Korean J.Plant.Res. 6(3) 170~174(2003)

# RAPD Pattern of Ginseng(*Panax ginseng* C.A. Meyer) Lines Containing High Level of Ginsenoside

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## ABSTRACT

**The important component for medical effect in ginseng is ginsenoside. Korea Ginseng & Tobacco Research Institute contains approximately 200 lines produced by inbred selection. It is assumed that ginseng lines containing high level of ginsenoside should be included in those lines. Besides, new breeding methods such as cell line selection *in vitro* and hairy root were recently developed. Therefore, this study was carried out to detect genes related to ginsenoside, and to use it for selection marker to select and distribute lines containing high level of ginsenoside. DNA was extracted from both ginseng roots and hairy roots, and the difference between the line containing high ginsenoside(KG101) and normal ginsenoside(KG103) were analysed. As a result, 28 out of 36 primers showed bands, and many primers showed band difference between ginseng lines. It is considered that the bands should be analysed using DNA sequence comparison to check if those are related to ginsenoside. In case of hairy roots of ginseng, almost no differences were found between two lines.**

***Key words*** **:** RAPD, PCR, ginsenoside, hairy root

## INTRODUCTION

Increasing concerns about human's health with improvement of their living standard, consumption of oriental medicinal plants is continually increased and their import from other country. The important medical component in ginseng is ginsenoside called saponin, which has been found for 29 ginseng species to date(Osamu, 1977; Sanada et al., 1974a; Sanada et al., 1974b; Furuya et al., 1970). At present, Korea Ginseng & Tobacco Research Institute contains approximately 200 lines which were obtained from different local areas and produced by inbred selection for 20 years(Kwon et al., 2001). It is assumed that several lines containing high ginsenoside should be present among the 200 lines. Recently, it has been developed for the cell lines *in vitro*. It has been found that crown gall tumor induced by plasmid of *Agrobacterium* has produced much more secondary metabolite than normal tissue(Hagimori et al., 1982; Yang et al., 1991; Choi and Yang, 1987). Especially, hairy root produced by *Agrobacterium rhizogenes* is fast to grow, easy to

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harvest, and contains much secondary metabolites(Yang et al., 2001). Therefore, hairy root is considered to be a good cell line for the production of medicine (Hamill et al.,1986; Yoshikawa and Furuya, 1987; Yang, 2003). To maintain and improve export of Korean ginseng to foreign countries even though Korean ginseng is more expensive than foreign ginseng, it is necessary to produce good Korean ginseng in quality that contains various kinds of and high level of ginsenosides. The purpose of this report is to screen genes related to ginsenoside using the ginseng lines of Korea & Tobacco Research Institute by RAPD analysis, and this will be used as a marker to select and distribute the lines containing high level of ginsenoside.

## MATERIALS AND METHODS

### Plant materials

The roots from KG101 selected by inbreeding and from KG103 containing high level of ginsenoside were used. In addition, KGHR-2 cell line containing low level of ginsenoside and KGHR-5 cell line containing high level of ginsenoside were used, which were the cell lines of hairy roots produced by the transformation of ginseng roots using Ri plasmid.

### DNA extraction

DNA extraction was carried out according to the procedure of Edwards et al. (1991). Approximately 0.3 g of plant samples was ground completely for 30 s in the 1.5 ml of eppendorf tube using the plastic tip fixed in the drill homogenizer. After grinding, 600 μl of DNA extraction buffer (2% CTAB, 1.5M NaCl, 20mM EDTA, 100mM Tris, pH 8.0, 2% soluble polyvinylpyrrolidone, 2% mercaptoethanol) was added and again ground by the plastic tip for 30 s followed by vortex for 30 s. This extract was centrifuged at 12,000 rpm for 3 min, and then 500 μl of supernatant was transferred to new eppendorf tube. The same amount (500 μl) of phenol:chloroform:isoamylalcohol(25:24:1) was added to the supernatant. After vortex for 30 s, it was centrifuged for 3 min, and 300 μl of supernatant was transferred to new tube. DNA was precipitated by addition of 200 μl of isopropanol and incubation of the mixture for 2 min at room temperature. DNA was recovered by centrifugation for 10 min at 12,000 rpm, washed with 70% ethanol, and dissolved in 100 μl of TE buffer (10 mM Tris-HCl pH 8.4, 1 mM EDTA).

