Chapter

Adaptive Evolution and Addressing the Relevance for Genetic Improvement of Sago Palm Commodity

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Abstract

Adaptive evolution implies evolutionary shifts within an organism which make it suitable and adaptable for its environment. Genetic resources of sago palm (*Metroxylon sagu* Rottb.) populations in Indonesia were explicated as follows: (1) Characters of sago palm in Indonesia were shown varied based on cpDNA markers and large variation based on RAPD markers. (2) Variation of starch production of sago palm correlated with *Wx* genes variation, (3) Distances barrier and geographies isolation in line of sago palm dispersions in Indonesia (4) Characteristics of genetic were observed does not related with vernacular names those were given by local people (5) Papua islands, Indonesia territorial is proposed the center of sago palm diversities, (6) Papua islands, Sulawesi islands and Kalimantan islands will be the provenance of the diversities (7) Genetic improvement of sago palm might enhanced using molecular marker that link to interesting genes by developing marker-assisted breeding.

Keywords: breeding, DNA, genetic, marker, *Metroxylon sagu*

1. Introduction

Information on adaptive evolution and genetic diversity of an organism are very important in supporting genetic improvement and germplasm conservation. Adaptive evolution implies evolutionary shifts within an organism which make it suitable for its environment. The improvements lead to improved chances of survival and reproduction. In order to conserve germplasm of an organism, information on genetic diversity is needed so that it can capture germplasm as a whole and efficiently in the implementation of germplasm conservation activities. In addition, information on the diversity of organisms needs to be documented to maintain information on the wealth and existence of certain types of an organism, including sago palm.

Several markers that can be used for accessing the diversity of an organism are morphology, protein, and DNA marker. Morphological and protein markers are not sufficiently used as indicators for measuring genetic characteristic because they are heavily affected by the surrounding factors. One of the markers that is not

influenced by the surrounding factors is a molecular marker. Thereby, in expressing adaptive evolution and genetic characteristics, it is necessary to be based on molecular markers. Disclosure of the genetic characteristics of organism such as plant in Indonesia will be better focused on molecular-based markers.

Several DNA markers that can be used for accessing adaptive evolution of an organism are: Simple Sequence Repeat (SSR) in the nuclear genome and chloroplast genome (cpSSR), Random Amplified Polymorphism DNA (RAPD), functional gene such as Waxy gene in sago palm, 5S, Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP), chloroplast DNA (cpDNA) such as *mat*K gene, and mitochondrial DNA (mtDNA) such *nad* gene. These molecular markers are widely used as markers to express adaptive evolution of plant.

SSR markers have been shown to have high polymorphisms in soybean and in apples [1–4], thereby, can be used for revealing the adaptive evolution of an organism. SSR is composed of 1–6 base pairs (bp) of repeated DNA sequences with varying amounts [5]. The polymorphic fragments (alleles) are produced from variations in the length of the SSR repeats which can be separated by electrophoresis to display the genetic profile of the genome and the organelle genome. SSR alleles are codominant monogenic inherited and can be distinguished between homozygous and heterozygous in segregated populations [1].

The advantages of SSR DNA markers or microsatellite markers in genome analysis are that SSR sequences are found in many eukaryotic genomes, high diversity, stable inheritance, co-dominant markers and high accuracy detection [6]. The RAPD marker is a technique that is widely used for genetic characterization because the RAPD technique is simpler than other techniques. Molecular markers related to the expression of certain genes are interesting molecular markers because it can be seen the variation of genes encoding certain characters, making it easier to trace genes that have specific expressions and are desired for the improvement of certain gene of organisms.

The *Wx* gene molecular marker is a marker related to the starch biosynthesis process and amplifies the plant DNA sequences that linked to the starch formation. The Waxy (Wx) gene in cereals and *amf* in potato is called isoform gene, Granule-bound starch synthase I (GBSS I) that it encodes starch synthesis [7, 8]. Furthermore, starch synthesis process is regulated by one of the key genes, those the *Wx* gene [9]. Starch from rice plants consists of amylopectin and amylose [10]. Furthermore, it was stated that the *Wx* gene regulates the level of amylose content in starch-producing plants such as wheat and rice [10–12]. The motive structure of the *Wx* gene was reported that it has a very conservative sequence [8] so it fulfills the requirements to be used as a marker. The *Wx* gene marker have been used in various types of crops, i.e. rice [13], barley [9], wheat [14, 15], and sago palm [16, 17].

