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Relationship between Geographical Distribution and Genetic Distance of Sago Palms in the Malay Archipelago

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Abstract Random amplified polymorphic DNA (RAPD) analysis was carried out to estimate the relationship between the geographical distribution and genetic distance of sago palms growing in the Malay Archipelago. The plant materials contained 38 sago palm (*Metroxylon sagu* Rottb.) populations, which included 16 spineless and 22 spiny populations. The polymerase chain reaction (PCR) was performed using nine 10-mer primers. A total of 77 PCR products were scored from all the primers. Out of 77 products, five were shared by all the populations and 72 were polymorphic. A cluster analysis using the UPGMA method was conducted. From the dendrogram based on RAPD data, two groups were found. The cluster of group B consisted of the populations collected in the eastern area of the Malay Archipelago. Group A included a sub-group consisted of the populations collected on Sulawesi and Mindanao and the other sub-group mainly consisted of the populations collected in the western area of the Malay Archipelago. The genetic distance of sago palm populations grown in the Malay Archipelago was considered to be related to geographical distribution. Moreover, the presence or absence of spines on the petiole and rachis did not correspond with genetic distance.

Key words: genetic distance, geographical distribution, RAPD, sago palm, the Malay Archipelago

マレー諸島におけるサゴヤシの地理的分布と遺伝的距離の関係

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要約　マレー諸島に生育するサゴヤシ（*Metroxylon sagu* Rottb.）の地理的分布と遺伝的距離の関係を検討するため，RAPD分析を行った．実験は，無刺16個体群，有刺22個体群を含む合計38個体群を供試材料とし，RAPD-PCRには９種類の10塩基プライマー用いた．全プライマーで合計77のPCR増幅産物が得られ，その内72が多型であった．平均距離法によるクラスター解析を行ったところ，供試個体群は２グループに分かれた．グループBはマレー諸島東部地域から採取した個体群により構成された．一方，グループAはインドネシアのスラヴェシ島とフィリピンのミンダナオ島で採取した個体

群から成るサブグループと，主にマレー諸島西部地域から採取した個体群からなるサブグループで構成された．本結果より，マレー諸島に生育するサゴヤシ個体群間の遺伝的距離は地理的分布と関連していることが窺われた．また，葉柄・葉軸上のトゲの有無と遺伝的距離には明確な関係が見られなかった．
キーワード　遺伝的距離，サゴヤシ，地理的距離，マレー諸島，RAPD

Introduction

The sago palm belongs to the genus *Metroxylon* and has often been classified into two species, *M. sagu* Rottb. and *M. rumphii* Mart., based on morphological characteristics - the absence or presence of spines on the petiole and rachis (Beccari 1918). Rauwerdink (1986) proposed that *M. rumphii* (the spiny type) should be synonymous with *M. sagu* (the spineless type). On the other hand, sago palms are classified in much more detail in informal classifications used by native people who have subsisted on sago (Yosida 1980), and it is therefore considered that various forms may exist.

Previously, the current authors reported that there was a large variation in sago palm production in the eastern archipelago of Indonesia, which was closely related to environmental factors, especially natural soil fertility (Ehara et al. 1995, Ehara et al. 2000). However, to determine the factors affecting starch production, the genetic diversity and the correspondence of sago palms growing at different sites should also be investigated. In this study, random amplified polymorphic DNA (RAPD) analysis was carried out to estimate geographical and genetic relationships among various sago palms growing in the Malay Archipelago.

Materials and Methods

Plant materials

The plant materials contained 38 populations of sago palm (*M. sagu* Rottb.) collected from 22 sites in the Malay Archipelago and one site in Papua New Guinea (PNG). Fig.1 shows the locations of collection area of the populations used in the present study. The vernacular names, sampling sites and morphological charac-teristics of the populations are shown in Table 1. Among the 38 sago palm populations, 16 populations were spineless and 22 populations were spiny. Three types of banding pattern on the back of the petiole and rachis were included: a weak black band, a brown band and the

Fig. 1 Location of collection area of plant materials used.

