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Intraspecific variations of the Yam (*Dioscorea alata* L.) based on external morphology and DNA marker analysis

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ABSTRACT

Intraspecific genetic relationship of 19 variation types of the Yam (*Dioscorea alata*) classified by their external morphological characteristics such as leaf and tuber shape were assessed by DNA using random and specific primer. Twenty two out of 113 primers (100 random[10-mer] primers, two 15 mer [M13 core sequence, and $(GGAT)_4$ sequence]) had been used in PCR-amplification. Only 12 primers, however, were success in DNA amplification in all of the analyzed plants, resulting in 93 randomly and specifically amplified DNA fragments. The analyzed taxa showed very high polymorphisms(69 bands, 71.0%), allowing individual taxon to be identified based on DNA fingerprinting. Monomorphic bands among total amplified DNA bands of each primer was low under the 50%. Similarity indices between accessions were computed from PCR(polymerase chain reaction) data, and genetic relationships among intraspecific variations were closely related at the levels ranging from 0.66 to 0.90. These DNA data were not matched well with those of morphological characters since they were divided into two major groups at the similarity coefficient value of 0.70. Therefore, Grouping of species into variation types by mainly morphological charactistics was suggested unreasonable.

Key Words : *Dioscorea alata*, PCR, polymorphism, monomorphism, intraspecific relationship, morphological characters

INTRODUCION

Yam is a perennial, climbing herb and distributed in temperate, subtemperate, mediterranean coast (Cronquist, 1981; Ohwi, 1984). Recently, Tuber part of yam has been used as good food and medical resources. Generally, useful parts for identification in external morphology of the genus *Dioscorea*(including Yam) were recognized the tuber and leaf shape, seed(Bary, 1877; Makino, 1888 and 1889; Matsuno, 1918; Burkill, 1960). But, intraspecific variations of external morphology of the Yam cultivars in Korea appeared continuously(Oh *et al*, 1996). Then, it is necessary to

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study about intraspecific variations.

In the past, cultivars were identified primarily based on horticultural, morphological, and physiological descriptions. In most cases, the descriptions and measurements varied considerably due to environmental fluctuation and human judgement(Torres *et al.*, 1993).

Of late, the ability to differentiate organisms based on difference at the DNA level has provided biologists with a new set of tools for taxonomic determination (Halward *et al.*, 1991; Zhang *et al.*, 1992, 1993; Kidwell *et al.*, 1994).

The genomic polymorphism has been reported in many species using RAPD technique(Hu and Quiros, 1991; Shah *et al.*, 1994; Shin *et al.*, 1995). The RAPD technique provides a faster and easier approach for exploring genetic polymorphism in molecular taxonomic studies (Williams *et al.*, 1990, 1993; Welsh and McClelland, 1990). For repositories with large collections, this technique represents an important advance towards detailed characterization of individual accessions at the molecular level(Melchinger *et al.*, 1991). Specific primers such as $(GATA)_4$, $(GGAT)_4$, were known to be very useful method for confirmation of genetic similarity in interpopulation and intraspecific variations at geographicaliy isolated species (Nybom, 1993). And PCR markers are also useful for genetic diagnostics and molecular taxonomic studies(Williams *et al.*, 1993; Stammers *et al.*, 1995; Seok and Choi, 1998).

This paper discusses the genetic relationships by PCR with arbitrary 10 and 15-mer oligonucleotide primers among 19 yam variation types pre-classified morphological characters such as leaf and tuber shape.

MATERIAL AND METHODS

1. *Plant materials*

Yam(*Dioscorea alata*) was classified to nineteen variation types based on the external morphology of leaf and tuber shape(Table 1). The leaf samples of 19 accessions were obtained from plants grown in the field and a greenhouse and stored at -80 oC until use(Table 1). Specimens have been maintained in the Herbarium of Department of Biology, Kangwon National University, Korea.

2. *DNA extraction and polymerase chain reaction*

For each taxa, a bulk of leaf samples were ground in liquid nitrogen. Grinding was continued after adding extraction buffer(0.2 M Tris-HCl pH 8.0, 0.05 M EDTA pH 8.0, 0.5 M NaCl, 0.5% SDS). An equal volume of chloroform : isoamyl alcohol(24:1) was immediately added, and the solution was mixed and incubated on ice for 5 min. After centrifugation at 13,000 g for 7 min, the supernatant was collected, into which 2 volumes of ethanol were added, mixed, and left in a freezer (-20℃) for one hour. After centrifugation at 13,000 g for 7 min, the supernatant was removed. The DNA pellet was washed twice in 70%(v/v) ethanol, dried in a vacuum dryer, and resuspended in TE buffer containing 10μg/ml RNAse. The presence of total genomic DNA was confirmed by electrophoresis on a 0.7%(w/v) agarose gel and quantified by measuring absorbance at 260 nm by a Beckman spectrophotometer.

