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GENETIC DIVERSITY OF COCONUT (*Cocos nucifera* L.) IN YAP STATE

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Abstract

Coconut is an important crop in the tropical zone. In this study, we collected leaflet samples from coconuts in Yap proper and the four inhabited islands of the Ulithi atoll. Then, we evaluated genetic diversity of coconuts grown in Yap using simple sequence repeats (SSRs). Two alleles were detected at the CNZ33 locus. At least eight alleles were detected at the CNZ29 locus. The number of alleles in samples from Yap at the CNZ29 locus was comparable to that detected in samples across the entire geographic range. The cause of high genetic diversity of coconut in Yap is discussed.

Keywords: coconut, genetic diversity, simple sequence repeats, Ulithi, Yap

Introduction

The coconut palm (*Cocos nucifera* L.) is an outbreeding perennial crop in the tropical zone. Owing to its importance for rural communities, it has been termed the 'tree of life'; from the roots to the fronds, all coconut palm constituents are utilized for either nutritional or non-food purposes (PERSLEY 1992; HERRAN et al. 2000). Many researchers have studied its morphological characteristics for coconut variety classification and for genetic diversity evaluation. However, morphological characteristics are more or less affected by environmental conditions, a fact which has impeded exact classification and evaluation.

Microsatellites, or simple sequence repeats (SSRs), are simple, tandemly repeated di- to tetra-nucleotide sequence motifs flanked by unique sequences. They are valuable as genetic markers because they are co-dominant, detect high levels of allelic diversity, and are easily and economically assayed by the polymerase chain reaction (PCR) (MCCOUCH et al. 1997). Coconut SSRs were detected by RIVERA et al. (1999); the primer pairs to amplify them by PCR were designed by TEULAT et al. (2000). According to TEULAT et al. (2000), SSRs provide the most informative means for evaluating genetic diversity in coconut populations.

TEULAT et al. (2000) evaluated genetic diversity in 31 individuals from 14 coconut populations across the entire geographic range (2–3 individuals per population) using SSRs. They collected samples from the Ivory Coast, Mozambique, Malaysia, the Philippines, Sri Lanka, Indonesia, Kiribati, Papua New Guinea, Tonga, the Solomon Islands, Fiji, and Panama. However, they did not collect any from the Federated States of Micronesia (FSM). In the present study, genetic diversity of coconuts in Yap State, located in westernmost FSM, was evaluated using SSRs.

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Materials and Methods

Sample collection

Coconut trees in Yap state are usually planted in yards, along roads and at the sea shore. We did not see coconut plantations there. Leaflets were collected from 150 coconut trees in Yap proper and the four islands in Ulithi atoll: Mogmog, Falalop, Asor, and Fassarai, through the courtesy of the coconut trees' owner. In the present study, 24 samples, of which several morphological characteristics such as trunk girth, length of the 5-leaf-scars interval, fruit shape, and nut shape were also surveyed, were examined: six were from Yap proper, six from Mogmog, four from Asor and four from Fassarai. Samples were stored at -50 °C in a deep freezer of a research ship until disembarkation at Kagoshima, Japan. There, they were stored at -80 °C in a deep-freezer until DNA extraction.

DNA extraction

Total DNA was extracted from frozen leaflet samples by a modification of the method of MUKAI and YAMAMOTO (1997). Briefly, 25 mg of leaflet per sample was ground to a fine powder with a mortar and pestle in liquid nitrogen. The powder was transferred to a 1.5 ml microcentrifuge tube containing 250 µl cold isolation buffer (10% polyethylene glycol, 0.35 M sorbitol, 0.1 M Tris-HCl (pH 8.0), 0.5% spermidine, 0.5% spermine, 0.5% β-mercaptoethanol). After vortexing, the tube was centrifuged at 15,000 rpm at 4 °C for 10 min. The supernatant was removed and 250 µl cold isolation buffer was poured. Next, the tube was centrifuged again under identical conditions. The supernatant was removed and the pellet was resuspended in 125 µl of lysis buffer (0.35 M sorbitol, 0.1 M Tris-HCl (pH 8.0), 0.5% spermidine, 0.5% spermine, 0.5% β-mercaptoethanol). After vortexing, 15 µl of 10% sarcosine was added and the tube was incubated 10 min at room temperature. Then, 150 µl of 2 × CTAB solution (2% CTAB, 0.1 M Tris-HCl (pH 9.5), 20 mM EDTA, 1.4 M NaCl, 0.5% β-mercaptoethanol) was added and the tube was incubated for 10 min at 65 °C. Next, 300 µl of chloroform/isoamyl alcohol (24:1. v/v) was added and the tube was laid down and shaken 15 min before centrifugation at 15000 rpm at 20 °C for 10 min. The upper aqueous phase was transferred to a 1.5 ml microcentrifuge tube. Then, 300 µl of cold isopropanol was added and the tube was inverted twenty times. The tube was placed for 20 min and was centrifuged at 6000 rpm at 4 °C for 5 min. The supernatant was removed and 500 µl of high salt TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 M NaCl) was added. The tube was incubated at 56 °C with gentle shaking until the pellet (mainly composed of DNA and RNA) at the bottom was completely dissolved; 300 µl of cold isopropanol was added and the tube was inverted twenty times. The tube was placed for 20 min and was centrifuged 6000 rpm at 4 °C for 5 min. The DNA was recovered as a pellet, washed with 70% ethanol twice and with 100% ethanol once, dried, and resuspended in 100 µl of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) at 56 °C until it was completely dissolved. Then, 30 µl of 10 mg/ml RNase was added and the tube was incubated 60 min at 42 °C. DNA concentration was measured using a spectrometer Lambda bio 20 (PerkinElmer, MA, USA). After that, the tube was stored at -20 °C until use.

