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Evaluation of Genetic Diversity among Korean Wild *Codonopsis lanceolata* by Using RAPD

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ABSTRACT

The introduction of molecular biology methodologies to plant improvement programs offers an invaluable opportunity for extensive germplasm characterization. We have applied the developed technique of random amplification of polymorphic DNA(RAPD) to the analysis of evaluating genetic diversity among Korean wild *Codonopsis lanceolata*. A total of 340 polymorpic bands were gernerated on agarose- and polyacrylamide-gel by 19 primers of abitrary sequence, grouped by cluster analysis using sample matching coefficients of similarity. Among of the samples, the minimum genetic distance value was obtained between sample no. 1(Girisan) and no. 2 (Girisan), and the largest value between sample no. 11 (Sulaksan) and no. 17 (Sulaksan). In separate cluster dendrograms based on agarose- and polyacryamide-gel, some differences were observed; In the case of agarose gel, 41 samples could be devided into 7 groups at below about 0.44 level of distance. However they were divided into 6 groups at below about 0.40 level of distance in the case of polyacrylamide gel. These results showed that polymophic data in agrose were not grouped to wild plant selected from each mountainous district, but polymorphic data in polyacrylamide gel were grouped to wild plant selected from each mountainous district except for wild plants selected from Sulaksan and Chiaksan. We believe that polyacrylamide-RAPD is a superior method for detecting DNA polymorphism compared to agarose-RAPD method.

Key words: DNA polymorphism, Cluster analysis, *Codonopsis lanceolata*, Genetic diversity, Polymerase chain reaction

INTRODUCTION

Codonopsis lanceolata is a perennial herb belonging to the family Campanulaceae. Its taproot is used in medicine or as a wild vegetable in south Korea, Japan and China. Recently it has been adopted as an ornamental crop. The genus of *codonopsis* is distributed with *Codonopsis lanceolata*, *C. pilosula*, *C. minima* and *C. ussuriensis* in Korea(Komarov, 1901-1907). Among them, *C. lanceolata* is consumed as medicine or wild vegetable. It have a strong self-incompatibility controlled by multi-alleles(de Netancourt , 1977). Thus the genes responsible for pollination reactions would be heterogenous in natural population. For these some reasons, we do not know precisely genetic bacgrounds for the wild *Codonopsis lanceolata* plants in Korea. Development of useful genetic markers is essential for the identification of plants, and in tern, for the selection, breeding and germplasm maintance of excellent strains. Recent advances in technique for DNA analysis and subsequent data analysis have greatly increased our ability to understand the genetic relationships among organisms at the molecular level. This technique uses short synthetic deoxyribonucleotides of random sequence as primers for PCR. While the PCR products are produced from random regions of the genome, they are specific and reproducible since they are primed from specific DNA sequences within the genome. Williams et al.(1990) and Welsh and McClelland (1991)

have described a random amplified polymorphic DNA (RAPD) analysis technique based on the amplification of multiple, random segments of the genome using arbitrary primers. RAPD analysis has proven valuable in plant genotype fingerprinting, population and pedigree analyses, phylogenetic studies, and genetic mapping (Hu and Quiros, 1991; Van Heusden and Bachman, 1992; Reiter et al., 1992). Many of these studies utilized RAPD analysis in agarose-gel electrophoresis. In the present article, we have utilized in combination with in agarose- or polyacrylamide-gel electrophoresis to efficiently detect unusually high levels of DNA polymorphism in Korean wild *C. lanceolata*.

