B I O D I V E R S I T A S
Volume 10, Number 4, October 2009
Pages: 168-174

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)

Genetic Relationship of Sago Palm (*Metroxylon sagu* Rottb.) in Indonesia Based on RAPD Markers

BARAHIMA ABBAS[1,♥], MUHAMMAD HASIM BINTORO[2], SUDARSONO[2], MEMEN SURAHMAN[2], HIROSHI EHARA[3]

[1]Faculty of Agriculture and Technology, State University of Papua (UNIPA), Manokwari 98314, Indonesia
[2] Faculty of Agriculture, Bogor Agricultural University (IPB), Bogor 16680, Indonesia
[3] Faculty of Bioresources, Mie University, 1577 Kurimamachiya,Tsu-city, Mie-Pref. 514-8507, Japan

Received: 8th March 2009. Accepted: 20th July 2009.

ABSTRACT

The areas of sago palm (*Metroxylon sagu* Rottb.) forest and cultivation in the world were estimated two million hectares and predicted 50% of that areas located in Indonesia. Distribution of sago palm areas in Indonesia is not evenly distributed as well as their diversities. Information of plant genetic diversities and genetic relationship is very important to be used for germ plasm collection and conservation. The objectives of research were revealed the genetic relationships of sago palm in Indonesia based on RAPD molecular markers. Fragments amplification PCR products were separated on 1.7% agarose gel, fixation in Ethidium Bromide, and visualized by using Densitograph. Genetic relationships of sago palm in Indonesia showed that sample in individual level were inclined mixed among the other and just formed three groups. Genetic relationship of sago palm population showed that samples populations from Jayapura, Serui, Sorong, Pontianak, and Selat Panjang were closely related each others based on phylogenetic analysis and formed clustered in one group, event though inclined to be formed two subgroups. Populations from Manokwari, Bogor, Ambon and Palopo were closed related each others, they were in one group. Genetic relationships in the level of island were showed sago palm from Papua, Kalimantan, and Sumatra closely related. Sago palms from Maluku were closed related with sago palm from Sulawesi whereas sago palm from Jawa separated from the others. Based on this observation we proposed that Papua as centre of sago palm diversities and the origin of sago palm in Indonesia. This research informed us the best way to decide sago palm places for germ plasm of sago palm conservation activity.

Key words: genetic relationships, population, sago palm, RAPD, Indonesia.

INTRODUCTION

Indonesia has the biggest sago palm (*Metroxylon sagu* Rottb.) forest and cultivation as well as its rich of genetic diversities. The areas of sago palm forest and cultivation in the world were predicted two million hectares and estimated 50% of that area located in Indonesia. Kertopermono (1996) reported that sago palm areas in Indonesia were larger than proposed by Flach (1983). According to measurement of Kertopermono (1996), sago palm areas in Indonesia were 1,528,917 ha and it was distributed into several locations in Indonesia. The locations of sago palm areas in Indonesia were observed in the previous studied, namely: Irian Jaya 1,406,469 ha, Ambon 41,949 ha, Sulawesi 45,540 ha, Kalimantan 2,795 ha, West Jawa 292 ha, and Sumatra 31.872 ha. The distribution of sago palm areas in Indonesia was not evenly distributed as well as their diversities. Flach (1983) predicted that sago palm diversities in Indonesia were found higher in Papua islands (New Guinea) than other islands in Indonesia.

Information of plant genetic diversities is very important to be used for germ plasm collection and conservation. When germ plasm conservation activity is done, an information on genetic diversities are needed, especially from the natural habitat to carried out germ plasm conservation efficiently. A popular DNA markers used for revealing genetic diversities and genetic relationships are Random Amplified Polymorphism DNA (RAPD) markers. The RAPD marker is one of many techniques used for molecular biology research. The advantages of RAPD markers are simpler in their preparation than other molecular markers. The other RAPD markers are easy applied for examining the diversities of organism (Powel et al., 1995; Colombo et al., 1998; Ferdinandez et al., 2001), because it is not using radioactive and relatively chief (Powel et al., 1995).

Research which carried out for revealing genetic relationships by using RAPD markers were reported for *Sorghum bicolor* L. (Agrama and Tuinstra, 2003),

♥ **Corresponding address:**
Jl. Gunung Salju Amban, Manokwari 98314, Papua Barat
Tel. +62-986-212095, Fax.: +62-.986-212095
e-mail: barahimabas@yahoo.com

Brassica oleracea L (Graci et al., 2001), and *Medicago sativa* L. (Mengoni et al., 2000). Whereas a study for genetic structure of population were reported for at *Acacia raddiana* Savi (Shrestha et al., 2002), *Pimelodus* spp. (Almeida et al., 2004), and *Primula elatior* (L.) Oxlip (Jacquemyn et al., 2004).

