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Comparison of ITS (Internal Transcribed Spacer) and 5.8S rDNA Sequences among Varieties and Cultivars in *Panax ginseng*

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Ginseng (*Panax* genus) is one of the most medicinally important genera and consists of highly regarded medicines. Among the species of *Panax*, the *ginseng* species is widely known to have most medicinal quality. *P. ginseng* has 3 varieties, Jakyung, Chunggyung and Hwangsook, discovered in nature with different colors of stem and fruit. Jakyung has two cultivars, Yunpoong and Chunpoong. Rigorous phylogenetic analysis of these varieties and cultivars has been conducted with sequencing of rDNA region. The sequences of ITS1, ITS2 of every varieties and cultivars within *P. ginseng* were identical. The sequence of 5.8S rDNA of Hwangsook variety were different from the sequences of 5.8S rDNAs of others by only one base pair at nucleotide position 14. In phylogenetic analysis and predicted RNA secondary structure study, it is assumed that evolution has proceeded from Hwangsook to other varieties recently.

key words: *Panax ginseng*, variety, cultivar, ITS, 5.8S

INTRODUCTION

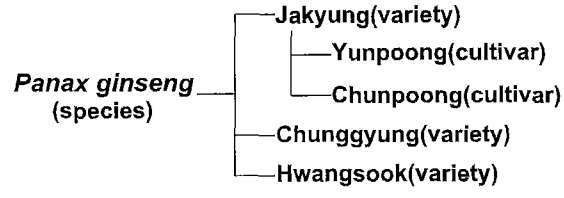
Ginseng (*Panax* genus) is one of the most medicinally important genera in the Orient, where almost every species of the genus has been used as a source of medicine.

Ginseng literally means the essence of man [20] and is known as the lord or king of herbs. China and Korea have used it for over 2000 years as a tonic, a stimulant, and a fatigue-resistance medicine [3].

Ginseng has many properties such as it enhances natural killer cell activity and antibody-dependent cell cytotoxicity in chronic fatigue syndrome patients [4], reduces lungs pathology [5], improves learning performance in mouse [6], potentiates vaccination against common cold and/or influenza [7], inhibits the development of reverse tolerance to the ambulatory-accelerating effect of morphine [8], prevents oxygen free radical injury [9], has anti-stress effect [10], inhibits mutagenesis [11], potentiates growth factor-mediated nerve fiber production [12], and has anti-ageing properties [13].

Ginseng genus (*Panax*) consists of 13 species in which eleven from eastern Asia and two from eastern North America. The phylogenetic studies of the thirteen species were done [1]. Three main species were cultivated widely due to its high medicinal quality. They are *P. ginseng* (ginseng), *P. quinquifolium* (American ginseng) and *P. notoginseng* (Sanchi) (10). Among the species within *Panax*, the *ginseng* species is widely known for having more medicinal quality (more ginsenosides) than any other species. So *Panax* is commonly referred as the ginseng genus [3].

P. ginseng species has 3 varieties, Jakyung, Chunggyung and Hwangsook. These varieties are discovered in nature with different colors of stem and fruit (Figure 1). And Jakyung has two cultivars, Yunpoong and Chunpoong. Yunpoong has thick root so is available for mass production of ginseng, whereas Chunpoong has long stem so makes the best quality commercial product. These 3 varieties and 2 cultivars are



(A)

	Color of Stem	Color of fruit	Number of stem
Jakyung	Violet	Red	1
Yunpoong	Violet	Red	a few
Chunpoong	Pale violet	Orange	1
Chungkyung	Green	Red	1
Hwangsook	Green	Yellow	1

(B)

Figure 1. Characterization of the varieties and cultivars of *P. ginseng*. (A) Tree-like description of varieties and cultivars of *P. ginseng*, (B) Differences of varieties and cultivars of *P. ginseng*.

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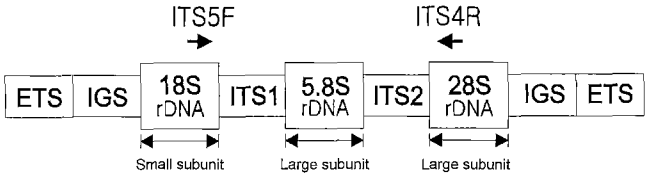

Figure 2. A map of Nuclear rDNA region and the position of primer sets used for PCR. Internal transcribed spacer (ITS), External transcribed spacer (ETS), Intergenic spacer (IGS).

commonly cultivated for medicine in Korea. But no rigorous or explicit phylogenetic analyses of these varieties and cultivars have been conducted.