Table 1 Plant mateerials used.

No.	Vernacular name	Location for collection	Morphological characteristics		
			Spine	Band†	Pith‡
1	Ambtrung 1	Batu Pahat, Johor (Jh1)	–	WB	W
2	Ambtrung 2	Batu Pahat, Johor (Jh1)	–	WB	W
3	Ambtrung 3	Batu Pahat, Johor (Jh1)	–	WB	W
4	Ambtrung 4	Batu Pahat, Johor (Jh1)	+	–	W
5	Ambtrung 5	Batu Pahat, Johor (Jh1)	+	–	W
6	Ambtrung 6	Batu Pahat, Johor (Jh1)	+	–	W
7	Ambtrung 7	Batu Pahat, Johor (Jh1)	+	–	W
8	Ambtrung 8	Batu Pahat, Johor (Jh2)	–	WB	W
9	Ambtrung 9	Batu Pahat, Johor (Jh2)	+	–	W
10	Rumbio 1	Padang, W. Sumatra (WS1)	–	WB	W
11	Rumbio 2	Padang, W. Sumatra (WS2)	–	WB	W
12	Rumbio 3	Padang, W. Sumatra (WS2)	–	WB	W
13	Sagu 1	Siberut, W. Sumatra (Sb1)	–	Br	W
14	Sagu 2	Siberut, W. Sumatra (Sb3)	–	Br	W
15	Gobia	Siberut, W. Sumatra (Sb2)	+	–	W
16	Marui	Siberut, W. Sumatra (Sb4)	+	–	W
17	Sagu 3	Bangka, S. Sumatra (Bn)	–	WB	W
18	Kiray	Bogor, W. Java (WJ)	–	WB	W
19	Roe 1	Konda, S.E. Sulawesi (SeS1)	–	WB	W
20	Roe 2	Totombe, S.E. Sulawesi (SeS2)	–	WB	W
21	Runggumanu 1	Totombe, S.E Sulawesi (SeS2)	+	–	W
22	Runggumanu 2	Lakomea, S.E. Sulawesi (SeS3)	+	–	W
23	Rui	Lakomea, S.E. Sulawesi (SeS3)	+	–	R
24	Molat 1	Seram, Maluku (Sr1)	–	WB	W
25	Tuni 1	Seram, Maluku (Sr2)	+	–	W
26	Ihur	Seram, Maluku (Sr3)	+	–	R[1)]
27	Tuni 2	Seram, Maluku (Sr3)	+	–	W
28	Tuni 3	Seram, Maluku (Sr3)	+	–	W
29	Molat 2	Seram, Maluku (Sr3)	–	WB	W
30	Makanaru 1	Seram, Maluku (Sr4)	+	–	W
31	Makanaru 2	Seram, Maluku (Sr4)	+	–	W
32	Tuni 4	Ambon, Maluku (Am1)	+	–	W
33	Tuni 5	Ambon, Maluku (Am2)	+	–	W
34	Makanaru 3	Ambon, Maluku (Am3)	+	–	W
35	Makanaru 4	Ambon, Maluku (Am3)	+	–	W
36	Saksak	Misamis Oriental, Mindanao (MO)	–	WB	W
37	Lumbio	Davao del Sur, Mindanao (DdS)	+	–	W
38	Wakar	East Sepik (ESp)	+	–	R[2)]

† Banding pattern on the back of the petiole and rachis, WB: weak black band, Br: brown band, –: bandless.
‡ Colour of pith, W: white pith, R: reddish pith.
1) Soerjono (1980). 2) Flach (1997).

absence of a band (i.e. bandless). Two types of pith colour, white and reddish, were also included.