METERIALS AND METHODS

DNA isolation

Young leaf tissue was cut into small pieces and frozen in liquid nitrogen. DNA was isolated from 1g of fresh tissue by the modified procedures of Gawel and Jarret(1991). The tissues were ground in liquid nitrogen and suspended in 15ml of extraction buffer(100mM Tris-HCl, pH 8.0, 20mM EDTA, 1.4M NaCl and 0.1% beta-mercaptoethanol). The slurry was incubated for 60min at 65℃. The lysate was extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and the aqueous fraction mixed with an equal volume of isopropanol. Precipitated DNA was collected by centrifugation at 14,000rpm for 10min. The pellet was washed with 80% ethanol containing 0.3M sodium acetate and redissolved in TE. Contaminating RNA was removed by digestion with 10㎍ RNase A for 30min at 37℃. The DNA was further purified by extracting twice with an equal volume of chloroform. The DNA was precipitated by the addition of 0.1 volume of 3M sodium acetate, 0.8 volume of propanol and recovered as before. The DNA concentration was determined by fluorometry using a Pharmacia DNA fluorometer and following procedures supplied by the manufacturer.

Amplification of DNA

PCR was carried out in 25㎕ reactions containing 30ng template DNA, 2㎛M primer, 150㎛M each deoxyribonucleotide triphosphate, 15mM MgCl2, 10mM Tris-HCl (pH8.3), 50mM KCl, 0.001% gelatin and 0.5U Taq DNA polymerase(Perkin Elmer Cetus). Amplification was for 45 cycles, each consisting of 94℃ for 1 min, 38℃ for 1 min and 72℃ for 2 min.

Gel electrophoresis

The PCR products(25㎕) were fractionated by combination with agarose- or polyacrylamide gel electrophoresis. Agarose-gel electrophoresis consisted of 2% agarose in 0.5 TBE buffer (0.045M Tris-borate and 1.0mM EDTA pH8.0) at 4V/3cm for 3h. Gels were stained in ethidium bromide(1 ㎍/ml) for 15 min and photographed over long wave UV light source. Polyacrylamide-gels consisted of 4% acrylamide(acrylamide : bisacrylamide, 37.5 : 1) in 0.5 x TBE buffer. Electrophoresis conditions were 1600V for 2h at room temperature. Gels were stained in silver nitrate (Promega silver staining kit) and following procedures supplied by the manufacturer.

Table 1. Collected region and sample number of *Codonopsis lanceolata* to RAPD analysis with 19 primers

Collected region	Sample number	Collected region	Sample number
Girisan(Hwaamsa)	1, 2, 3	Girisan(Kure)	4, 5, 6, 7
Girisan(Bemsakol)	8, 9, 10	Sulaksan(Hankeyoung)	11, 12, 13
Sulaksan(Ansan)	14, 15	Sulaksan(Jumbo)	16, 17, 18
Juwangsan	18, 19, 20, 21, 22, 23	Dukyusan(Jangki)	24, 25
Dukyusan(Muju)	26, 27	Dukyusan(Koje)	28, 29, 30
Chiaksan(Sangwonsa)	31, 32, 33	Chiaksan(Namtebong)	34, 35, 36, 37
Keyoungsan	38, 39, 40, 41		

Data analysis

RAPD profiles were scored visually. Variation among samples was evaluated from pair-wise comparisons of the proportion of shared fragments divided by the total number in the profiles being compared. This method was equivalent to calculating Nei and Li's(1979) index of genetic similarity(Sxy) for RFLP comparisons. Relationships among samples were evaluated with a phenetic cluster analysis, using the unweighted pair group method for arithmetic averages(UPGMA), and plotted in a phenogram as described by Sneath and Sokal(1973) using the numerical taxonomy and multivariate analysis system for the IBM PC version 1.60(Applied Biostatistics). Parsimony analysis was done using the PAUP computer program (Swofford).

