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Full Length Research Paper

Random amplified polymorphic DNA (RAPD) analysis and RAPD-derived sequence characterized amplified regions (SCAR) marker development to identify Chinese and Korean ginseng

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***Panax ginseng*, a family Araliaceae, is an herbaceous medicinal plant with thickened roots containing pharmacologically active triterpenes, and distributed in North East Asia. Korean and Chinese ginseng are same species but consumers require strict identification of the place of origin of Chinese and Korean ginseng because of highly different prices of roots. Here, we investigated the intraspecific population of Korean and Chinese ginseng by random amplified polymorphic DNA (RAPD) and developed RAPD-derived sequence characterized amplified regions (SCAR) marker. RAPD analysis using two UBC (University of British Columbia) primers (519 and 534) resulted in high polymorphic to detect the genetic differences between Korean and Chinese ginseng population but highly monomorphic among the individuals within the population. Chinese ginseng specific PCR products using a 534 primer were isolated and sequenced. From the PCR reaction using SCAR primer (CG965) designed from 965 bp of sequence, population of Chinese ginseng was determined as a clear band but Korean ginseng did not produce any amplified band. Conclusively, two RAPD primers (519 and 534) were effective to detect heterogeneity of ginseng population growing in Korea and China, and a RAPD-derived SCAR marker can be used for rapid identification of Chinese ginseng population among Korean ginseng one.**

Key words: *Panax ginseng,* random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCAR) marker.

INTRODUCTION

Panax ginseng C. A. Meyer belongs to Araliaceae and has been used as an important traditional medicine (Yun, 2001; Shibata, 2001). *P. ginseng* is distributed geographically in different countries in Korea, China, and Russia. Wild and wild-cultivated *P. ginseng* is much higher price than field-cultivated ginseng and being increasing demand with high medicinal values (Choi et al., 2006). Moreover, Korean wild and wild-cultivated ginseng is highly priced compared to the Chinese ginseng in Korea. Thus the disguised foreign ginseng is a serious problem in the Korean ginseng markets. It is very difficult to discriminate the difference between the intraspecific population between Korean and Chinese ginseng by using methods other than molecular analysis. Recently, there are many reports to discriminate the interspecific ginseng using various molecular technologies such as RAPD (Shaw and But, 1995; Um et al., 2001; Shim et al., 2003; Cui et al., 2003), PCR-RFLP (Ngan et al., 1999; Fushimi et al., 1997) and gene sequences (Komatsu et al., 2001). However, little was done to determine the intraspecific population of *P. ginseng*, especially for detection of ginseng roots produced in different countries. Kim et al. (2005) and Ahn et al. (2009) reported the detection of Chinese and Korean ginseng population using amplified fragment length polymorphism (AFLP)

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analysis and microsatellite marker, respectively. Compared to the AFLP and simple sequence repeat (SSR), RAPD analysis is a very simple and rapid method because of cheap agarose gel loading of PCR products (Williams et al., 1990). This method was applied to understand the genetic relationship and diversity (Bai et al., 1997; Boehm et al., 1999; Kim et al., 2003; Cha et al., 2003), and DNA identification of commercial ginseng samples (Mihalov et al., 2000). Paran and Michelmore (1993) developed a technique known as sequence-characterized amplified regions (SCAR). SCAR markers have been derived from RAPD and AFLP markers, and have proven useful in identifying the plants at intra- and/or inter-specific level. SCAR as PCR based genetic markers is a genomic DNA fragment that is identified by PCR amplification using a pair of specific oligonucleotide primers (Paran and Michelmore, 1993). The conversion of RAPD or AFLP markers into SCARs based on the marker sequence information significantly improves the reproducibility and reliability of PCR assays (Paran and Michelmore, 1993). SCAR markers are highly advantageous for quick and easy assessment (Paran and Michelmore, 1993). Molecular authentication of *Panax* species is developed for the identification of ginseng population by AFLP, RFLP, RAPD and microsatellite marker (Ha et al., 2002; Ngan et al., 1999; Reunova et al., 2010; Hon et al., 2003). Wang et al. (2001) developed the SCAR marker from RAPD fragments to authenticate *P. ginseng* and *Panax quinquefolius* species. The simple and rapid identification of population-specific DNA markers of *P. ginseng* would be of great importance to discriminate the domestic population of *P. ginseng*. In this study, we developed the polymorphic RAPD markers to detect the heterogeneity of ginseng population growing in Korea and China, and a population-specific SCAR marker (named CG965) for Chinese ginseng was obtained from sequences of RAPD PCR products. PCR amplification using specific primers clearly demonstrated the specific band for Chinese population of *P. ginseng*.

