | |
|----|-----------|------------------------------|-------------|------------------|------------|---------------------|-------------------------|
| | | Spine type | Spear Color | Young Leaf Color | Leaf color | Young Petiole Color | Petiole |
| 1 | SP001 | Spiny | RHS67A | RHS67A | RHS142A | RHS67A | RHS154A |
| 2 | SP002 | Spineless | RHS67A | RHS67A | RHS142A | RHS67A | RHS142A |
| 3 | SP003 | Spineless | RHS142A | RHS150C | RHS142A | Strips 154B & 153D | Strips RHS150C & RHS33D |
| 4 | SP004 | Spineless | RHS142A | RHS150C | RHS142A | 150C | 142A |
| 5 | SP005 | Spineless | RHS142A | RHS150C | RHS142A | 150C | 142A |
| 6 | SP006 | Spineless | RHS142A | RHS150C | RHS142A | Strips 154B & 153D | Strips RHS150C & RHS33D |
| 7 | SP007 | Spineless | RHS142A | RHS150C | RHS142A | RHS67A | 154A |
| 8 | SP008 | Spineless | RHS67A | RHS67A | RHS142A | RHS67A | 154A |
| 9 | SP009 | Spineless | RHS67A | RHS67A | RHS142A | RHS67A | 154A |
| 10 | SP010 | Spineless | RHS67A | RHS67A | RHS142A | RHS67A | 154A |
| 11 | SP011 | Spiny | RHS142A | RHS150C | RHS142A | RHS154A | 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



Figure 1. Morphological characteristic in the russet stages of eleven sago palm accessions. A types are accession SP002, SP008, SP009, and SP010; B types are accessions SP003, SP004, SP005, SP006, SP007; C types are accessions SP001; and D types are accessions SP011. Photographs were documented by Abbas *et al.* 2015.

2. Genetic Characteristic Based on *nad-2* gene Markers

Amplification of mtDNA by using a primer pair of mitochondrial *nad-2* gene from the 11 accessions sago palm has been successfully carried out. Sequence alignment of 11 sago palm accessions were resulting eight accessions that have precise of nucleotide sequences and three accessions that have less precise of nucleotide sequences. Accessions that have less precise of their nucleotide sequences are SP009, SP010, and SP011, it may be caused by DNA contamination. Sago palm accessions that have precise of nucleotide sequences of eight accessions is SP001, SP002, SP003, SP004, SP005, SP006, SP007, and SP008. Nucleotide sequences of 8 sago palm accessions based on *nad-2* gene markers have been registered in the GenBank with registration number: KY849956, KY849957, KY849958, KY849959, KY849960, KY849961, KY849962 respectively.

Nucleotide sequences based on *nad-2* gene of sago palm accessions were presented in Table 1. Nucleotide arrangements of sago palm accession number SP001, SP002, SP003, SP004, SP005, SP006, SP007, and SP008 were detected no differences among others according to their nucleotide sequences. This cases hypothetically caused by conservative of mitochondrial mechanism in sago palm that it is following to another common crops. 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 (Boestch *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 genome. Genes associated with mitochondrial genome were generally known their functional as energy regulation in biological metabolism. Chen *et al.* (2017) reported that the mitochondria is responsible as primary source of cellular energy for growth, development, and reproduction of organism.

Table 1. DNA Sequences of mitochondrial genome associated with *nad-2* gene of 8 sago palm accessions

