APPLICATION OF RANDOMLY AMPLIFIED POLYMORPHIC DNA(RAPD) ANALYSIS METHOD FOR CLASSIFICATION AND BREEDING OF THE KOREAN GINSENG

Lim, Y.P.*, C.S. Shin, S.J. Lee, Y.N. Youn, and J.S. Jo

College of Agriculture, Chung Nam National University, Taejon, 305 - 764, Korea

ABSTRACT

Korean ginseng has been widely used as medicine from ancient times in Asia. Current breeding efforts in Korea include the individual plant selection and the subsequent pure - line isolation, and considerable number of lines with desirable traits have thus been isolated. However, there were rare data on genetic maker and its analysis for selection of superior varieties. For taxonomic characterization and development of genetic markers for ginseng breeding, molecular biological methods including the RFLP and RAPD methods were applied.

Cytoplasmic DNA of ginseng was analyzed for RFLP analysis. However, there is no different pattern among the chloroplast DNA or mitochondrial DNA of variants.

In the case of RAPD analysis, the band patterns using 4 of 10 RAPD primers show the distinctive polymorphism among 9 ginseng variants, and lines, and Similarity Index(SI) on polymorphism was calculated for the extent and nature of these variabilities in ginseng. The sequences of 4 selected primers were TGCCGAGCTG, AATCGGGCTG, GAAACGGGTG, and GTGACGTAGG. By SI based on the polymorphic band patterns, Chungkyung - Chong and Hwangskoog - Chong, and Jakyung - Chong 81783 and Jinjakyung of Russia showed the most close SI. The data of KG101 coincided with the fact that it was released from Hwangskoog - Chong, and Jakyung - Chong 81783 and Jinjakyung of Russia showed the most close SI. The data of KG101 coincided with the fact that it was released from Hwangskoog - Chong by breeding process. The data of Jakyung strains indicated the significant variation among the strains. From these results, RAPD analysis method could be succesively applied to the classification and genetic analysis for breeding of Korean ginseng.

INTRODUCTION

In contrast to most other crops, in ginseng, little effort has yet been exerted for varietal improvement and only the mixed native varieties are available for commercial cultivation. Such a lack of varietal differentiation in ginseng is mainly due to long generation time and difficulty in growth management. Current breeding efforts in Korea include the individual plant selection and the subsequent pure - line isolation, and considerable number of lines have thus been isolated. However, there is rare data on genetic marker and its analysis for selection of superior varieties.

For the isolation of plant species and varieties, there were many methods used, for example, morphological (Eames, 1961), cytological (Stebbins, 1971), anatomical (Radford *et al.*, 1974) and physioecological methods (Kruckeberg, 1969). In addition biochemical method used by isozyme (Gibbs, 1974) and molecular biology with DNA level (Hamrick and Allard, 1975 ; Mabry, 1976) were recently developed the isolation of plant varieties.

Recently, the development of genetic maker using Restriction Fragments Length Polymorphism (RFLP) technique was used in the isolation among varieties and strains of crops. This technique was firstly used in human genetics and then now it was widely made RFLP maps in the plants, for example, *Arabidopsis* (Nam *et al.*, 1989) and corn (Burr *et al.*, 1983). However, RFLP method was taken several difficult points ; (1) dangerous process based on the Southern blot techniques using radioisotope ; (2) so many times needed ; (3) too expensive to examine, etc. These reasons were interfered with freely basic research on the molecular genetics.

To overcome the previous difficulties, Polymerase Chain Reaction (PCR) technique was applied. Random Amplified Polymorphic DNA (RAPD) analysis method was proper to make genetic maker (Mullis and Fakiina, 1987 ; Saiki *et al.*, 1988). The RAPD used oligonucleotide 10mer which was randomly synthesized, and was able to take 4^{10} primers. A few primers among these million primers could be make used to find DNA polymorphism which were more come out than RFLP method (Williams *et al.*, 1990). This RAPD method have some advantages e.g., easier, quicker, need small amount of DNA and less dangerous than RFLP (Waugh and Powell, 1992).

The review will concern about the progress of molecular biological techniques including the RFLP and RAPD methods for ginseng classification and development of genetic makers. We tried to analysis several kinds of ginseng by using RFLP and RAPD, and select the proper primers for analysis.