Ten arbitrary primers(10-mer, UBC, Canada and Operon, USA) and two 15-mer oligonucleotide primers, M13 core sequence and (GGAT)4, were used for polymerase chain reaction(PCR) based on the protocol of Williams et al.(1990), with minor modifications. PCR was performed in a volume of 25μl reaction solution containing 1x KCl buffer, 4mM dNTPs, 50ng of DNA, 0.8 unit of Taq polymerase(Promega), 0.2μM of primer. Twenty μl of mineral oil was added over the reaction solution. PCR was performed in a DNA Thermal Cycler(Perkin Elmer Cetus 9600). DNA was amplified using the following program; 10-mer : 94℃ for 1 min, 35℃ for 1 min, 72℃ for 2 min, 45 cycles; 72

Table 1. External morphology of *Dioscorea alata* accessions used in this study

Characters / Accessions	Leaf shape	Leaf base	Leaf apex	Tuber shape
A	cordate	cordate	acuminate	round
B	cordate	widely-cordate	acuminate	round
C	widely-elliptical	auriculate	acuminate	round
D	cordate	sagittate	acuminate	round
E	ovate	cordate	acuminate	round
F	cordate	cordate	acute	shortly-elongated
G	deltoid	widely-sagittate	acuminate	round
H	reniform	cordate	obtuse	elongated
I	deltoid-ovate	cordate	acuminate	round
J	deltoid	widely-sagittate	acuminate	shortly-elongated
K	deltoid	sagittate	acuminate	subround
L	deltoid	widely-sagittate	acuminate	round
M	subcordate	cordate	acuminate	elongated
N	subcordate	widely-cordate	acuminate	long-elongated
O	widely-elliptical	auriculate	acuminate	long-elongated
P	ovate-cordate	cordate	acuminate	round
Q	cordate	widely-cordate	acuminate	round
R	deltoid	widely-cordate	acuminate	shortly-elongated
S	deltoid	widely-sagittate	acuminate	subround

℃ for 10 min, 1 cycle, 15-mer : 94℃ for 30 second, 55 ℃ for 30 second, 72℃ for 1 min, 45 cycles; 72℃ for 10 min, 1 cycle. Amplified DNA products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining the gels with ethidium bromide. The gel was photographed under UV light with Polaroid film 667. A PCR tube containing all components but genomic DNA was run as a control with each primer in order to check for contamination. To check the molecular weights of each DNA fragments separated on the gel, 3Kb ladder DNA and Double digested lambda markers was used for comparison.

3. *Phylogenetic relationship*

Amplified and separated DNA fragments appeared as bands on agarose gels were scored as presence (1) or absence (0) of bands for each of the 19 accessions using 12 primers. Only reproducible bands were considered, and faint bands which appeared unstable in multiple runs were ignored (Hashizume et al., 1993). In some cases no band was detected possibly due to insufficient homologies between primers and the DNA template. These were counted as missing values due to the possibility that they arose by the failure of the PCR caused by some other variations in the reaction. Similarity coefficients were calculated based on the following equation(Nei and Li, 1979).

$$S = \frac{2n_{xy}}{n_x + n_y}$$

The NTSYS program (Exeter Software) was used to produce a phenogram for which the UPGMA (unweighted pair-group method with arithmetic average) was employed(the NTSYS tree program).

Table 2. List of primers and their sequences used in PCR analysis

Primers	Sequence (5′ → 3′)	Primers	Sequence (5′ → 3′)
UBC-31	CCG GCC TTC C	OPB-08	GTC CAC ACG G
UBC-65	AGG GGC GGG A	OPB-11	GTA GAC CCG T
UBC-82	GGG CCC GAG G	OPE-15	ACG CAC AAC C
UBC-95	GGG GGG TTG G	OPE-20	AAC GGT GAC C
OPB-03	CAT CCC CCT G	M13	GAG GGT GGN GGN TCT
OPB-05	TGC GCC CTT C	$(GGAT)_4$	GGA TGG ATG GAT GGA

RESULTS AND DISCUSSION

1. *PCR analysis*

The phylogenetic relationship of *Dioscorea alata*, 19 varation types were investigated at DNA level using DNA fingerprinting method. Twenty-two out of the 113 primers(10-mer and 15-mer) were tried amplification with the analyzed plants. However, only 12 primers were in success of amplification in all of the taxa. Effective PCR requires that bases at the 3 end of the primer must provide a perfect match to the DNA segment to be amplified, while mismatching of other regions of the primer can be tolerated(Sommer and Tautz, 1989). Generally, base composition of primers influences the ability of DNA amplification (Williams et al., 1990), and higher ratio of G+C content has been shown to be positively correlated with ability and strength of DNA amplification (Fritsch et al., 1993). A low G+C content primer annealing tends to be less stable and more variable, possibly promoting more mismatching between the primer and DNA templates resulting in more non-specific amplification. Mismatching 101 primers except for 12 primers