MATERIALS AND METHODS

Sago palm samples were collected from several islands in Indonesia. A total 100 samples of sago palm were collected from six islands and nine populations of sago palm centre in several islands in Indonesia. Location and geographical range of the selected sago palm stands were presented in Figure 1. The populations and the numbers of samples that were used in this experiment were presented in Table 1. Leaf samples were collected and preserved by using silica gel granules in zip lock plastic according to previous reported procedures (Chase and Hill, 1991). Isolation and extraction of total DNA from dried sago palm leaf samples were conducted using procedures as described in Qiagen DNA extraction kit (Qiagen, 2003). The total DNA was stored in -20°C in freezer until ready for using.

PCR Amplification

RAPD primers used in this research were as follows: P01 (GCG GCT GGA G), P02 (GTG ACG CCG C), P04 (CGT CTG CCC G), P06 (TTC CGC GGG C), P17 (ATG ACG ACG G), OPG02 (GGC ATC GAG G), OPA04 (AAT CGG GCT G), OPAB04 (GGC ACG CGT T), OPAA17 (GAG CCC GAC T), and OPAB18 (CTG GCG TGT C). PCR mixtures and cycles condition were followed procedures described by Ehara et al. (2003) which has a little bit modification such as 0.12 µM, 0.63 U Ampli Taq Gold™, 10 ng DNA genome, 1.7% agarose gels for separating amplification fragments, and visualization by using Densitograph, Bioinstrument ATTA.

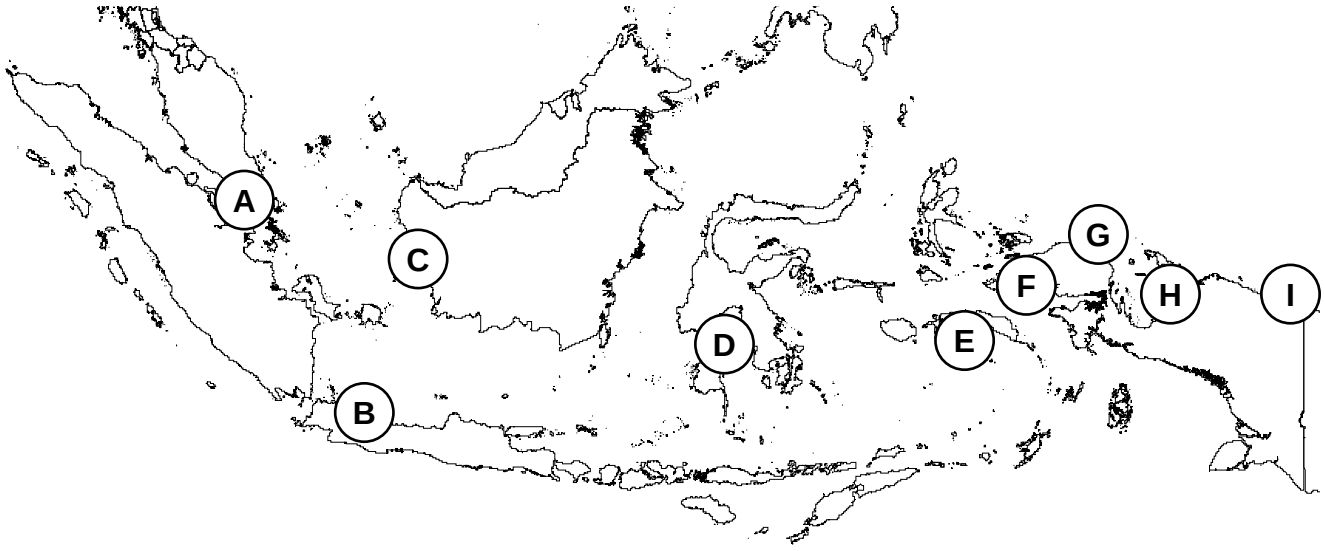

Figure 1. The map of sampling sites of sago palm used (scale 1: 39,800,000). The cycles represent the population sampling. A. Selat Panjang, B. Bogor, C. Pontianak, D. Palopo, E. Ambon, F. Sorong, G. Manokwari, H. Serui, I. Jayapura.