RESULTS

RAPD polymorphism in agarose gel

The total nineteen 12-base primers were tested in each of 41 wild Korean *Codonopsis* samples to detect and characterize RAPD polymorphism(Table 2). All nineteen primers contained 60-70% (G+C), and none of the base sequences had self-complementary ends. The primer sequences were arbitrary and produced repeatly using the same DNA. Of the primers tested, five produced amplification products that were monomorphic across all samples or were polymorphic but not reliable(Fig. 1-A). The remaining fourteen primers revealed a total of 72 polymorphisms that could be scored reliably in all 41 samples. The number of polymorphic amplification products revealed by each of these primers ranged from 3 to 8 (Fig. 1-B, 1-C). The size of the polymophic amplification products ranged from

Table 2. Synthetic deoxyribonucleotides used as a primers for amplification of *Codonopsis lanceolata*

Primer number	Nucleotide sequence (5' to 3')	Total number of fragments amplified	
		Agarose gel	Polyacrylamide gel
1	ACCAGGCCAA	16	80
2	ATCTGTGTGG	8	nt
3	CACAGGTTCT	6	nt
4	CAGTCCTAGG	7	nt
5	CTAAGCCATG	11	70
6	CTTGGGTTGG	13	68
7	AAAGGCAACC	6	nt
8	GATCGACACT	9	nt
9	ACGGACTGGA	9	nt
10	GTGGCTCTGA	12	55
11	CAGGCCCTTC	11	nt
12	AATCGGGCTG	7	nt
13	CAGCACCCAC	16	72
14	GACCGCTTGT	6	nt
15	GTTGCGATCC	5	nt
16	GGTGACGCAG	18	75
17	GTCCACACGG	13	nt
18	CAAACGTCGG	9	nt
19	TCGGCGATAG	6	nt
Total		188	420

nt:not tested

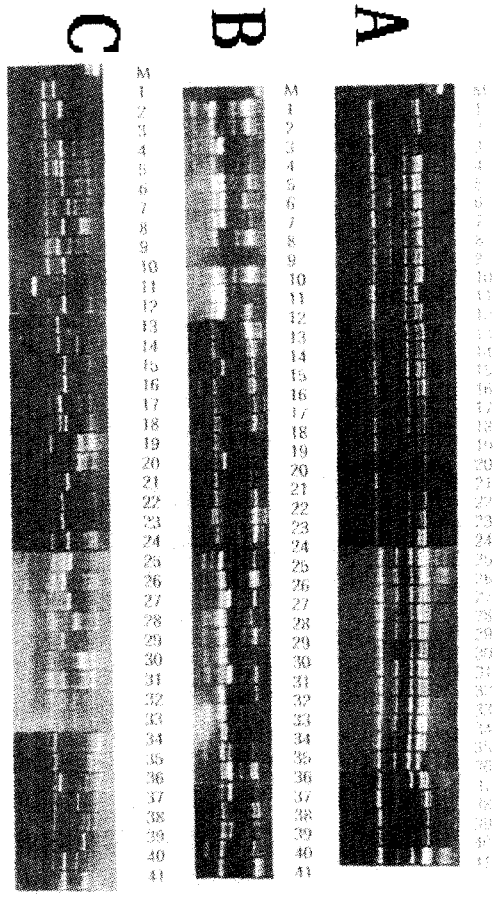

Fig. 1. Agarose gel electrophoresis pattern of RAPD-amplified among 41 Korean wild *C. lanceolata*. DNA samples were amplified using primer 5(CTAAGCCATG)(A), 10(GTGGCTCTGA)(B) and 17(GTCCACACGG)(C), and seperated on a 2% agarose gel as described in the methods. The number of the lane indicates the sample number as in Table 1. Line M: molecular weight marker(*Hind*III-digested lamda DNA).