Large numbers of insertions and deletions in the genome can be detected using agaros gel separation techniques. A technique that is more suitable for small changes in DNA sequences, such as mutations or small deletions or insertions, is fragment analysis using sequencer tools. The technique can detect a change in the size of one base in a DNA fragment. The use of a separation technique that is able to distinguish the differences of one base pair makes it possible to detect the genetic diversity of sago palm that occur at the individual and population. The estimation of adaptive evolution that occurs over a long period of time (hundreds to thousands of years) can be determined based on the chloroplast Simple Sequence Repeat (cpSSR) marker and barcode *mat*K gene in the cpDNA genome. The barcode *mat*K gene was commonly use in the vascular plant, such as Dipterocarpaceae [18], Arecaceae [19]

and in the species of sago palm also [20]. The variation that occurs in a relatively short period of time can be determined based on RAPD markers and other markers used to investigate the nucleus genome.

2. Adaptive evolution of sago palm

Diversity is a reflection adaptive evolution in an organism. Variations within a population and inter species that are affected by the occurrence of adaptive evolution. Adaptive evolution of sago palm can be measured by using various markers. The characteristics of sago palm in Indonesia were shown widely varies in morphological phenotypic. It was reported that around Sentani, Jayapura there are 15 varieties [21]. These varieties show variation in a broad sense, not only in morphological characters, but also in their adaptation to the environment (tolerant to fire and waterlogging). Furthermore, the variation of sago palm in Papua is very large based on morphological phenotypic, there are 96 varieties based on vernacular name [22]. The variation base on morphological phenotypic may differences from another population and location because morphological characters are strongly influenced by environmental factors. Observing the variation of sago palm need a marker that are not influenced by the environment so that they can reflect the actual state of plant variation. Markers developed in a wide variety of organisms including plants, namely chloroplast genome molecular markers (cpDNA) and nuclear genome molecular markers (RAPD, Wx gene expression, and others).

The cpDNA molecular marker is a very conservative molecular marker, so it is very suitable to be used to estimate long-term adaptive evolution for a particular organism. The cpDNA locus mutation rates was estimated between 3.2 x 10–5 and 7.9 x 10–5 [23]. Apart from this, cpDNA sequences are conservative in comparison to nuclear genome because they do not undergo recombination in the genome and uniparental inherited [24, 25]. Based on the information found in the chloroplast genome, it is a difference that occurred hundreds or thousands of years ago.

The cpDNA markers were developed in plants showed that the cpDNA of sago palm varied, the total 10 haplotypes were found throughout Indonesia territorials [26]. Seven haplotypes were found on the island of Papua and three haplotypes were found apart from the island of Papua and two haplotypes were found on several islands (sharing haplotypes). Based on highly conservative cpDNA criteria, the variations in cpDNA detection were reflect conditions hundreds or thousands of years ago. It is hypothetically that gene flow of sago palm since ancient times moving from one island to another in various ways. It was found that only two haplotypes experienced displacement. This phenomenon was corresponded of *Pinus silvestris* L. and *Abies alba* Mill referred to as the refugee population [27, 28].

Base on the largest number of haplotypes were found on several islands where sago samples were taken, the island of Papua is the center of sago diversity because the island of Papua has the highest number of cpDNA haplotypes. Large amount of diversity is found in natural populations [29]. Based on this statement, it can be said that the sago palm in Papua is a natural population (not refers to a migrant population). When talking about the source of diversity, the islands of Papua, Sulawesi and Kalimantan are the sources of diversity of sago palm because it has a specific haplotype. Large number of haplotypes reflects the high variation or diversity in a population [28] and differences in cpDNA

haplotypes in each population reflect differences in genetic entities (sources of variation) [29].

Based on the developed molecular markers of the chloroplast genome (cpDNA) and nucleus genomes, it was revealed that individuals with different local names within and between populations were generally not different. This indicates that the environmental influence on the appearance of the morphological phenotype is very large because the local name given by the local community is based on morphological phenotypic and local language. In Papua alone, there are a lot of regional languages which make the local names for the sago palm too many. People in Jayapura (West, Central, and East Sentani) give local names for one type of sago palm which differs from one another [30]. If the grouping and naming of sago palm varieties is based on local names, there will be a very large number of vernacular names comparing from the real thing. It was documented that in Papua there are 96 vernacular name of sago palm [31]. Furthermore, the farmers indicated that there are 21 varieties in Sentani and Scientist only recognized 15 varieties out of 21 varieties based on morphological phenotypic [21]. Based on this information, it reflects confusion and there is an overlap in the naming of varieties, which makes the classification and number of varieties recorded larger than the real thing. Cases like these are make molecular markers play an important role for clarification as well as correction of varieties number.