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, 49, 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, 9, 20, 21, and 22
	Sorong	8, 13, 17, 28, 69, 70, 71, 72, 74
Maluku	Maluku	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

Data analysis

Dissimilarity matrix was calculated by using distance coefficient. The dissimilarity matrix was employed to construct phylogenetic 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 (Rohlf, 1998). Bootstrap analysis with permutation 10,000 times were performed by using software Tools for Genetic Analysis (TFPGA 1.3). Ordinate analysis calculated by using Multidimensional Scaling (MDS) and performed by using NTSYS 2.02 Package (Rohlf, 1998).

RESULTS AND DISCUSSIONS

RAPD Polymorphism

Polymorphisms of RAPD amplification fragments by using ten RAPD primers and performed in the PCR tools were resulted 86 numbers of polymorphic fragments and two to seven genotype numbers per population. Samples DNA Fragments resulted by PCR were shown in Figure 2. High numbers of RAPD polymorphisms and genotypes were found in this observation. These results were similarly with genetic diversity of sago palm in the previous study, by Ehara et al. (2003) by using RAPD markers utilizing small amount individual sago palm samples from Indonesia and Malaysia. Fig 2 showed that the performance samples of DNA bands were amplified by using 10 primer sets. Numbers of fragment DNA band were amplified from each primer, and it was ranging from 6 to 12 polymorphic bands per primers and no monomorphic DNA band was observed. The averages polymorphic DNA bands were calculated 9 per primer. Primer P17 was resulted the highest numbers of polymorphic DNA bands that was 12 DNA bands, whereas primers OPA04 and P06 produced the lowest numbers of polymorphic DNA bands that were produced 6 polymorphic DNA bands per primers. Base pairs sizes of DNA bands produce by 10 primer sets were ranging from 150 bp (base pairs) to 1800 bp. Overall primers used in this observation were suitable for studying genetic of sago palm. The previous of this observation applied more than 100 RAPD primers sets.

Genetic relationships in the level of individuals

Genetic relationships in individual levels showed that the samples divided into three groups based on phylogenetic construction (Figure 3) and three clusters based on multidimensional scaling analysis (Figure 4). Numbers of individual samples associated in group I were the sample number 2, 10, 13, 15, 16, 17, 20, 21, 22, 23, 33, 34, 39, 40, 42, 43, 44, and 62; group II were the sample number 6, 9, 14, 24, 25, 26, 27, 41, 49, 51, 58, 75, 95, and 97; group III were the sample number 1, 3, 4, 5, 7, 8, 11, 12, 18, 19, 28, 29, 30, 31, 32, 35, 36, 37, 38, 45, 46, 47, 48, 50, 52, 53, 54, 55, 56, 57, 59, 60, 61, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91`, 92, 93, 94, 96, 98, 99, and 100. The individual samples in group I and group III were associated individual samples from overall populations. Group II individual samples associated with population from Jayapura, Serui, Manokwari, Ambon and Pontianak. These grouping were similarly with sago palm grouping by Ehara et al. (2003) which divided sago palm samples from Indonesia and Malaysia into two groups and sub group based on RAPD markers. Papua islands in Indonesia were shown that individual samples divided into three groups also based on cp-DNA markers (Barahima et al., 2005). Based on our observation, we proposed that sago palm in Indonesia classified into three groups. Individuals grouping in the phylogenetic construction were based on genetic distances, grouping methods, and coefficient used or bootstrapping levels. In our observation showed that the different genetic markers used did not change grouping pattern of sago palm. Some cases in the molecular analysis, the dissimilarities grouping pattern, by using the same markers or different markers, were found frequently in the studied of genetic relationships (Ishikawa et al., 1992; Viard et al., 2001; Panda et al., 2003).