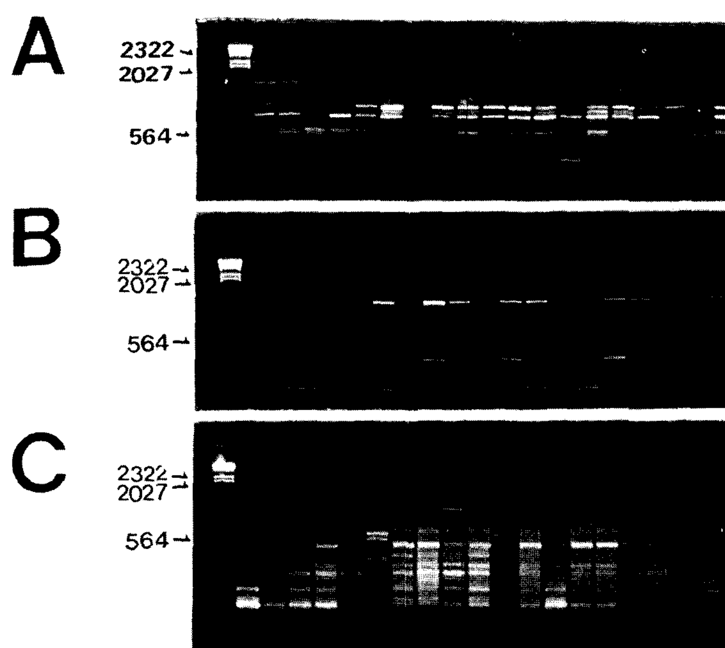

Fig. 1. RAPD profiles of the analyzed plants. The sequence of each primer in A(OPB-03), B(OPE-20). C(M13) is shown in Table 2.
M. DNA Marker, Lane 1-19. each variation type in Table 1.

amplified in all analyzed plants was considered due to a feature of the primer sequence. But, twelve primers tried for this study, successfully amplified the genomic DNAs, and G+C contents of all the primers were higher than 60% (Table 2).

The 12 primers out of 115 primers tried gave rise to a total of 93 randomly amplified DNA fragments. Each primer amplified 4(UBC-95, [GGAT]4) to 12 (M13) DNA fragments with a size ranging between 200 and 2320 bp. Specific primer in Tomato, (GGAT)4, was known to proved to be the most efficient probe for cultivar identification, showing high levels of between- and low levels of within-cultivar variation(Weising *et al.*, 1995). But, in this study, it was not identified in all analyzed taxa. Another specific primer M13 core sequence, one of the most frequently used minisatellite probes for DNA fingerprinting in plants and fungi, has the most amplified bands among primers amplified in all analysed taxa(Fig. 1).

Of the 93 PCR products generated 29.0%(24 bands) were monomorphic in all varieties. Many band appeared in most of the varieties and were absent in only a few varieties. The remaining 69 bands(71.0% of the total products score) were polymorphic amongst the 19 Yam varieties. This amounts to an average of 5.9 polymorphic bands per primer. This is a relatively high level of polymorphism expressed by arbitrary primers compared to reports of other RAPD studies such as in Rice(Ko *et al.*, 1994), sweet potato(Connolly *et al.*, 1994). Monomorphic band in the primer UBC-31 and UBC-95 was not appeared in all analyzed plants, wheares, appeared in monomorphic bands 4 amplified DNA fragments of (GGAT)4.

2. *Genetic similarity and phylogenetic relationship*

The indices of genetic similarity among variation types are presented as a phenogram in Fig. 2. The 19 variation types showed high levels of similarity indices ranging from 0.66 to 0.90. This analysis clearly distinguished the two major groups ; Group I, including 8 variation types, with 0.74-0.89 similarity between one another. And two sub-clusters at similarity index 0.74 which can be identified. Group II, eleven other variation types which are relatively more distinct, expressing an 0.70-0.90 similarity index between one another. Among them variation type J, K, L was

Table 3. Number of amplified DNA bands separated on agarose gel electrophoresis at each primer