Molecular markers of the chloroplast genome and nucleus genome developed on sago palm detected that sago palm in various islands in Indonesia experienced high diversities as seen from the varying values of genetic diversity: $\sum$H, HE, S, G, $\hat{H}$, V$\hat{H}$, π, πn, and P. This means that in a population there are individuals who are very different from one another. In general, it can be interpreted that the sago palm scattered in various islands in Indonesia, even though the samples from the island of Java with the Wx gene marker and samples from the islands of Ambon and Java with the nucleus genomic SSR markers are not differentiated. This is probably due to the discriminatory focus of each molecular marker that is different from one another. The Wx gene marker focuses its discrimination on genes encoding the biosynthesis of amylose. If the DNA sequence of the *Wx* gene in the population sample did not vary like the population sample from Bogor, then the amylose content did not vary either. Various *Wx* gene alleles determine the amylose content in starch-producing plants [10, 12, 32].

Based on the codominant molecular markers (*Wx* genes and nucleus genomic SSR) used, it shows that the level of heterozygosity of sago palm in various populations in Indonesia varies in terms of the ratio of heterozygous and homozygous values. Based on the *Wx* gene marker, it shows that the samples from the Palopo and Bogor populations are all heterozygous, in contrast the SSR markers of the nuclear genome of the individual samples from the Ambon and Bogor populations are all homozygous. This phenomenon reflects the degree of individual heterozygosity depending on the particular character observed. The heterozygous diversity of the *Wx* gene was relevant to the quality and quantity of plant starch production which also varied. Starch content of sago palm varied as well as the accumulated dry matter [21]. Variations in the *Wx* gene in wheat caused variations in the viscosity of the resulting starch production [15]. The heterozygosity values based on the nucleus genomic SSR markers also varied, although they were not as high as the heterozygosity values of the *Wx* gene markers [16]. SSR markers when designed based on SSR sequences information of the plant genome under study will produce high levels of polymorphism. Previous studies on various types of plants have shown that SSR markers are commonly used to measure adaptive evolution because of their high rates of polymorphism [33–36].

Genetic hierarchy and genetic differentiation based on chloroplast genome markers and nucleus genome indicate that sago samples with cpDNA markers and *Wx* genes differentiate at individual and population levels [16, 19, 26, 37]. Furthermore, samples with RAPD markers experience differentiation at the individual and population levels [16, 26]. The levels of genetic hierarchy observed were individual, population, and island levels [38]. On the other hand, the SSR marker of the nucleus genome was only a sample between populations from the island of Papua which experienced differentiation. This difference is strongly influnced by the nature and the degree of polymorphism of the genetic markers used. The conservative genetic markers such as *mat*K gene markers and mitochondrial *nad2* gene markers tend to show lower levels of polymorphism and only at lower levels of genetic hierarchy are significantly different [20, 37]. Low levels of polymorphism between populations and did not experience genetic differentiation in Pinaceae using the cpSSR marker, but with the RAPD marker, high polymorphism and genetic differentiation were found [39]. Furthermore, the cpDNA characters that evolved in the cotton genus were low [40].

Genetic relatedness of the population based on phylogenetic constructs shows that the SSR molecular marker of the nucleus genome divides the sample into two groups, the cpDNA and RAPD molecular markers divide the samples into three groups, and the *Wx* gene molecular marker divides the sample into four groups [17]. The variations that occur may be due to the different nature and focus of discrimination for each molecular marker used. This case is something that is often encountered in various kinds of molecular markers. Previous studies have shown that different molecular markers infer variability, genetic relatedness, and adaptive evolution of individual or population variations [40–42]. Furthermore, the genetic variation in *Pseudotsuga menziesii* (Mirb.) Franco. using univarentally inherited (cpSSR) and biparental inherited (isozyme and RAPD) molecular markers concluded that the level of polymorphism and differentiation of cpSSR markers was lower than that of isozyme and RAPD markers [39].

Based on molecular markers of cpDNA, RAPD, Wx genes, SSR nucleus genome, cpDNA *mat*K gene, and mitochondrial *nad*2 gene, it shows that sago palm in Indonesia are diverse [17, 19, 37]. The relevance of genetic diversity generated by molecular markers of the chloroplast genome and nucleus genome with the morphological diversity that has been revealed by sago plant researchers is that they both reveal that sago palm in Indonesia are diverse, but the level of diversity based on genetic markers is lower than that based on morphological markers [43]. The variation of sago palm in Papua is very large based on morphological phenotypics, namely that in total there are 96 varieties found from eight locations (Salawati, Waropen, Sentani, Kaureh, Wasior, Inanwatan, Onggari, and Windesi) in Papua and west Papua Province [22]. It was reported three varieties of sago palm in Kendari, Southeast Sulawesi [21]. Furthermore, it was documented 11 varieties of sago palm in Southeast Sulawesi, North Sulawesi and North Ambon based on morphological characteristics [44]. Genetic diversity based on the molecular markers that have been disclosed classifies sago palm in Indonesia from two to four groups. It was reported that sago palm is divided into two clusters and two sub-clusters [45]. The Morphological performance of Sago palm forest is shown on **Figure 1** and the morphological performance at the russet growth is shown on **Figure 2**.

