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Research article

Ginsenoside profiles and related gene expression during foliation in *Panax ginseng* Meyer

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ABSTRACT

Panax ginseng is one of the most important medicinal plants in Asia. Triterpene saponins, known as ginsenosides, are the major pharmacological compounds in *P. ginseng*. The present study was conducted to evaluate the changes in ginsenoside composition according to the foliation stage of *P. ginseng* cultured in a hydroponic system. Among the three tested growth stages (closed, intermediate, and opened), the highest amount of total ginsenoside in the main and fine roots was in the intermediate stage. In the leaves, the highest amount of total ginsenoside was in the opened stage. The total ginsenoside content of the ginseng leaf was markedly increased in the transition from the closed to intermediate stage, and increased more slowly from the intermediate to opened leaf stage, suggesting active biosynthesis of ginsenosides in the leaf. Conversely, the total ginsenoside content of the main and fine roots decreased from the intermediate to opened leaf stage. This suggests movement of ginsenosides during foliation from the root to the leaf, or vice versa. The difference in the composition of ginsenosides between the leaf and root in each stage of foliation suggests that the ginsenoside profile is affected by foliation stage, and this profile differs in each organ of the plant. These results suggest that protopanaxadiol- and protopanaxatriol (PPT)-type ginsenosides are produced according to growth stage to meet different needs in the growth and defense of ginseng. The higher content of PPT-type ginsenosides in leaves could be related to the positive correlation between light and PPT-type ginsenosides.

1. Introduction

Panax ginseng Meyer is a slowly growing perennial herb belonging to the Araliaceae family. It has been cultivated for its highly valued roots and used in traditional medicine as a natural adaptogen for >1000 yr [1]. Ginseng has numerous pharmacological effects on humans, including anticancer [2–4], antidiabetic [5,6], immunomodulatory [2,7], neuroprotective [2], radioprotective [8], antiamnestic [2], and antistress [9] properties. Most of the medicinal effects of ginseng have been attributed to triterpene saponins, which are referred to as ginsenosides. More than 40 ginsenosides have been isolated and identified from white and red ginseng, showing different biological activities based on their structural differences [10–15]. Two types constitute >80% of the identified ginsenosides: protopanaxadiol (PPD)-type saponins (sugar moieties are attached to the β-OH at C-3 and/or C-20) such as ginsenosides Rb1, Rb2, Rc, and Rd, and protopanaxatriol (PPT)-type saponins (sugar moieties are attached to the α-OH at C-6 and/or β-OH at C-20) such as ginsenosides Re, Rg1, and Rf [16].

The cultivation of *P. ginseng* is difficult due to the long duration (4–6 yr) needed for cultivation, and due to plant diseases such as red skin and root rot. Furthermore, ginseng needs to be cultivated under special conditions to meet its requirements of about 30% full sunlight. High exposure to light (50% solar radiation) decreases the levels of ginsenosides in *Panax pseudoginseng* [17], while exposure to >36% sunlight has been reported to cause photobleaching and leaf death in *P. ginseng* plants [18]. Although there have been many studies on the production of ginsenoside using tissue and cell cultures, the productivity has been low. To meet the demand for safe agricultural products of high quality, the cultivation of ginseng by hydroponics was developed in Korea [19,20]. This technique involves a shorter period by cultivation in a greenhouse in which variables such as light, temperature, moisture, and carbon dioxide content can be controlled. Hydroponic systems can produce

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ginseng roots that are pesticide free and ginseng leaves with high ginsenoside contents [19,20].

Ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry. Different parts of the plant contain distinct ginsenoside profiles [2], which may exhibit different pharmacological activities. Although the *P. ginseng* root has been the main component in medicinal uses of ginseng, recent studies have revealed that the leaf and root hair contain higher ginsenoside levels than the root [21]. Ginseng berries contain ginsenoside levels that are 4.8 times higher than the levels in cultivated 4-yr ginseng roots, with the levels of the ginsenoside Re being 28 times higher in the berry than in the root [22,23]. Ginsenoside content in the root and root hair increases with age in *P. ginseng* plants from 1 yr to 5 yr, but it decreases with age in the leaves, except there is no alternation in the 3-yr-old stage [21]. Although several studies have evaluated the ginsenoside content in different parts of the plant at different ages, there have been no studies investigating the ginsenoside profile of plants in different foliation stages. The present study was conducted to investigate the changes in ginsenoside composition in the leaves and root of 3-yr-old ginseng plants cultivated by hydroponics according to their foliation stage.