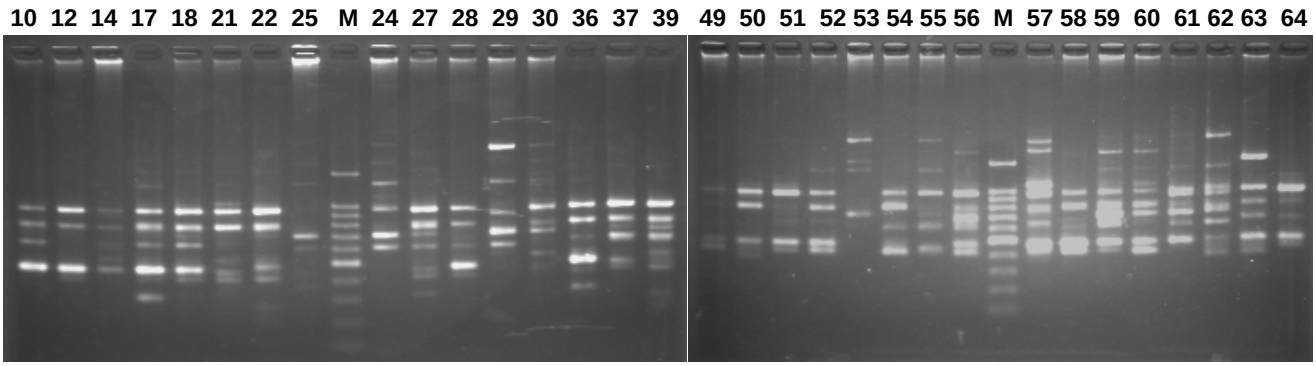

Figure 2. Performance of RAPD fragment by using OPAA17 primers on 1.7% agorose gels. Marker (M) and the number of well (10 to 64) indicated number of sago palm samples.

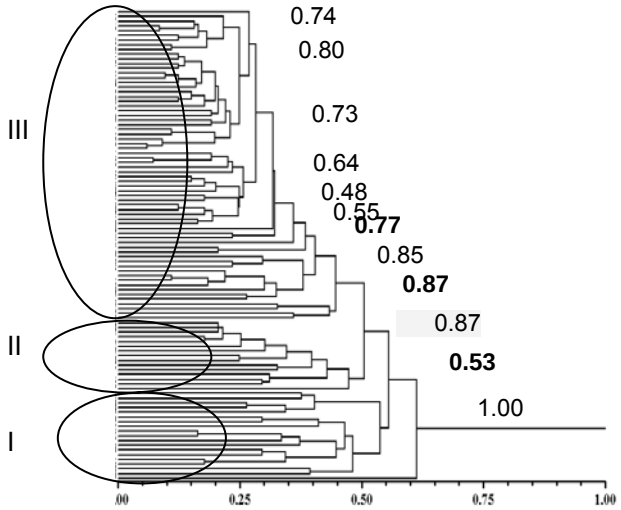

Figure 3. Phylogenetic of samples in the level of individuals based on 86 loci and 10 RAPD primers of 100 individuals samples by using UPGMA clusters and bootstrap by using 10,000 permutations.

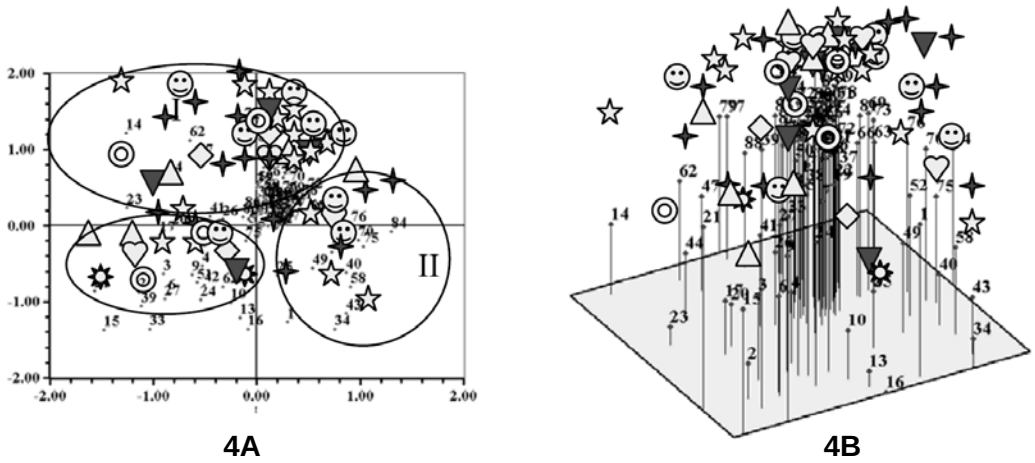

Figure 4. Ordinate analysis of individual level by using MDS based on 86 loci, 10 RAPD primers, and 100 individuals of sago palm. Two dimension scales (4A) and three dimensional scales (4B). Individual samples from Jayapura (☆), Serui (✦), Manokwari (△), Sorong (▼), Ambon (◇), Palopo (♡), Potianak (☺), Bogor (✿), Selat Panjang (◎).