In eucaryotes, the ribosomal RNA coding gene (rDNA) found in the nuclear genome generally consists of tandem repeats of three RNA genes that are transcribed as a single unit and code for the 18S, 5.8S and 28S RNA (Figure 2). The 18S and 28S rRNA genes are also known as nuclear small and large subunit rDNA sequences, respectively. Internal transcribe spacers (ITS) flank the 5.8S rDNA region [14]. The ITS regions are co-transcribed with the genes for 18S, 5.8S, 28S rRNAs, but not translated.

The coding rDNA sequences evolve relatively slowly so are useful for studying distantly related organisms, whereas the non-coding rDNA (ITS) sequences evolve faster than the coding sequences so may vary among species and populations [15-19]. So the ITS region is a suitable target to investigate the plant phylogenetic relationships of same species because of the rapid evolution. Jorgensen and Cluster [20] pointed out that the sequences of 5.8S subunit are conserved at the same level as other coding regions while the sequences of ITS are much more variable.

Recently the phylogenetic relationship of *Panax* genus was studied but intra-specific phylogenetic study of *P. ginseng* was not. In this study the ITS regions of 3 varieties and 2 cultivars of *P. ginseng* were sequenced and their intra- and inter-specific phylogenetic relationship were studied.

MATERIALS AND METHODS

PCR-amplification

The shoots of 3 varieties and 2 cultivars of *P. ginseng* were collected and ground in liquid nitrogen. The DNA's from the ground samples were isolated and purified with Plant DNA isolation kit (NucleoSpin Plant, Macherey-Nagel). The oligonucleotide primers used for amplification of ITS region were synthesized by Bioneer, Inc., Chungwon, Republic of Korea. Primers annealing at the 5' and 3' ends of the ITS region were ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4R (5'-TCCTCCGCTTAT-TGATATGC-3') respectively. PCR amplification was performed in a final reaction volume of 100 μl, and the reaction mixture contained each primer at a concentration of 1.5 μM, 50 ng isolated DNA, each deoxynucleoside triphosphate at a concentration of 200 μM, 1.5 mM $MgCl_2$, 10x reaction buffer (Biogen., Taejon, Republic of Korea) and 1 Unit of *Taq* DNA polymerase (Biogen., Taejon, Republic of Korea). Each reaction mixture was overlaid with mineral oil, and the PCR was run for 36 cycles with a DNA Thermal Cycler (Perkin Elmer Co.) The following thermal profile was used for the PCR: denaturation at 96°C for 30 sec, primer annealing at 55°C for 30 sec, and extension at 72°C for 1 min. The final cycle included extension for 10 min at 72°C to ensure full extension of the products. The PCR products were analyzed by electrophoresis of a 5 μl aliqot through a 1.0% (wt/vol) agarose gel (Agarose, CALEDON) , stained with ethidium bromide, and visualized by UV transillumination. The DNA's from all the samples were PCR-amplified and purified with PCR-product purification kit (Suprec-02, Dakara).

Phylogenetic analysis and RNA secondary structure Analysis

Purified PCR-amplified DNAs were used as templates for sequencing. Sequencing was done with Perkin Elmer ABI377 autosequencer and the same primers for PCR-amplification were used. The DNA sequence comparison was done with ClustalX and phylogenetic analysis was done with ClustalX and treeview. The RNA secondary structures of rDNA region of *P. ginseng* were predicted with RNAdraw.

RESULTS

Characterization of ITS Sequences of P. ginseng

Total 619 base pairs of rDNA region were PCR-amplified and sequenced (Figure 3). The lengths of sequences of ITS 1, 5.8S rRNA, ITS 2 were 221, 164, 234 base pairs. These

