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Molecular authentication of *Panax ginseng* and ginseng products using robust SNP markers in ribosomal external transcribed spacer region

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ABSTRACT

Panax ginseng and *Panax quinquefolius* are the most widely used *Panax* species, but they are known to have different properties and medicinal values. The aim of this study is to develop a robust and accurate DNA marker for identifying *P. ginseng* and the origins of ginseng products. Two single nucleotide polymorphism (SNP) sites specific to *P. ginseng* were exploited from nuclear ribosomal external transcribed spacer (ETS) region. Based on the SNP sites, two specific primers were designed for *P. ginseng* and *P. quinquefolius* respectively. *P. ginseng* can be easily discriminated from *P. quinquefolius* by amplifying the two specific alleles using multiplex allele-specific PCR. Favorable results can also be obtained from commercial ginseng products. The established method is highly sensitive and can detect 1% of intentional adulteration of *P. quinquefolius* into *P. ginseng* down to the 0.1 ng level of total DNA. Therefore this study provides a reliable and simple DNA method for authentication of the origins and purities of ginseng products.

1. Introduction

Panax ginseng (Korean ginseng) and *Panax quinquefolius* (American ginseng) are the most widely used *Panax* species, and the products of these two ginsengs have attracted worldwide consumption. Ginsenosides are the major bioactive constituents in both species, but they are known to have different properties and medicinal values [1,2]. *P. ginseng* is considered to be "warm" and used in "yang-deficient" conditions, whereas *P. quinquefolius* is "cool" and is mainly used in "yin-deficient" conditions [3]. However, American ginseng is sometimes adulterated in commercial Korean ginseng products as material by some dishonest merchants to reduce production costs, as American ginseng has much higher total ginsenosides content [4]. Therefore, it is essential to develop effective methods to authenticate the origin of commercial ginseng products to safeguard public health as well as consumers' rights.

Although several efforts have been made for discrimination of Korean ginseng from American ginseng, authentication of the origin of *P. ginseng* products is not an easy task. First, the sterilization and extraction treatments in processing red ginseng extracts will result in a high degree of DNA degradation, therefore DNA molecular markers such as RAPD (random amplified polymorphic DNA), ISSR (inter-simple sequence repeat), SSR (simple sequence repeat), and AFLP (amplified fragment length polymorphisms) may get unfavorable identification results. Second, with the development of new *P. ginseng* cultivars [5], the current DNA molecular markers cannot identify the ginseng origins accurately. The chemical analysis method [6,7] encounters the same problem because ginsenoside profile used for authentication markers may be affected by commercial processing. In the present study, we describe a robust and reliable method for authentication of the origin of *P. ginseng* products, by using two SNP markers exploited from the external transcribed spacer (ETS) region of ribosomal DNA.

2. Materials and methods

2.1. Materials and DNA isolation

Samples of ginseng plants list in Table 1 were provided by *Korean Ginseng Center for Most Valuable Products & Ginseng Genetic Resource Bank*. The plant roots were frozen in liquid nitrogen and ground into fine powders. Genomic DNA was isolated using a plant DNA isolation kit (Exgene Plant SV mini, GeneAll), according to manufacturer's instructions. Commercial products of Korean ginseng and American ginseng were purchased from local market and USA, respectively. Ginseng commercial samples were used directly in DNA isolation procedure.

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Table 1
Plant samples used in this study.

Ginseng samples	Voucher	Localities
Chunpoong[a]	GB001	Kochang, Korea
Yunpoong[a]	GB002	Kochang, Korea
Gopoong[a]	GB003	Kochang, Korea
Sunpoong[a]	GB004	Kochang, Korea
Gumpoong[a]	GB005	Kochang, Korea
Sunwon[a]	GBD048	Daejeon, Korea
Chungsun[a]	GBD073	Daejeon, Korea
Sunweon[a]	GBD043	Daejeon, Korea
Sunhyang[a]	GBD058	Daejeon, Korea
Damaya[b]	GB090	Jilin, China
Ermaya[b]	GB091	Jilin, China
Biantiao[b]	GB092	Jilin, China
P. quinquefolius	GB099	Wisconsin, USA
P. quinquefolius	GB100	Minnesota, USA
P. quinquefolius	GB101	Iowa, USA
P. quinquefolius	GB102	Shandong, China
P. quinquefolius	GB103	Daejeon, Korea
P. notoginseng	GB031	Yunnan, China
P. notoginseng	GB032	Yunnan, China
P. notoginseng	GB033	Yunnan, China
P. notoginseng	GB034	Yunnan, China

[a] Cultivars of Korean *P. ginseng*.
[b] Landraces of Chinese *P. ginseng*.