250 to 1810bp, with an average of 1030bp. Common bands in 41 samples were scored as present(1) or absent(0) and the data was used to calculate values of genetic distance between all the samples. The genetic distance value was estimated using distance matrix and parsimony numerical taxonomic methods on the 72 polymorphic amplification products scored. Among the comparison methods used, coefficients of similarity ranged from about 0.04 for the most colsely related samples to 0.60 for those more distantly relately. Cluster analysis of the genetic distance values was carried out to generate dendrograms indicating relationships

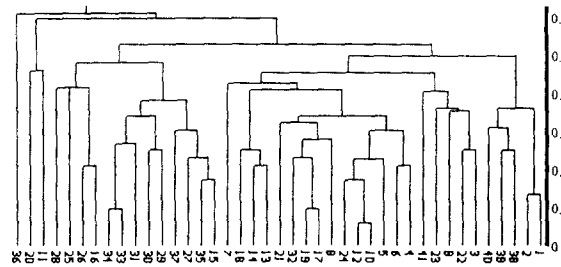

Fig. 2. Dendrogram obtained from the UPGMA cluster analysis based on Euclidean distance by using the first three principal component scores in agarose gel of 41 Korean wild *C. lanceolata* by principal components analysis.

among the Korean wild plants. Fourty one samples could be devided into 7 groups at below about 0.44 level of distance: group I, samples no. 1, 2, 38, 39, 40, group II, samples no. 3, 22, 9, 23, 41, group III, sample no. 4, 6, 5, 10, 12, 24, 8, 17, 19, 32, 21, 13, 14, 18, 7, group IV, samples no. 15, 35, 27, 37, 29, 30, 31, 33, 34, group V, samples no. 16, 26, 25, 28, group VI, samples no. 11, 20, group VII, sample no. 36 (Fig. 2). Grisan wild plants selected to 10 popupations belonged to Group I, II and III, Sulaksan wild plants selected to 8 populations belonged to group III, IV, V and VI, Juwangsan wild plants selected to 6 populations belong to group II, III and VI, Dukyusan wild plants selected to 7 populations belonged to group III and IV, Chiaksan wild plants selected to 6 populations belonged to group III, IV and VII, Keyungsan wild plants selected to 4 populations belonged to group I and II.

RAPD polymorphism in polyacrylamide gel

In order to detect efficiently high levels of DNA polymorphism among Fjourty one samples, we have adapted a polyacrylamide gel with RAPD analysis. Analysis of 420 reproducible amplified DNA sequences was possible from a total of 6 gels and 6 primers applied to 41 samples. Among the DNA bands scored, 63.8% were polymorphic (Fig. 3). Cluster analysis of the genetic distance values was carried out as in agarose-gel. Among of the samples, the minimum genetic distance value was obtained between sample no. 1 (Girisan) and no. 2 (Girisan), and

