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Advances in Agronomy, Plant Breeding and Horticulture

Vol. 7 (2), pp.28 - 34, April, 2019

Appraisal hereditary variety and relationship of sago palm (*Metroxylon sagu* Rottb.) in Indonesia dependent on explicit articulation quality (Wx qualities) markers

Sebastien Christian and Swennen Rony

Faculty of Bioresources, Mie University, Japan.

Abstract

Starch production of sago palm based on physical and chemical properties were observed in a large variation in the previous study. The important gene markers used for the assessment of the genetic variation and relationships which is related to the starch production is wx gene markers. The generating data based on the Wx gene markers were described in 8 alleles and 14 genotypes on sago palm in Indonesia. The sizes of alleles range from 100 to 700 bp. Many vernacular names were given by local people where the population is located. However it has different names, but it is genetically the same. The vernacular names do not accurately describe the types of the sago palm. The genetic relationships of sago palm were observed in a higher variation in the level of individual sampling stages, then followed by the populations sampling and the islands sampling stages. Sago palm samples were clustered into four groups in the level of individuals, three groups in the level of populations, and two groups in the level of islands. The existence of the specific genotypes in both population, Serui in the Papua islands and Palopo in the Sulawesi islands are considered to be germplasm resources. The Papua islands have the largest genotype; therefore, it is proposed to be the center of genetic diversity of sago palm in Indonesia.

Keywords: Sago palm, wx gene, population, genetic variation, genetic relationship.

INTRODUCTION

The most important product of sago palm is starch, which contains a large amount of carbohydrates and is used for many purposes. Capabilities of sago palm accumulated starch in their trunk measured up to 200 to 220 kg per palm (Jong, 1995). Starch from sago palm contains 84.7% carbohydrates which consist of 73% amylopectin and 27% amylose (Wiyono and Silitonga, 1989). Sudradjat (1985) reported that rendement of sago palm extraction can be reached at 38.23% which consist of 73 to 86% starch, 0.18 to 0.22% lipid, and 0.42 to 0.44% protein. Azudin and Noor (1992) demonstrated that the starch compositions of sago palm in Malaysia are 0.55 to 0.93%protein and 0.01 to 0.20% fibers.

Starch qualities and quantities that result from varieties of sago palm were reported variously, so that it might reflect the genes encoding starch biosynthesis in sago palm differently. Starch production of plant is regulated by ADP-glucose phosphorylase (AGPase) enzyme. AGPase was identified as the main enzyme for starch biosynthesis and carbohydrate polymerization (Li et al., 2002; Smidansky et al., 2002). Starch biosynthesis in tubers identified depends on AGPase enzyme which stimulated starch synthesizes (Tiessen et al., 2002). Alfa Glucan Water Kinase enzyme is the main enzyme for regulation of starch phosphorilation so that starch that is formed by plant can be mobilized and transported to the sources of the starch accumulation (Blennow et al., 2002). Singh et al. (2002) isolated four cDNA clones which encoded two large subunits of AGPase (CagpL1and CagpL2) and two small subunits AGPase (CagpS1 and CagpS2). Shurunken2 (Sh2r6hs) gene that encoded AGP large subunit in corn is transferred to the wheat (*Triticum aestivum* L.) to increase seeds and

Table 1. The populations and the numbers of sample used.

Island	Population	Numbers of sample
Papua	Jayapura	6, 7, 9, 11, 14, 24, 27, 34, 35, 46, 49, 50, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100
	Serui	1, 3, 5, 12, 18, 25, 26, 38, 43, 44, 47, 48, 73, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85
	Manokwari	2, 4, 19, 20, 21, and 22
	Sorong	8, 13, 17, 28, 69, 70, 71, 72, 74
Ambon	Ambon	10, 41, 45
Sulawesi	Palopo	36, 37, 39, 40
Kalimantan	Pontianak	51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68
Jawa	Bogor	15, 16
Sumatra	Selat Panjang	23, 29, 30, 31, 32, 33, 42

biomasses of the wheat production (Smidansky et al., 2002).

Waxy (Wx) gene in cereals and *amf* gene in potatoes were named GBSSI isoform genes because they encode starch production (Edwards et al., 1996) and *iAGPLI*-1, *iAGPLI*-2, and *iAGPLI*-3 genes were identified as genes encoding isoform of AGPase large subunit (Harn et al., 2000). The Wx genes reported regulation of amylose content levels in the plant resulted in starch such as wheat and rice (Wickramasinghe and Miura, 2003; Wanchana et al., 2003; Sharma et al., 2002; Zhang et al., 2011). The Wx genes structure motives are very conservative sequences (Gamer et al., 1998) so that the Wx genes fulfil requirement for using it as markers. The Wx genes were found, functioning as markers in several species of plant such as: *T. aestivum* L. (Shariflou and Sharp, 1999; Boggini et al., 2001; Saito et al., 2009; Huang and Babel, 2011), *Oriza sativa* L. (Bao et al., 2002), and barley (Domon et al., 2004).