DNA isolation and RAPD analysis

A newly expanded leaflet of a young palm among each population was cut into small pieces and either freeze-dried or silica-dried (Chase and Hrold 1991). Total genomic DNA was isolated from the dried leaflet tissue using either the CTAB protocol (Doyle and Doyle 1987) or the ISOPLANT protocol (Nippon Gene). The extracted total genomic DNA was purified using the QIAquick PCR purification Kit (Qiagene). The polymerase chain reaction (PCR) was performed using nine 10-mer primers (Table 2) selected from the screening of primers in the previous study (Ehara et al. 1997) and the preliminary experiment. Amplification reaction volumes were 25 μ l, which included 2.5 μ l of 10 × reaction buffer (PCR Gold Buffer, Applied Biosystems), 1.5 μ l of 25 mM $MgCl_2$, 2 μ l of 2.5mM dNTP, 3.3 μ l of 1.52 μ M primer (final 0.2 μ M), 0.2 μ l of 5U/ μ l Taq polymerase (AmpliTaq Gold, Applied Biosystems), 1.25 μ l of DMSO (5 %) and 1 μ l of DNA solution (final 2ng for P01, P02, P04, P06, P17 or 25 ng for OPG02, OPAA17, OPAB04 and OPAB18). Amplification was performed in a GeneAmp PCR system 2400 (Perkin Elmer) programmed as follows: 1 cycle of 10 min at 95℃; 45 cycles of 1 min at 93℃, 2 min at 42℃, 2 min at 72℃; followed by 7 min at 72℃. A portion of DNA from the amplification reactions was electrophoresed in a 1.5 % agarose gel containing 1 × TBE buffer [89 mM Tris-borate, 2mM EDTA (pH 8.0)] at 100V for 30 min, and stained with ethidium bromide and visualised using a UV transilluminator. The sizes of the amplified fragments were calculated using a 'Lane & Spot Analyzer'(ver. 6, Atto). The total DNA of every population was amplified twice, and the reproducible products were rated as the population's own products.

Genetic distance and cluster analysis

Each population's product was considered to be a unit character and the populations were scored for the presence or absence of a product. Genetic similarity (S) between all pairs of populations was calculated according to Nei and Li (1979), and the similarity indices were converted into dissimilarity: $D = -\ln(S)$. The dissimilarity indices were regarded as the genetic distance and a distance matrix between the 38 populations was calculated. A cluster analysis by the unweighted pair-group method using arithmetic average (UPGMA) was conducted with a 'PHYLIP' (ver. 3.6) software package (Felsentein 2001), and a rooted dendrogram was constructed. The cluster dendrogram was drawn up using the program 'Tree View' (ver. 1.6.5) developed by Page (2001). The standard errors (SE) of branching points were calculated according to Nei et al. (1985).

Table 2 Sequence of primers and the number of scored bands for each primer.

Primer	Sequence	G (%)	C (%)	Scored bands	RAPDs
P01	5'-GCGGCTGGAG-3'	60	20	9	8
P02	5'-GTGACGCCGC-3'	40	40	5	4
P04	5'-CGTCTGCCCG-3'	30	50	6	4
P06	5'-TTCCGCGGGC-3'	40	40	9	9
P17	5'-ATGACGACGG-3'	40	10	11	10
OPG02	5'-GGCACTGAGG-3'	50	20	11	11
OPAA17	5'-GAGCCCGACT-3'	30	40	10	10
OPAB04	5'-GGCACGCGTT-3'	40	30	9	9
OPAB18	5'-CTGGCGTGTC-3'	40	30	7	7
Total				77	72

P01, P02, P04, P06 and P17 (selected by Ehara et al. 1997).
OPG02, OPAA17, OPAB04 and OPAB18 (Operon Technology).