PCR and electrophoresis

PCR amplification was performed following a modified method of TEULAT et al. (2000). The 20 µl of reaction mix contained 5 ng template DNA, 0.4 µM forward primer, 0.4 µM reverse

primer, 0.1 mM of each dNTP and 0.3 U Amplitaq Gold (a modified taq polymerase) in the incubation buffer provided by the enzyme manufacturer (Applied Bio-Systems, CA, USA). Two pairs of primer sets designed by TEULAT et al. (2000) for amplification of microsatellite sequences, CNZ29 and CNZ33, were used in the present study. Forward and reverse primers of CNZ29 were TAAATGGGTAAGTGTTTGTGC and CTGTCCTATTTCCCTTTCATT, respectively, while those of CNZ33 were TTGCCCTATGACATTAAAAGA and GAGGTCAAAGTTATTTCCGAT, respectively. Amplification was achieved using a GeneAmp PCR System 9700 (Applied Bio-Systems) programmed as follows: cycle 1, 10 min at 95 °C; cycles 2-41, 1 min at 94 °C, 2 min at 54 °C, 3 min at 73 °C. Then, 4 µl aliquots were loaded onto polyacrylamide gels (6% acrylamide/bisacrylamide 29:1 in 1 × TAE buffer) and electrophoresed at 130 V constant voltage for 2.5 h. Amplification products in gels were detected by ethidium bromide staining, viewed under ultraviolet light, and photographed using Fas-III (Toyobo Co., Ltd., Osaka, Japan). Their molecular weights (number of base pairs) were estimated by comparison with Marker 10, a standard DNA size marker provided by Nippon Gene (Toyama, Japan).

Results and Discussion

TEULAT et al. (2000) identified two alleles (sequences for which repeat unit times mutually differ), 152 bases and 169 bases, at the CNZ33 locus; they also found 13 alleles ranging from 102 to 180 bases at the CNZ29 locus in 31 individuals from 14 coconut populations across the entire geographic range (2-3 individuals per population). We detected two alleles in our 24 samples at the CNZ33 locus. Though the exact base numbers were not examined, the long allele (L: the slow band in Fig. 1) and the short allele (S: the fast band in Fig. 1) were thought to correspond to the 169-base allele and 152-base allele, respectively. The ratio 5LL:10LS:9SS fitted to 4.17 : 11.67 : 8.16, the expected ratio of gene constitution of a random mating population (χ^2=0.4900, P=0.78). This result is consistent with the fact that coconuts are usually cross-pollinating. Though we did not examine all samples, we detected at least eight alleles at the CNZ29 locus (Fig. 2). This result indicated that the number of alleles in the samples from Yap state at the CNZ29 locus were comparable to that detected in samples across the entire geographic range. All samples examined were heterozygous. This result also confirms the coconut's cross-pollinating behavior.

We carried out a brief interview with residents in Mogmog Island about the local classification of coconuts. According to this interview, coconuts with a special shape had names associated with their places of origin. A popular coconut was called *lu* in the Ulithi language. One with a flat bottom was called *lul moros*, indicating a coconut brought from New Guinea. A large round variety was called *lul hadohobey*, which means a coconut brought from an outer island in Palau. A small round one was called *lu satwal*, indicating a coconut brought from Satwal, a Yap island close to Chuuk state. This local coconut nomenclature suggests that coconut germplasm has been introduced from many places. Typhoon Ophelia destroyed most plants of the Ulithi atoll in 1960. Subsequently, coconuts and some other crops were introduced mainly from Yap proper. Therefore, few coconuts grown in Ulithi atoll are indigenous. However, since there have been myriad exchanges among islands in Yap state or the Caroline islands (KUWAHARA 2001), some coconuts of Yap proper are thought to be introduced from other islands. If so, exchange and introduction can be a source of high genetic diversity of coconuts in Yap state. Recent DNA-marker based studies (LEBRUN et al. 1998; TEULAT et al. 2000) have shown that coconut genetic diversity is higher on the Pacific islands than in other regions. These studies support the