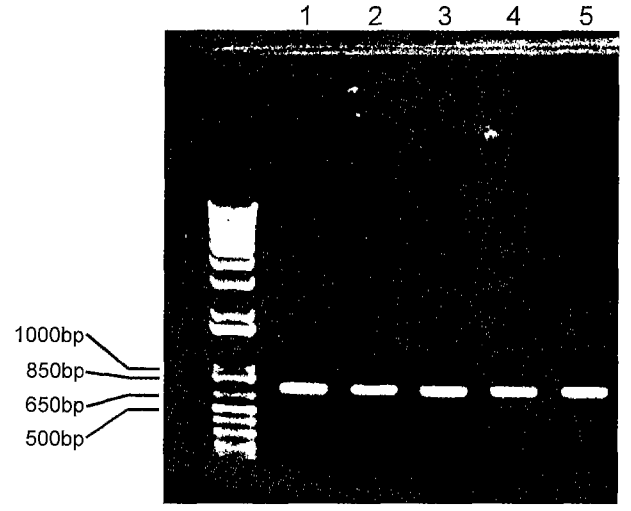

Figure 3. PCR products of ITS regions of *Panax ginseng*. Lane 1: *Panax ginseng* Jakyung variety; Lane 2: *Panax ginseng* Yunpoong cultivar; Lane 3: *Panax ginseng* Chunpoong cultivar; Lane 4: *Panax ginseng* Chunggyung variety; Lane 5: *Panax ginseng* Hwangsook variety.

	Sequences of 5.8S rDNA	GenBank Accession No.
Jakyung (variety)	1CAAACGACTCTCGACAACGG20	In this paper
Yunpoong (cultivar)	CAAACGACTCTCGACAACGG	In this paper
Chunpoong(cultivar)	CAAACGACTCTCGACAACGG	In this paper
Chungkyung (variety)	CAAACGACTCTCGACAACGG	In this paper
Hwangsook (variety)	CAAACGACTCTCGGCAACGG	In this paper
P. bipinnatifidus	CAAACGACTCTCGGCAACGG	U41679
P. japonicus	CAAACGACTCTCGGCAACGG	U41702
P. major	CAAACGACTCTCGGCAACGG	U41683
P. notoginseng	CAAACGACTCTCGGCAACGG	U41685
P. omeiensis	CAAACGACTCTCGGCAACGG	U41692
P. pseudoginseng	CAAACGACTCTCGACAACGG	U41694
P. quinqufolius	CAAACGACTCTCGGCAACGG	U41688
P. sinensis	CAAACGACTCTCGGCAACGG	U41703
P. stipuleanatus	CAAACGACTCTCGGGAACGG	U41696
P. triolius	CAAACGACTCTCGGCAACGG	U41698
P. wangianus	CAAACGACTCTCGGCAACGG	U41691
P. zingiberensis	CAAACGACTCTCGGCAACGG	U41700

Figure 4. Comparison of the sequences of 5.8S rDNA of 3 varieties and 2 cultivars. Different base substitutions were observed at nucleotide position 14.

results coincided with those of Wen and Zimmer [1].

The sequences of ITS1, ITS2 of each varieties and cultivars within *P. ginseng* was identical. The sequence of 5.8S rDNA of Hwangsook variety was different from the sequences of 5.8S rDNAs of others within *P. ginseng* by only one base pair at nucleotide position 14 (Figure 4), which was confirmed by silver sequencing (Figure 5).

Phylogenetic Analysis

Phylogenetic tree was drawn with ClustalX and Treeview (Figure 6). This figure shows that Hwangsook variety has the intermediate position between other *P. ginseng* species and other different species.

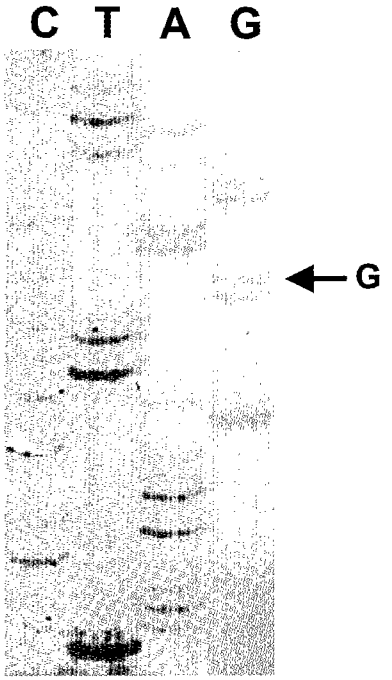

Figure 5. The photograph of a sequencing gel about substitution site in Hwangsook variety.

