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RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS OF WILD SWEET POTATOES AND YAMS PICKED IN THE YAP ISLANDS

Satoru TAURA, Tadashi ABE, Michio ONJO, Yoshitaka SAKAMAKI,
Maki YAMAGUCHI, Mika TAWARA, Atsushi NISHITANI and Kotaro KAWABE

Abstract

Several leaves of wild sweet potatoes (*Ipomoea* spp.) and yams (*Dioscorea* spp.) were picked in various parts of the Yap islands. After extraction of DNA from those materials, polymerase chain reactions (PCR) were done using the selected primers for the both plants classifications. Results of random amplified polymorophic DNAs (RAPD) analysis, 4 groups found in 27 wild sweet potatoes lines. Most of lines were included in *I. triloba* group. In this study, existence of *I. trifida* group (cross compatible to sweet potato, *I. batatas*) recognized. In yams, total 29 lines divided into 5 clusters, consisting of 9 groups. High variability existed.

Key words: Classification, RAPD, wild sweet potato, yam, Yap islands

Introduction

Tubers (taro, yam, sweet potato etc.) were staple foods in the Yap islands. They gradually were replaced by cereals. Still, they are important crops, especially taro and yam. Taro is cultivated at the hollow called taro patch located near farmer's house. It takes 4 to 5 years to harvest them. Yams are cultivated in the forest where small trees are cut off around planting sites. Yam vines are twined about a bamboo pole stood against big tree. Both crops were transmitted to the Yap islands together with mankind transmission from Asia. They have a long history. While, sweet potatoes are planted at house garden and roadside. Many different kinds in leaf shape and color are observed in the one site. Those sweet potatoes are kept roughly as compared with taro and yam. And most of lines are introduced during the war or after the war. Sweet potato originated in middle South America spread to the world through the three major routes (YEN, 1974). Its are Kamote, Kumara and Batata routes. Yap located on the end of Kamote route. This crop also has a long history. But, it is difficult to distinguish newly introduced ones and old ones. So, we took notice of wild spices of sweet potato. Sweet potato has several wild spices. It is said that *Ipomoea trifida* among them is ancestor of sweet potato, *Ipomoea batatas* because of cross compatibility. We interest in variation of wild sweet potato on the end of transmission route.

Yams are cultivated from temperate to tropical zone in Asia, Africa and Oceania. About 10 spices have been used for starch crop (COURSEY, 1967). Classifications of the cultivated spices have been studied. We investigated how much variations are existed in the Yap islands.

On this study, we need rapid and easy method to be known about genetic variation. Random Amplified Polymorphic DNAs (RAPDs) analysis by the polymerase chain reaction (PCR) is

useful method for this kind of study. RAPD analysis of wild sweet potatoes and yams in the Yap islands were done.

Materials and Methods

Leaf samples

Wild sweet potato (*Ipomoea* spp.): Twenty seven leaf samples were picked in various parts of the Yap islands (Fig. 1). Four typical spices of wild sweet potatoes were added as a control (Table 1.). *I. purpurea* group (K150 and K148), *I. triloba* group (K121 and 7926), *I. trifida* group (K221 and 7930) and not classified group (8203 and K4) .

Yams (*Dioscorea* spp.): Yam leaf samples were collected from cultivated ones by farmer mainly. Twenty nine leaf samples including two wild types were picked in various parts of the Yap islands (Fig.1). As a control, *D. domitorium*, *D. bulbifera* (2 lines), *D. alata* (Alata and Solo), *D. opposita* (Shinshu zairai and Yamanoimo), *D. japonica* (Jinen-jo) and Nepal wild (not