2.2. PCR amplification of ETS region

PCR amplification of ribosomal ETS region was performed on ginseng plant DNA samples. Oligonucleotide primers ETSF (5′-TTTGCAAGTCGTGTGAGTTG-3′) and ETSR (5′-AGACAAGCATATGACTACTGGCAGG-3′) specific for the ribosomal ETS region were designed according to the 26S–18S ribosomal DNA intergenic spacer region of *P. ginseng* (GenBank accession EU232126). The 20 μl PCR reaction mixture consist of 10 ng of template DNA, 0.5 μM of each primer, and 10 μl of 2X Premix DNA polymerase (Genotech). PCR amplification was performed using 1 predenaturation cycle of 4 min at 94 °C, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were analyzed on a 1.0% agarose gel stained with ethidium bromide.

Table 2
Oligonucleotide sequences of primers used in this study.

Primer name	Nucleotide sequence (5′ → 3′)
ETSF	TTTGCAAGTCGTGTGAGTTG
ETSR	AGACAAGCATATGACTACTGGCAGG
AgF	GTGTTGGCATAGTGTACGT**T**A (A → T)
PgF	AGAGCAGTAAGCCTTGGAA**A**AT (C → A)

2.3. DNA sequencing and analysis

The PCR products were purified with a PCR DNA Purification Kit (GeneAll), as described in the manufacturer's instructions. DNA was sequenced in both directions on an automatic DNA sequencer (ABI PRISM 3700, USA), by using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). DNA sequences were assembled using SeqMan software, Multiple sequence alignments were conducted using the Clustal W 2.0 program [8].

2.4. Design of allele-specific primers

Two primers AgF and PgF, specific to American ginseng and Korean ginseng respectively, were designed based on the SNP sites detected in ETS region. The substitutions of T for A in primer AgF and of A for C in primer PgF were additional mismatches introduced deliberately (Table 2), to ensure required allelic specificity [9,10]. Primer ETSR was used as the corresponding reverse primer of AgF and PgF. The orientations of these three primers are shown in Fig. 1.

2.5. Multiplex allele-specific PCR

Multiplex allele-specific PCR was used for authentication of *P. ginseng* and *ginseng* products. For authentication of ginseng plants, the 20 μl PCR reaction mixture consist of 10 ng of template DNA, 0.5 μM of ETSR, 0.5 μM of PgF, 0.15 μM of AgF, and 10 μl of 2X Premix DNA polymerase (Genotech). PCR amplification was performed using 1 predenaturation cycle of 4 min at 94 °C, 35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 for 30 s, and a final extension at 72 °C for 5 min. The annealing temperature range from 65 °C to 68 °C was tested in the experiment to check the robustness of this method.

For authentication of the mixture of *P. ginseng and P. quinquefolius*, the total amount of mixed template DNA in the 20 μl PCR reaction mixture was set to 0.1 ng. Seven mixed DNA samples con-

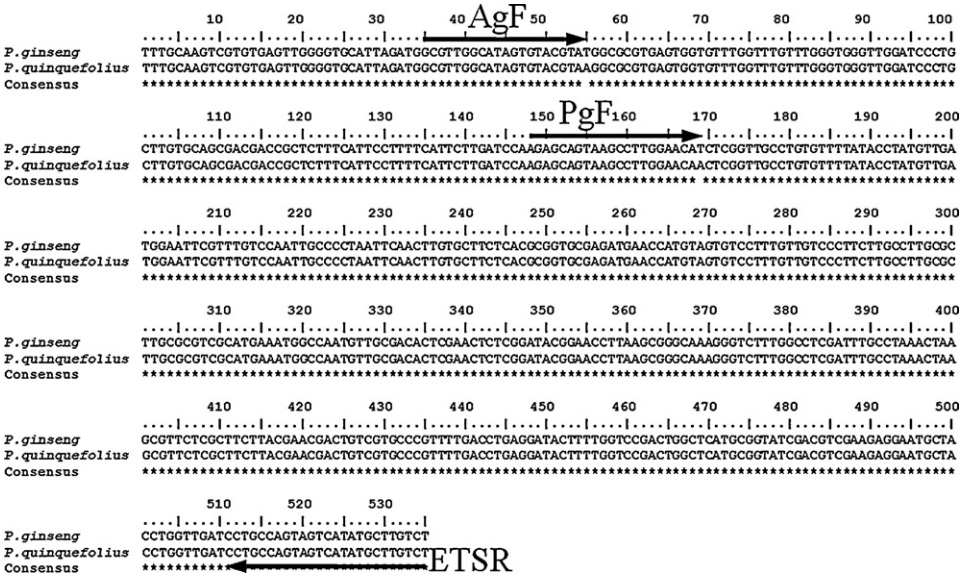

Fig. 1. Comparison of the ETS sequences of *P. ginseng* and *P. quinquefolius*.