Molecular Authentication and Genetic Polymorphism of Korean Ginseng (*Panax ginseng* C. A. Meyer) by Inter-Simple Sequence Repeats (ISSRs) Markers

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Molecular authentication and genetic polymorphism of Korean ginseng cultivars and accessions were investigated using ISSR (inter-simple sequence repeat amplification) markers. Five primers among 56 produced clear and reproducible DNA fragments among seven cultivars and accessions. A total of 43 bands ranging from 250 bp to 1,700 bp from five primers were scored. Average number of bands per primer was 8.6 and only nine bands were polymorphic across the six *Panax ginseng* from Korea. Especially Chunpoong cultivar exhibited the highest level of polymorphism, whereas other accessions did not showed almost any polymorphism. Consequently, these ISSR markers will be available to differentiate Chunpoong cultivar from other major Korean ginseng cultivars and accessions, such as Yunpoong, Hwangsukjong and Jakyungjong, at the DNA level.

Key words – *Panax ginseng*, molecular authentication, genetic polymorphism, ISSR marker

Ginseng (*Panax* spp., Araliaceae) is a herbaceous perennial plant and is cultivated for its highly valued root in Korea, northeastern China, the northern United States, and Canada [3]. Recently, ginseng has been used as a medicinal herb in western medicines and its application (or utilization) is expanding, probably because of a growing interest in alternative treatments of them as medicines [2]. In Asia, where it is considered as a universal therapeutic agent due to its adaptogenic properties, ginseng is believed to improve vitality into old age and extend the human life span [6]. It has shown behavior modifying effects, stress and fatigue reducing effects, positive influences on the cardiovascular, nervous, reproductive system, endocrine and metabolic activity, as well as cancer reducing and immune stimulating effects [7,17].

The ginseng plant is associated with various names and these many varieties are commercially available. This can cause considerable confusion and misinterpretation. The two major species are included in ginseng; one is *Panax ginseng* C. A. Meyer, referred to Asian ginseng, which is distributed primarily in Asia and East-Siberia and the other is *Panax quinquefolium* L., commonly called American ginseng. The later species grows wild in rich at cool wooden areas in the US such as Minnesota. Because of recent intense harvesting in the US, the plant has been labeled an endangered species under regulations on collection and sale [8]. A non-Panax species of the ginseng family is *Eleutherococcus senticosus* Maxim. or *Acanthopanax senticosus*, which is also known as Siberian ginseng [19].

Schaal *et al.* [20] reported that investigations of population genetic diversity and the structure of populations within species might not only illustrate the evolutionary process and mechanism but also provide a useful information for biological conservation. In recent, various molecular markers, such as RFLP, RAPD, AFLP, SSR and ISSR, have provided an efficient tool for breeders who desire to obtain an accurate measurement for the genetic variation within or among species. Especially ISSR marker is based on PCR technique and has the simplicity like RAPD marker. ISSR markers involve the PCR amplification of DNA using single primers composed of microsatellite sequences. These primers target microsatellite regions that are abundant throughout the eukaryotic genome [14,22,23]. ISSR markers have been successfully employed to reveal genetic variation in several crops [1,12,13], to characterize genome diversity [13], and to construct genetic linkage maps [16].

The aims of this study are (1) to investigate the genetic variation within major cultivars and accessions and (2) to develop molecular markers for differentiating both cultivars, Chunpoong and Yunpoong, developed from Korea

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Ginseng & Tobacco Research Institute, from four accessions, Hwangsukjong and Jakyungjong collected from farmers fields, of Korean ginseng at the DNA level.

Materials and Methods

Materials and DNA extraction

Superior cultivars and accessions of Korean ginseng, Chunpoong, Yunpoong, Hwangsukjong and Jakyungjong, collected from various farmers fields including the Kumsan region in Korea, were used for ISSR analysis (Table 1). Samples (100 mg of fresh leaves and dried roots), which were mixed with each five individuals per accession, were frozen in liquid nitrogen and ground in a mortar to become a fine powder. DNA was extracted using QIAGEN DNeasy Plant Kit according to the manufacturer instructions [4,5].

