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Discrimination of Potato Varieties by Random Amplified Polymorphic DNA Analysis

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ABSTRACT This study was carried out to discriminate potato cultivars and breeding lines by specific molecular markers using random amplified polymorphic DNA (RAPD) analysis. The genotypes of potatoes used for analysis were eight cultivars and five breeding lines. Some of those show much phenotypic resemblances among them because 'Jopung', 'Daekwan70', 'Gawon', and 'Daekwan72' have immediate parental relationship with 'Superior', 'Irish Cobbler', 'Namsuh', and 'Atlantic', respectively. So, there are many difficulties to distinguish the varieties by the morphological characteristics. Three URP primers, URP2, URP4, and URP8 were selected for promising primers to discriminate potato genotypes or cultivars. The three URP primers were shown very high reproducibility because of the relatively high annealing temperature and long primer size. Although the results of similarity analyses did not always reflect the genetic relationship between potato varieties, the reproducible pattern of amplified DNA bands by URP primers showed possibility for molecular markers for discrimination of potato genotype or cultivar.

Additional key words: DUS test, genetic diversity, *Solanum tuberosum*, URP

Introduction

Potato (*Solanum tuberosum* L., 2n=4x=48) is one of the most popular vegetables and cultivated in many countries of the temperate and subtropic area. Morphological variations have been often observed in cultivated potatoes because F1 is undergone the genetic crossover of diploid gamates. Recently, enforced Seed Industry Law requires the rapid and accurate identification of new variety to prove the originality and to protect the breeder's right. In case of potato, newly bred varieties has registered every year and more than thousand cultivars were registered in world wide (Kawchuck et al., 1996). Such things make difficulties to confirm of distinctness new variety's identity. It is prescribed in the Seed Industry Law that potato cultivars should be discriminated with DUS (distinctness, uniformity and stability) test, that based on for registration of new variety to the NVL (National Varietal List). But the procedure have several limitations by various morphological markers, by personal deviation of investigator, and by relatively long period of time to observe the discriminative characteristics etc. (Kim et al., 1998). So, development of genetic markers has been recognized for one of the ideal methods. Many of the genetic analysis methods including RAPD (Demeke et al., 1993; Williams et al., 1990) and AFLP analysis (Vos et al., 1995; Weising et al., 1989) were developed and widely used for variety identification etc. Especially, RAPD has been used for variety identification (Demeke et al., 1993; Yae and Ko, 1995), determination of genetic diversity (Demeke et al., 1996; Lee and Kim, 2000; Vierling and Nguyen, 1992), and taxonomic studies (Adams and Demeke, 1993; Demeke et al., 1992). The results of RAPD analysis is so independent on environmental influences, tissue types and development stages that it should provide greater reproducibility than isozyme pattern (Demeke et al., 1996). Though having some defect such as the lackness of stringency, RAPD is more rapid and easier than other molecular analysis (Demeke et al., 1996). The repetitive genome sequences have different size and high level of genetic diversity among the inter and intra species. The URP primers (Patant No. 97-016981) were randomly designed from repetitive sequence of Korean Red Rice (*Oriza sativa* L.). The URP primers have been applied to prove the genetic diversities of various species. This study was carried out to develop the genetic markers for distinction new potato varieties, to prove the originality of newly bred potato cultivars, and to construct database of gene pools by using URP primers.

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Materials and Methods

Plant materials

Leaf tissues of potato varieties were sampled from field for breeder's seed and *in vitro* cultured plantlets as a pathogen free stock at National Alpine Agricultural Experiment Station in Pyongchang, Korea. The potatoes used for RAPD analysis were seven cultivars and eight breeding lines including gene pools. The origin and breeding parents were shown as table 1.

Preparation of potato total DNA

Potato total DNA was isolated by modified method of Teresa et al. (1995). One hundred mg of leaf tissues from young and full-expanded potato leaves was homogenized in 750 μl of microprep buffer [DNA extraction buffer (0.35 M sorbitol, 0.1 M tris base, 5 mM EDTA (pH 7.5) : nuclei lysis buffer (0.2 M tris base, 50 mM EDTA, 2 M NaCl, 2% (w/v) CTAB : 5% (w/v) sarkosyl=2.5 : 2.5 : 1)]. Then incubated in 65℃ water bath for 30−120 min. The sample tubes were filled with chloroform : isoamyl alcohol (24 : 1), mixed by mild vortexing and centrifuged at 10,000 rpm for 5min. The total DNAs were harvested from upper aqueous phase by ethanol precipitation and resuspended in 50 μl TE buffer (pH 8.0).