Genetic relationships in the level of populations

Phylogenetic construction show that sago palm samples in the population levels was divided into two groups, those were group I and II. The group I was inclined to form two sub groups because bootstrap value was high (0.99) in one of finger phylogenetic (Figure 5) and two clusters based on MDS analysis (Figure 6). The group I included population sample from Jayapura, Serui, Sorong, Pontianak, and Selat Panjang. The group II was associated population sample from Manokwari, Ambon, Palopo, and Bogor. The group I will be divided into two sub groups. The subgroup I included population from Jayapura, Serui, and Sorong and the subgroup II included population from Pontianak and Selat Panjang. The genetic relationships in the level of population showed the same pattern with individual levels, even though samples in the level of population just inclined to form three groups, but solid pylogenetic construction only showed two groups (Figure 4). Variation levels were detected in this observation similarly with genetic variation of *Cynara scolymus* L. by using RAPD markers (Lanteri et al*.* 2001) and *Medicago sativa* L. (Mengoni et al*.* 2000). The differences of relationships among population probably were caused by out breeding, so that populations become different. Population differences may owing to pollen migration (Latta and Mitton 1997). Generally, pollination of sago palm occurred a cross pollination since male and female flower mature in different of time period (Jong, 1995). Cross pollination process in sago palm may cause population different.

Association sample population from Jayapura, Serui, Sorong, Pontianak, and Selat Panjang to form one group in the phylogenetic construction probably owing to sago palm interchange from one population

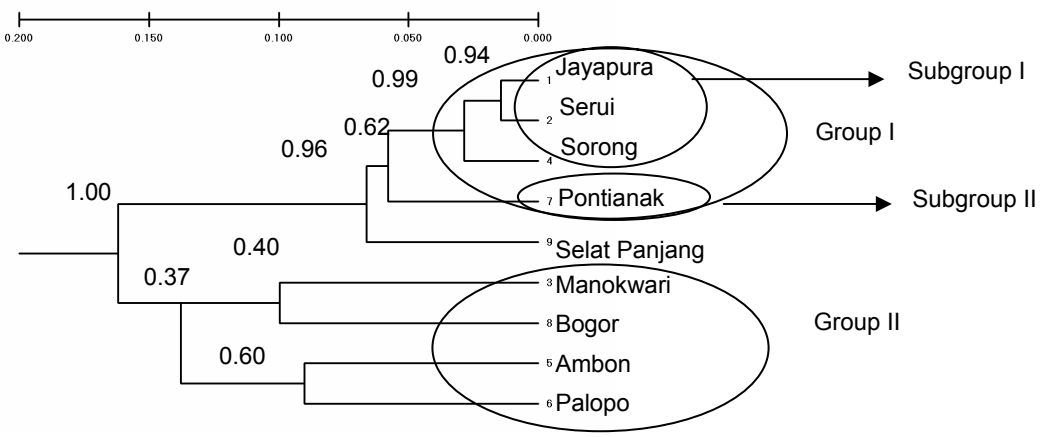

Figure 5. Phylogenetic of samples in the level of populations based on 86 loci and 10 RAPD primers of 100 individuals samples by using UPGMA clusters and bootstrap by using 10,000 permutations.

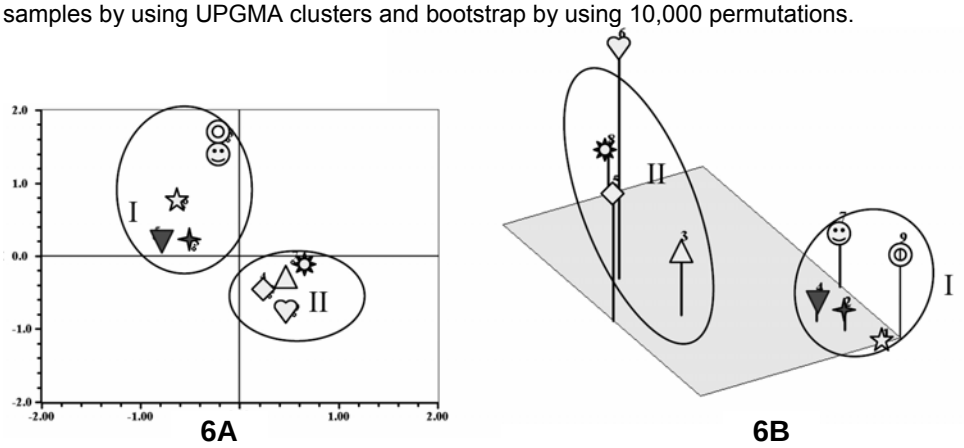

Figure 6. Ordinate analysis of population level by using MDS based on 86 loci, 10 RAPD primers, and 100 individuals of sago palm. Two dimension scales dimension (6A) and three dimensional scales (6B). Populations from Jayapura (☆), Serui (✦), Manokwari (△), Sorong (▼), Ambon (◇), Palopo (♡), Potianak (☺), Bogor (✡), Selat Panjang (◎).

