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Screening of Primers for RAPD Analysis of Spiny and Spineless Sago Palm in Indonesia

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インドネシアのトゲサゴとホンサゴの RAPD 分析に用いるプライマーのスクリーニング

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Introduction

Sago palms belong to the genus *Metroxylon* and are classified into two species, *M. rumphii* Mart. and *M. sagu* Rottb., based on morphological characteristics, namely, spiny and spineless types (Barrau 1959; Takamura 1989). Three subspecies of spiny type and one species of spineless type are recognized in Indonesia (Takamura 1990). In contrast, sago palms are classified in more detail in folk classifications used by Indonesian people who have subsisted on sago (Yoshida 1980). Although it is considered that there are many varieties in not only the spiny type but also the spineless type, there are few studies on genetic variation in sago palm.

Ehara et al. (1995a, b) reported that there were large variations in starch yield of sago palm and growth environments, particularly in soil fertility, in east islands of Indonesia. There was a positive correlation between starch content of pith and stomata density of abaxial side of leaflet in sago palms, and the stomata density and exchangeable Ca content in soil (Ehara et al. 1995b). Starch productivity of sago palm would be closely related with soil fertility. However, to determine the factors relating the starch productivity, the genetic variation and the correspondence of sago palm growing at different sites should be investigated.

Randomly amplified polymorphic DNA (RAPD) markers generated by the polymerase chain reaction (PCR) can be used to differentiate between morphologically indistinguishable strains and varieties (Welsh and McClelland 1990; Welsh et al. 1991; Goodwin and Annis 1991). DNA profiles based on arbitrarily primed PCR are both time and cost effective (Herdrick 1992). Furthermore, the availability of markers will aid in mapping genes coding for agronomically important characters. In this study, we conducted the screening of primers to get polymorphic DNA markers by RAPD-PCR using two species of sago palm.

Materials and Methods

Extraction of DNA

Mature leaflet from young plant of Tuni (*M. rumphii* Mart.) and Molat (*M. sagu* Rottb.) was harvested at Suli, Ambon Island, and Kairatu, Seram Island in Maluku of Indonesia, respectively. Leaflet tissue was cut into small pieces and freeze-dried. The freeze-dried tissue was then ground with crys-

tal sand to a fine powder. The powdered tissue (0.5 g) was transferred to a sterile tube containing 10 ml of DNA extraction buffer [50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% (w/v) SDS, 1% (v/v) 2-mercaptoethanol] and incubated at 65 °C for 15 min. This mixture was then added with 10 ml of 3 M sodium acetate (pH 5.2) and kept on ice for 30 min. After centrifugation at 3500 rpm for 20 min, the supernatant was decanted into an equal volume of isopropanol and kept at room temperature. The sample was centrifuged at 3500 rpm for 20 min, and the precipitate was dissolved in 1 ml of TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. The DNA solution was re-precipitated with two volumes of ethanol and rinsed with 3 ml of 70% (v/v) ethanol. After air-drying, the DNA was resuspended in 0.5 ml of TE buffer. Three tenth mililiter of the DNA solution was treated with 1μl of RNase (10 μg/ml) at 55 °C for 30 min, which was then added with an equal volume of chloroform : iso-amyl alcohol (24:1) and mixed gently for 10 min. After centrifugation at 3000 rpm for 10 min, the aqueous phase was decanted into 1/10 volume of 3 M sodium acetate and two volumes of ethanol and centrifuged at 8000 rpm for 5 min. The precipitated DNA was washed with 1 ml of 70% (v/v) ethanol, air-dried, resuspended in 100 μl of TE and stored at 4 °C. DNA concentration was measured with a Milton Roy spectro-photometer (Spectronic 3000 Array) at 260 nm.