PCR and electrophoresis

PCR was performed in a volume of 20 μl containing 1.0 unit of Taq polymerase, 1.5 mM MgCl2, 0.25 mM dNTPs, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 100 pmole primer and 200 ng template DNA. Amplication was performed in a Perkin Elmer Thermal Cycler 480 (Perkin-Elmer, USA). URP random primers (Korean Patent No. 97-016981) were purchased from Seolin Scientific Co., Ltd. Seoul, Korea. These primers were designed from repetitive sequence of Korean Red Rice (*Oriza sativa* L.). Amplification was performed for 40 cycles at 94℃ for 1 min, 55℃ for 1 min, and 72℃ for 2 min, for denaturation, annealing, and extension, respectively. Following the final cycle, amplified DNA product was completed with a 10 min 72℃ extension. Reaction products were resolved by electrophoresis in 1.2% (w/v) agarose gel at 5.0 volt/cm for 100 min or 6.0% (w/v) of denaturing polyacrylamide gel with 7.5 M urea at 55 W constant power condition for 3 hours. The DNA bands in agarose and polyacrylamide gel were stained with EtBr and silver nitrate, respectively.

Genetic analysis

Amplified DNA fragments were scored as 1 (band present) or 0 (band absent) to calculate simple matching coefficients (S_{sm}= No. of monomorphic bands / No. of total bands for similarity analysis (Sokal and Michener, 1958). For each group, estimates of similarity between all pairs of varieties were summarized in matrix form and similarity matrices were performed to graphically summarize associations among the varieties. Dendrograms based on a dissimilarity index ($d=1-S_{sm}$) were generated using the unweighted pair-group method with arithmetic averages (UPGMA, Sneath and Sokal, 1973). Calculations of the similarities and statistics were carried out by using the NTSIS-pc program based on Nei's estimation of similarity (Nei, 1972).

Results and Discussion

In most plants, only 20−40% of the genome consists of unique or single copy DNA sequences. And highly repetitive sequence DNA is usually present at 10^5~10^7 copy per genome (Hughes, 1996). The repetitive genome have different size and high level genetic diversity among the inter and intra species (Weising et al., 1989; Zhao and Kochert, 1992). In some crops, repetitive sequences were used for verification of genetic diversity among the varieties (Kawchuk et al., 1996; Provan et al., 1996; Rus-Kortekaas et al., 1994). The single sequence repeats (SSRs) or microsatellite were reported as a useful marker for the detection of genetic variation (Akkaya et al. 1992; Kawchuck et al. 1996). But the URP primers that used in this

Table 1. List of potato varieties for RAPD in this study and their immediate parents.

Varieties Name	Abbreviation	Parents	Origin	Released Year
Namsuh	NS	(Sroeken×Superior)×Wheeler	Korea	1995
Jopung	JP	Resy×Superior	Korea	1995
Dejima	DJ	Hokaido31×Unzen	Japan	1978
Gawon	GW	Daekwan52×Konahubuki	Korea	2000
Daekwan48	D48	R521-7×Alamo	Korea	-
Daekwan69	D69	Unknown[z]×Katahdin	Korea	-
Daekwan70	D70	Irish Cobbler×Katahdin	Korea	-
Daekwan72	D72	Atlantic×Unknown[z]	Korea	-
Alpha	AL	Paul Kruger×Preferent	Holland	1992
Superior	SUP	B96-56×M59.44	USA	1951
Irish Cobbler	IC	Bud-mutant of Early Rose	USA	1960
Atlantic	AT	B5141-6×Wauseon	USA	1969
Shepody	SP	F58050×Bake King	Canada	1969

[z]Collected from Inje, Kangwon as a native potato species in Korea

Table 2. The list of 12 URP primers used in RAPD of potato varieties.