| Accession SP001 (1304 bp) | Accession SP002 (1304 bp) |
|--|--|
| 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac | 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac |
| Accession SP003 (1304 bp) | Accession SP004 (1304 bp) |
| 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac | 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac |
| Accession SP005 (1304 bp) | Accession SP006 (1304 bp) |
| 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac | 1 tcggaaccgc aggagatagt tgcccatcat acggctcacc aacttcactt gcctctaagg 61 ggggctcgcg cgggcaggt tcggatcact tacaatacac ggctctacga aggggttagg 121 agcgtttca agatgattct ttctttgctg agacgaaaaa ggaaccatt ttctcgactg 181 gaaaatggga gtctgtttg tctactttat ccatccccc ctatcaaat gatcaaaaag 241 gaaggtgagc ttgcttcta ttccctgtt tgatctttc catctctgcc ccgcttccat 301 gtgggcagag acccctgtag agaatgaaga ggggccaagg atcttctct caagagtgtc 361 tctcgaggct ccaactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct 481 ctgtaaatgt gtgagccaa gtgtagtgtg gtgtagtgt aggcacttct agggcccttc 541 ccggctactg gatactcca gtgctcggg tactacggac cctctgccat ccattgcagc 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa 661 tcgaaatcga ttcttttta gatatccgga tagatggatg gatctatct tctattcata 721 tatatttgc aagaagcccc aaatccttga ttggccagg aaacaaagca ctgctttggg 781 cccaggaagc gaagggaaag agctcggctg ctctctccc acacttctt atttctcctg 841 gcccttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta ttcttcait 901 ggacggttcg gatggacttc gccgttctt cccaacgaaa atggaaaagg ctgtatcaca 961 tcgagatgic gattcgtttt ccgccccaaa tgagatgggg aattagtcac ctctgtccct 1021 tcatcttct gaattgaatc gaggccccgc ccggctcgcg tcttccaac aaccgacggg 1081 gagcacctca gtatacgatc gcgcgcagta actgggagtc ctattacacc ggcggccaac 1141 ttccattcac caaaccagg ttcatctcgt gtatgtattg tggactcgt caaggatatg 1201 gactcgacgg ttgatgtatc agactcgacc ctgtcttcc tagcatgcat tccatccgt 1261 gtcgcaactg attcggtaag ctacgtgtcc ggtgcacgga aaac |

| Accession SP008 (1304 bp) | Accession SP009 (1304 bp) |
|---|---|
| 1 tccgaaccgc aggagatagt tgcctcatc acggtcacc aactcactt gecttaagg | 1 tccgaaccgc aggagatagt tgcctcatc acggtcacc aactcactt gecttaagg |
| 61 ggggctcgt cccggcaggt tcggatcact tacaatacac ggctctacga aggggttagg | 61 ggggctcgt cccggcaggt tcggatcact tacaatacac ggctctacga aggggttagg |
| 121 agcgittca agatgattct tctttgtcg agacgaaaaa ggaaccatt tttcgcactg | 121 agcgittca agatgattct tctttgtcg agacgaaaaa ggaaccatt tttcgcactg |
| 181 gaaaatggga gtctgtttg tctactttat ccatcccct ctatcaaaat gatcaaaaag | 181 gaaaatggga gtctgtttg tctactttat ccatcccct ctatcaaaat gatcaaaaag |
| 241 gaaggtgagc ttgcttcta ttccgtgtt tgatctttc catctctgcc ccgcttccat | 241 gaaggtgagc ttgcttcta ttccgtgtt tgatctttc catctctgcc ccgcttccat |
| 301 gtggcgagag acccctgtag agaatagaaga ggggccaagg atcttctct caagagtgc | 301 gtggcgagag acccctgtag agaatagaaga ggggccaagg atcttctct caagagtgc |
| 361 tctcgaggct ccactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg | 361 tctcgaggct ccactctct cctgaataa gtaaggctcc gtagcctgg gctgagatgg |
| 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct | 421 ggataaggag tcaggattga agcccccaac gttctgccag acactggaca ggggttagct |
| 481 ctgaaatgt gttagagcaa gtgtagtgtg gtgtagtgt aggcacttct aggcccttc | 481 ctgaaatgt gttagagcaa gtgtagtgtg gtgtagtgt aggcacttct aggcccttc |
| 541 ccggctactg gatcactca gtgcttggg tactacggac cctctgccat ccattgcagc | 541 ccggctactg gatcactca gtgcttggg tactacggac cctctgccat ccattgcagc |
| 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa | 601 agagccgttt catgagcggg ggggctaagc gcagttctt gaatcaaac ttgaatgaaa |
| 661 tcgaaatcga tcttttita gatatccgga tagatggatg gatctatct tctattcata | 661 tcgaaatcga tcttttita gatatccgga tagatggatg gatctatct tctattcata |
| 721 tatatttgc aagaagcccc aaatcctga ttggccagg aaacaaagca ctgctttggg | 721 tatatttgc aagaagcccc aaatcctga ttggccagg aaacaaagca ctgctttggg |
| 781 cccaggaagc gaaggaatg agctcggctg ctctctccc acacttctt atttctcct | 781 cccaggaagc gaaggaatg agctcggctg ctctctccc acacttctt atttctcct |
| 841 gccgttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta tttcttcat | 841 gccgttccg catgcgcttc gcgcgccatt ggcgcttgc tctctctta tttcttcat |
| 901 ggacggctg gatggactc gccgttctt cccaacgaaa atggaaaggg ctgtatcaca | 901 ggacggctg gatggactc gccgttctt cccaacgaaa atggaaaggg ctgtatcaca |
| 961 tcgagatgt gttagctttt ccgccccaaa tgatggggg aattagtcac ctctgtctt | 961 tcgagatgt gttagctttt ccgccccaaa tgatggggg aattagtcac ctctgtctt |
| 1021 tcatctttt gaattgaatc gaggccccgc ccggctcgcg tegtccaac aaccgacggg | 1021 tcatctttt gaattgaatc gaggccccgc ccggctcgcg tegtccaac aaccgacggg |
| 1081 gagcactca gtatcagatc gcgcgagta actgggagtc ctattacacc ggcggccaac | 1081 gagcactca gtatcagatc gcgcgagta actgggagtc ctattacacc ggcggccaac |
| 1141 tccattcac caaaccagg ttcactcgt gtatgtattg tggactcgt caaggatag | 1141 tccattcac caaaccagg ttcactcgt gtatgtattg tggactcgt caaggatag |
| 1201 gactgacgg ttgatgtac agactgacc ctgtcttcc tagcatgat tccatccct | 1201 gactgacgg ttgatgtac agactgacc ctgtcttcc tagcatgat tccatccct |
| 1261 gtgcgaactg attcggtaag ctactgtctc ggtgcacgga aaac | 1261 gtgcgaactg attcggtaag ctactgtctc ggtgcacgga aaac |