to another population which carried by people. In this research we do not know exactly, when sago palm came of exchange and where sago palm population originated. Based on sago palm diversities and natural stand we found that the largest variation and the largest natural stand in the population from Papua. Sago palm population from Jayapura we found the largest variation and the largest vernacular name was given by local people. Matanubun et al. (2005) reported that there were 96 sago palm varieties in Papua based on morphology characteristic and Yamamoto (2005) reported that there were 15 sago palm varieties in Jayapura based on morphological characters. Population from Jayapura has the largest variation of sago palm. Based on that data, we can estimate that the population origin of population in group I came from Jayapura population. Populations formed in group II, we predicted also caused by interchange individual of sago palm in the past through people mobilization from one place to another place. Therefore, the population in one group such as group II have average genetic distance closed each others. We have no sufficient data to estimate the population origin in group II. This research give us an information for the best way to chose sago palm places for germ plasm of sago palm conservation activities.

Genetic relationships in the level of islands

The genetic relationships of sago palm in the level of island showed that it also formed three groups as shown on individual levels. Sago palm sample from Papua, Kalimantan and Sumatra were observed and show genetic distance closed each others, and formed Group I. Sample from Ambon and Sulawesi formed Group II, and sample from Jawa formed Group III in the phylogenetic construction. The genetic relationships based on phylogenetic construction (Figure 7) and MDS analysis (Figure 8) showed that samples in island levels were closely related between samples from Papua, Kalimantan and Sumatra. Samples from Sulawesi islands were closely related with samples from Ambon. Samples from Jawa island were separated with samples from the others island based on RAPD markers. There was very interesting phenomenon, at which we should pay

attention, samples in the island levels formed the same group with samples from other islands, which have distances far away each other. Those shown by Papua island were in the same group with Sumatra island (Figure 7) at group I. This phenomenon may be occurred owing to samples in individual levels from Papua have genetic distances more closely than individual samples from Sumatra, which made total genetic distance between Papua and Sumatra closed each others. If we estimated through migration aspects, probably individual of sago palm from Papua mixed with sago palm individual from Sumatra in the past by people mobilization/migration. During the Dutch colonization in Indonesia, people already moved from Sumatra to Papua or the other way around, with probably people carrying sago palm plant and growing at new places for anticipating food crisis in the future. Features of sago palm in Papua have highest variation, largest sago palm forest, many wild types, and semi cultivated. Sago palm features in another island in Indonesia Such as Sumatera, Kalimatan, Jawa, Sulawesi, and Maluku were found sago palm cultivated, semi cultivated, low variation, no wild types, and no sago palm forest. Therefore we estimated the origin of sago palm in Indonesia come from Papua. The genetic distances of sago palm from Papua were assayed closed with sago palm from Sumatra. Probably, sago palm from Papua moved to Sumatra which carried out by people when they moved from Papua to Sumatra in the past and formed a new population in the new places. This prediction may occur because RAPD markers which used did not show as conservative as cpDNA markers which uniparental inherited (Ishikawa et al., 1992; Savolainen et al., 1995). RAPD markers are molecular nuclear genome which related with DNA recombinant process and biparentally inherited (Viard et al., 2001). Therefore, RAPD markers are molecular markers which it have no longer conservative periods time rather than cpDNA markers. In the previous studies at different plants showed that higher variation were found by using nuclear genome markers (RAPD, AFLP, ISSR, and nuclear SSR), then using chloroplast genome markers such as cpDNA markers (Hultquist, 1996; Viard et al., 2001; Cronn et al., 2002; Panda et al., 2003).

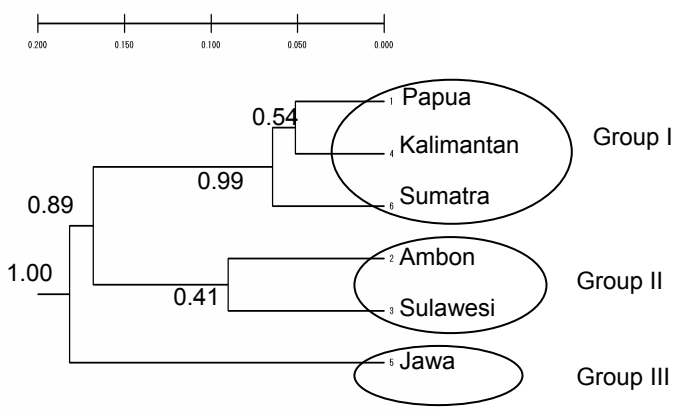

Figure 7. Phylogenetic of samples in the level of islands based on 86 loci and 10 RAPD primers of 100 individuals samples by using UPGMA clusters and bootstrap by using 10,000 permutations.