No.	Products[z]	Sequences
1	URP 1	5'-ATCCAAGGTCCGAGACAACC-3'
2	URP 2	5'-CCCAGCAACTGATCGCACAC-3'
3	URP 3	5'-GTGTGCGATCAGTTGCTGGG-3'
4	URP 4	5'-AGGACTCGATAACAGGCTCC-3'
5	URP 5	5'-GGCAAGCTGGTGGGAGGTAC-3'
6	URP 6	5'-ATGTGTGCGATCAGTTGCTG-3'
7	URP 7	5'-GGTGAACAGTGAGATGAACC-3'
8	URP 8	5'-TACATCGCAAGTGACACAGG-3'
9	URP 9	5'-AATGTGTGGCAAGCTGGTGG-3'
10	URP 10	5'-GATGTGTTCTTGGAGCCTGT-3'
11	URP 11	5'-GGACAAGAAGAGGATGTGGA-3'
12	URP 12	5'-GGCATTCTACCACCACAAGT-3'

[z]Accession numbers of SRILS UniPrimer Kit.

study, was randomly designed from repetitive sequence of rice genome but did not contained SSRs or microsatellite sequences (Table 2). In case of potato, various genetic analysis techniques for determination of genetic variants were developed including isozyme (Quirois and McHale, 1985), RAPD (Demeke et al., 1993; Hosaka et al., 1994), and AFLP analysis (Kim et al., 1998). Seven domestically recommended varieties including 'Superior' and six breeding lines or varieties including 'Daekwan70' and 'Daekwan72' were used for RAPD analysis by URP primers (Table 1). We examined the all of URP primers produced commercially and selected the URP2, URP4, and URP8 primer for discrimination of potato genetic diversity (Figs. 1 and 2).

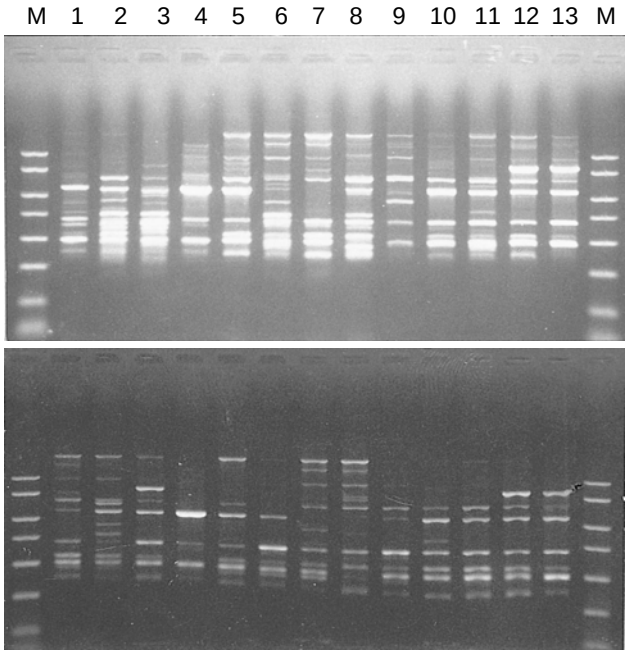

Fig. 1. Pattern of RAPD fragments generated with primer URP2 (upper) and 4 (lower) for the 13 potato varieties.
M, 1kb ladder; 1, NS; 2, IC; 3, AT; 4, DJ; 5, SP; 6, SUP; 7, JP; 8, D48; 9, GW; 10, D69; 11, D70; 12, AL; 13, D72.