ISSR analysis

56 ISSR primers supplied by BIONEER Technologies Inc. (Daejeon, Korea) were used for the analysis. The ISSR analysis was carried out using the following mixture: genomic DNA (1 ng/μl) 5 μl, primer (5 μM) 2 μl, dNTPs (250 μM total) 2 μl, *Taq*-polymerase (5U/μl) 0.2 μl, 10× buffer 2.5 μl, distilled water 13.3 μl, for a total of 25 μl reaction mixture. The *Taq*-polymerase and other reagents were purchased from BIONEER (Korea). Amplification reactions were carried out on the DNA Thermal Cycler (BIOMETRA) subjected to an initial two minutes at 94℃, followed by 35 cycles of 30 seconds at 94℃, 30 seconds at 50℃, 60 seconds at 72℃, and a final 5 minutes at 72℃.

The amplification products were separated by electrophoresis on 2.0% agarose gels, stained with ethidium bromide, and photographed under UV light using Alpha Image TM (Alpha Innotech Co., U.S.A).

Results and Discussion

ISSR marker was applied for the investigation of genetic diversity within Korean ginseng. A total of 56 ISSR primers were screened to select optimum primers both polymorphism and reproducible patterns. After primer screening, five primers (Table 2) which produced clear and reproducible fragments were selected for further analysis.

A total of 43 bands ranging from 250 bp to 1,700 bp were scored, corresponding to an average of 8.6 bands per primer, of which 20.9% (nine in total bands) were polymorphic across the six Korean ginseng. Chunpoong cultivar exhibited the highest level of polymorphism (seven in nine polymorphic bands), whereas other accessions almost no polymorphism among Korean ginseng accessions. Fig. 1 showed an example of the polymorphic bands amplified by primer ISSR-809, ISSR-845, ISSR-852, and ISSR-880 within cultivars and accessions of Korean ginseng. These ISSR

Table 1. Names and collective sites of Korean ginseng cultivars and accessions used for ISSR analysis

Entry No.	Cultivars or accessions	Collective sites	Characteristics
1	Chunpoong	Kumsan, Kyeonggi province	Red ginseng
2	Yunpoong	Ansung, Kyeonggi province	White ginseng and high yielding
3	Hwangsukjong	Ansung, Kyeonggi province	Yellow berry
4	Jakyungjong	Ansung, Kyeonggi province	Local accession
5	Jakyungjong	Kumsan, Chungnam province	Local accession
6	Jakyungjong	Punggi, Kyeongbuk province	Local accession
7	American ginseng	Imported from America	Disease resistance

Table 2. Selected primers sequence and the size of polymorphic bands generated by ISSR markers

Primers	Sequence [a]	Molecular size of polymorphic bands (kb)
ISSR-809	AGAGAGAGAGAGAGAGG	1.1 (CP[b])
ISSR-845	CTCTCTCTCTCTCTCTRG	1.2 (CP), 1.5 (CP)
ISSR-848	CACACACACACACACARG	0.7, 0.75 (JK[c]), 0.85 (CP)
ISSR-852	TCTCTCTCTCTCTCTCRA	1.3 (CP), 1.8 (CP)
ISSR-880	GGAGAGGAGAGGAGA	1.0 (CP)

[a]: R=A, G; Y=C, T.
[b]: Specific bands of Chunpoong (CP) cultivar.
[c]: Specific band of Jakyungjong (JK) accession.