MATERIALS AND METHODS

Ginseng materials and extraction of genomic DNA

Korean *P. ginseng* roots were collected from the various places of Kangwon-do province (mainly Inje-, Hwacheon-, and Hongcheon-gun) of South Korea. Wild mountain ginseng roots were collected from Seorak Mountain of Kangwon-do province. Chinese *P. ginseng* roots were collected from Jilin province (Chang Bai Mountain) of China.Total genomic DNA was extracted as previously described by Edwards et al. (1991). The samples were ground into fine powders after freezing with liquid nitrogen. Total genomic DNA was extracted from approximately 200 mg of ginseng roots using the MagExtractor (TOYOBO, Japan). DNA concentration was measured with an UV spectrophotometer (Shimazu Co., Japan).

RAPD analysis

PCR reaction were performed in 25 µl volume made in DNA free water containing 10 to 20 ng of genomic DNA, 10 pmol primer (100 different primers obtained from The University of British Columbia, Canada), 0.2 mM of each dNTP, 2 mM $MgCl_2$ and 0.5 unit of Taq DNA polymerase (EX Taq, TaKaRa, Japan). Reactions were run on a DNA thermal cycler (ABI applied biosystems, Singapore). Cycling conditions were 94°C for 3 min; followed by 40 cycles of denaturation at 94°C for 1 min, annealing temperature at 35°C for 2 min, polymerization at 72°C for 2 min and final extension at 72°C for 5 min.

Cloning, DNA sequencing and SCAR primer design

RAPD reactions were performed in a PCR system 9700 (ABI applied biosystems, Singapore). Taq DNA polymerase-generated RAPD bands were excised from agarose gels with a razor blade and the DNA extracted using the QIA quick Gel Extraction kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). Ligation of the PCR product was cloned into pGEM-T Easy vector (Promega) and introduced into competent *E. coli* according to manufacturer's instructions. 1 ml LB medium was added immediately following electroporation and incubated for 1 h at 37°C with shaking, after which a 200 µl aliquot was plated onto LB agar plates containing ampicillin, IPTG and X-gal and incubated overnight at 37°C. Up to six white colonies from each transformation reaction were streaked onto plates to produce single colonies.

Plasmid DNA was extracted from overnight cultures of transformed bacterial cells, and samples was diluted 1:50 in sterile water. 10 µl aliquots of the diluted miniprep were mixed with 6 µl sterile water, 10 × buffer (Roche), and 2 µl *Eco*RI restriction enzyme (Roche) and the reactions incubated at 37°C for 1 h. The entire reaction was electrophoresed on 1% agarose gel and stained with ethidium bromide. Two sequencing reactions were set up using 1 µg plasmid DNA combined with 4 µl of M13 forward and reverse primer, respectively. These reactions were brought up to a total volume of 18 µl with sterile water. DNA sequencing was performed using pUC/M13 primers on an automated sequencer (Applied Biosystems 3700, USA). The software Encyclon was used for sequence analysis and SCAR marker design. Melting temperature, GC contents and molecular weight were verified using oligo software, and then primers were commercially synthesized.

PCR analysis with SCAR primers

The designed SCAR primer pairs (forward: 5´-ATGAACCCGCCGCCGCCTGCACC-3´ and reverse: 5´-TATGAACCCGCCGCCGCCTGCAC -3´) were used to test Korean and Chinese ginseng. Testing was done to ensure amplification of the band with the exact molecular weight and determine optimal annealing temperature. The PCR reaction to amplify the SCAR marker was performed as follows: 94°C for 3 min, 40 cycles of 94°C for 30 s, 62°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 5 min. The PCR products were run on a 1.5% (w/v) agarose gel and stained in ethidium bromide as stated previously. The presence or absence of the SCAR band was visually scored and compared with samples.