RNA secondary structure prediction

The RNA secondary structures of *P. ginseng* were predicted with RNA draw (Figure 7). The putative ITS 1, 5.8S rRNA, ITS 2 RNA secondary structures of *P. ginseng* was folded into a compact formation. The RNA secondary structure of 5.8S rRNA of Hwangsook variety was somewhat different from those of

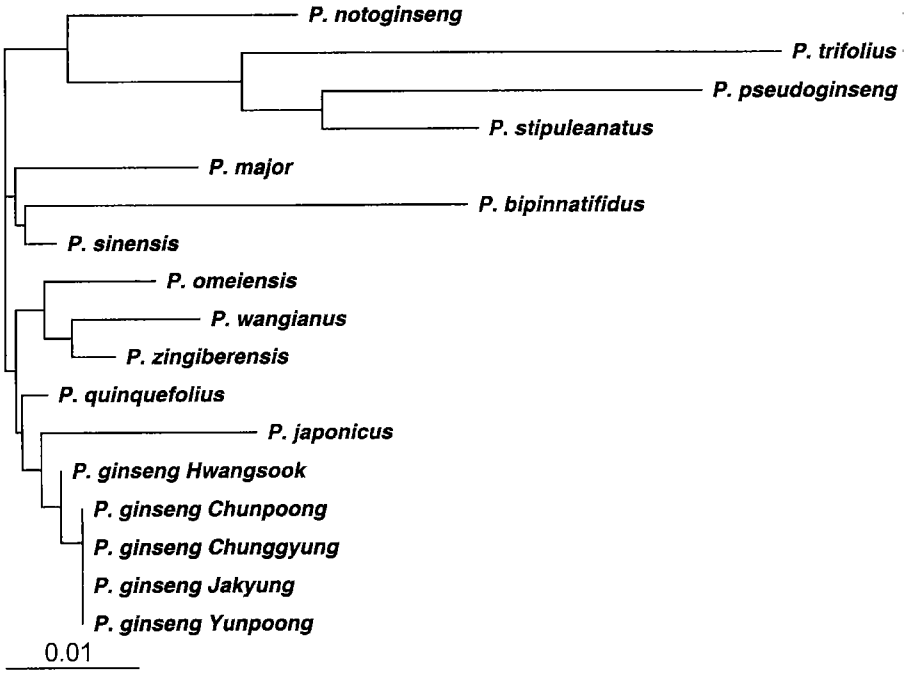

Figure 6. The phylogenetic tree of the varieties and cultivars within *Panax* species.