Based on the molecular markers that have been used on sago palms, nothing has been associated with the morphological characters. The same thing was also that spineless and spiny of sago palm was not related to genetic distance based on RAPD markers [45]. It is believed that spine and spineless in sago palm is controlled

Figure 1.
Morphological performance of sago palm forest.

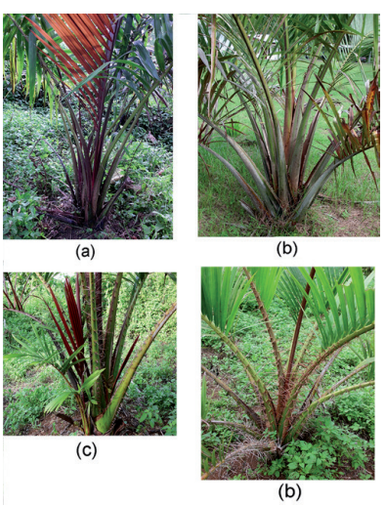

Figure 2.
Morphological performance of sago palm in the russet growth. Spineless with purple color of the young leaf (A), spineless with green color of the young leaf (B), spiny with purple color of the young leaf (C), and spiny with green color of the young leaf (D).

by certain genes, so that there are certain nucleotide sequences in the sago palm genome that undergo transcription and translation processes in the spine formation process. Molecular markers encoding the characteristics of sago palm can be designed if desired by reverse transcription of sequences encoding protein synthesis for spiny formation in sago.

Genetic relatedness based on the phylogenetic constructs of each tested molecular marker shows that the distribution of the level of sample similarity according to the size of genetic distance is not limited by location and geographical isolation because samples from one island to others islands blending with each other [17]. The blending of Stylosanthes sp. obsessions from various regions in the dendrogram construction indicates that the obsessions have geographic distribution [46]. It was reported that, if there is distance and geographic isolation in the long term, the population from one region to another will experience differentiation or adaptive evolution as happened in Brassicaceae [47]. Furthermore, Scientist documented that population differentiation of O. rifipogon affected by distance or geographic isolation [36].

3. Genetic assessment of sago palm

3.1 Random amplified polymorphism DNA (RAPD) marker

RAPD polymorphisms amplified on the PCR machine produced polymorphic fragments and the number of genotypes of each population. RAPD polymorphisms and high number of genotypes are a reflection of plant genetic diversity and adaptive evolution based on RAPD markers [38]. This result is in line with the diversities of sago palm revealed by using RAPD markers on several samples from Indonesia and Malaysia [48].

Population genetic diversity shows that the population samples from Papua have the highest of polymorphic sites number (S), the moderate of pairwise differences values (π), and the highest percentage of haplotype polymorphic compared to other populations from several islands in Indonesia [38]. Genotype diversity equal to one means that no identical genotype is found in a population sample. The value of $\hat{H}$ of individual samples at the island level all shows number of one, which means that one sample of individuals with another sample of individuals differs from one another based on the RAPD markers. Sago progenies obtained from semi-cultivated sago populations showed genetic differences among the progeny tested [43]. The varying values of S, π, and $\hat{H}$ indicate the genetic variation values of sago palm population in Indonesia. In the previous studies of sago palm by using RAPD markers showed that the sago palm diversities among individual was recorded also high [48] and other scientist reported 15 varieties of sago palm based on morphological characters in around Sentani lake [21].

The genetic hierarchy was estimated based on Analyses Molecular of Variance (AMOVA) calculations. The AMOVA calculation value shows that 89.35% of the total variety of samples is contributed by individuals with very significantly different with probability (P) values, 6.58% and 8.4% variance is contributed among populations [17]. The rates of diversities and adaptive evolution were detected in sago palm, those related to the genetic diversity of *M. sativa* L. by using RAPDs marker [47] as well as *Cynara scolymus* L. [49]. The statistical test method used to reveal the differentiation that occurs at the population and island level is also found to be used to reveal the differentiation that occurs at the population level in various types of plants, such as in *M. sativa* L [47], in *Acacia radiana* [50], and in *Primula elatior* (L.) Oxlip [51].