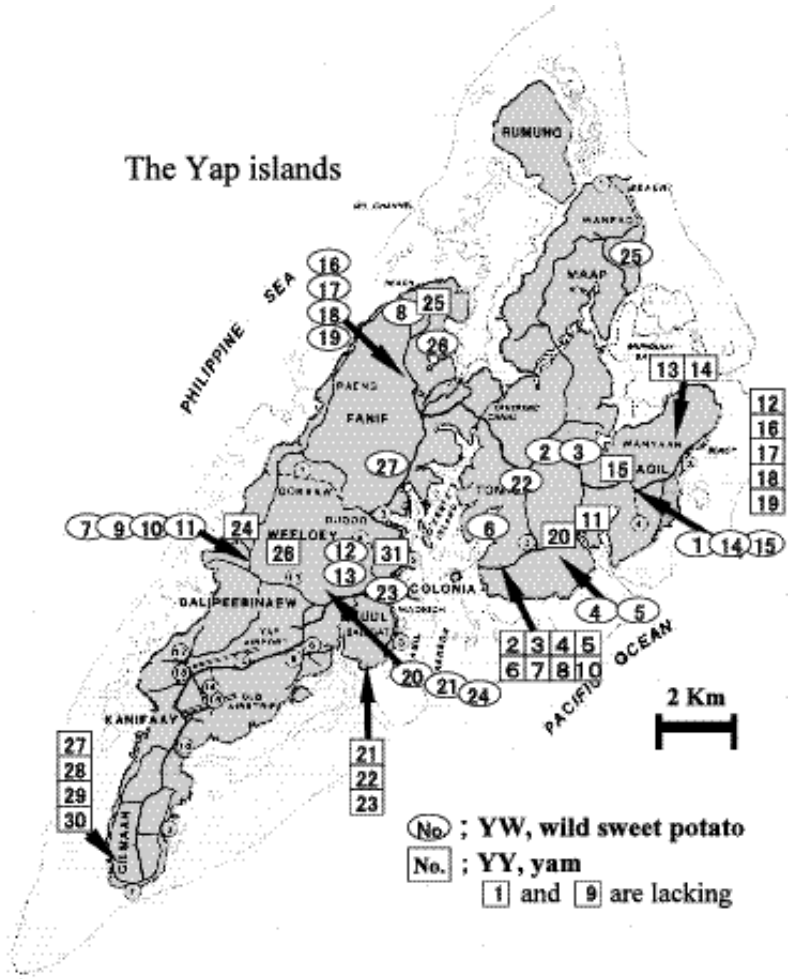

Fig. 1. Picking sites of wild sweet potatoes and yams in the Yap islands.

classified) were tested (Table 2.). Picked leaves were stored - 80℃ before DNA extraction.

DNA extraction

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

PCR and electrophoresis

PCR was performed in a 0.2 ㎖ tube for use on a GeneAmp PCR systems 9700 (PE Biosystems). The reaction consisted of 20 ng of DNA, 10 pmol primer, 2.5 ㎕ 10 x PCR buffer, 200㎛M of each dNTP and *Taq* polymerase (TAKARA *Taq*) in 25 ㎕ volume. Used primers for wild sweet potato were CMN-A17, A19, A47, D1, D2, D3 (Bex) that were selected for classification of its diploid spices (Sakai, unpublished). In yam, OPA-2, C-15, C19, C-20, E-12, W-7, X-1, X-9 (Operon) were used (Shiwachi *et al.* , 2000). Those are possible to classify into *D. alata*, *D.opposita* and *D. japonica*. The thermocycler was set at 94℃ for 5 min. and repeated 30 sec. at 94℃, 1 min. for annealing, and 2 min. at 72℃. That annealing temperature was degraded 4℃ every 3 cycles from 56℃ to 44℃, and the reaction kept on 35 cycles at 40℃. This was followed by a final cycle of 72℃ for 7 min. The amplified DNA sample with dye was loaded on 1% agarose and 0.5 % synergel (Diversified biotech) in 1 x TAE buffer at 60 V for 70min, 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 or two samples were picked up from each group. About 16 PCR samples including control samples were tested again for reconfirmation of relationship between them. Polymorphic bands were scored 1 for present or 0 for absent. Cluster analysis using flock average method (UPGMA) was done by STATISTICA soft wear.

Results and Discussions

Wild sweet potatoes

Total 27 samples were tested and divided into 4 groups, *I. triloba* group, *I. trifida* group, 8203 group and group of Yaps own, different from control plants (Table 1. and Fig. 2.). *I. purpurea* group wasn't recognized in Yaps samples (Fig. 3.). Most of samples belong to *I. triloba* group. In this group, seeds set and seedling spread every where of roadside (YW 4, YW