Results and Discussion

A total of 77 PCR products were scored from all the primers. Out of 77 products, five were shared by all the populations, and 72 were polymorphic among the 38 populations. The number of RAPDs by primer are shown in Table 2. The number of RAPDs produced by each primer varied from a minimum of four

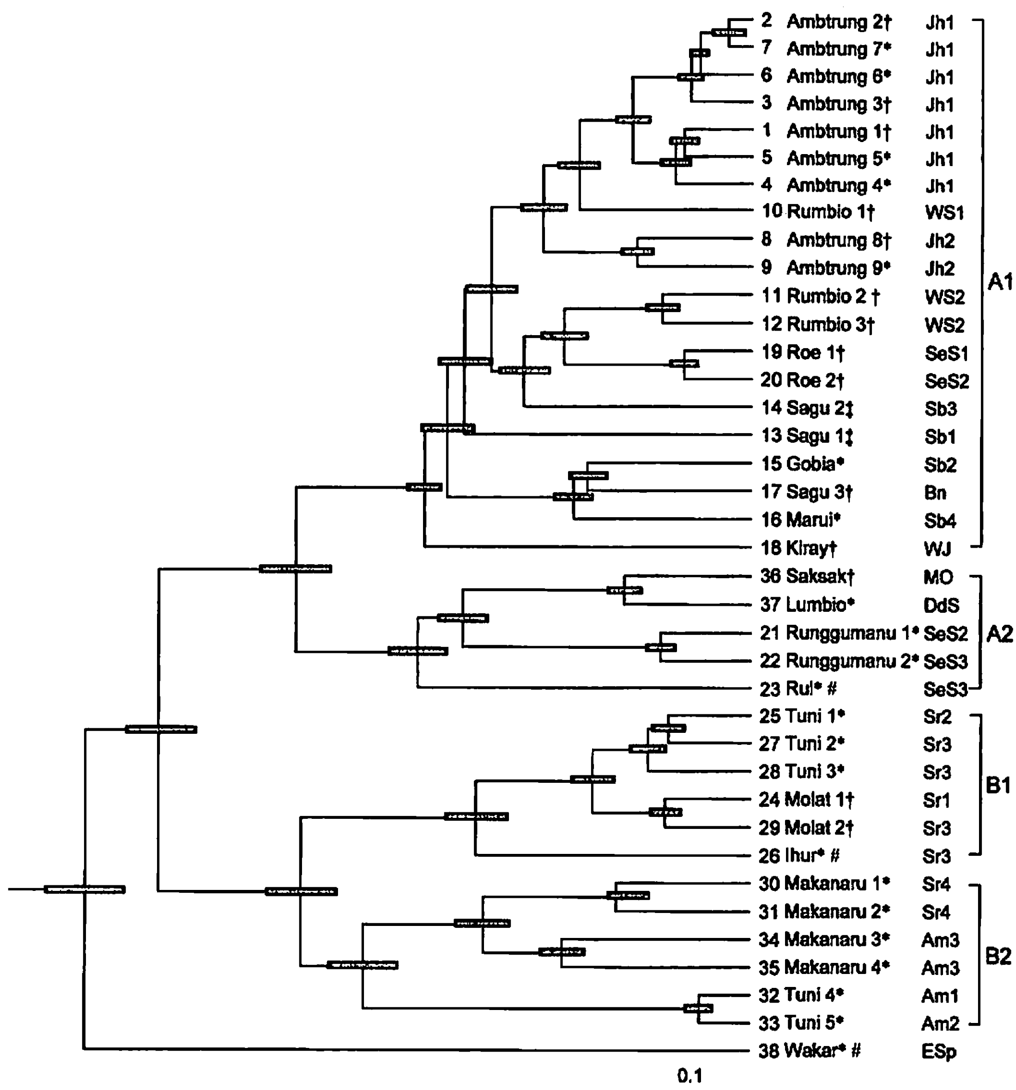

Fig. 2 UPGMA dendrogram based on RAPD data. Asterisk indicates spiny population.
*: spiny population, †: weak black, ‡: brown band, #: reddish pith.

amplified by P02 and P04 to a maximum 11 amplified by OPG 02.