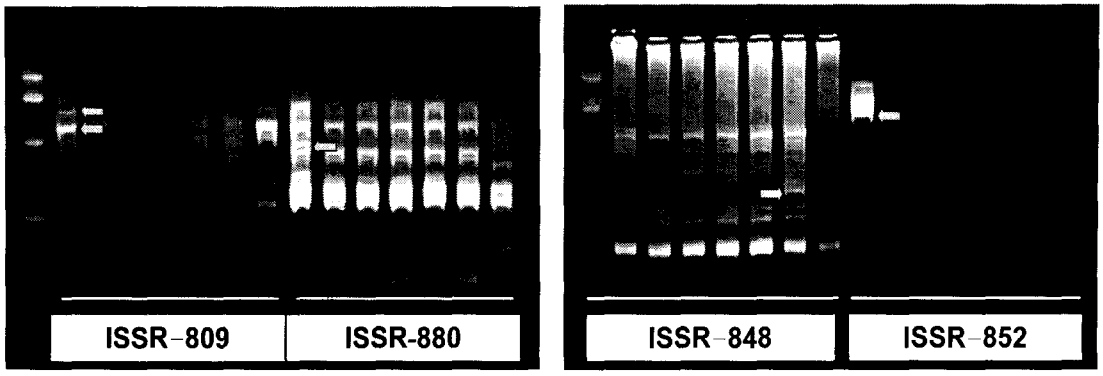

Fig. 1. ISSR profiles amplified from DNA of Korean ginseng cultivars and accessions using ISSR-809, ISSR-848, ISSR-852 and ISSR-880 primers. M, 100 bp marker. The order of lanes within each cultivar and accession is the same as that in Table 1. Arrows indicate specific DNA bands among cultivars and accessions mentioned in the text.

primers showed specific bands for Chunpoong accession, which could be useful for discriminating from other ginseng accessions. The molecular weights of specific bands were 1.1 kb, 1.2 kb, 1.3 kb, and 1.0 kb, respectively. However, the result generated by ISSR-848 no band near 0.8 kb in Chunpoong and showed Jakyungjong specific band at 0.7 kb.

In order to develop convenient and reproducible methods for identification and classification of *Panax* species at the DNA level, various molecular techniques have been applied for those molecular biological researches. Fushimi *et al.* [9] demonstrated that PCR-Restriction fragment length polymorphisms (PCR-RFLP) and Mutant allele specific amplification (MASA) analysis were convenient for identifying three Ginseng drugs. Using conserved plant sequences as primers, the DNA sequences in the ribosomal ITS1-5.8S-ITS2 region were amplified and determined for six *Panax* species, *P. ginseng* C. A. Meyer, *P. quinquefolius* L., *P. notoginseng* F. H. Chen, *P. japonicus* C. A. Meyer, *P. trifolius* L. [18]. Ho and Leung [11] reported that several novel repetitive DNA sequences from *P. ginseng* were used to differentiate *P. ginseng* from *P. quinquefolius*, and should be useful for evolutionary studies of these distinct species. Shaw and But [21] demonstrated that PCR approach may be used as a means to authenticate the concerned *Panax* species and analysis of the degree of similarity of the fingerprints confirmed that *P. ginseng* is more closely related to *P. quinquefolius* than to *P. notoginseng*. The method of direct amplification of length polymorphism (DALP) was applied to authenticate *P. ginseng* and *P. quinquefolius* [10]. However, genetical and molecular biological researches for identification of cultivars and accessions within *P. ginseng* have been very rare.

To identify the variation of the RAPD patterns for *P. ginseng*, 20 different random primers were applied to cultivars and accessions of *P. ginseng* genomic DNA. However, it did not detect polymorphic bands (data not shown), implying that RAPD analysis did not provide an excellent information for detection of genetic variation within Korean ginseng subspecies. This result was in accordance with the data on RAPD variation in *P. ginseng* [15,24].

Both cultivars, Chunpoong and Yunpoong were developed through comparative cultivation of several lines selected by pure line separation from local races at Korea Ginseng & Tobacco Research Institute in 1999. However, molecular markers, which are available for cultivar identification of Korean ginseng, have not been developed.

Therefore, ISSR markers derived from this study will be used for available tool for the identification of Chunpoong from other major Korean ginseng cultivars and accessions, Yunpoong, Hwangsukjong and Jakyungjong, at the DNA level.

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초록 : ISSRs 마크에 의한 고려 인삼의 분자적 인증과 유전적 다형현상

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ISSR마크를 사용하여 고려 인삼의 품종 및 계통간 분자적 인증과 유전적 다형현상을 조사하였다. 56개의 ISSR 프라이머중 5개가 일곱 품종 및 계통간 명확하고 재현성이 높은 DNA 분절을 나타내는 최적 프라이머로 선택되었다. 전체 43 밴드는 250 bp - 1,700 bp의 분자량을 가지며 프라이머당 8.6개의 밴드를 나타내었다. 고려 인삼에서 다형현상 정도는 20.9%였다. 특히 천풍 품종이 가장 높은 다형현상을 나타낸 반면 다른 품종은 거의 다형현상을 나타내지 않았다. 결론적으로 DNA 수준에서 ISSR마크로 천풍이 다른 고려 인삼의 품종 및 계통인 연풍, 황숙종, 자경종과 구분에 이용될 수 있음이 판명되었다.