Primers	Total bands	Monomorphic bands	Polymorphic bands	% polymorphic bands
UBC-31	8	0	8	100
UBC-65	10	3	7	70.0
UBC-82	9	4	5	55.0
UBC-95	4	0	4	100
OPB-03	7	1	6	85.0
OPB-05	10	1	9	90.0
OPB-08	5	1	4	80.0
OPB-11	10	3	7	70.0
OPE-15	6	3	3	50.0
OPE-20	8	1	7	87.0
M13	12	3	9	75.0
(GGAT)4	4	4	0	0
Total	93	24	69	72.0

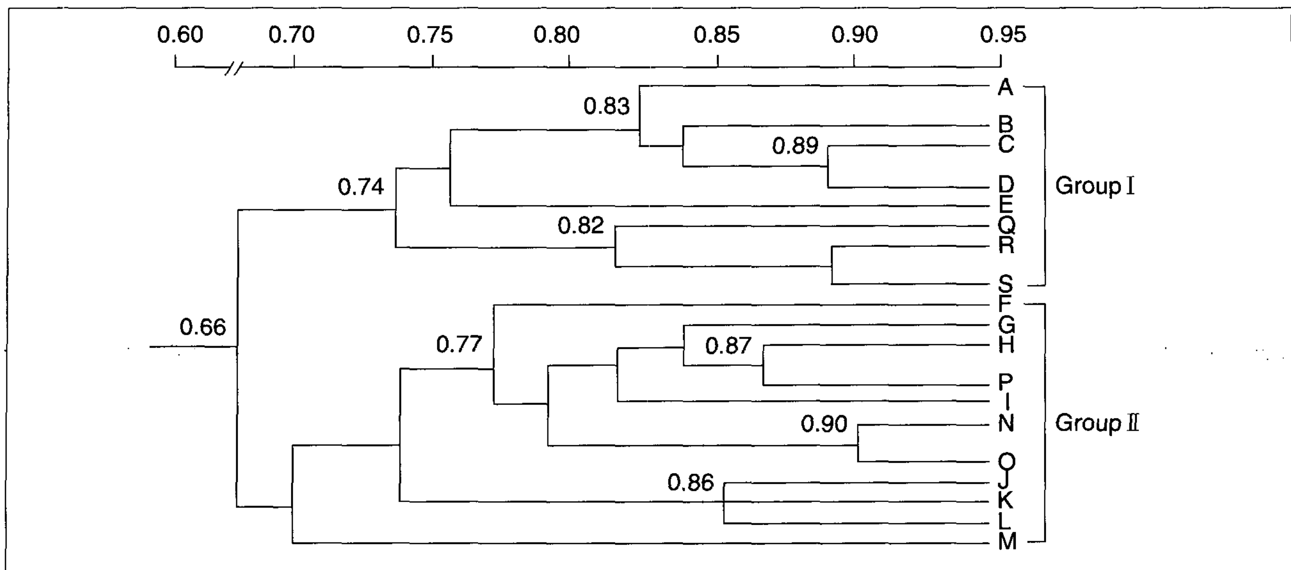

Fig. 2. Phenogram by UPGMA of *Dioscorea alata* 19 variation types based on the analysis of amplified DNA fragments produced by 12 arbitrary primers.

identical at similarity index 0.86. And also, type M was distinghished from other 10 variation types at similarity index 0.70(Fig. 2).

This phenogram was not identifical to the variation groups by external morphology such as leaf shape and tuber shape. Actually, morphological variations in the field and greenhouse appeared very seriously (especially, leaf shape, tuber shape), and variation patterns appeared seriously in the DNA size after amplification in the intraspecies. Yoo et al.(1997) investigated intraspecific variations of Epimedium koreanum and Allium victorialis var. platyphyllum by DNA polymorphism. In this study, genetic diversity of each species was not identifical to the morphological characteristics. Then, morphological variation types or population was considered 'ecotypes' that is adaptation forms in environment condition. Morphological variations and DNA polymorphism of Yam were considered as identical in this study. But this study was conducted only 19 vatiation types, and native Yam was not distributed in Korea. Thus, complete comparative analysis of phylogenetic relationship was somewhat limited in our study. Other approaches for molecular taxonomy such as RFLP and gene sequencing are undergoing for the resolution of these systematic problems.

CONCLUSION

This study has confirmed the close genetic relationship of the Yam (*Dioscorea alata*), 19 variation types the classified by external morphology. All of the 19 variation types which were studied had a similarity 0.66-0.90. The analyzed taxa showed very high polymorphism (71.0%), thus the individual taxon except for J, K, L was able to be identified by the RAPD patterns with ease. But, this data was not identified compared with the morphological characters. So, complete comparative analysis of phylogenetic relationship was somewhat limited in our study. Other approaches for molecular taxonomy such as RFLP and gene sequencing are undergoing for the resolution of these systematic problems.

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