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RESEARCH ARTICLE

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Identification and Analysis of Differentially Expressed Genes in Mountain Cultivated Ginseng and Mountain Wild Ginseng

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Abstract
Ginseng is one of th[illegible]ost widely [illegible]bal medicines in the world. Wild ginseng is thought to be m[illegible]ive than cultivated ginseng in chemoprevention; however, little has bee[illegible]sport[illegible]e differences between wild and cultivated ginseng. In the present [illegible]dy[illegible] used suppressive subtractive hybridization to identify wild gin[illegible]ecific g[illegible]s. One of the clones isolated in this screen was the *NRT2* gene[illegible]sign[illegible]d *pNR*[illegible] a high-affinity nitrate transporter. Real-time reverse tra[illegible]iption[illegible]olymerase[illegible]hain reaction results showed that *pNRT2* expression was [illegible]ign[illegible]t[illegible]p[illegible]d in wild ginseng compared with cultivated ginseng. However, *pNRT2* [illegible]A levels were similar between mountain cultivated ginseng and mountain wild ginse[illegible] Nitrate is an important nitrogen source for plant growth, and its soil [illegible]vels can va[illegible]in wild environments; thus it is conceivable that *pNRT2* expression [illegible]regulated in wild ginseng and may be an important marker of wild ginseng.

1. Introduc[illegible]n

Ginsen[illegible]of th[illegible]ost[illegible]idely used herbal medicine[illegible]n the[illegible]orld. It[illegible]enefits to general health inc[illegible]e po[illegible]ffects on the endocrine, cardiovascu[illegible]mune, a[illegible]d central nervous systems, and preventi[illegible]f fatigue, oxidative damage, mutagenicity, and ca[illegible] [1–4]. Most of the pharmacological actions of ginseng are attributed to ginsenosides, which are triterpenoid saponins [5,6]. The physiological benefits and medicinal effects of the various ginsenosides differ and can even be oppositional [7]. Cultivated ginseng currently accounts for most of the ginseng on the market.

Mountain wild ginseng grows in natural environments in the mountains, and mountain cultivated ginseng, which is grown in forests and mountains, can be considered to mimic mountain wild ginseng. Wild mountain ginseng is considered superior to regular cultivated ginseng and contains higher levels of certain ginsenosides [8], although minimal differences in total ginsenoside content between wild and cultivated ginsengs have been reported by some studies. Ginsenoside levels are consistently lower for the more intensively cultivated crops, but growth was consistently higher [9]. In both Korea and China, wild ginseng is widely assumed to be more effective than cultivated ginseng in chemoprevention.

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However, little has been reported on the differences between wild ginseng and cultivated ginseng.

Nitrate is an important plant nutrient and acts as a signal for plant growth; however, its soil levels can vary by three to four orders of magnitude [10]. Nitrate is the primary nitrogen source available in soil, and can act directly to regulate both shoot-root allocation and modify the root system architecture [11]. Consequently, plants have evolved regulated, energy-dependent systems for nitrogen uptake using both high-affinity and low-affinity transporters [12]. Genes that encode representatives of each type of transporter have been identified and fall into two families: NRT1 (nitrate transporter 1) and NRT2 (nitrate transporter 2) [13]. These genes are induced in response to nitrate in the environment and are regulated by internal signals including nitrogen metabolites and shoot demand for nitrogen. The evidence to date indicates that NRT2 transporters contribute specifically to the nitrate-inducible, high-affinity nitrate uptake system, whereas NRT1 transporters contribute more broadly to nitrogen uptake and show both inducible and constitutive expression [14].

In this study, we identified and analyzed genes that are differentially expressed between regular cultivated ginseng and mountain cultivated and wild ginseng. Three mountain cultivated ginseng-specific genes were cloned, and their expression was analyzed by real-time reverse-transcription polymerase chain reaction (RT-PCR).

2. Materials and Methods

2.1. Ginseng specimens used for analysis

The cultivated ginseng (CG) roots used in this experiment were 6 years old (Figure 1A). Mountain cultivated ginseng (MCG) roots were 10 years old (seeded in 1999) and the plants were grown at ChonBangNong in Chochiwon, Korea (Figure 1B). The wild-simulated ginseng (MWG) roots were collected on Chilbo Mountain in July 2007. They were approximately 20–40 cm long, 10–20 g (dried weight), and about 20–50 years old (Figure 1C).

2.2. RNA isolation and purification

The ginseng was ground in liquid nitrogen using a mortar and pestle, and RNA was isolated using an RNeasy Plant RNA Isolation kit (Qiagen GmbH, Hilden, Germany). RNA concentration was estimated by measuring its absorbance at 260 nm. The RNA was treated with DNase I (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions before cDNA synthesis with SuperScript III reverse transcriptase (Invitrogen) and random hexamers.

2.3. Suppressive subtractive hybridization

Suppressive subtractive hybridization (SSH) was performed to isolate differentially expressed genes using the PCR-Select cDNA Subtraction Kit (Clontech Laboratories, San Francisco, CA, USA) according to the manufacturer's protocol. SSH consists of six steps: cDNA synthesis, restriction enzyme digestion, adaptor ligation, two rounds of hybridization, and PCR. The cDNA fragments, derived from the SSH forward subtractive library (tester: mountain cultivated ginseng; driver: cultivated ginseng), were cloned into the pEC-T vector (KOMA Co., Seoul, Korea). Clones containing inserted fragments were identified using the colony PCR method (Table 1).

2.4. Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed to determine the differential expression of genes in the SSH library; β-actin was used as a control. The gene-specific primers for RT-PCR are listed in Table 1. Total RNA (2 μg) was reverse transcribed with the First Strand cDNA Synthesis Kit (Invitrogen), and 1.0 μL cDNA was used as a template for PCR. PCR amplification was performed under the following conditions: 95°C for 5 minutes, followed by 30 cycles of 95°C for 45 seconds, 54°C for 30 seconds,

Figure 1 (A) Cultivated ginseng, (B) mountain cultivated ginseng, and (C) mountain wild ginseng were analyzed for differential gene expression.