MATERIALS AND METHODS

Chloroplast and mitochondrial DNA isolation and analysis

The experiment was conducted with leaves and roots of 1, 2, or 5 years old plants of *Panax ginseng* cultured at Korea Ginseng and Tobacco Research Institute. The tobacco and maize were used as a control for chloroplast and mitochondrial DNA, respectively. Detailed experimental protocol of chloroplast and

mitochondrial DNA isolation were reported by Lim and Choi (1990) for mitochondrial DNA, and Lee *et al.* (1990) for chloroplast DNA.

RAPD analysis

The ginseng variants used Chungkyung - Chong, Hwangskoog - Chong, KG101 selected by the pure - line breeding method, and 6 kinds of Jakyung - Chong strains, that is Jinjakyung, Jakyung - Chong 81783, Jakyung - Chong 847913, Jakyung - Chong 79742, Jinjakyung of Russia and Mimaki of Japan. *Taq Polymerase* for PCR was bought from Korea Biotech Inc. Oligodeoxynucleotide primers (10mer set) were purchased from Operon Company, USA. Agarose, tris, EDTA and other chemicals were bought from Boehringer Mannheim and Sigma.

DNA amplification was done by Perkin Elmer Cetus DNA Thermal Cycler. For the extraction of genomic DNA, total DNA was extracted by urea extraction method which was modified the Dellaporta *et al.*'s method (1984) after grinding the ginseng roots with liquid nitrogen. This DNA extraction was treated with RNase, then, after purified by phenol/chloroform method amount of DNA was measured by spectrophotometer.

PCR was acted with *Taq* polymerase and added reaction condition, then DNA was treated with 5 pM of 10mer primer and 50 ng of genomic DNA at 94℃ for 2 min after that, 45 times process which were consisted that there were at 94℃ for 1 min, at 35℃ for 1 min and at 72℃ for 2 min. Finally these DNAs were acted at 72℃ for 3 min. This products were confirmed by 1.2% agarose gel electrophoresis after EtBr treatment. Polymorphic banding patterns of ginseng strains were scored and analyzed by the Similarity Index (SI) of Cluster Analysis in Statistical Ecology by IBM personal computer.

RESULTS AND DISCUSSION

RFLP analysis using organelle DNA

Four variants of Korean ginseng were only released by ginseng breeding program, i.e. Chungkyung - Chong (green - stem variant), Jakyung - Chong (violet - stem variant), Hwangskoog - Chong (yellow - berry variant), and Deung Hwangskoog - Chong (reddish yellow - berry variant). For the comparison among the variants of ginseng, RFLP technique was applied. RFLP technique was the classification method using the restriction digested patterns of nuclear or cytoplasmic DNA. Recently, RFLP technique was applied to the development of classification and genetic map construction of varieties and strains of major crops.

Cytoplasmic DNA was small in size, so we could analyze easily the restriction band patterns of DNA. Fig. 1 showed electrophoresis pattern of *Hind*III digested mitochondrial DNA of Jakyung - Chong, Chungkyung - Chong and Hwangshook - Chong. There was no different pattern among them. Fig. 2 showed the electrophoretic pattern of *Bam*HI digested chloroplast DNA of Korea ginseng variants, Jakyung - Chong and Hwang-

Fig. 1. Agarose gel electrophoretic patterns of *Bam*HI digested ctDNA of Korean ginseng variants, Jakyung - Chong (Lane 1), and Hwangsook - Chong (Lane 2).

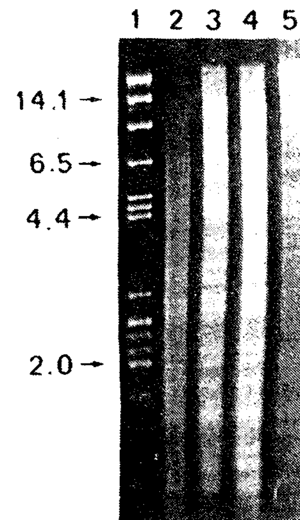

Fig. 2. Agarose gel electrophoresis patterns of *Hind*III digested mtDNA of korean ginseng Jakyung - Chong (lane 2), Chungkyung - Chong (lane 3), Hwangskoog - Chong (lane 4), and maize (lane 5). Lane 1 is marker.

shook - Chong. Also, there was no different between them. Therefore, application of RFLP method on cytoplasmic DNA had limit to classify the ginseng variants.