### RAPD analysis

PCR amplifications were performed on GeneAmp PCR system 2400 (Perkin Elmer, USA) in a total volume of 20 μl containing 50 ng of template DNA, 10 pmol of primers, and AccuPower Premix-Top (Bioneer, Korea). Thirty six primers synthesized by UBC (Universityof British Columbia, Canada) were used for PCR analysis. PCR amplification was performed with the following profile: 2 min denaturation at 94℃ and 45 cycles of 1 min denaturation at 94℃, 1 min annealing at 37℃, 1 min extension at 72℃, followed by a final extension at 72℃ for 10 min.

## RESULTS AND DISCUSSION

### RAPD banding pattern of ginseng roots

The difference between the ginseng lines containing normal ginsenoside (KG101) and high ginsenoside (KG103) was analysed using UBC PCR primers. Twenty eight kinds of primers out of 36 kinds of total primers showed bands. Amplified bands were shown in the KG103 line using UBC-6, 105, 149, 159, 174, 177 primers, whereas no bands in the KG 101 line. On the contrary, amplified bands were found in KG101 line using UBC-29, 33, 125, 157, 181 primers (Fig. 1, Table 1). Especially, there were differences in major bands when UBC-29, 33, 125, 157, 181 primers were used (Fig. 1-2, Table 1). The bands showing major differences will be analysed to search for the relation

Table 1. Characteristics of banding pattern on KG101 and KG103 according to selected primers

| UBC Primer Number | Band Shown | | Molecular Weight (Kb) | Number of Band |
|---|---|---|---|---|
| | KG101 | KG103 | | |
| 6 | | x | 3 | 5 |
| 105 | | x | 2.5 | 3 |
| 149 | | x | 0.75 | 4 |
| 159 | | x | 1 | 3 |
| 174 | | x | 2.5 | 3 |
| 177 | | x | 0.7 | 5 |
| 29 | x | | 1.5 | 2 |
| 33 | x | | 3 | 3 |
| 125 | x | | 1 | 1 |
| 157 | x | | 1.6 | 5 |
| 181 | x | | 1.8 | 3 |
| 218 | x | | 1.4 | 4 |
| 250 | x | | 1 | 1 |
| 211 | | x | 2.5 | 4 |
| 220 | | x | 1.5 | 4 |
| 225 | | x | 1.8 | 3 |
| 239 | | x | 0.8 | 3 |
| 248 | | x | 1.7 | 5 |

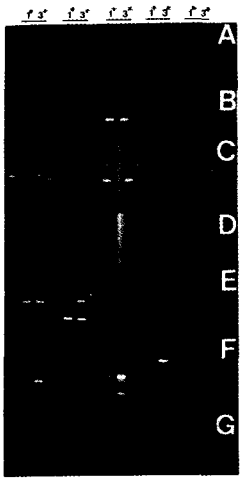


Fig. 1. RAPD banding pattern of ginseng roots containing normal(KG101; 1*) or high level(KG103; 3*) of ginsenoside according to different primers. A: UBC-1, 2, 3, 4, 6, B: UBC-12, 13, 17, 23, 25, C: UBC-29, 30, 33, 34, 63, D: UBC-77, 82, 83, 89, 100, 102, E: UBC-193, 105, 106, 125, 127, F: UBC-137, 147, 149, 150, 155, G: UBC-157, 159, 174, 177.