Genetic relatedness among individual shows that the sago palm were classified into three groups based on the dendrogram construction. Group I include sago palm from all the populations as well as group III, while Group II includes sago palm from Jayapura, Serui, Manokwari and Ambon. This is related to the grouping of sago palm that it was reported in the previously study and divided sago palm from Indonesia and Malaysia into two groups and subgroups based on RAPD markers [48]. Previous sago genetic studies that focused on the Indonesian archipelago showed that sample individuals were divided into four groups based on RAPD markers [52]. Grouping of individuals in a dendrogram is largely determined by the genetic distance used, the method of grouping, and the desired bootstrap coefficient or rate. The differences between the groupings based on the cpDNA markers and the RAPD markers observed in previous studies are common in genetic relatedness studies [24, 39, 41].

Genetic relatedness among population shows a clustering pattern similar among individual. Genetic relatedness based on the dendrogram sample construction at the island level shows that the samples from the island of Papua are more closely related to the samples from Sumatra and Kalimantan, the samples from the island of Sulawesi are closely related to the samples from Ambon, and the samples from

the island of Java are separate from other islands based on the RAPD marker [38]. Here there is something interesting to observe because the sample at the island level forms a group together with samples from other islands that are far away, such as the sample from the island of Papua which forms a group together with the sample from the island of Sumatra. When examined from the migration side, it is possible that individual sago palm from Papua population have mingled with sago palm from the island of Sumatra. This phenomenon is possible because the molecular markers (RAPD) used are not as conservative as the cpDNA molecular markers that are uni-parental inherited [24, 25]. The RAPD marker is a nucleus genomic molecular marker associated with the DNA recombination process and is biparental inherited [39] so that the RAPD marker is a molecular marker that has a relatively short conservative time (one generation) compared to the cpDNA molecular marker. Previous studies suggest that higher variation is found using nucleus genomic markers rather than cpDNA markers [39–41, 53].

3.2 Gene encoding starch biosynthesis (waxy genes)

Polymorphisms of *Wx* gene markers that were found of 8 polymorphisms alleles and 14 genotypes of the *Wx* genes [16]. The polymorphism detected in sago palm was in line of the polymorphism in *Triticum aestivum* L. by using the *Wx* (SunI) gene markers [14]. The number of alleles and genotypes of sago palm at the level populations and islands varies as well as their frequency [17]. The Wx gene variations found in sago are similar with the *Wx* gene variations on wheat [15]. Furthermore, Scientist were reported a high Wx gene polymorphism in barley [9] and in rice [13]. This phenomenon indicates that the source of the *Wx* genes diversity is the Papua islands Papua and Sulawesi islands because these islands are found genotypes that are not found on other islands [16]. If the center of diversity is the object of attention, then the island of Papua is the center of diversity of the *Wx* genes because the most genotypes of *Wx* genes are found on the island of Papua [17].

The genetic diversities of *Wx* genes that was observed to the sago palm from various islands were shown varied. The genetic diversities calculation results showed varying values except for samples from Jawa [17]. The sago palm variations were detected, those a reflection of sago palm variations that it occurs in the several islands in Indonesia [16]. The *Wx* gene is one of many genes that it is regulated biosynthesis process for resulting starch of plants, including sago palm. If the *Wx* gene has high variations that will be resulting various quantity and quality of starch. In the previous studies were reported the quantities of starch accumulation of sago palm range from 28 to 710 kg trunk^{-1} [45] and starch accumulation of sago palm trunk^{-1} will be depend on the varieties [21]. The *Wx* gene was one of the genes that influenced of starch synthesis in rice endosperm [54]. Two alleles of the Wx gene that is Wxa and Wxb gene were reported regulating to increase *Wx* protein and amylose content [10]. *Wx* allelic pulp in wheat showed a significantly different reduction in amylose content [12] and recombinant inbred line (RIL) of wheat that has integrated three *Wx* genes in their genome was reported resulting high quality starch than wheat RIL which did not contain the three *Wx* genes [32].

The heterozygote values of sago palm in the populations that was observed were shown variation from 0.52–1.00 with a low standard deviation of 0.0000–0.0014 [17]. The heterozygosity variations were indicated variations in the *Wx* gene in the genome of sago palm. The key gene that influences starch synthesis in rice endosperm is the *Wx* gene [54]. Variation of the *Wx* gene causes a variation in the viscosity of starch production in wheat (Boggini et al. 2001). The *Wx*a and

*Wx*b alleles were found to regulate quantitative levels of *Wx* protein as well as amylose content [10].