RESULTS AND DISCUSSION

RAPD analysis

Wild and mountain-cultivated plants were collected from

Table 1. The sequence and the number of polymorphic bands produced from the selected primers for RAPD analysis.

Name of primer	Sequence of primer (5' to 3')	Total no. of amplification (B)	No. of polymorphic (A)	Polymorphism (A/B 100) (%)
UBC 519	ACCGGACACT	6	1	16.6
UBC 534	CACCCCCTGC	5	4	80
UBC 536	GCCCCTCGTC	10	6	60
UBC 540	CGGACCGCGT	9	6	66.6
UBC 548	GTACATGGGC	3	0	0
UBC 594	AGGAGCTGGC	4	2	50
Total bands scored		37	19	51.3

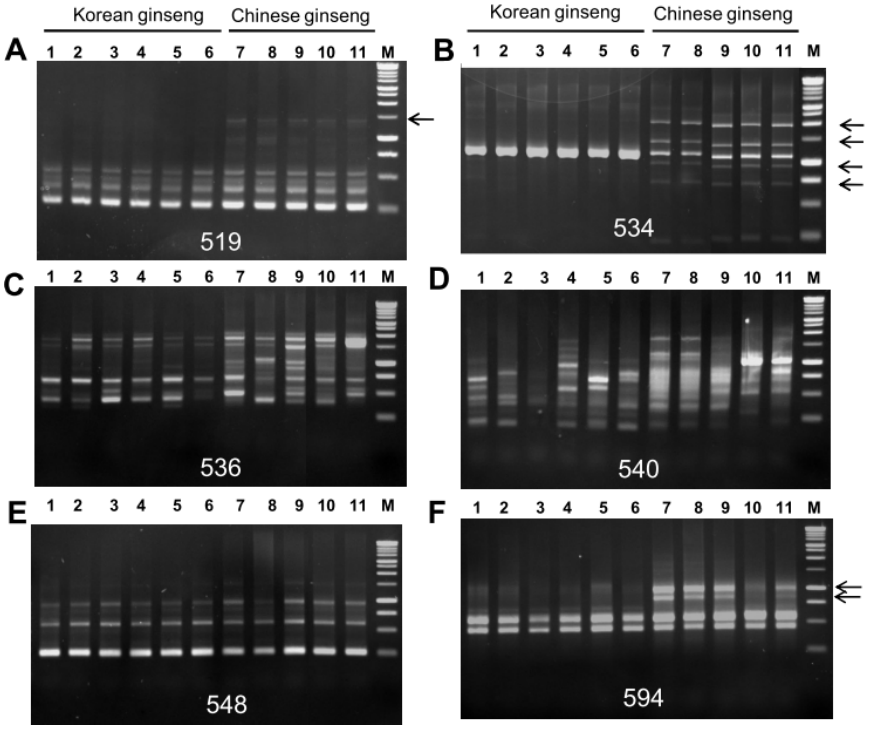

Figure 1. Profile of PCR products obtained from RAPD analysis using UBC 519 (A), UBC 534 (B), UBC 536 (C), UBC 540 (D), UBC 548 (E), and UBC 594 (F). Lane 1 to 6: Korean ginseng (Korean mountain cultivated ginseng), Lane 7 to 11; Chinese ginseng (Chinese mountain cultivated ginseng), M: 1 kb ladder. Arrows indicate Chinese ginseng specific bands.

Korea and China and were used for RAPD analysis. Among 100 random primers tested, yielded molecular heterogeneity between Korean and Chinese ginseng population was gained from six primers (Table 1). DNA amplification fragments of Korean and Chinese ginseng amplified by 6 primers was seen between 250 to 2,000 bp and 3 to 10 bands were created in each primer.