PCR

Twenty four arbitrary 10-mer primers (Table 1) were used for the PCR. Amplification reaction vol-

Table 1 List of primers and their products

No.	Sequence	G (%)	C (%)	Number of DNA bands in the gel		Presence (1) or absence (0) of RAPD marker
				Tuni	Molat	
1	5'-GCGGCTGGAG-3'	60	20	7	8	1
2	5'-GTGACGCCGC-3'	40	40	3	4	1
3	5'-CTCGGGTGGG-3'	60	20	2	5	1
4	5'-CGTCTGCCCG-3'	30	50	1	1	0
5	5'-AGTAGACGGG-3'	50	10	8	10	1
6	5'-TTCCGCGGGC-3'	40	40	5	10	1
7	5'-GAATTTCCCC-3'	10	40	0	2	1
8	5'-ATACAGGGAG-3'	40	10	0	0	0
9	5'-GCGGTTGAGG-3'	60	10	6	6	1
10	5'-ATCTGGCAGC-3'	30	30	3	3	0
11	5'-CGCACCGCAC-3'	20	60	1	6	1
12	5'-GAGTCACGAG-3'	40	20	5	6	1
13	5'-CTGGCGGCTG-3'	50	30	4	4	0
14	5'-CGTGGGCAGG-3'	60	20	2	3	1
15	5'-TAGCCGTGGC-3'	40	30	0	0	0
16	5'-AACGGGCAGC-3'	40	30	7	9	1
17	5'-ATGACGACGG-3'	40	20	4	4	1
18	5'-AACGGGGGAG-3'	60	10	0	0	0
19	5'-AGAATCCGCC-3'	20	40	2	2	0
20	5'-AGGACGTGCC-3'	40	30	4	5	1
21	5'-CTCCTCCCCC-3'	0	80	1	4	1
22	5'-GAAACAGCGT-3'	30	20	5	4	1
23	5'-CCAAGATGCT-3'	20	30	4	4	0
24	5'-TGCTGGCTTT-3'	30	20	1	7	1

umes were 25 μl, each containing 2.5 μl of 10 × reaction buffer, 2 μl of 2.5 mM dNTP, 3.3 μl of 1.52 μM primer, 0.2 μl of 5 U/μl *Taq* polymerase (AmpliTaq, Perkin Elmer), and 1 μl of DNA solution. Amplification was performed in a Takara PCR Thermal Cycler MP programmed as follows: 1 cycle of 1 min at 94 °C; 45 cycles of 1 min at 93 °C, 2 min at 42 °C, 2 min at 72 °C; followed by 7 min at 72 °C. A portion of DNA from the amplification reactions was electrophoresed in a 1.5% agarose gel containing 1 × TBE buffer [89 mM Tris-borate, 2 mM EDTA (pH 8.0)] and 0.5 mg/l ethidium bromide at 100 V for 35 min, and visualised by UV transilluminator.

Prior to the screening of primers, the optimal DNA concentration for PCR reaction was determined using primer No. 4; 1-, 10-, 100-, and 1000-fold dilutions of the DNA solution prepared as above.

Table 2 List of amplified products in basepairs

Primer	Products	
	Tuni	Molat
No. 1	1100, 900, 800, 750, 600, 500, 300	1100, 950, 900, 800, 750, 600, 450, 300
No. 2	1000, 700, 250	1400, 1000, 700, 250
No. 3	750, 500	1050, 950, 750, 500, 200
No. 4	550	550
No. 5	1250, 950, 800, 750, 600, 500, 400, 300	4000, 2600, 1250, 950, 800, 750, 700, 500, 400, 300
No. 6	2100, 1200, 750, 550, 500	2350, 1850, 1400, 1150, 900, 750(2), 700, 500, 300
No. 7	no amplified	1050, 500
No. 8	no amplified	no amplified
No. 9	850, 650, 550, 450, 400, 300	850, 650, 550, 450, 300, 200
No. 10	650, 500, 200	650, 500, 200
No. 11	700	1450, 1150, 950, 900, 650, 450
No. 12	950, 700, 600, 450, 400	1050, 750, 550, 450, 400, 200
No. 13	900, 550, 400, 300	900, 550, 400, 300
No. 14	350, 300	750, 350, 300
No. 15	no amlified	no amplified
No. 16	1050, 850, 700, 550, 450, 350, 300	1050, 850, 700, 650, 550, 500, 450, 350, 300
No. 17	1000, 600, 400, 250	1000, 800, 650, 250
No. 18	no amplified	no amplified
No. 19	450, 350	450, 350
No. 20	1100, 650, 500, 400	900, 750, 650, 500, 400
No. 21	300	1000, 900, 800, 350
No. 22	900, 700, 600, 450, 350	900, 700, 600, 450
No. 23	1200, 800, 500, 200	1200, 800, 500, 200
No. 24	800	2300, 2100, 1500, 900, 800, 550, 400