The genetic hierarchy calculation using AMOVA shows that individuals and populations was estimated significantly different [17]. The differentiation values based on the chi-square test at the population and island level were found sago palm differentiated that occurs at the population and island level [17]. The detected variance is an indicator that the *Wx* gene varies both at the individual level and at the population level. Previous studies of sago palm using different markers also showed that sago palm varied both in terms of quantity and quality of production [21, 45]. The allelic levels of *Wx* genes and their interactions in starch-producing plants were reported increasing quality and quantity of starch production [10, 11, 32], and [55]. It is predicted that the *Wx* gene variation in sago palm is one of the genes that determines the variation in the quantity and quality of sago starch yields [16]. The sample diversity at the inter-island population was not significantly different based on the AMOVA value as was the sample at the inter-island population. This phenomenon indicates that the variation of the *Wx* gene in sago palm is more caused by variations at the individual and populations, not due to the isolation of different distances and geographic differences due to the low FCT value of 0.06044 [17].

Genetic relationship among individual shows that sago palm are grouped into four groups based on dendrogram construction [17]. The division into four groups was strengthened by the MDS test which showed the sample was distributed in four quadrants. The data illustrates that certain individuals are not grouped based on population origin but rather mixed with each other with different population origins and different local names [16]. This description implies that local names are not appropriate when used as a reference for determining the number of species or varieties of sago palm without the support of other data such as molecular data. In the vicinity of Sentani Lake, the local community revealed that there were 21 types of sago palm based on morphology and scientist found only 15 species based on the same marker [21].

Genetic relationship of sago palm in the population level shows that sago palm from the populations of Jayapura, Serui, Sorong, and Pontianak are closely related and form group I, samples from populations from Manokwari, Palopo, and Selat Panjang cluster to form group II, then groups III and IV only formed from one population. The grouping of the population into four groups is also strengthened by the MDS test which shows the population sample is distributed in four quadrants [16]. Previous studies have discussed the genetic relationships of populations using various markers [39, 47, 49, 51]. Populations contained in one group are closely related, on the other hand, populations in different groups are not closely related. The differences in a population is thought to be caused by outbreeding so that the population experiences differentiation. Population differentiation can be caused by pollen migration [56]. In general, it can be interpreted that there is a tendency for sago palm in Indonesia to be differentiated inter-island and among island based on the *Wx* gene marker. Differentiation can be caused by evolutionary processes, georaphic isolation, distance isolation, genetic drift and gene flow. Population differentiation is caused by evolution, natural selection, migration, and genetic drift [57] and the differentiation of Cruciferae due to gene flow [58].

3.3 Chloroplast DNA (cpDNA) marker

Based on cpDNA markers, various polymorphic and haplotypic alleles were found in sago palm. Studies related to the use of the NTCP21 and NTCP22 markers

in potato have also demonstrated allele polymorphisms in potato [59]. Locus rpl1671, NTCP21, and NTCP22 on sago were detected in three haplotypes out of 10 haplotypes which were specific haplotypes in populations from Jayapura and one specific haplotype each for populations from Serui, Palopo, and Pontianak [26]. The specific haplotype phenomenon is also found in several types of plants i.e. Cunninghamia spp. [60], *Pinus sylvestris* L. [27], and *Alyssum* spp. [29]. The specific haplotypes were found in a population, those indicated the source of diversities in a population. The specific haplotypes of sago palm were found in the populations of Papua, Sulawesi, and Kalimantan indicated the provenance of the diversities, while the most haplotypes of sago palm diverse is the population from Jayapura then followed by the sago palm population from Serui [17]. The large number of haplotypes reflects the high variation in a population in line of the *Abies alba* Mill population [28]. The differences in chloroplast haplotypes in each population reflect differences in genetic entities or sources of variation [29]. The number of haplotypes that were found to be present together in each population is an indication that genetic similarities among individual in a population. It is hypothetically that the sago palm migration by carrying of people. Four haplotypes of 10 haplotypes of sago palm were found in to two or more populations, which means that only four haplotypes were found migration through various kinds of intermediaries. The same thing was also found in *P. sylvestris* L. and *A. alba* Mill. referred to as the refugial population [27, 28].

Population genetic diversity shows that the population from Papua has the highest number of haplotypes ($\sum$H), the number of polymorphic sites (S), and the highest percentage of haplotype polymorphics compared to other populations. A value (HE) equal to one means that no haplotype numbers are the same in individual samples in a population (single haplotype individuals) as happened in the population from Bogor. This is similar with individual haplotype on *P. sylvestris* L [27]. Previous studies on sago palm using RAPD markers showed that sago plant diversity at the individual level was also high [48, 52].