3. Genetic Distances and Phylogenetic Construction Based on *nad-2* gene

Genetic distance of sago palm and others related plant based on *nad-2* gene markers were performed by blasting method and using Mega 6 software. Pairwise differences of sago palm accession and others related plant were presented in the Table 2. The genetic distances among sago palm accession were calculated no differences based on *nad-2* gene markers, but differences among others related plant which producing a large amount of starch. Otherwise, it was reported that among sago palm accessions were calculated different by using *atp6-2* gene marker (Abbas *et al.*, 2015). The use of different molecular markers might resulted 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 and dioecious species, whereas gynodioecious species 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 *nad-2* gene marker. Barr *et al.* (2007) was reported that the mitochondrial plant is possessing low substitution rates and no recombination

Phylogenetic construction of sago palm and others related plant based on *nad-2* gene markers showed that the phylogenetic divided into three major cluster. Sago palm, coconut, and date palm in is being in the same cluster into cluster 1. All of sago palm accessions are being in the same sub cluster in the cluster 1, not separated to the other branch of phylogenetic. Cereal plants that was 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 gen 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. *nad-2* gene might related to energy resources for accumulating starch in the stain part of plant. The NAD and NADP pyridine nucleotide pools were known play critical roles for regulating energy-producing in the catabolic process (Blacker and Duchon, 2016).