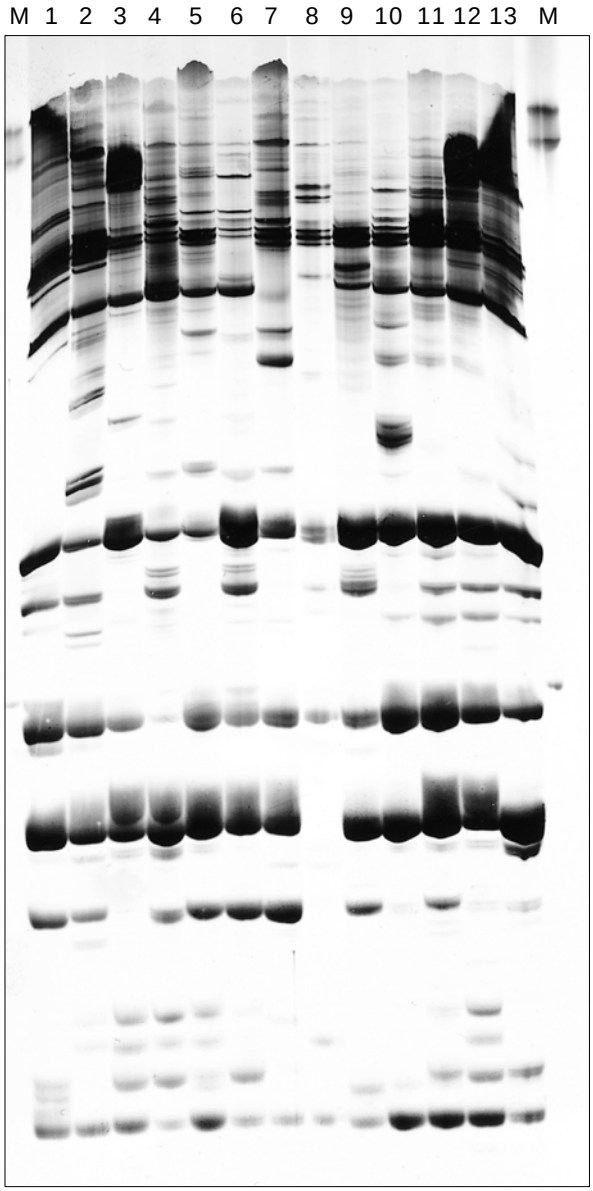

Fig. 2. 6% polyacrylamide gel electrophoresis of RAPD fragments generated with primer URP 8 for the 13 potato varieties.
M, 1kb ladder; 1, NS; 2, IC; 3, AT; 4, DJ; 5, SP; 6, SUP; 7, JP; 8, D48; 9, GW; 10, D69; 11, D70; 12, AL; 13, D72.

URP primers were shown very high reproducibility because of the high annealing temperature above 55℃. So, they were known to be very desirable to examine the specificity between inter and intra species by preliminary studies. Some of these genotypes have many resemblances in plant characteristics each other because 'Jopung', 'Daekwan72', 'Gawon', and 'Daekwan70' have immediate parental relationship with 'Superior', 'Atlantic', 'Namsuh', and 'Irish Cobbler' respectively. So, there are many difficulties to distinct the varieties by the morphological characteristics. Amplified DNA showed polymorphism when URP primers were used (Fig. 1). When URP2 primers were used, 11 among 15 bands showed polymorphism and made us possible to

Table 3. Similarity matrix of the pattern of polymorphic DNA bands generated by URP primers using Nei's estimation (Nei, 1972).

NS	IC	AT	DJ	SP	SUP	JP	D48	GW	D69	D70	AL	D72
1.000												
0.639	1.000											
0.667	0.694	1.000										
0.500	0.806	0.667	1.000									
0.667	0.861	0.722	0.667	1.000								
0.694	0.722	0.528	0.806	0.639	1.000							
0.750	0.778	0.583	0.583	0.806	0.778	1.000						
0.722	0.694	0.778	0.500	0.722	0.528	0.694	1.000					
0.750	0.667	0.528	0.694	0.583	0.833	0.722	0.583	1.000				
0.778	0.694	0.667	0.500	0.722	0.639	0.861	0.722	0.806	1.000			
0.722	0.750	0.611	0.611	0.667	0.750	0.806	0.611	0.694	0.778	1.000		
0.639	0.889	0.806	0.861	0.806	0.722	0.722	0.639	0.611	0.639	0.750	1.000	
0.806	0.778	0.639	0.639	0.694	0.833	0.889	0.694	0.722	0.806	0.917	0.778	1.000

