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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS OF YAMS COLLECTED IN THE YAP ISLANDS AND THE ULITHI ISLANDS.

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Abstract

Yam (*Dioscorea* spp.) leaf samples were collected from cultivated crop in the Yap islands (Yap proper) and the Ulithi islands (Mogmog, Asor, Fassarai and Falalop). Most of the materials have been identified based on morphological characters as *D. alata* L., but some other species were found, as well. For genetic analysis, total DNA was obtained by SDS method and polymerase chain reaction (PCR) was done to amplify the segment of DNA generated in the presence of RAPD primers. Within the 36 samples, due to RAPD markers band polymorphism, 14 band patterns were detected. These results suggest that the presence of large genetic diversity in *D. alata* from the state of Yap and that RAPD markers might work well in detecting it.

Keywords: classification, RAPD, yam, Yap islands, genetic resources

Introduction

Tubers (taro, yam, sweet potato etc.) were staple foods in the state of Yap. The weights of tubers transferred to cereals gradually. Still, they are important crops, especially taro and yam. Taro is cultivated at the hollow called the taro patch located near the farmer's house. From planting time to harvest takes 4 to 5 years. Yams are cultivated in the forest where small trees are cut down at planting sites. Yam vines are twined about a bamboo pole stood against a big tree. Yam and taro were transmitted to the state of Yap along with mankind transmission from Asia. So that we accept the crops have a long history in this area.

Yams as crop exist from temperate to tropical zones in Asia, Africa and Oceania. About 10 spices have been used for starch (COURSEY 1967). Classifications of the cultivated species have been done by identifying morphological characters. Recently, classification using DNA markers has been tried. The existence of *D. alata, D. opposita and D. japonica* is also confirmed by Random Amplified Polymorphic DNAs (RAPDs) analyses (SHIWACHI et al. 2000). Yam breeding programs need a rapid and easy method to detect genetic variations of geographically different materials. The RAPD analysis by the polymerase chain reaction (PCR) was successfully used on many crops. So RAPD analyses of yams collected in the state of Yap were done.

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Materials and Methods

Leaf samples

Yam leaf samples were collected from cultivated ones by farmers. Most samples are identified *D. alata* by morphological characters. A total of 36 samples were collected including 3 different species. Table 1 shows collecting sites and control plants used for testing (*D. bulbifera* as Airyam, *D. alata* as Arata, and *D. japonica* as Jinen-jo). Collected leaf samples were stored at

Table 1. Collected Yams (*Dioscorea* spp.) in theYap islands and the Ulithi islands

Lines and species		Site (Island, District, Village)			Band pattern
YYA 2	*D. alata*	Yap	Tomil	Thol	7
YYA 3	*D. alata*	Yap	Tomil	Thol	1
YYA 5	*D. alata*	Yap	Tomil	Thol	11
YYA 6	*D. alata*	Mogmog			2
YYA 7	*D. alata*	Mogmog			N.D
YYA 8	*D. alata*	Mogmog			2
YYA 9	*D. alata*	Asor			13
YYA 10	*D. alata*	Asor			N.D
YYA 11	*D. alata*	Fassarai			1
YYA 12	*D. alata*	Fassarai			3
YYA 13	*D. alata*	Fassarai			2
YYA 14	*D. alata*	Mogmog			N.D
YYA 15	*D. alata*	Mogmog			N.D
YYA 16	*D. alata*	Mogmog			4
YYA 17	*D. alata*	Yap	Gagil	Leabinau	1
YYA 18	*D. alata*	Yap	Gagil	Leabinau	6
YYA 19	*D. alata*	Yap	Rull	Ngof	7
YYA 20	*D. alata*	Yap	Rull	Dachngar	8
YYA 21	*D. alata*	Yap	Maap	Waned	10
YYA 22	*D. alata*	Yap	Maap	Waned	7
YYA 23	*D. alata*	Yap	Maap	Waned	5
YYA 24	*D. alata*	Yap	Maap	Waned	8
YYA 25	*D. alata*	Yap	Maap	Waned	7
YYA 26	*D. alata*	Yap	Maap	Waned	6
YYA 27	*D. alata*	Yap	Tomil	Thol	N.D
YYA 28	*D. alata*	Yap	Tomil	Thol	10
YYA 29	*D. alata*	Yap	Tomil	Thol	10
YYA 30	*D. alata*	Yap	Tomil	Thol	4
YYA 31	*D. alata*	Yap	Tomil	Thol	7
YYA 32	*D. alata*	Yap	Maap	Waned	6
YYA 33	*D. alata*	Mogmog			N.D
YYA 34	*D. liebretsiana*	Falalop			9
YYA 35	*D. liebretsiana*	Mogmog			9
YYA 36	*D. esculenta*	Mogmog			12
YYA 37	*D. bulbiferea*	Mogmog			14
YYA 38	*D. esculenta*	Mogmog			12
Airyam	*D. bulbiferea*				
Arata	*D. alata*				
Jinen-jo	*D. Japonica*				