RAPD analysis

Four of 10 RAPD primers showed the distinctive polymorphism among 9 ginseng variants and strains, and were selected for more detailed polymorphic analysis (Table 1). The sequences of 4 selected primers were TGCCGAGCTG (Primer #2) AATCGGGCTG (Primer #4), GAAACGGGTG (Primer #7), and GTGACGTAGG (Primer #8). But, other 6 primers were not showed the amplified DNA and the difference among variants and strains. Frisch *et al.* (1993) reported similarly results which showed or not. Munthali *et al.* (1992) supposed that amount of DNA and amplified conditions were important to form PCR products in these kinds of unshowed primers.

Table 1. The sequence of selected RAPD primers

Primer No.	Sequence
Primer #1	CAGGCCCCTTC
Primer #2	TGCCGAGCTG
Primer #3	AGTCAGCCAC
Primer #4	AATCGGGCTG
Primer #5	AGGGGTCTTG
Primer #6	GGTCCCTGAC
Primer #7	GAAACGGGTG
Primer #8	GTGACGTAGG
Primer #9	GGGTAACGCC
Primer #10	GTGATCGCAG

All primers produced several common bands among the strains. However, when primer #2 was applied (Fig. 3), the electrophoregram showed the specific band at 1.8kb region in Chungkyung - Chong, Hwangskoog - Chong, and KG101, and at 1 kb region in Jakyung - Chong 847913. In primer #4 (Fig. 4), 1.1 kb band was shown in Chungkyung - Chong, Hwangskoog - Chong, KG101, and Jakyung - Chong 79742. 1.6 kb, 800 bp, 650 bp, and 400 bp bands were in all strains. When the primer #7 applied (Fig. 5), 700 bp band was appeared in Jakyung - Chong 81783 and Jinjakyung of Russia and 450 bp band in Jakyung - Chong 81783. Eight hundred bp band was detected in all samples. With primer #8 (Fig. 6), 800 bp band was observed only in Mimaki of Japan, comparing that all strains were showed 1.1 kb and 0.9 kb band. These DNA polymorphism might be important that kinds of primer and sequences of DNA were important to amplify DNA (Rieseberg *et al.*, 1992). Reiter *et al.* (1992) reported that RAPD marker was important to decide the genetic model. Amplified DNA segments used by 10 - mer random primer might be essential to decide the genetic model of ginseng strains as genetic marker.

Polymorphic banding patterns of ginseng strains were scored and analyzed by the Similarity Index of Cluster Analysis

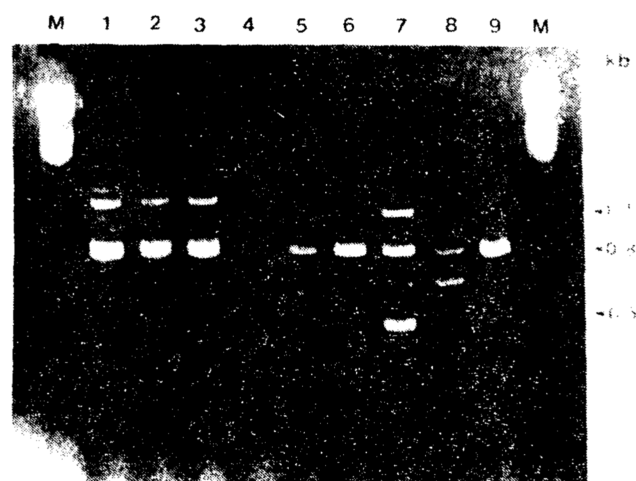

Fig. 3. RAPD amplification products of genomic DNA from 9 variants and strains of Korean ginseng (*Panax ginseng*) using primer #2. Lane 1 : Chungkyung - Chong, Lane 2 : Hwangskoog - Chong, Lane 3 : KG101, Lane 4 : Jinjakyung - Chong, Lane 5 : Mimaki, Lane 6 : 847913, Lane 7 : 81783, Lane 8 : Jinjakyung - Chong of Russia, Lane 9 : 79742, Lane M : Moleculer marker.