There were 19 bands that showed polymorphism among 37 DNA bands that were amplified in total 6 UBC primers. It was shown that about 51.3% of the total amplified DNA fragments had genetic polymorphism (Table 1). For example, Chinese ginsengs specific band (arrows in Figure 1) was shown in UBC 519, 534 and 594 but there was no band in Korean ginseng (Figure 1A). Several Chinese ginseng specific bands (arrows) were clearly detected using UBC 534 (Figure 1B). In case of UBC 536 and 540 (Figures 1C to D), Korean and Chinese ginseng showed no clear difference but high polymorphic among the samples in both Korean and Chinese ginseng but this might be useful for determining the individual linage because of high polymorphic among samples. In UBC 548 and 594 (Figure 1E to F), there is polymorphism between Korean and Chinese ginseng, considered as different population.

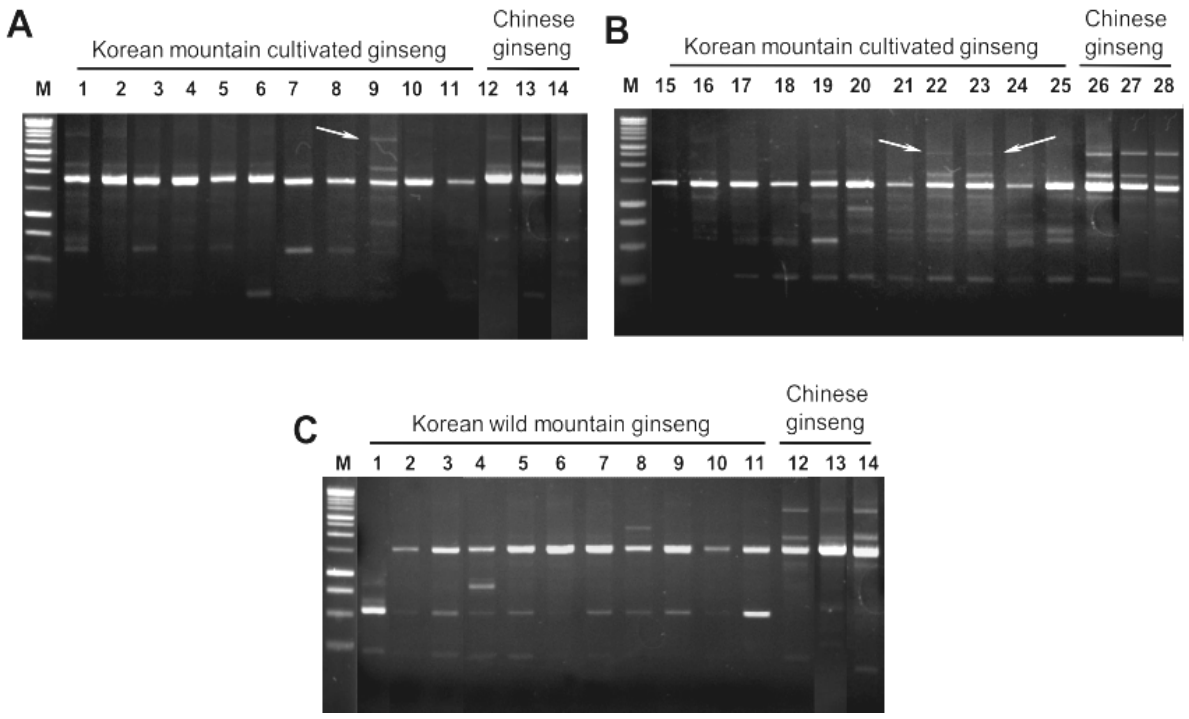

Figure 2. Profile of PCR products obtained from RAPD analysis using the UBC 534 among the ginseng produced in Inje-gun. A and B: Korean mountain cultivated ginseng (1 to 11 and 15 to 25: Individual samples of Korean mountain cultivated ginseng; 12 to 14, and 26 to 28: Individual samples of Chinese mountain cultivated ginseng), C: Korean wild mountain ginseng (1 to 11: Individual samples of Korean mountain cultivated ginseng; 12 to 14: Individual samples of Chinese mountain cultivated ginseng). M: 1 kb ladder. Arrows indicates Chinese ginseng specific bands.