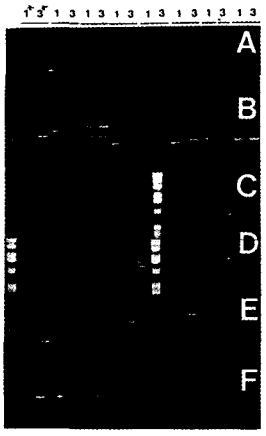


Fig. 2. RAPD banding pattern of ginseng roots containing normal(KG101; 1*) or high level(KG103; 3*) of ginsenoside according to different primers. A: UBC-181, 184, 190, 198, 199, 203, 204, 208, B: 211, 213, 218, 219, 220, 222, 225, 226, C: 228, 230, 231, 232, 234, 237, 239, D: 240, 241, 243, 244, 245, 246, 248, E: UBC-249, 250, 251, 253, 254, 262, 264, 266, F: UBC-270, 273, 275, 276, 280, 282, 283, 285.

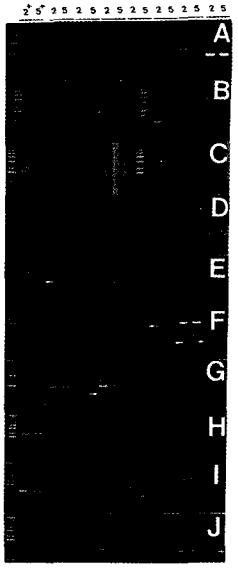


Fig. 3. RAPD banding pattern of ginseng hairy root cell lines containing low(KGHR-2; 2*) and high(KGHR-5; 5*) level of ginsenoside. A: UBC-1, 2, 3, 4, 6, 12, 13, 17, B: UBC-23, 25, 29, 30, 33, 34, 50, C: UBC-63, 64, 67, 70, 71, 72, 73, D: UBC-77, 82, 83, 89, 100, 102, 103, 105, E: UBC-106, 125, 127, 137, 147, 149, 150, 155, F: UBC-157, 159, 174, 177, 181, 184, 190, 198, G: UBC-199, 203, 204, 208, 211, 213, 218, 219, H: UBC-220, 222, 225, 226, 228, 230, 231, 232, I: UBC-234, 237, 239, 240, 241, 243, 244, 245, J: UBC-246, 248, 249, 250, 251, 253, 254, 262.

with ginsenoside in the near future.

**RAPD banding pattern of hairy roots**

When ginseng plants were used for RAPD, there was big differences between individuals. Therefore, hairy roots of ginseng were used, since they can reduce the differences between the individuals. PCR amplification was successful using most of the 79 selected primers except 10 kinds of primers showing no bands or bands difficult to confirm (Fig. 3). However, no distinct differences were found between the two cell lines except in UBC primers 198 and 208. A little banding difference was found using UBC primers 106, 181 and 194. Therefore, the amplified bands using UBC primers 198 and 208 will then be analysed by sequencing after cloning.

RAPD analysis developed by William et al(1990) uses oligonucleotide primers consisting of 9-10 number of bases and amplify plant genomic DNA randomly. The amplified PCR product is analysed by gel electrophoresis and banding pattern, which is widely used because of its simplicity in analysis(Joao and Monteiro, 1994; Kim, 1996). The RAPD analysis was performed in our experiment to select the ginseng lines containing high level of ginsenoside. Amplified bands were found in 28 PCR primers out of 36 primers. Out of 23 PCR primers showing bands, banding differences were found in 11 primers. It is not clear whether or not the banding differences are related to the ginsenoside genes, and we are going to sequence the bands showing the differences followed by homology search. In addition, more ginseng lines need to be analysed using PCR primers. If the marker for high level of ginsenoside is developed, it will be used not only for the breeding of new cultivar, but also for the mass production of good ginseng in quality by farmers. The study for the production of secondary products using the hairy root culture is currently underway because of the advantages of hairy roots such as genetic and biochemical stability, fast growth, the synthetic ability on useful products showing the same characteristics as parental plants(Hwang et al., 1989; Yang et al. 1996; Yoshikawa and Furuya, 1987). Not much differences were found between the two cell lines of hairy roots, however, different banding pattern was found using 5 different UBC primers that will be analysed for DNA sequences. If we can find the genes related to ginsenoside, the genes will be transformed to ginseng hairy roots. As the results, we expect mass production of ginseng containing high level of ginsenoside in a short time.