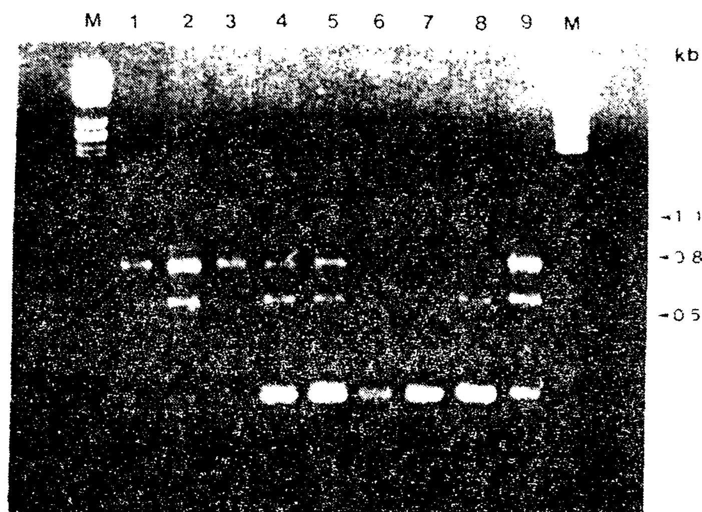

Fig. 4. RAPD amplification products of genomic DNA from 9 variants and strains of Korean ginseng (*Panax ginseng*) using primer #4. Lane 1 : Chungkyung - Chong, Lane 2 : Hwangskoog - Chong, Lane 3 : KG101, Lane 4 : Jinjakyung - Chong, Lane 5 : Mimaki, Lane 6 : 847913, Lane 7 : 81783, Lane 8 : Jinjakyung - Chong of Russia, Lane 9 : 79742, Lane M : Moleculer marker.

in Statistical Ecology by IBM personal computer 486DX. When Similarity Index (SI) was calculated, Fig. 7 showed that close relationship between Jakung - Chong and Hwangskoog - Chong, and between Jakyung - Chong 81783 and Jinjakyung of Russia were 0.11 and 0.08 in SI, respectively, KG101 which was released

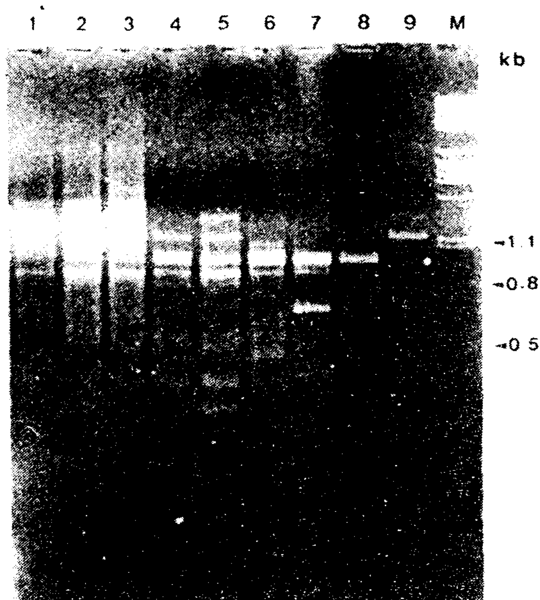

Fig. 5. RAPD amplification products of genomic DNA from 9 variants and strains of Korean ginseng (*Panax ginseng*) using primer #7. Lane 1 : Chungkyung - Chong, Lane 2 : Hwangskoog - Chong, Lane 3 : KG101, Lane 4 : Jinjakyung - Chong, Lane 5 : Mimaki, Lane 6 : 847913, Lane 7 : 81783, Lane 8 : Jinjakyung - Chong of Russia, Lane 9 : 79742, Lane M : Moleculer marker.

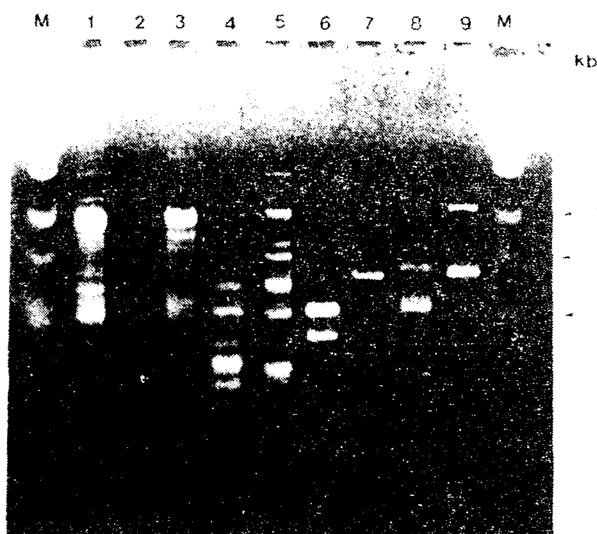

Fig. 6. RAPD amplification products of genomic DNA from 9 variants and strains of Korean ginseng (*Panax ginseng*) using primer #8. Lane 1 : Chungkyung - Chong, Lane 2 : Hwangskoog - Chong, Lane 3 : KG101, Lane 4 : Jinjakyung - Chong, Lane 5 : Mimaki, Lane 6 : 847913, Lane 7 : 81783, Lane 8 : Jinjakyung - Chong of Russia, Lane 9 : 79742, Lane M : Moleculer marker.