Table 1. Wild sweet potatoes picked in the Yap islands and used control plants

Lines	Site and species	Band patterns
YW 1	Looraag	Yap own[1)]
YW 2	Tamargil	Yap own
YW 3	Tamargil	Yap own
YW 4	Maa	*I. triloba*
YW 5	Maa	*I. triloba*
YW 6	Machalead	*I. triloba*
YW 7	Kanif	Yap own
YW 8	Runuw	*I. triloba*
YW 9	Kanif	*I. trilfida*
YW 10	Kanif	*I. triloba*
YW 11	Kanif	*I. triloba*
YW 12	Madeqdeq	*I. triloba*
YW 13	Madeqdeq	*I. triloba*
YW 14	Looraag	*I. triloba*
YW 15	Looraag	*I. triloba*
YW 16	Fanif	*I. triloba*
YW 17	Fanif	*I. triloba*
YW 18	Fanif	*I. triloba*
YW 19	Fanif	*I. triloba*
YW 20	Agri. Exp. Sta.[2)]	*I. triloba*
YW 21	Agri. Exp. Sta	*I. triloba*
YW 22	Maa	*I. triloba*
YW 23	Colonia	*I. trilfida*
YW 24	Agri. Exp. Sta	*I. triloba*
YW 25	Wacholab	8203
YW 26	Runuw	*I. triloba*
YW 27	Maqweach	*I. triloba*
K 150	*Ipomoea purpurea*	*I. purpurea*
K 148	*Ipomoea purpurea*	*I. purpurea*
K 121	*Ipomoea triloba*	*I. triloba*
7926	*Ipomoea triloba*	*I. triloba*
K 221	*Ipomoea trilfida*	*I. trilfida*
7930	*Ipomoea trilfida*	*I. trilfida*
8203	unknown	8203
K 4	unknown	8203

1): band pattern different from controls
2): Agricultural experiment station

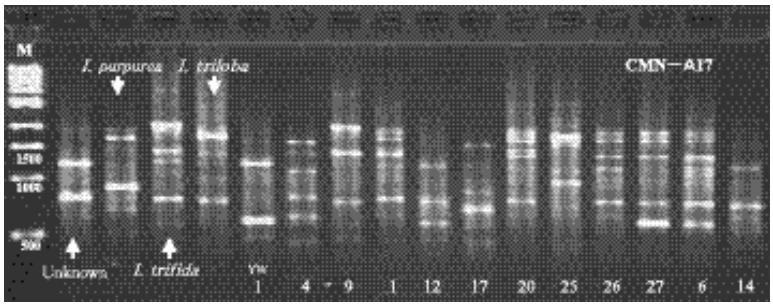

Fig. 2. DNA polymorphisms of wild sweet potatoes detected by amplification of total DNA using CMN-A17 primer.

6 and YW 20). These plants were similar to *I. triloba* in shape of leaf and flower. This kind of *I. triloba* was called "Giliy" in the Yap islands and used for medicine or food formerly. Other plants in the *I. triloba* group showed lignification in part of stem (YW 11, YW 12 and YW 14). Those plants intertwined with fernbrake that grow in barren and damp clayey soil, and born flowers but didn't set seeds. Two of 27 samples were found to belong to *I. trifida* (YW 2 and YW 9). *I. trifida* is origin of sweet potato, because only *I. trifida* is able to cross to *I. batatas*. Yap islands locate the end of Kamote route on the sweet potato distribution. It is interesting that *I. trifida* groups exist in the Yap islands far from the origin.

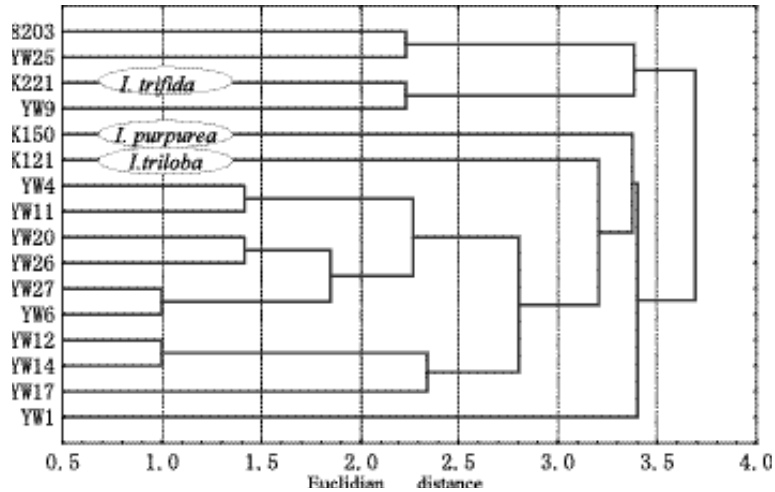

Fig. 3. Cluster diagram for wild sweet potatoes picked in the Yap islands using RAPD maker.