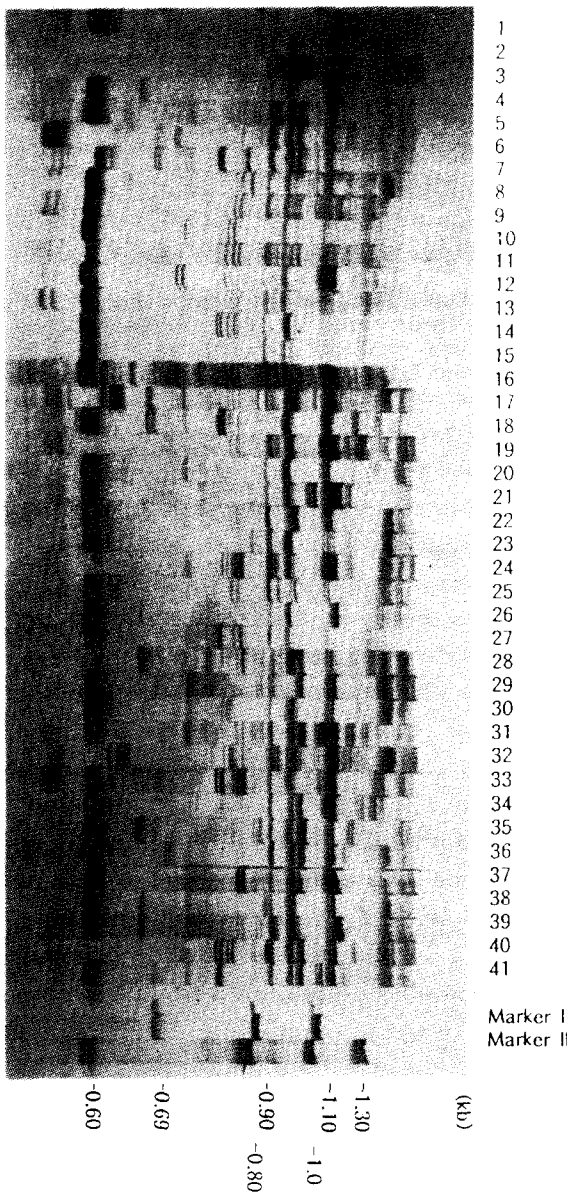

Fig. 3. Polyacrylamide sequence gel electrophoresis pattern of RAPD-amplified among 41 Korean wild *C. lanceolata*. DNA samples were amplified using primer 1(ACCAGGCCAA), and seperated on a 4% polyacrylamide sequence gel as described in the methods. The number of the lane indicates the sample number as in Table 1. Line M: low molecular weight marker(Promega).

the largest value between sample no. 11 (Sulaksan) and no. 17 (Sulaksan). Fourty one samples could be divided into 6 groups at below about 0.40 level of distance

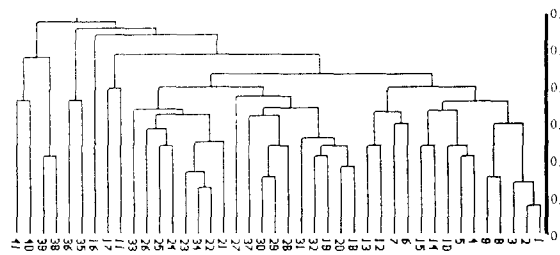

Fig. 4. Dendrogram obtained from the UPGMA cluster analysis based on Euclidean distance by using the first three principal component scores in polyacrylamide sequence gel of 41 Korean wild *C. lanceolata* by principal components analysis.

(Fig.4). About 81%(33/42 plants) of samples belonged to group I and II. The samples from Girisan and Keyungsan belonged to group I, and VI, respectively, and Juwangsan and Dukyusan wild plants belonged to group II. However, Sulaksan and Chiaksan wild plants belonged to group II, III, IV and V.

DISCUSSION

The most commonly used gel matrix for the resolution of RAPD-based polymorphism is agarose. The complexity of patterns obtained with polyacrylamide gel suggests that a large number of different DNA bands are contained within a single band resolved on agarose. Consequently, the use of polyacrylamide enhances greatly resolution of the multiple amplification products (Caetano-Anolles et al. , 1991). Within the addition of a polyacrylamide sequence gel, added resoultion of DNA sequence polymorphisms is achieved(Fig. 3). Polyacrylamide sequencing gel electrophoresis is designed to allow the resolution of sequence differences among fragments of similar or identical size(Ismail D. et al., 1993). These methods for detecting DNA polymorphism has been successfully used in a number of plant and animal systems(John Welsh et al., 1990). It should be pointed out, howerver, that other difficulties inherent in the PCR technique that influence reproducibility are not necessarily reduced by modifying the gel system. Comparisons of agarose-(2%) and polyacrylamide- sequence (4%)gel electrophoresis of identical RAPD products are shown in Fig. 1 and 3. Little or no visible polymorphism

among *Codonopsis* samples was detected in agarose. However polyacrylamide was enhanced detection of DNA polymorphism. Also, cluster analysis of the genetic distance values was carried out to generate dendrograms indicating relationships among the Korean wild plants. These results showed that polymophic data in agrose were not grouped to wild plant selected from each mountainous district, but polymorphic data in polyacrylamide gel were grouped to wild plant selected from each mountainous district except for wild plants selected from Sulaksan and Chiaksan. From our results, the evolution of *Codonopsis* seemed to be done to north-moutain from south-mouth in Korea. This imformation will help to realize more potential of germplasm diversity and breeding programs in *C. lanceolata.*