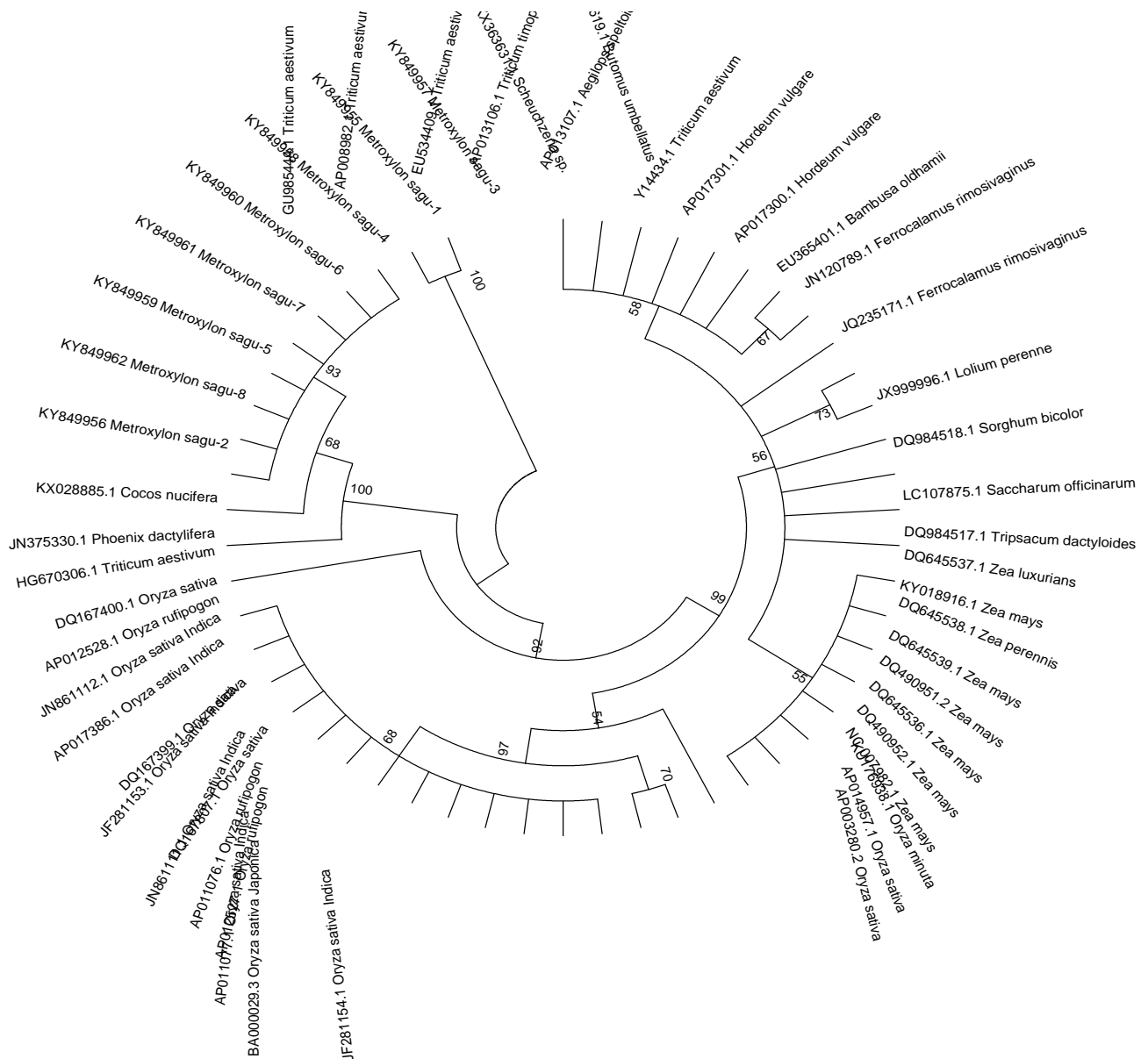


Figure 2. Phylogenetic construction of sago palm accessions and other plant producing carbohydrate based on *nad-2* markers

Conclusions

Morphological characteristics in the russet stages of sago palm accessions were divided into four phenotypic and several accessions show phenotypically the same. Based on *nad-2* gene was shown that the sago palm accessions have the same genetic constitution among the others. This assessments show that morphological phenotypic were not correlated with genetic arrangement of sago palm accession based on *nad-2* gene. Phylogenetic constructions of sago palm accession and other related plant based on *nad-2* gene markers were described that sago palm closer to the coconut and date palm and then following others plant producing large amount carbohydrates.

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