Yams

29 samples were tested and showed 9 different band patterns (Table 2. and Fig. 4). The band pattern 1 (YY5) was the largest group and was found in all the islands. The group belongs to *D. alata* and *D. opposita* that are typical cultivated species in tropical zone. Second largest group (YY2 and band pattern 4) showed different band patterns from used as control plants'. The YY2 group is the farthest one on genetic distance among the tested materials (Fig. 5). Two wild types picked at roadside were located in same group, *D. domitorium* and *D. japonica* cluster. In

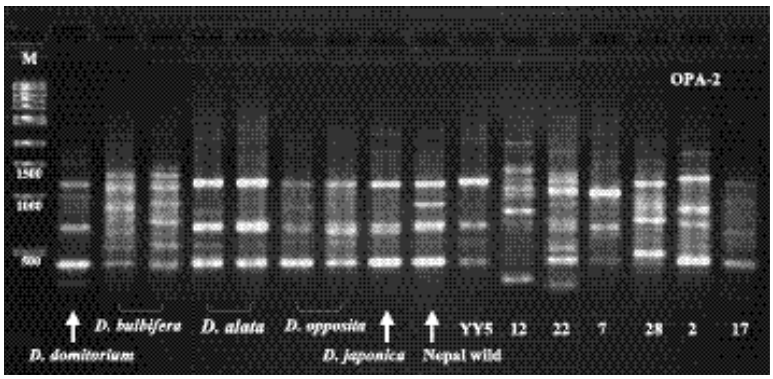

Fig. 4. DNA polymorphisms of yams detected by amplification of total DNA using OPA-2 primer.

Table 2. Yams (*Dioscorea spp.*) picked in theYap islands and used control plants

Lines and species	Site	Band patterns and clusters	
YY 2	Doomchuy	4	I
YY 3	Doomchuy	3	III
YY 4		4	
YY 5	Doomchuy	1	II
YY 6	Doomchuy	1	
YY 7 (wild)		2	V
YY 8	Doomchuy	4	
YY 10	Doomchuy	4	
YY 11	Doomchuy	4	
YY 12	Doomchuy	5	II
YY 13	Thol	4	
YY 14	Tamargil	5	
YY 15	Fitilaw	3	
YY 16	Amun	1	
YY 17	Amun	9	
YY 18	Tamargil	7	III
YY 19	Tamargil	2	
YY 20	Tamargil	1	
YY 21	Tamargil	1	
YY 22	Maa	8	V
YY 23	Qatliw	1	
YY 24	Balabata	7	III
YY 25	Balabata	6	
YY 26	Okaw	9	
YY 27	Runuw	1	
YY 28	Kanif	6	IV
YY 29	Anoth	1	
YY 30	Anoth	7	
YY 31 (wild)	Anoth	2	
D. domitorium	Anoth		V
D. bulbiferea	Madeqdeq		IV
D. bulbiferea (Airyam)			
D. alata (arata)			II
D. alata (Soloyam)			
D. opposita (Shinsyu zairai)			II
D. opposita (Yamanoimo)			
D. Japonica (Jinen-jo)			V
Unknown (Nepal wild)			IV

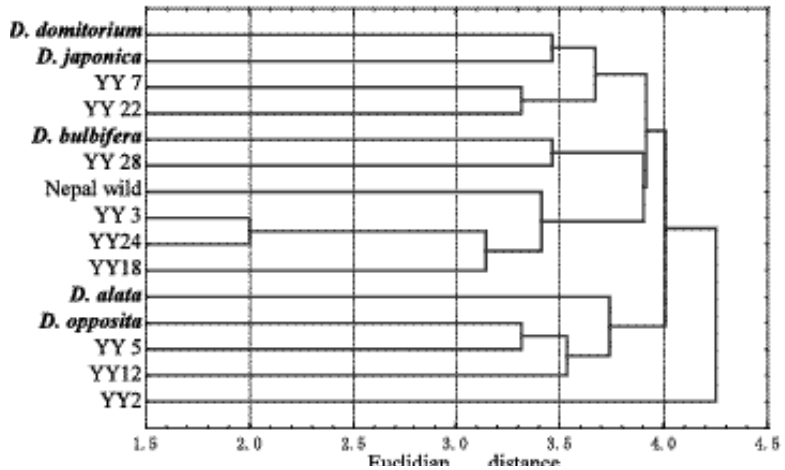

Fig. 5. Cluster diagram for yams picked in the Yap islands using RAPD maker.

result, 29 samples were divided into 5 clusters on 3.8 Euclidian distance.

In this survey, variation of yams in the Yap islands were recognized high and many different kinds of yams were cultivated in one site.

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