LITERATURE CITED

Burr, B, E bola, S.V., Burr, F.A. and Beckman, J. S. 1983. The application of restriction fragment length polymorphism to plant breeding . In : Genetic Engineering Principles and Methods, Vol. 5(ed.), Plenum Press, p45

Barone, A., Ritter, E., Schachtschabel, U., Debener, T., Salamini, F. and Gebhardt, C. 1990. Localisation by restriction fragment length polymorphism mapping in potato of a major dominant gene conferring resistance to the potato cyst nematode Globodera rostochiesis. Gen. Genet., 224:177-182.

Dallas, J. F. 1988. Detection of DNA fingerprints of cultivated rice by hybridization with a human minisatellite DNA probe. Proc. Natl. Acad. Sci. 85:6831-6837.

de Nettancout, D. 1977. "Incompatibility in Angiossperms", p230. Springer-Verlag, Berlin, Heidelberg, New York.

Demeke T., Adams Rp, Chibbar R. 1992. Potential texonomic use of random amplified polymorphiic DNA(RAPD): a case study in Brassicca. Theor. Appl. Genet. 84 990-994.

Devos K.M. and Gale M.D. 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet. 84:567-572.

Hu J. and Quiros C. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505-511

Ismail D., Sally M., Morris L. and Herbert O. 1993. Pedigree assessment using RAPD-DGGE in cereal crop species Theor. Appl. Genet. 85:446-455

Jeffreys, A., Wilson V. and Thim S.I. 1985. individual-specific fingerprints of human DNA, Nature 314:67-73.

Jaccard P. 1908. Nouvelles recherches surla distribution florale. Bull Soc Vaudoise Sci Nat 44:223-270.

John W. and Michael M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nuclelic Acids Res Vol. 18(24):7213-7218.

Matsuyama T., Motohashi, R. Alkihama T. and Omura. M. 1992. DNA fingerprinting in Ctrrus cultivars Japan J. Breed: 42:155-159.

Nybom, H. and Schaal B. A. 1990, DNA fingerprinting applied to paternity analysis in apples(Malus domestic) Theor. Appl. Genet. 79:763-768.

Nei M. and Li W. 1979. Mathermatic model for studying genetic variation in terms of restrictions endonucleases. Pros. Natl. Acad. Sci. USA 76:5269-5273.

Nybom, H. and Schaal B. A. 1990. DNA fingerprints reveal genotypic distributions in natural populations blackberries and raspberies. Amer.J. Bot. 77:883-888

Rafalski J. A. Tingey S. V. and Williams, J. G. K. 1991. RAPD markers - a new technology for genetic mapping and plant breeding. Agbiotech. News and Inforrmation, 3(4):645-648

Reiter RS, Williams J., Feldmann K., Rafalski JA., Tingey SV. and Scolink P. 1992. Global and local genome mapping in Arabidopsis thaliana by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477-1481.

Roeder, G.S. and Fink, G.R. 1983. In Mobile Genetic Elements, ed, Shapiro, J.A.(Academic Orlando FL) pp 300-328.

Sneath P.H.A. and Sokal R.R. 1973. Numerical taxonomy. Freeman London

Van Heusden A.W. and Bachmann K. 1992. Genotype relationships in Microseris-Elegans (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers

RAPDs. Plant Syst. Evol. 179:221-233.

Welsh J. and McClelland M. 1991. Genomic fingerprints produced by PCR with consensus tRNA gene primers. Nucleic Acids Res 19:861-866

Williams J. G., Kubelik A. R., Livak K. J., Rafalski J. A., and Tingey S. V. 1990. DNA polymorphisms amplified by agitrary primers are useful as genetic markers. Nucl. Acids Res., 18:6531-6534.