Mirron et al. (2002) stated that the biosynthesis of starch in *Lycopersicon* sp. varied, consequently starch biosynthesis genes in the sago palm genome are expected to vary as well. The objectives of this study is to reveal the genetic variation and genetic relationship of sago palm in Indonesia based on Wx gene markers.

MATERIALS AND METHODS

Sample collection

A total of a 100 samples of sago palm were collected from several populations of sago palm in Indonesia. The populations and the numbers of samples that where used in this study are presented in Table 1. Leaf samples were collected and preserved using silica gel granules in ziplock plastic bags according to previous procedures reported (Chase and Hill, 1991). DNA extraction and purification of dried leaf samples was performed using the Qiagen DNA extraction kit following the instructions of the manufacturer.

PCR amplification

Wx gene primers used in this research as follows: forwards (5'-ttggcacacagctctcattc-3'), reverse (5'-ggccttggtaggcaatgtta-3'). The primer pairs were designed based on alignment sequences accession AF079258, X65183, X03935, and X58435 of NCBI Gene Bank Websites by using Software Primer-3 and synthesized by Qiagen. PCR mixtures and cycles condition were followed by 25 µl total volume which contain: 2.5 µl 10 × PCR buffer contained 15 mM $MgCl_2$ (Perkin Elmer), 0.5 µl dNTP 2 mM (GeneAmp^R mix), 10 µg BSA, 50 pmol primer forward and reverse, 0.42 U Ampli Taq Gold^{TM} (Applied Biosystems) and 10 ng genomic DNA. PCR cycles condition is as follows: initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min (8 level touchdown) at 63°C first cycles, decreased 1°C per cycles until it reached 55°C, then 27 cycles at 5 5°C, extension for 2 min at 72°C, and end extension for 8 min at 72 °C at the end of 35 cycles. PCR amplification fragments product were separated on 2% agarose gels by electrophoresis, staining was done using Ethidium Bromide and visualization by using Densitograph apparatus.

Data analysis

The dissimilarity matrix was calculated by using distance co-efficient. Dissimilarity was employed for clustering individuals, populations, and islands state of the sago palm in Indonesia by the Unweighted Pair-Group Method Arithmetic Average (UPGMA), using the Sequential Agglomerative Hierarchical Nested Cluster Analysis (SAHN-clustering, Sneath and Sokal, 1973) and TREE program from NTSYS-pc, version 2.02 packages (Rohalf, 1998). Bootstrap analysis of 1,000 permutation times were performed with the aid of software Tools for Genetic Analysis (TFPGA 1.3). Exact test were calculated using chi-squares (X^2) and performed using TFPGA 1.3 software (Miller, 1997).

RESUTS AND DISCUSSION

Wx gene variation

Eight polymorphic alleles and fourteen genotypes of Wx genes were observed in the sago palm genome. The alleles ranging from 100 to 700 bp were detected on agarose gels. The samples of polymorphic fragments are presented in Figure 1. Polymorphism levels detected in sago palm were similar to the levels found in the *T. aestivum* L. genome by Shariflou and Sharp (1999) using a Wx gene (Sun1) primer. Wx gene variations observed in the sago palm genome were similar to those found by

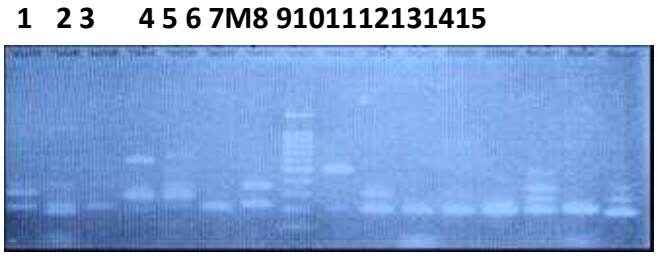

Figure 1. Samples of PCR amplification fragment results based on Wx gene marker.

Boggini et al. (2001) in the wheat genome. Using single nucleotide genotyping of the Wx gene revealed high polymorphism in barley (Domon et al., 2004) and high polymorphism in rice (Bao et al., 2002; Tran et al., 2011), using a microsatellite marker of Wx genes.