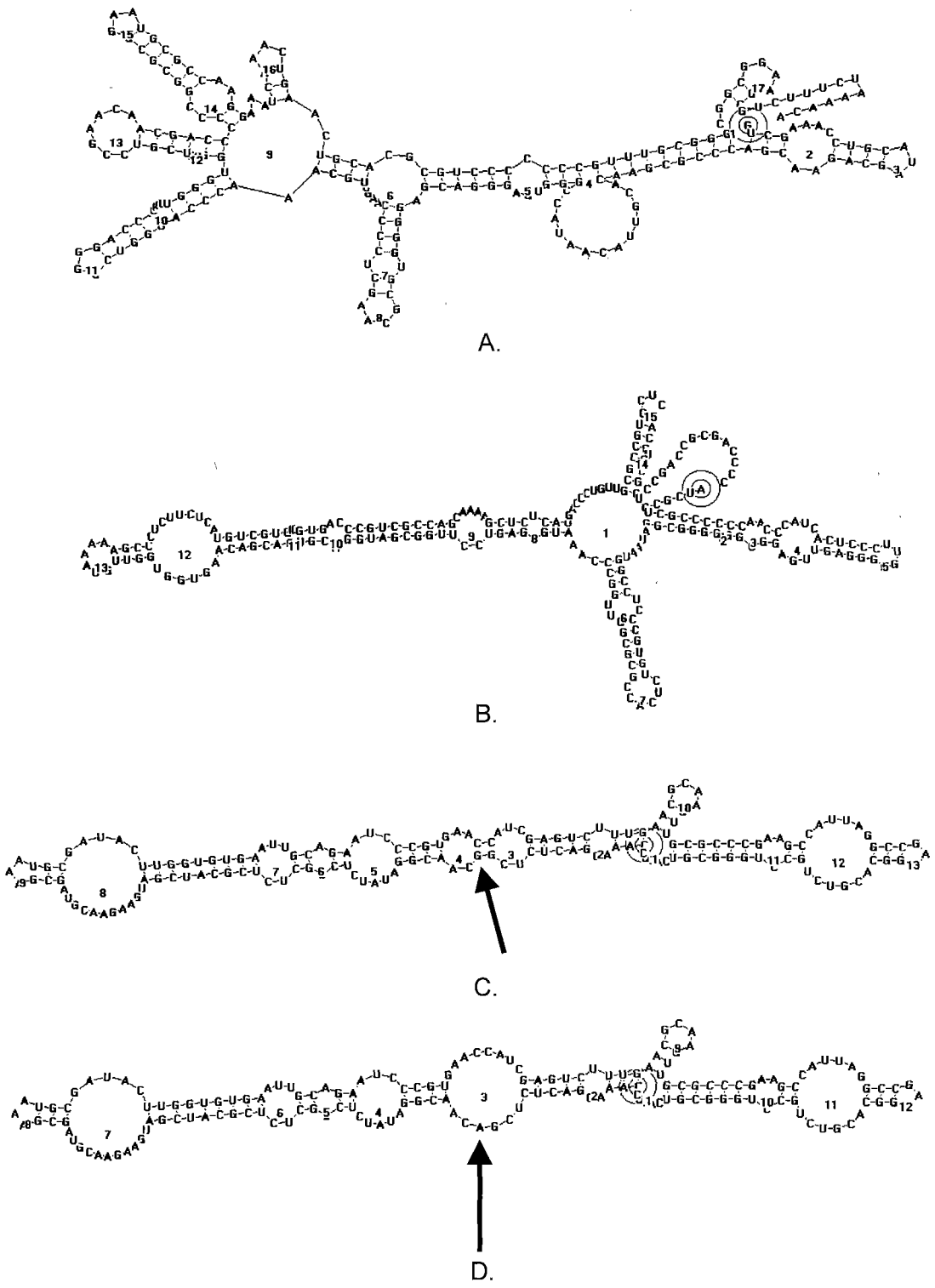

Figure 7. Predicted RNA secondary structures of *P. ginseng*. A. RNA of ITS 1 of *P. ginseng*; B. RNA of ITS 2 of *P. ginseng*; C. 5.8S rRNA of *P. ginseng* Hwangwook variety; D. 5.8S rRNA of *P. ginseng* except Hwangwook variety.

others within *P. ginseng*. The substituted base G of Hwangsook variety could make base pair so made stem between loop 3 and 4, but differentiate base A of other *P. ginseng* species could not make base pair so formed a loop (loop 3).

Discussion

Characterization of ITS Sequences of P. ginseng

In fact, this one base-substitued site is specific for *P. ginseng*

except Hwangsook. If the specific primer is synthesized for this site, the *P. ginseng* except Hwangsook can be discriminated from other *Panax* species. This technique is needed for discrimination of ginseng powder in commercial purposes. But *P. pseudoginseng* has the same base like the *P. ginseng* species.

There is another site, which is specific for the *P. ginseng*. At nucleotide position 170 of ITS 2, the specific base for the *P. ginseng* T. Other *Panax* species have C or A. At nucleotide position 116 of ITS 1, the *P. ginseng* has the base A and other *Panax* species have C or T. Only *P. Japonicus* has a base A just like the *P. ginseng*.

Phylogenetic analysis

In phenotypic characteristics, Hwangsook is the most different from other *P. ginseng* species, and this phenotypic characteristic is congruent with the result of rDNA sequencing. Based on chromosome numbers and geographical distribution Yang [21] argued that *P. ginseng*, *P. japonicus*, *P. quinquefolius* and *P. wangianus* constitute the phylogenetically more derived group. Based on phylogenetic tree Hwangsook variety is assumed to be more primitive than others within *P. ginseng* and the *P. ginseng* is assumed to evolve and diverged recently.

RNA secondary structure prediction

Mutation analyses in *Saccharomyces cerevisiae* have shown that some sequence regions in ITS 2 are necessary for processing of the 45S rRNA precursor [22]. Although the *S. cerevisiae* ITS1 structure is not as tightly folded as is ITS2, some domains also appear to play a major role in the production of mature 17S rRNA [23].

If any ITS region is very conservative, this may mean that these regions are under evolutionary pressure to maintain the RNA secondary structure involved in the post-transcriptional processing of rRNA. The ITS region of the varieties and cultivars within *P. ginseng* is very conservative and their RNA secondary structures are folded tightly, so this region may be under evolutionary pressure. When evolution proceeds from Hwangsook to other varieties, the base G changed to base A and the stem changed to loop. Because this loop is not terminal loop they may escape the evolutionary pressure.

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