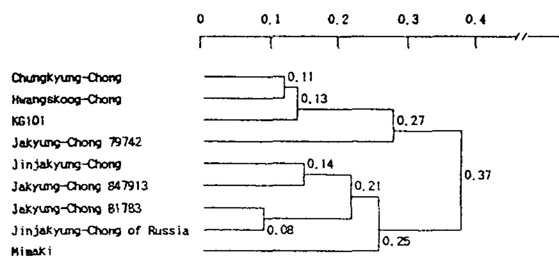

Fig. 7. Phenogram of clustering pattern for 9 variants and strains of Korean ginseng (*Panax ginseng*)

from Korea Ginseng and Tobacco Research Institute, was closely related with the group of Chungkyung - Chong and Hwangskoog - Chong as SI of 0.13. This result confirmed that KG101 was originated from Hwangskoog - Chong by breeding process. In the other band, Jakyung - Chong lines indicated the significant variation among the other strains. For the identification of Jakyung - Chong lines it should by analyzed with more samples cultivated in Korea. From these results, RAPD analysis method might be successively contributed to genetic analysis for breeding of Korean ginseng, and the classification between native and introduced ginseng.

REFERENCES

Burr, B., S.V. Ebola, F.A. Burr, and J.S. Beckman. : The application of restriction fragment length polymorphism to plant breeding. In : Genetic Engineering Principles and Methods, vol 5(ed.), Plenum Press : 45 (1983).

Dellaporta, S.L., Wood, J., and Hicks. J.B. : Plant Molec. Biol. Reporter 1 : 2 (1984).

Eames, A. : Morphology of the Angiosperms. McGraw - Hill. New York (1961).

Fritsch. P., M. A. Hanson, C.D. Spore, P.E. Pack. and L.H. Rieseberg. : Mol. Biol. Rep. 11(1) : 10 (1993).

Gibbs, R.D. : Chemotaxonomy of Flowering Plants. 4 Vols. McGill - Queens University Press. Montreal (1974).

Hamrick, J.L., and R. W. Allard. : Evolution, 29 : 438(1975).

Kruckeberg, A. R. : Taxon, 18 : 92 (1969).

Lee, J.H., Y.P. Lim, and K.T. Choi. : Korean J. Ginseng Sci. 17 : 39(1993).

Lim, Y.P. and K.T. Choi. : Korean J. Ginsneg Sci. 14 : 310 (1990).

Mabry, T.J. : Plant Syst. Evol. 126 : 79 (1976).

Mullis, M., B.V. Ford - Lloyd, and H. J. Newbury. : PCR Methods and Application 1 : 274 (1992).

Nam, H.G., J. Giraudat, B. den Boer, F. Moonan, W.D. V. Loos, G.M. Hauge, and H.W. Goodman. : The Plant Cell 1 : 699 (1989).

Radford, A.W., W.C. Dickison, J.R. Massey, and C.R. Bell : Vascular Plant Systematics, Harper and Row, New York (1974).

Reiter, R.S., J.G. K. Williams, K. A, Feldmann, J.A. Rafalski, S.V. T.ngey, and P.A. Scolnik. : Proc. Natl. Acad. Sci. 89 : 1477 (1992).

Rieseberg, L.H., H. Choi, R. Chan, and C.D. Spore. : Heredity (1992).

Saiki, R. K., D.H. Gelfand, S. Stoffel, S.J. Schraf, R. Higuchi, G.T. Horn, and H.A. Erlich. : Science 239 : 487 (1988).

Stebbins, G.L. : Chromosomal Evolution in Higher Plants. Addison - Wesley, Reading, Mass (1971).

Waugh, R., and W. Powell. : Trends in Biotechnology 10 : 186 (1992).

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S.V. Tingey. : Nucl. Acids Res. 18 : 6531 (19 90).