The distribution of genotypes in Table 2 shows that the populations are made up of a specific genotype. It is found with the population of Serui in the Papua island and with the population of Palopo in the Sulawesi island. These facts indicate that sago palm variation originated from both Papua islands and Sulawesi islands due to the existence of specific genotypes in their populations. Therefore, both of these islands are considered to be the sources of sago palm germplasm in Indonesia. Papua islands, were the study was carried out reveals that the largest numbers of genotype exist in their population based on Wx gene markers, so it is considered to be the centre of sago palm genetic diversity in Indonesia.

Genetic relationships in the individuals level

Matrix genetic distance data in the individual levels were not supplied because the matrixes were too large (10 pages). Clustering analyses of the individual levels was based on 8 alleles and 14 genotypes of Wx genes by using the UPGMA and Nei`s genetic distances. The clustering analyses of sago palm in the individual level were performed under four groups (Figure 2). Numbers of individual samples associated in the first Group were incorporated in the samples numbers 4, 21, 36, 45, 46, 50, and 55. Group II were incorporated in the samples numbers 2, 5, 7, and 13. Group III were incorporated in the sample numbers 10, 18, 30, 40, 41, 54, 60, 61, 62, 63, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, 84, 85, 86, 88, 89, 90, and 92. Group IV contained the sample numbers 1, 3, 6, 8, 9, 11, 12, 14, 15, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 37, 38, 39, 42, 43, 44, 47, 48, 49, 51, 52, 53, 56, 57, 58, 59, 64, 69, 77, 83, 87, 91, 93, 94, 95, 96, 97, 98, 99, and 100. The individual samples in group 1 were associated with sample populations from Jayapura, Manokwari, Ambon, Palopo and Pontianak. The indivi-dual samples in Group II were associated with sample populations from Jayapura, Serui, Manokwari, and Sorong. Overall population in the Group II were incorporated in all of the populations from Papua islands only. The individual samples in group III were associated with sample population from Jayapura, Serui, Ambon, Palopo, Pontianak, and Selat Panjang. The individual samples in Group IV was associated with samples population from overall populations, except population from Ambon.

The data showed individual samples do not form groups based on the population or island origin, but individual samples incorporated from several populations with different vernacular names are mixed together to form a group. The sago palm in Indonesia has a lot of vernacular names because it is given by local people who use their own language. However, it may actually be same type in the other location but the name would be diffrent. These features indicated that the vernacular names are not suitable for describing sago palms types without supporting molecular data. Around Sentani Lake, Jayapura, the local people claim 21 types of sago palm based on morphological characteristic, other scientist found 15 types only based on morphological charac-teristics (Yamamoto et al., 2005).

Genetic relationships in the level of populations

Genetic distance among populations of sago palm were calculated in the range of 0.0023 to 0.2859 and genetic identities ranged from 0.7513 to 0.9977 (Table 3). A wide range of the genetic distance of the population indicated a great variation. However, the variation that existed in the population's level was smaller than the variation that occurred at the individual levels. The smallest population pairwise differences were observed in the population of Serui and Pontianak and the largest populations pairwise differences were observed in the population of Ambon and Bogor. This means that the sago palm population in Serui and Pontianak are close related, while the sago palm population in Ambon and Bogor are far related.

Clustering analyses in the population level of sago palm in Indonesia show that sago palm is divided into three groups based on Wx genes markers. Group I incorporated population sample from Jayapura, Serui, Sorong, and Pontianak. Group II incorporated population sample from Manokwari, Palopo, and Selat Panjang. Group III incorporated population sample from Bogor and Ambon (Figure 3). The genetic relationships of popu-lation by employing various genetic markers were reported frequently in the previous study (Mengoni et al., 2000; Jacquemyn et al., 2004; Lanteri et al., 2001; Viard et al., 2001). Populations incorporated in the same group were more closely related than the population stated in the different group. Variation of the populations maybe triggered by out breeding. Generally, sago palm cross pollinate due to pollen grain maturity in different times with ovary maturity. Latta and Mitton (1997) published the differentiation of the population caused by Pollen

Table 2. Variation of reproducing bands by PCR in a total 100 sago palm samples based on Wx gene markers.