## ACKNOWLEDGEMENTS

This work was supported by a research grant for fresh professor from Kyung Hee University at 2003.

## REFERENCES

Choi, K.T. and D. C. Yang. 1987. Characteristics of the growth of ginseng tumor callus. Korean J. Ginseng Sci. 11(1) : 56-65.

Edwards, K., C. Johnston, C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19 : 1349.

Furuya, T., H. Kojima, K. Syono and T. Ishill. 1970. Isolation of Panaxatriol from *Panax ginseng* callus. Chem. Pharm. Bull. 18(11) : 2371-2372.

Hagimori, M., T. Matsumoto and Y. Obi. 1982. Studies on the production of digitalis cardenolides by plant tissue culture. Plant Physiol. 69: 653-656.

Hamill, J.D., A.J. Parr, R.J. Robins and M.J.C. Rhodes. 1986. Secondary product formation by cultures of *Beta vulqaris* and *Nicotiana rustica* transformed with *Agrobacterium rhizogenes*. Plant Cell Reports. 5: 111-114.

Joao S., A.A. Monteiro. 1994. Taxonomy of protuguese tronchuda cabbage and galega kale landraces using morphological characters, nuclear RFLPs, and isozyme analysis : A review. Euphytica 79 : 115-126.

Kamada, H., N. Okamura, M. Satake, H. Harada, and K. Shimomura. 1986. Alkaloid production by hairy root cultures in *Atropa belladonna*. Plant Cell Reports. 5: 239-242.

Kim, N.S., K.M. Shim, K.M. Yoon, Y.B. Shin, Y.S. Cho, M.Y. Eum and Y.G. Cho. 1996. Polymorphism and hhylogenetic analyses in rices cultivated in Korea by RAPDs and RFLPs. Korean J. Genetics 18(3) : 211-224.

Kwon, W. S. and M. K. Lee. 2000. Breeding Process and Characteristics of Yunpoong, a New Variety of *Panax ginseng* C.A. Meyer. Korean J. Ginseng Sci. 24(1): 1-7.

Osamu, T. 1977. Recent studies on the chemistry of ginseng saponins. Korea. J. Ginseng Sci. 2(1): :9-15.

Sanada, S., N. Kondo, J. Shoji, O. Tanaka and S. Shibata. 1974. Studies on the saponins of ginseng. I. Structures of ginsenoside -Ro, -Rb1, -Rb2, -Rc and -Rd. Chem. Pharm. Bull. 22(2): 421-428.

Sanada, S., N. Kondo, J. Shoji, O. Tanaka and S. Shibata. 1974. Studies on the saponins of ginseng. II. Structures of ginsenoside -Re, -Rf and -Rg2. Chem. Pharm. Bull. 22(10): 2407-2412.

Williams, J., A. Kubelick, K. Livak, J. Rafalski and S. Tingey. 1990. Necleic Acids Res. 18: 6531.

Yang, D. C., K. J. Yang, Y. E. Choi. 2001. Production of red ginseng specific ginsenosides(Rg2, Rg3, Rh1 and Rh2) from *Agrobacterium*-transformed hairy roots by heat treatment. J. of Photoscience 8(1): 19-22.

Yang, D. C., K. T. Choi and D. C. Yang. 1991. Pattern of soluble protein, reducing sugar and ginsengosides in transformed calli of ginseng(*Panax ginseng* C. A. Meyer). Korean J. Ginseng Sci. 15(2): 124-130.

Yang, Deok-Chun. Induction of ginseng hairy roots and their possible application to large scale culture. 2003. Korean J. Plant. Res. 6(1): 1-6.

Yoshikawa, T. and T. Furuya. 1987. Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogenes*. Plant Cell Reports. 6: 449-453.

(Received Oct. 23, 2003)
(Accepted Dec. 10, 2003)