Analysis of polymorphism

To confirm the usefulness of developed UBC 534, a total of 22 roots of mountain cultivated ginseng sampled from regions of Kangwon province and 11 roots of wild mountain ginseng from Seorak Mountain of Kangwon province were analyzed with RAPD. A specific band similar DNA fragments to Chinese ginseng was shown in 3 samples (number 9, 22, and 23 in Figures 2A to B) among total 22 mountain cultivated ginsengs when PCR was done by using UBC 534 (Figures 2A to B). This observation suggests that Korean growers might purchase some seeds of Chinese ginseng and cultivate them in Korean although the facts could not be reported clearly. Practically, some amount of Chinese wild ginseng seeds are imported by Korean growers because seeds of Korean wild ginseng are very difficult to be purchased because of rareness. On the other hand, there were no Chinese ginseng specific bands in 11 wild mountain collected Korean ginseng roots but each sample of roots showed higher polymorphic DNA fragments than mountain cultivated ginseng roots (Figure 2C). This result indicates that wild-collected mountain ginseng has higher ratio of polymorphism than the mountain cultivated ginseng. Most of seeds for cultivation in mountain are sourced from field cultivated ginseng seeds but rarely from wild mountain ginseng.

In USA, about 21 wild populations of *P. quinquefolius* are grown in wild of Gerogia, Maryland, North Carolina and West Verinia (Cruse-Sanders and Hamrick, 2004). Boehm et al. (1999) reported that the population of *P. quinquefolius* naturally grown wild ginseng in North Carolina, Pennsylvania and Wisconsin are clearly distinguished from field (artificially) cultivated ginseng by RAPD analysis. Grubbs and Case (2004) reported that there are highly monomorphic within the same population but highly polymorphic among the population of wild ginsengs in *P. quinquefolius*. In this work, polymorphism was higher in wild mountain ginseng than field cultivated ginseng (Figure 2). This result suggests that wild ginseng can be used as a good material for breeding because wild grown ginseng is highly polymorphic and can be survived for several decades at harsh mountain environment.

Authentication of Korean *P. ginseng* from Chinese *P. ginseng* and *P. quinquefolius* were already reported in our group using polymorphism of AFLP (Kim et al., 2005) and SSR (Ahn et al., 2009). However, the AFLP analysis method requires a complicated technique and takes a long time (about one week to be resulted). SSR analysis also needed to load the PCR products in toxic acrylamide gel. Therefore, the UBC 534 primer for RAPD analysis can be used for easy and quick detection of Chinese ginseng from Korean ginseng roots.

1 ATGAACCCGC CGCCGCCTGC ACCTGCTGCG AGCAAGCCTG CAGCAAAACC TGCCGCTGCC
61 AAATCTGTAG CAGCCAAGCC TGCGGCAAAA AAACCGGTCG CAGCTAAAAA GCCGGCCGCC
121 GCTAAACCGC CGGTCACTGC TCCTGAGGCA AAACCTGAAG CTACGGTTTA GCCCATAGCA
181 ACTTCAGAAG AGGCTTGCCC GCGATGCAGA CACCATCGCT GGCAAGCCTC TTTCTATCCG
241 GGATGGGCGT TAAAGCATCA GCTTGACGAT GGATTCCGAA GGGTCGCGGG ACTTGCCGGC
301 TTTTTTCAGT TCTTGCAGAT AATCAGCCCA CAACTCCTCT TGGCGCGCTC CGAGCTGGTA
361 GAGGTACTCC CAGGTAAACA GGCCGCTGTC GTGACCGTCA TCGAAGGTTA TTTTCAACGC
421 ATATTGGCCC GCGGGTTCTA TCTTGCTCAG GCCTACGTTG ATTTTCCCGA CTTGCAGAAT
481 CGGATTGCCG TGGCCCTGGA CTTCGGCGGA GGGCGAGTGC ACGCGCAGAA ACTCTGCAGC
541 CAGGTGGTAC TGCTCGCCGG AGGGGTATTT GAGCGTGAGG GTTTTAGAGG CTTTGTGCAG
601 GTTGATGGCG TTGGGGATTG GCGCTGGAGT AGGGCTCATT TGGACAAAAC CTTTTTGTCA
661 TCACACACGC TGTAGCCGCG GCCACAGGTT GCGATTGCCA ACCGGGTTGG CGCTGGATCC
721 GGGGGATCGC AGCAGGCTTT TGGCCTTATC GCAGCCTTTG ACAGCGGCTA CAGGTTTGGT
781 GAGGGCTTAC AAAATATAGC GCGAGAGGTC TTCGTTCTCG GCCAGTTCGC CCAGGTGGCT
841 GTTCACGTAT TCAGCGTCAA TGCGGATCGG CTCGTTTTTT TGTGCGCTGG CGATATCACC
901 GGCGCTGAAA GACACCTCTT CAAGCAAACG CTCAAGCAAT GGGTGCAGGC GGCGGCGGGT
961 TCATA