The genetic hierarchy based on cpDNA was estimated by using analysis of molecular variance (AMOVA) was calculated of differentiation level of population samples at the inter-island level (−3.88% and FCT = −0.03884), between populations within islands (8.49% and FSC = 0.08177), and Papua and others (5.05% and FCT 0.05054) which is low with the probability value not significantly different. High percentage values of variance were observed at the level among individuals (95.39% and FST = 0.04610) and between populations (5.91% and FST = 0.05914) with significantly different probability values [26]. The same thing was also found in *P. sylvestris* L., namely the percentage value of variance between populations (3.24%) with a significantly different probability [27]. The negative value observed at the inter-island level indicates that the sample island level does not contribute to the total measured variance. This phenomenon resembles the tetraploid alfalfa population [47]. Negative correlation coefficients have a biological significance in that the samples at the inter-island level are more closely related than those at the island level [61]. Based on this, it indicates that island or geographic differences do not cause variations in the chloroplast genome, even though the distance between one island and another is far (hundreds to thousands of kilometers). The variation between individuals and between populations contributed 95.39% and 5.91% to the total variety and was significantly different [18]. The results observed were similar with Abies species that was only a small variance value between populations (6.10%), high proportion of variance within the population or between individuals (74.66%) [62]. A low trend of genetic variability between populations is also found in Pinaceae [39] and species other than pine [63].

Genetic differentiation based on the Fst value shows that among the populations being compared, only the population from Jayapura is significantly different from the population from Palopo and Pontianak [26]. These populations based on cpDNA markers each have a genetic entity, which means that the diversity that occurs in this population has appeared separately since ancient times (thousands of years ago). The genetic differentiation of samples at the population level based on the X^2 test shows that the population originating from Jayapura is different from the population originating from Serui, Manokwari, Sorong, Palopo, Pontianak, and Selat Panjang and the population originating from Serui is different from the population originating from Pontianak but not different from other populations [17]. Based on the X^2 test at the population level, it indicates that the source of sago plant diversity at the population level is the population from Jayapura, Serui, and Pontianak. Genetic differentiation of samples at the island level based on the X^2 test shows that samples originating from the island of Papua are different from samples from the islands of Sulawesi and Kalimantan [26]. This is in line with the specific haplotypes found on the three islands. For this reason, it is suggested that the sources of sago palm diversity based on the samples tested are the islands of Papua, Sulawesi and Kalimantan. This data is also consistent with the grouping of sago palm samples through phylogenetic construction at the island level which divides the samples into three groups, namely the Papua group, the Sulawesi group and the Kalimantan group. This indicates that the source of the diversity of sago palm in Indonesia, apart from being on the island of Papua, is also found in other islands, namely Sulawesi and Kalimantan [26].

The genetic relationship of the samples at the individual level shows that the samples are classified into three groups based on the phylogenetic construction. Sago palm relationship studies previously show that sago palm originating from the Malay Archipelago and several samples of sago from Indonesia clustered into two groups and two sub-groups based on the RAPD markers [48]. The sago relationship study focused on the Indonesian archipelago, but with a larger number of samples, showed that the sample individuals were divided into four groups based on RAPD markers [52]. The discrepancy in the division of the number of groups (groups) between the groupings based on cpDNA markers and RAPD markers that was observed in previous studies is something that is often found in studies of genetic relationship using molecular markers. Previous genetic related studies which showed that different molecular markers led to different groupings of certain plants by using cpDNA, RAPD and isozyme markers in Pseudotsuga spp. (Pinaceae) [39], cpDNA and inter-SSR (ISSR) markers in the nucleus genome on *Brassica oleraceae* L. plants using [41], and using cpDNA and mitochondrial DNA on apple plants [24].