Island	Population	Number of sample	Wx gene variation			
			Alleles	Genotypes	Frequency	Relative frequency
Papua	Jayapura	27	5	G01	3	0.111
				G02	1	0.037
				G03	10	0.370
				G06	5	0.185
				G07	1	0.037
				G10	4	0.148
				G12	2	0.074
				G14	1	0.037
	Serui	24	6	G01	2	0.083
				G03	7	0.292
				G04*	1	0.042
				G05	1	0.042
				G06	11	0.458
				G10	2	0.083
	Manokwari	6	6	G02	1	0.167
				G03	1	0.167
				G07	1	0.167
				G08	1	0.167
				G10	1	0.167
				G14	1	0.167
	Sorong	9	5	G01	2	0.222
				G02	1	0.111
				G05	1	0.111
				G06	4	0.444
				G12	1	0.111
Ambon	Ambon	3	3	G06	1	0.333
				G07	1	0.333
				G13	1	0.333
Sulawesi	Palopo	4	4	G01	1	0.250
				G09*	1	0.250
				G11	1	0.250
				G13	1	0.250
Kalimantan	Pontianak	18	5	G01	2	0.111
				G03	4	0.222
				G06	9	0.500
				G08	1	0.056
				G10	1	0.056
				G14	1	0.056
Jawa	Bogor	2	2	G01	2	1.000
Sumatera	Selat Panjang	7	5	G01	3	0.429
				G06	1	0.143
				G08	1	0.143
				G11	1	0.143
				G12	1	0.143

Notes: *specific genotypes. G04 is genotype just found in the population of Serui in the Papua island. G09 is genotype just found in the population of Palopo in the Sulawesi island.

Table 3. Nei`s genetic distance (below diagonal) and Nei`s genetic identities (above diagonal) in the level of population based on Wx gene markers.

Pop	1	2	3	4	5	6	7	8	9
1	*****	0.9895	0.9811	0.9860	0.8571	0.9473	0.9868	0.9028	0.9803
2	0.0106	*****	0.9587	0.9946	0.8925	0.9221	0.9977	0.8600	0.9605
3	0.0191	0.0422	*****	0.9592	0.8675	0.9685	0.9608	0.9141	0.9709
4	0.0141	0.0054	0.0417	*****	0.8864	0.9209	0.9942	0.8911	0.9706
5	0.1542	0.1138	0.1421	0.1206	*****	0.8896	0.9063	0.7513	0.8232
6	0.0541	0.0811	0.0320	0.0825	0.1170	*****	0.9288	0.9117	0.9453
7	0.0133	0.0023	0.0400	0.0058	0.0984	0.0739	*****	0.8744	0.9664
8	0.1022	0.1508	0.0898	0.1153	0.2859	0.0925	0.1342	*****	0.9508
9	0.0199	0.0403	0.0295	0.0298	0.1945	0.0562	0.0342	0.0505	*****

Notes: Population (Pop) from Jayapura (1), Serui (2), Manokwari (3), Sorong (4), Ambon (5), Palopo (6), Pontianak (7), Bogor (8), and Selat Panjang (9).

Table 4. Nei`s genetic distance (below diagonal) and Nei`s genetic identities (above diagonal) in the level of islands based on Wx gene markers.

Islands	Papua	Ambon	Sulawesi	Kalimantan	Jawa	Sumatera
Papua	*****	0.8794	0.9411	0.9947	0.8910	0.9759
Ambon	0.1285	*****	0.8896	0.9063	0.7513	0.8232
Sulawesi	0.0607	0.1170	*****	0.9288	0.9117	0.9453
Kalimantan	0.0054	0.0984	0.0739	*****	0.8744	0.9664
Jawa	0.1154	0.2859	0.0925	0.1342	*****	0.9508
Sumatera	0.0244	0.1945	0.0562	0.0342	0.0505	*****

grain migration. It is commonly understood that cross pollination will impact a high variation of the population.

Genetic relationship in the level of islands

The genetic distances of sago palm were calculated ranging from 0.0054 to 0.2859 and genetic identities were ranged from 0.7513 to 0.9947 (Table 4). The smallest pairwise differences were reflected in sago palm samples from Papua islands and sago palm samples from Kalimantan islands. The largest genetic pairwise differences were contained in sago palm samples from Ambon islands and sago palm sample from Jawa islands. These indicates that genetically, sago palm in the Papua islands and sago palm in the Kalimantan islands have a close relationship while sago palm in the Ambon islands and sago palm in the Jawa islands don not share a close relationship relationship. Clustering analyses of sago palm samples were divided into two groups (Figure 2, 3 and 4). Group I incorporated samples from Papua and Kalimantan island. Group II incorporated samples from Ambon, Sulawesi, Jawa, and Sumatera islands. These indicated that the sago palm samples in the islands possess low level differentiation when compareed with the individual and population levels.

Generally, the sago palms in a leveled island are inclined to be different among other islands based on Wx gene markers. The differentiation is hypothetically caused by evolution process, geographical isolation, distance isolation, genetic drift and gen flow. Hartl and Klark (1989) reported that the differentiation is caused by evolution, natural selection, migration, and genetic drift. Furthermore, Mayer et al. (1994) documented the differentiation of *Cruciferae* caused by gen flow.