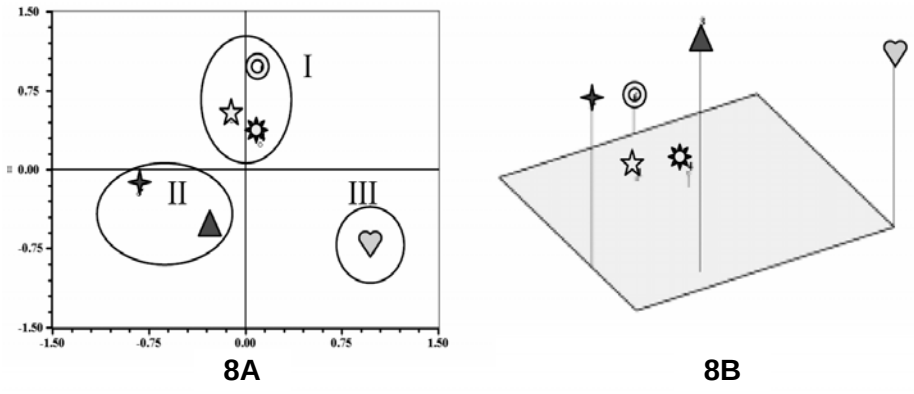

Figure 8. Ordinate analysis of island level by using MDS based on 86 loci, 10 RAPD primers, and 100 individuals of sago palm. Two dimension scales dimension (8A) and three dimensional scales (8B). Samples from Papua (☆), Ambon (✦), Sulawesi (▲), Kalimantan (✡), Jawa (♡),Selat Panjang (◎).

CONCLUSIONS

Genetic relationships of sago palm in Indonesia showed that sago palm in individual level were inclined to mix among the others, and just formed three groups. Sago palm population from Jayapura, Serui, and Sorong were closely related; sago palm from Manokwari, Bogor, Ambon, and Palopo were closely related; and sago palm from Pontianak was closely related with sago palm from Selat Panjang. In the level of Islands which has long geographical distance showed that sago palm from Papua island closed related with sago palm from Kalimantan and Sumatra island. Sago palm from Ambon closely related with sago palm from Sulawesi, and sago palm from Jawa island not formed cluster with sago palm from the other islands. Thus, we proposed that Papua is as centre of sago palm diversity, and the origin of sago palm in Indonesia. This research informed us the best way to decide sago palm places, for germ plasm and sago palm conservation activity.

REFERENCES

Agrama, H.A and M.R. Tuinstra. 2003. Phylogenetic diversity and relationship among sorghum accessions using SSRs and RAPDs. *African Journal Biotechnology* 2 (10): 334-340.

Almeida, F.S.D, L.M.K. Sodre, and E.P.B. Contel. 2004. Population structure analysis of **Pimelodus maculates** Pisces, Siluriformes) from the Tiete and Paranapanema Rivers (Brazil). *Genetic and Molecular Biology* 26 (3): 301-305

Barahima, A, M.H. Bintoro, Sudarsono, M. Surahman, and H. Ehara. 2005. Haplotype diversity of sago palm in Papua based on chloroplast DNA. *In:* Karafir, Y.P., F.S. Jong, and V.E. Fere (eds). *Sago Palm Development and Utilization. Proceeding of the Eighth International Sago Symposium in Jayapura, Indonesia*. Japan Society for the Promotion Science, Jayapura, 4-6 August 2005.

Chase, M. and H. Hill. 1991. Silica gel: an ideal material for field preservation of leaf samples. *Taxon* 40: 215-220.

Colombo, C., G. Second, T.L. Valle, and A. Charrier. 1998. Genetic diversity characterization of cassava cultivars (**Manihot esculenta** Cranz.) RAPD markers. *Genetic and Molecular Biology* 21: 69-84.

Cronn, R.C., R.L. Small, T. Haselkorn, and J.F. Wendel. 2002. Rapid diversification of the cotton genus (**Gossypium**: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany* 89 (4): 707-725.

Ehara, H., S. Kosaka, N. Shimura, D. Matoyama, O. Morita, H. Naito, C. Mizota, S. Susanto, M.H. Bintoro, and Y. Yamamoto. 2003. Relationship between geographical distribution and genetic distance of sago palm in Malay Archipelago. *Sago Palm* 11: 8-13.

Ferdinandez, Y.S.N., D.J. Somers, and B.E. Coulman. 2001. Estimating the genetic relationship of hybrid bromegrass to smooth bromegrass and medow bromegrass using RAPD markers. *Plant Breeding* 120: 149-153.