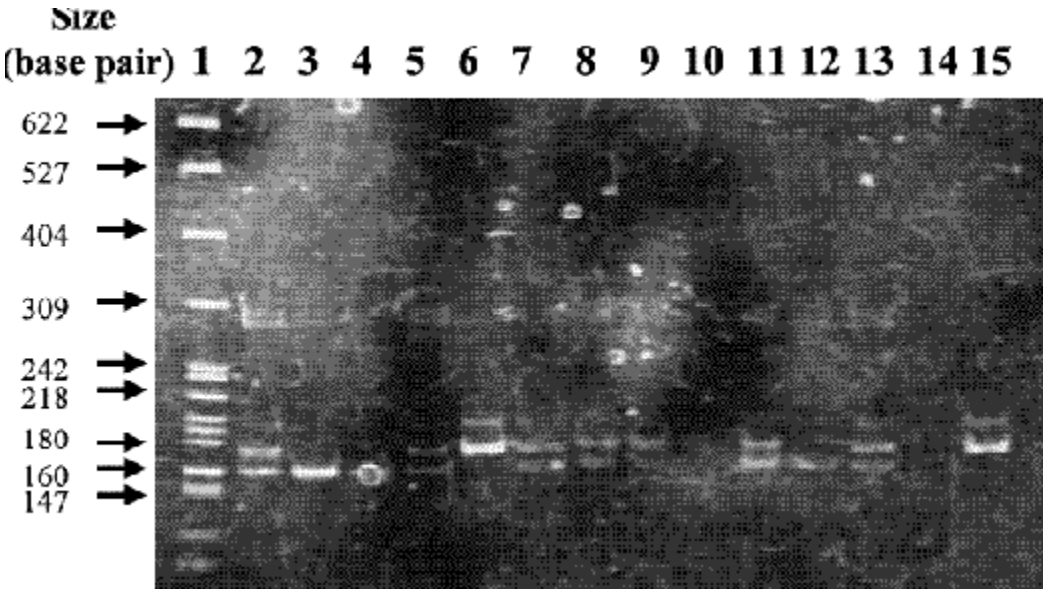

Fig. 1. An example of allelic polymorphism of coconut samples at the CNZ33 locus. Lane 1, size marker: lanes 2–15 PCR products of coconut samples. Estimated genotypes are: lane 2 LS, lane 3 SS, lane 4 SS, lane 5 LS, lane 6 LL, lane 7 LS, lane 8 LS, lane 9 LS, lane 10 unknown, lane 11 LS, lane 12SS, lane 13 LS, lane 14 unknown, lane 15 LL.

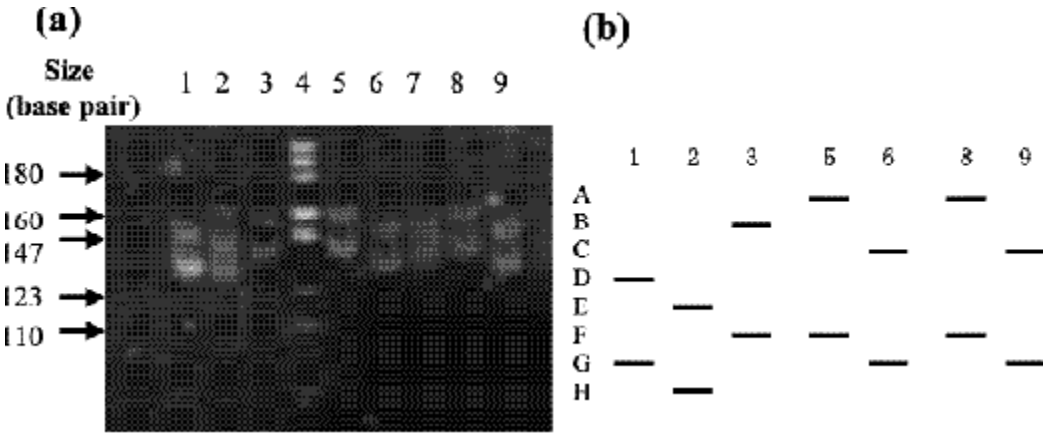

Fig. 2. An example of allelic polymorphism of coconut samples at the CNZ29 locus. Lane 1–3 and 5–8 PCR products of coconut samples, lane 4 size marker. (a) Polyacrylamide electrophoresis of SSRs of coconut samples at the CNZ29 locus. (b) Interpretative drawing of (a), indicating that there are eight alleles in the seven samples examined in this electrophoresis. The genotype of the lane 7 was unknown.

hypothesis that coconuts originated from somewhere on the Pacific islands and dispersed to other regions (HARRIES 1990). Our result is consistent with LEBRUN et al. (1998) and TEULAT et al. (2000).

To evaluate genetic diversity more exactly, we are increasing the number of DNA samples

and kinds of SSRs to be examined. Comparison of morphological and genetic diversity is being undertaken.

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