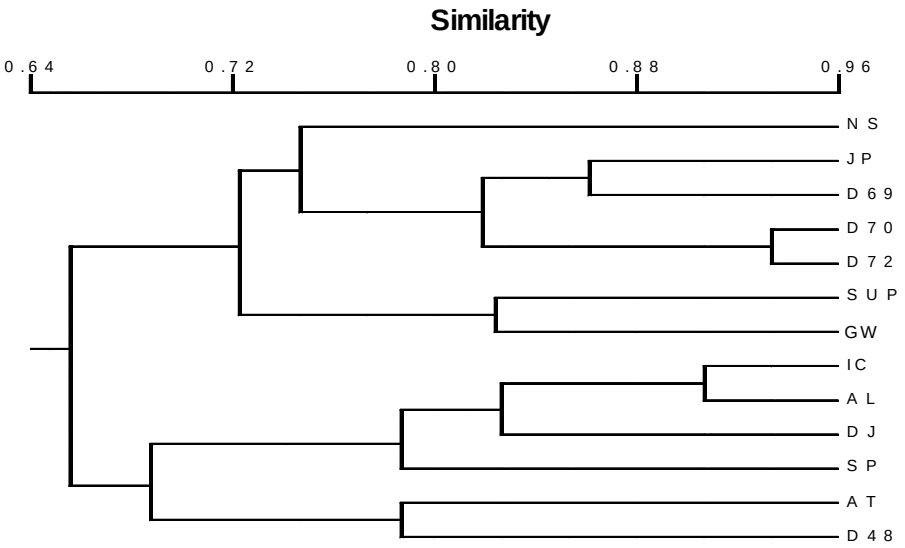

Fig. 3. Phenogram of 13 potato varieties generated by UPGMA cluster analysis of the similarity values given in Table 3.

distinct 13 varieties and lines. DNA polymorphisms on 6% PAGE of PCR products by URP8 were shown the distinguished polymorphic band pattern but the excessive band emergence in large size bands so delicate that the exact band scoring could not perform by silver staining procedure (Fig. 2). The similarity dendrogram by DNA band profiles amplified by URP2 and 4 had little genetic relationship between 'Irish Cobbler' and its offsprings 'Daekwan70' and 'Atlantic' (Fig. 3). 'Superior' had similarity to 'Namsuh' and 'Jopung' with reflection of pedigree relationship. This is coincided with the results of Demeke et al. (1996). These results indicated that varieties within close kinship can often be as genetically diverse as varieties with no immediate relationship. The ability to distinguish between genotypes using RAPD patterns is associated with the allelic state at each locus and results from the independent segregation of discrete genetic factors according to Mendellian genetic principles (Demeke et al., 1996; Quiros et al., 1993).

The UPOV (International Union for the Protection of New Varieties of Plants, 1986) guide line and Seed Industry Law regulate that the new potato cultivars should be discriminated with DUS test. It was thought that the RAPD by using URP primers is useful technique for discrimination of newly bred cultivars, breeding lines, and genetic resources of potato. And also this technique has a possibility for supportive or complementary method for confirm the distinctness of newly bred potato cultivar by DUS test.

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RAPD에 의한 감자 품종의 구분

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초 록

감자 DNA의 다형성을 통한 품종 및 육종 계통의 구분이 가능한 특이적 분자 마커를 찾기 위해 URP primer를 이용한 RAPD 분석을 실시하였다. 8 가지의 감자 품종과 5가지의 감자 육종계통을 분석 재료로 이용하였으며, 이들 중 '조풍', '대관70호', '가원'과 '대관72호'는 '수미', '남작', '남서'와 '대서'를 각각 모본으로 이용되어 육성되었으며, 다양한 표현형질에서 유사성을 가지고 있다. 따라서 생육단계별 표현형질들 만으로는 각각을 구분하기 곤란한 경우가 있다. 벼(*Oriza sativa*)의 repetitive sequence로부터 고안된 URP primer는 비교적 높은 annealing 온도를 가지며, 20 mer의 비교적 길이가 긴 RAPD용 primer로써 증폭산물의 다형성과 재현성이 높아 다양한 생물종의 구분이 가능한 것으로 보고되고 있다. 국내에서 상품화되어 시판되는 URP primer 들 중에 URP2, URP4, URP8 등이 감자에 적용될 수 있을 것으로 확인하였다. 전기영동된 DNA 밴드들의 유사성을 분석해본 결과 유전적 유사도와는 차이를 나타내고 있었다. 그러나 높은 재현성을 갖는 URP primer들에 의해 증폭된 DNA 밴드의 다형성은 감자의 종내변이를 구분할 수 있는 분자 마커로서 이용할 수 있을 것으로 확인되었다.

추가 주요어 : DUS test, 유전적 다형성, *Solanum tuberosum*, URP