Flach, M. 1983. *The Sago Palm. Domestication, Exploitation, and Product*. Rome: FAO Plant Production and Protection.

Graci, A., I. Divaret, F.M. Raimondo, and A.M. Chevre. 2001. Genetic relationships between Sicilian wild populations of **Brassica** analyses with RAPD markers. *Plant Breeding* 120: 193-196.

Hultquist, S. J., K.P. Vogel, D.J. Lee, K. Arumuganathan, and S. Kaeppler. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of Switchgrass, **Panicum virgatum** L. *Crop Science* 36: 1049-1052.

Ishikawa, S., S Kato, S. Imakawa, T. Mikami, and Y. Shimamoto. 1992. Organelle DNA polymorphism in apple cultivars and rootstocks. *Theoretical and Applied Genetics* 83: 963-967.

Jacquemyn, H., O. Honnay, P. Galbusera, and I.R. Ruiz. 2004. Genetic structure of forest herb **Primula elatior** in a changing landscape. *Molecular Ecology* 13: 211-219.

Jong, F.S. 1995. *Research for the Development of Sago Palm (***Metroxylon sagu** *Rottb.) Cultivation in Sarawak*, *Malaysia*. Kuching, Sarawak: Department of Agriculture, Malaysia.

Kertopermono, A. P. 1996. Inventory and evaluation of sago palm (**Metroxylon** sp.) distribution. *Sixth International Sago Symposium.* Pekan Baru, 9-12 December 1996.

Lanteri, S., I.D. Leo, L. Ledda, M.G. Mameli, and E. Portis. 2001. RAPD variation within and among population of globe artichoke cultivar 'Spinoso sardo'. *Plant Breeding* 120: 243-246.

Latta, R.G. and J.B. Mitton. 1997. A comparison of population differentiation across four classes of gene marker in limber pine (**Pinus flexilis** James). *Genetics* 146: 1153-1163.

Matanubun, H., B. Santoso, M. Nauw, A. Rochani, M.A.P. Palit, D.N. Irbayanti, and A. Kurniawan. 2005. Feasibility study of the natural sago sago forest for the establishment of the commercial sago palm plantation at Kaureh District, Jayapura, Papua, Indonesia. *Sago Palm Development and Utilization. Proceeding of the Eighth International Sago Symposium in Jayapura, Indonesia*. Japan Society for the Promotion Science, Jayapura, 4-6 August 2005.

Mengoni, A., A. Gori, and M. Bazzcalupo. 2000. Use of RAPD and micro satellite (SSR) variation to assess genetic relationships among populations of tetraploid alfalfa, **Medicago sativa***. Plant Breeding* 119: 311-317.

Panda, S., J. P. Martin, and I. Agunagalde. 2003. Chloroplast and nuclear DNA studies in a few members of the **Brassica oleracea** L. group using PCR-RFLP and ISSR-PCR markers: a population genetic analysis. *Theoretical and Applied Genetics* 106: 1122-1128

Powel, W., C.O. Castillo, K. J. Chaluers, J. Provan, and R. Waugh. 1995. Polymerase chain reaction based-assays for the characterization of plant genetic resources. *Electrophoresis* 16: 1726-1730.

Rohlf, F. J. 1998. *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System*. Version 2.02. New York: Exter Sift Ware..

Savolainen, V., R. Corbaz, C. Moncousin, R. Spchiger, and J.F. Manen. 1995. Chloroplast DNA variation and parentage analysis in 55 apples. *Theoretical and Applied Genetics* 90: 1138-1141.

Shrestha, M. K., A.G. Goldhirsh, and D. Ward. 2002. Population genetic structure and the conservation of isolated population of *Acacia raddiana* in the Negev Desert. *Biological Conservation* 108: 119-127.

Sneath, P. H. and R. R. Sokal. 1973. *Numerical Taxonomy.* San Francisco: Freeman.

Viard, F., Y.A.E. Kassaby, and K. Ritland. 2001. Diversity and genetic structure in populations of **Pseudotsuga menziesii** (Pinaceae) at chloroplast micro satellite loci. *Genome* 44: 336-344.

Yamamoto Y, Yoshida T, Miyazaki A, Jong FS, Pasolon YB, and Matanubun H. 2005. Biodiversity and productivity of several sago palm varieties in Indonesia. *Proceeding of Eighth International Sago Symposium in Jayapura, Indonesia.* Japan Society for the Promotion Science, Jayapura, 4-6 August 2005.