Results and Discussion

Various DNA concentrations for PCR reaction were tested. Although DNA solution of 1- and 10-fold dilution did not give amplified products, 100- and 1000-fold diluted solutions gave amplified bands in Tuni. DNA concentrations in the reaction mixture using the 100- and 1000-fold diluted solutions were 800 and 80 pg/μl, respectively. In the case of Molat, DNA solution of 100-fold dilution did not give amplified products, but 1000-fold diluted solution gave amplified bands. DNA concentration in the reaction mixture using the 1000-fold diluted solution of Molat was 148 pg/μl. Thus, DNA solution of 1000-fold dilution was used for the screening of primers.

Table 1 shows the sequences of the primers used and the result of the PCR amplifications. G/C contents of all primers were more than 50%. Primer Nos. 8, 15, and 18 did not give amplified products in Tuni and Molat. Primer No. 7 gave amplified bands in only Molat. The remaining 20 primers gave amplified bands in both Tuni and Molat. Among the 21 primers that gave amplified products, 16 displayed different banding patterns between Tuni and Molat. List of amplified products in basepairs is shown in Table 2. The amplified bands ranged from 4 kb to 200 bp.

The result shows that 88% of the primers tested produced amplified bands, 76% of which were polymorphic. To improve the reproducibility of these polymorphic bands and to investigate genetic variability in a species and/or subspecies are the subjects of further study. At least, some of these polymorphic bands can be used as RAPD markers to estimate genetic variation in sago palm. The use of RAPD analysis in the identification and characterization of sago palm varieties will be of considerable help to investigate the correspondence of sago palms grown at different sites.

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References

Barrau, J. 1959 The sago palm and other food plants of Marsh Dwelers in the South Pacific Islands. Econ. Botan. 13: 151–159.

Ehara, H., C. Mizota, S. Susanto, S. Hirose and T. Matsuno 1995a Sago palm production in eastern islands of Indonesia. — Variations in morphological characteristics and growth environment —. Jpn. J. Trop. Agr. 39 (extra issue 1): 11–12.

Ehara, H., C. Mizota, S. Susanto, S. Hirose and T. Matsuno 1995b Sago palm production in eastern islands of Indonesia. — Variations in starch yield and soil environment —. Jpn J. Trop. Agr. 39 (extra issue 2): 45–46.

Goodwin, P. H. and S. L. Annis 1991 Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified poly-morphic DNA assay. Appl. Environ. Microbiol. 57: 2482–2486.

Herdrick, P. 1992 Shooting the RAPDs. Nature 355: 679–680.

Takamura, T. 1989 Sago Palm. In: Plant Genetic Resources. (Matsuo, T. ed.) 4: 1330–1331.

Takamura, T. 1990 Present research activities and the problems on sago palm. Jpn. J. Trop. Agr. 34: 51–58.

Welsh, J. and M. McClelland 1990 Fingerprinting genoms using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213–7218.

Welsh, J., C. Petersen and C. McClelland 1991 Polymorphisms generated by arbitrary primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res. 19: 303–306.

Yoshida, S. 1980 Folk classification of the sago palm (*Metroxylon* spp.) among the Galela. Senri Ethnological Studies 7: 109–117.