N.D = Not detected

-80 °C before DNA extraction.

DNA extraction

Total DNA was isolated from frozen leaf sample by modified SDS method, about 1 g leaf tissue per sample ground in liquid nitrogen. To remove polyphenol, sample was vortexed with 5 ml of washing buffer (100 mM HEPES, 0.1% Polyvinylpyrrolidone K-30, 4% 2-Mercaptoethanol) and then centrifuged at 18,000 × g for 3 min. This washing process was repeated at least 4 to 5 times up to take out stickiness. The precipitant was solved with 5 mℓ extraction buffer (15% sucrose, 50 mM Tris-HCl, 50 mM EDTA, 500 mM NaCl, pH 8.0) and centrifuged at 4,600 × g for 5 min. The precipitant was resolved with 2 mℓ of resuspension buffer (20 mM Tris-HCl, 10 mM EDTA, pH 8.0) and incubated at 70 °C for 15 min within 250 μℓ of 10% SDS. 1 mℓ of 7.5 M ammonium acetate was added and cooled on ice for more than 30 min. After centrifuge for 15 min. at 18,000 × g, the supernatant was transferred to a new cube. Equivalent cold isopropanol was added and centrifuged for 15 min. at 18,000 × g. DNA pellet was washed in 70% ethanol and dried and dissolved in 500 μℓ TE buffer. 10 μg / μℓ RNAase was added and incubated at 55 °C for 10 min. Lastly, DNA solution was centrifuged for 5 min. at 18,000 × g. The supernatant was transferred a 1.5 μℓ cube and stored - 20 °C until use.

PCR and electrophoresis

PCR was performed in a 0.2 mℓ tube for use on a GeneAmp PCR systems 9700 (PE Biosystems). The reaction consisted of 20 ng of DNA, 10 pmol primer, 2.5 μℓ 10 × PCR buffer, 200 μM of each dNTP and Taq polymerase (TAKARA *Taq*) in 25 μℓ volume. Primers used for yam were OPA-2, C-15, C-19, C-20, E-12, W-2, W-7, X-1, X-9 and Y-9 Operon (SHIWACHI et al. 2000). It was possible to classify these into *D. alata*, *D.opposita* and *D. japonica*. The thermocycler was set at 94 °C for 5 min. and repeated 30 sec. at 94 °C, 1 min. for annealing, and 2 min. at 72 °C. That annealing temperature was degraded 4 °C every 3 cycles from 56 °C to 44 °C, and the reaction kept on 35 cycles at 40 °C. This was followed by a final cycle of 72 °C for 7 min. The amplified DNA sample with dye was loaded on 1% agarose and 0.5% synergel (Diversified biotech) in 1 × TAE buffer at 60 V for 70 min., and stained by ethidium bromide. The electrophoresed gel was photographed under UV light.