Based on cpDNA, the sources of sago diversity in Indonesia are predicted to come from three islands, namely Papua, Sulawesi and Kalimantan. It is suspected that from these three islands, individual sago palm experienced migration in line with migration and population mobilization in Indonesia that had occurred hundreds of years ago. This assumption is reinforced by haplotype data, phylogenetic analysis, and genetic hierarchies which show that samples at the inter-population level and between individuals are significantly different, which means that there are one or more different individuals or populations. Although the source of diversity is found in three islands, if the number of haplotypes is the size of the center of diversity, then the island of Papua is the center of diversity of sago palm in Indonesia because that island is found in the largest number of haplotypes compared to other islands. Apart from the highest number of haplotypes, on the island of Papua, the wild relatives of sago palm

are found. If the data obtained is linked to the incidence of sago distribution in Indonesia, it is strongly suspected that only four haplotypes experienced migration from one population to another, which were then given different local names. The sago population with a specific name for the origin of the Papua region which groups together with populations from other places with other names is also a reflection that in the past these sago populations were only one then experienced joint migration with the migration of people from one island to another or from one population to another. If population migration events have occurred hundreds of years ago and are thought to have caused sago palm to spread from sources of diversity to form new populations or join old populations on islands that are sources of diversity, it is still possible because the measure of similarity is cpDNA, which has very conservative sequences [64], a very low mutation rate of between 3.2×10^{-5} and 7.9×10^{-5} [23], is not recombinant [24, 64] and are inherited uniparental [25, 39].

3.4 Genetic improvement by using marker-assisted breeding (MAB)

The development of genetics and technology molecular has facilitated our understanding of the genetics underlying the traits sought by plant breeding. The development of molecular markers allows plant breeding to develop faster and more advanced in producing superior organisms. The benefits of DNA markers are for germplasm characterization, selection of desired traits from genomic regions involved in the expression of traits of interest, and single gene transfer. The application of selection using efficient and effective markers to improve polygenic properties certainly requires new technology. Genetic improvement of sago palm may use transformation agrobacterium-mediated and particle bombardment. Successfully introgression *bar* and *gus* gene into sago palm genome [65]. The embryogenic callus was the most appropriate transformation material compared to the via callus, the embryoid stage and the shoots initiated by using *Agrobacterium*-mediated. The transformation of the gene gun demonstrated greater efficiency of transformation than those transformed with Agrobacterium when targets were bombarded once or twice with 280 psi helium pressure at a distance of 6 to 8 cm [65]. Therefore, economics interesting genes may introgression into sago palm genome in the future.

The purpose of MAB is to enhance certain characteristics in plant or animal breeding programs. Strategy for rapidly integrating a targeted gene into a wheat genotype in only two generations and restoring 97% or more of the recurrent genotype of the parent by using MAB [66]. Deconvolution of ancestry offers a first step towards selection of suitable admixture profiles at the seed or seedling level, which will support marker-assisted breeding aimed at introgressing wild Vitis species while maintaining the desirable characteristics of elite *V. vinifera* cultivars [67]. Marker-assisted backcrossing can be used in plant breeding to integrate traits into elite cultivars while minimizing the transfer of unwanted alleles from the donor genome [68]. This method includes the selection of foreground as well as context. Foreground selection refers to offspring screening and selection based on the presence or absence of a particular allele associated with a feature of interest. Conversely, selection of offspring on the basis of genomic ancestry estimates is the history selection.

The MAB needs to be developed to accelerate and increase the success of the breeders to produce superior seeds. Recently, breeders were developed abundant MAB linked with specific characters of plant genetics. Simple sequence repeat (SSR), namely Md-PG1$_{SSR}$10kd tightly linked with fruits texture of apple [69] and microsatellites RM5926 and AP5659–5 were developed for detecting rice

blast resistance genes, those markers tightly linked with *Pi*-1 and *Piz*-5 genes respectively [70]. Marker-Assisted Introgression of b-carotene hydroxylase was developed for detecting b-Carotene Rich in maize hybrid [71]. Furthermore, Muthusamy et al. (2014) stated that B-carotene concentration among crtRB1-introgressed inbred ranged from 8.6 to 17.5 mg/g - a maximum increase up to 12.6 times over recurrent parent. In comparison to 2.6 mg/g in the original hybrid, the reconstituted hybrids formed from improved parental inbred also showed enhanced kernel *b-carotene* as high as 21.7 mg/g [71]. This study may use as a model for increasing quality starch that resulting of sago palm and other plant in the current time and in future time.

4. Conclusions

Genetic resources of sago palm in Indonesia were explicated as follows: (1) Characters of sago palm in Indonesia were shown varied based on cpDNA markers and large variation based on RAPD markers. (2) Variation of starch production of sago palm correlated with *Wx* genes variation, (3) Distances barrier and geographies isolation in line of sago palm dispersions in Indonesia (4) Characteristics of genetic were observed does not related with vernacular names those were given by local people (5) Papua islands, Indonesia territorial is proposed the center of sago palm diversities, (6) Papua islands, Sulawesi islands and Kalimantan islands will be the provenance of the diversities (7) Genetic improvement of sago palm might enhanced using molecular marker that link to interesting genes by developing marker-assisted breeding.

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