Conclusion

The Wx gene markers were used for assessment of genetic variation and genetic relationships of sago palm in Indonesia to show high variation. The generated data based on the Wx gene markers described 8 alleles and 14 genotypes of sago palm in Indonesia. The size of alleles ranged from 100 to 700 bp. Many vernacular names were given by local people where the population located has different name but are genetically the same. The vernacular names do not accurately represent the variation. The genetic relationships of sago palm were observed according to the higher variation in the level of individual sampling stages, then followed by the populations sampling and the islands sampling stages.

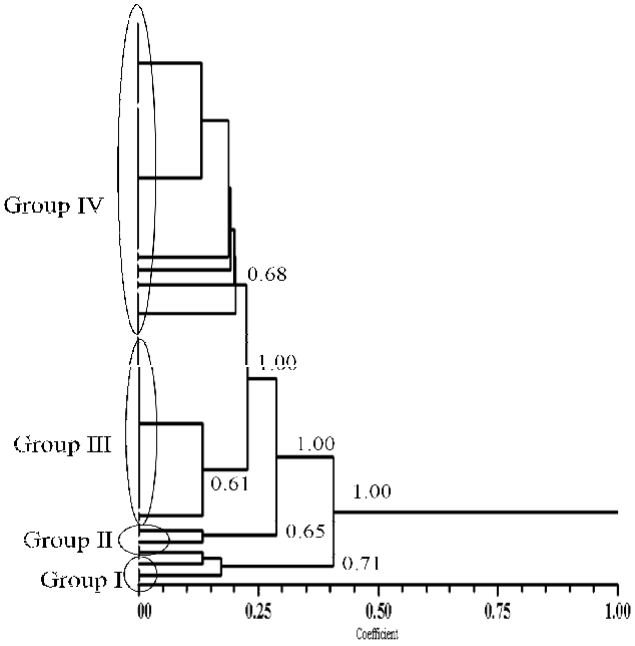

Figure 2. Clustering analyses of a total 100 samples based on UPGMA and bootstrapping by 1000 permutations generated by Wx gene pair primers.

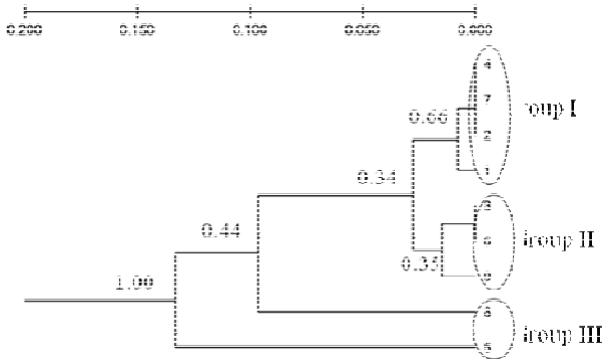

Figure 3. Clustering analyses of a total nine populations based on UPGMA and bootstrapping by 1000 permutations generated by Wx gene pair primers. Population from Jayapura (1), Serui (2), Manokwari (3), Sorong (4), Ambon (5), Palopo (6), Ponianak (7), Bogor (8), Selat Panjang.

Sago palm samples were clustered into four groups in the level of individuals, three groups in the level of populations, and two groups in the level of islands. The existence of the specific genotypes in both population, Serui in the Papua islands and Palopo in the Sulawesi islands is considered to be germplasm resources and the Papua islands have the largest genotype which is considered to be the center of genetic variation in Indonesia.

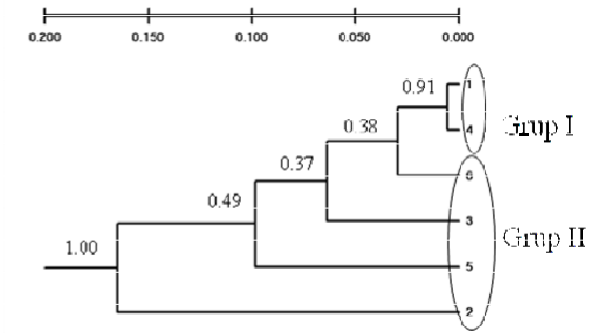

Figure 4. Clustering analyses of a total six islands of sago palm sample origin based on UPGMA and bootstrapping by 1000 permutation generated by Wx gene pair primers. Samples from Papua Island (1), Ambon (2), Sulawesi (3) Kalimantan (4), Jawa (5), Sumatera (6).

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