2. Materials and methods

2.1. Ginseng materials

Samples were obtained from 3-yr-old ginseng plants hydroponically cultured in perlite and peat moss and grown at 23 ± 2°C under white fluorescent light (60–100 μmol/m^2/s) in a controlled greenhouse (kindly provided by i-farm in Yeoju, Korea). For the ginsenoside analysis and RNA extraction, the plant leaves, main roots, and fine roots were sampled at different stages during foliation (Fig. 1).

2.2. Analysis of ginsenosides by HPLC

First, 0.8 g milled powder from heat-dried leaves, main roots, and fine roots was soaked in 80% methanol at 80°C. After the liquid evaporated, the residue was dissolved in water and extracted with water-saturated *n*-butanol. The butanol layer was then evaporated to produce a saponin fraction. Each sample was dissolved in methanol (1 g/5 mL) and then filtrated through a 0.45-μm filter for HPLC analysis. The HPLC separation was carried out on an Agilent 1260 series HPLC system (Agilent, Palo Alto, CA, USA), equipped with an autosampler and an UV detector using a C18 column (4.6 mm × 50 mm, 1.8 μm; Zorbax Eclipse Plus, Agilent). Gradient elution was used using solvent A (100% acetonitrile) and solvent B (100% water) at 38°C using the following gradient program: 0–4 min, 19% A (isocratic); 4–9 min, 19–25% A; 9–20 min, 25–40% A; 20–25 min, 40–56%; 25–28 min, 56–70% A; 28–29 min, 70–100% A; 29–35 min, 100% A (isocratic); 35–36 min, 100–19% A; 36–42 min, 19% A (isocratic). The flow rate was kept at 1.2 mL/min, the sample injection volume was 5 μL, and UV absorption was measured at 203 nm. Quantitative analysis was performed using a one-point curve method using external standards of authentic ginsenosides.

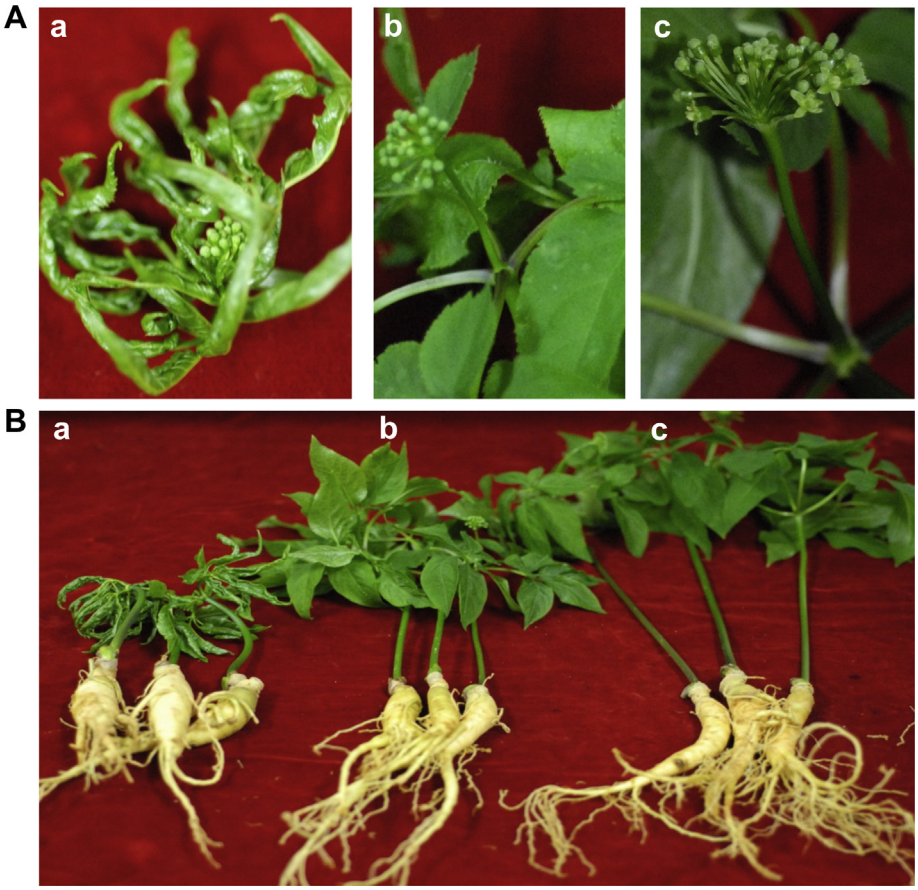

Fig. 1. Phenological growth stage of ginseng during foliation. Three-year-old ginseng plants hydroponically cultured in perlite and peat moss were sampled. For the ginsenoside analysis and RNA extraction, the leaf, main root, and fine root were sampled at different stages during foliation, including the (a) closed, (b) intermediate, and (c) opened leaf stages. (A) Closed-up leaf and inflorescence. (B) Whole plants during foliation stage.