RAPD analysis

All DNA samples were tested and divided into some groups by RAPD band patterns. One sample was picked from each group. About 16 PCR samples including 3 controls were tested again for reconfirmation of relationship between them.

Results and discussions

Total 36 samples were tested. Most of them did not show any amplified DNA bands because of the weakness of the SDS methods. And then, phenol treatment was applied to all samples. Some samples (YYA7, 10, 14, 15, 27 and 33) gave no bands, so they could not be used in classification.

Table 1 and Fig. 2 showed 14 different band patterns that were detected by OPA-2 in this test. In this survey, the *D. alata* species was mainly collected. *D. alata* characterized by about 1200 bp fragment was found in Arata which is accepted as model population for this *D. alata* species. Other examples differ in length or position. These can be accepted as signs of genetic

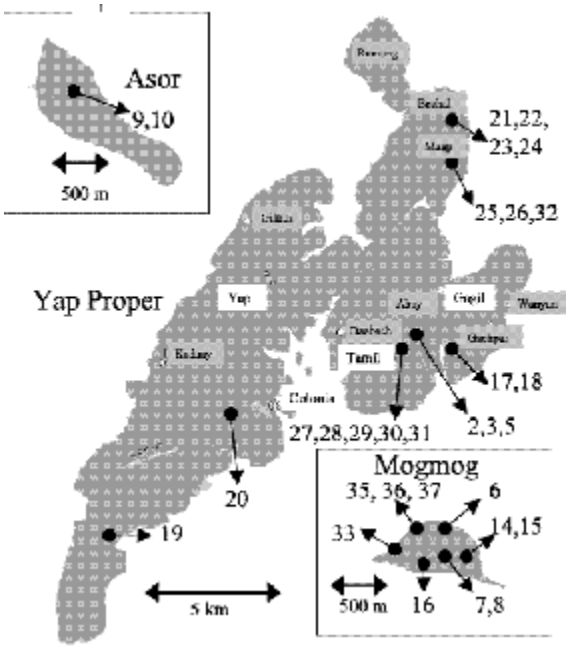

Fig. 1. Collecting sites of yams in Yap, Asor and Mogmog islands.

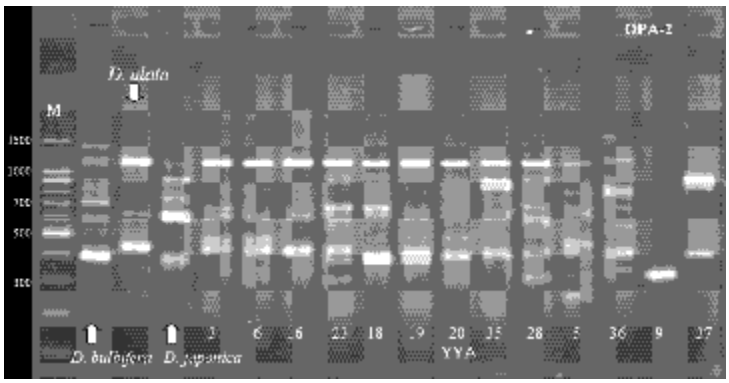

Fig. 2. DNA polymorphisms of yams detected by amplification of total DNA using OPA-2 primer

diversity. Those samples with no the band of 1200 bp (YYA 9, 36 and 37) were considered as other species. According to morphological characters, YYA 36 was identified as *D. esculenta* and YYA37 as *D. bulbiferea*. The sample YYA37 morphologically relates to D. bulbiferea which shows a different band pattern, so this may mean genetic variation can be found also in *D. bulbiferea*.

Genetic variation of yams in the Yap islands and Ulithi islands can be easily detected by RAPD analysis. The OPA-2 primer seems to be fitted for detecting genetic diversity in the *D. alata* species.

References

COURSEY, D. G. 1967. *Yams*. 230 pp. London: Longmans.
SHIWACHI, H. et al. 2000. *Jpn. J. Trop. Agr.*, 44: 229–237.