The dendrogram constructed by the UPGMA method is shown in Fig. 2. From the dendrogram based on RAPD data, two main groups were found. Group A included two sub-groups, and sub-group A1 consisted of nine populations from Johor on the Malay Peninsular, eight populations from Sumatra and the surrounding islands, one population from West Java and two populations from Roe (Roe 1, 2) in Southeast Sulawesi, Indonesia, and sub-group A2 consisted of three populations from Southeast Sulawesi in Indonesia and two populations from Mindanao in the Philippines. The cluster of sub-group A1 mainly consisted of the populations occurring in the western area of the Malay Archipelago. The cluster of group B consisted of 12 populations from the eastern area of the Malay Archipelago, i.e. eight populations from Seram and four populations from Ambon in the Maluku Islands (the Moluccas), Indonesia. Six populations from Seram (Tuni 1, 2, 3; Molat 1, 2; Ihur) formed sub-group B1 and the other two populations from Seram (Makanaru 1, 2) and four populations from Ambon

(Makanaru 3, 4; Tuni 4, 5) formed sub-group B2. Wakar, a population from PNG, appeared outside the two main groups in the dendrogram. It was therefore considered that the genetic distance of sago palms was related to geographical distribution.

In the previous report, six populations in sub-group B2 were appeared close to three populations from Southeast Sulawesi (Runggumanu 1, 2; Rui) and two populations from Mindanao (Saksak; Lumbio) in the dendrogram (Ehara et al. 2002). In the present study, the populations in sub-group B2 were considered to be close to the populations from Seram (Tuni 1, 2, 3; Molat 1, 2; Ihur in sub-group B1) rather than the other populations. From the current result, the closer relationship between geographical distribution and genetic distance of sago palms in the Malay Archipelago became apparent. However, there should be an exception as Roe from Southeast Sulawesi in Indonesia. We cannot explain currently how a land race as Roe from Southeast Sulawesi appeared in the cluster of sub-group A1. Sometimes a sucker (off shoot) of sago palm has been exchanged as a gift for the arrival of baby in Southeast Sulawesi, and then the transplanted sago palm would be harvested to earn money for the child who grew up. The distribution of sago palm could be influenced by not only natural factors but also some living customs of inhabitants, cultural factors. We should consider both natural dispersal and historical plant migration to investigate the correspondence of sago palms growing at different sites.

Each cluster included both spineless and spiny sago palm populations. The dissimilarity between the spineless population and the spiny population was not as large as that within different spineless populations or within different spiny populations. For instance, the dissimilarity between Ambtrung 2 (spineless) and Ambtrung 7 (spiny) from Johor on the Malay Peninsular was apparently small compared that within between the other pairs of spineless populations or spiny populations. Consequently, the presence or absence of spines on the petiole and rachis was not considered to correspond with genetic distance. This result supports the proposal that spiny and spineless sago palms should be synonymous as *M. sagu* (Rauwerdink 1986). Ehara et al. (1998) reported that spine emergence had also been observed in seedlings produced from seeds of spineless sago palm. Jong (1995) reported the opposite case that not only spiny seedlings but also spineless seedlings grew from seeds of spiny sago palm. Considering these results, some types of sago palm can be lumped as one species regardless of the presence or absence of spines in seedlings.

Moreover, two populations having a brown band on the back of the petiole and rachis (Sagu 1 and Sagu 2 from Siberut near West Sumatra in Indonesia) were included in sub-group A1. Three populations showing reddish pith colour, Rui from Southeast Sulawesi, Ihur from Seram in the Maluku Islands, Indonesia and Wakar from PNG occurred in sub-group A2, group B1 and outside the two main groups, respectively. However, neither the banding pattern on the back of the petiole and rachis, nor the pith colour showed a clear relationship with genetic distance in the present study.

Through this study, it can be concluded that the genetic distance of sago palm populations growing in the Malay Archipelago is closely related to geographical distribution, and the presence or absence of spines on the petiole and rachis do not correspond with genetic distance.

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