Figure 3. Sequence of the species specific DNA fragment of Chinese ginseng. Total length of the fragment is 965 bp. Designed primers for PCR analysis are underlined and it was named to CG965.

Table 2. Sequences of primers used for the SCAR marker.

SCAR marker	Sequence (5' to 3')
CG965	F: ATGAACCCGCCGCCGCCTGCACC R: TATGAACCCGCCGCCGCCTGCAC

Isolation of RAPD fragment for SCAR marker design

RAPD fragments of PCR product using UBC 534 primer for Chinese ginseng was unique (arrows in Figure 1B) in Chinese ginseng and not conserved in Korean ginseng. The Chinese ginseng specific fragments were cloned and sequenced. A 965 bp DNA fragment was obtained. Forward and reverse oligonucleotide primers (23 mers) (underlined in Figure 3.) were designed from 965 bp sequence to amplify this RAPD fragment. BLAST results revealed that the sequences had no homology with known plant nucleotide sequences at sequence-similarity levels. The nucleotide sequences of the primer pairs used for amplifying the SCAR fragments are shown in Table 2. The primer pairs were named as CG965 for amplification of DNA at 965 bp fragments, respectively.

PCR amplification using SCAR primers

PCR amplification was performed using SCAR primer (CG965) and DNA samples of Korean ginseng and Chinese ginseng. RAPD fragments using SCAR primer (CG965) showed a clear band (965 bp) in Chinese ginseng by PCR but no band in Korean ginseng (Figure 4). The optimized protocol is described as follows. Each reaction consisted of 2 µl genomic DNA, 2 µl of both the forward and reverse primers, 8.5 µl sterile water and 12.5 µl DNA master mix (Promega). The optimized amplification program requires an initial denaturation of 3 min at 94°C; 40 cycles at 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min.

Although, we previously reported the authentication of *P. japonicus* and *P. notoginseng* by AFLP (Choi et al., 2008; Kwon et al., 2009), this report is the first report for SCAR marker development for the detection of Chinese ginseng among Korea ginseng by PCR.

Conclusion

RAPD analysis using UBC 534 primer clearly determined the Chinese ginseng among Korean ginseng population.

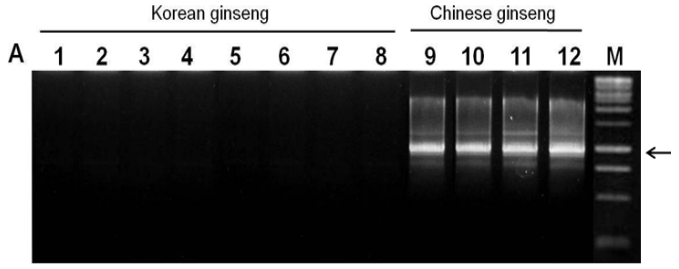

Figure 4. PCR analysis using the CG965 primer pair. Lane 1 to 8: Korean ginseng (Korean mountain cultivated ginseng), Lane 9 to 12; Chinese ginseng (Chinese mountain cultivated ginseng), M: 1 kb ladder. Arrows indicates expected amplified Chinese ginseng specific bands (965 bp) by CG965 primer pair.

A specific SCAR marker for detection of Chinese ginseng was obtained from sequencing of RAPD fragments. PCR amplification using CG965 specific primers clearly demonstrated the unique band only in Chinese ginseng and not Korea ginseng. The UBC 534 RAPD markers and RAPD-derived SCAR marker (CG965) will be used for rapid authentication of Chinese ginseng among Korean ginseng population.

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