Research Article
J Ginseng Res Vol. 36, No. 4, 430-441 (2012)
http://dx.doi.org/10.5142/jgr.2012.36.4.430

JGR Journal of Ginseng Research

Effects of Natural Bioactive Products on the Growth and Ginsenoside Contents of *Panax ginseng* Cultured in an Aeroponic System

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This study was conducted to evaluate the effects of natural bioactive products such as Manda enzyme (T1), Yangmyeongwon (T2), effective microorganisms (T3), and Kelpak (T4) on the growth and ginsenoside contents of *Panax ginseng* cultured in an aeroponic system using a two-layer vertical type of nutrient bath under natural light conditions. The growth of ginseng plants showed specific characteristics according to the positions in which they were cultured due to the difference of light transmittance and temperature in the upper and lower layers during aeroponic culture in a two-layer vertical type of system. The growth of the aerial part of the leaves and stems of ginseng plants cultured in the lower layer (4,000 to 6,000 lx, 23°C to 26°C) of the nutrient bath was observed to be superior to that of the ginseng plants cultured in the upper layer (12,000 to 15,000 lx, 25°C to 28°C). The leaf area was significantly larger in the treatment of T2 and T4 (46.70 cm^2) than with other treatments. Conversely, the values of the root weight and root diameter were higher in ginseng plants cultured in the upper layer of the nutrient bath. The root weight was significantly heavier in the treatment of T4 (6.46 g) and T3 (6.26 g) than with other treatments. The total ginsenoside content in the leaves and roots was highest in the ginseng plants cultured by the treatment of T1, at 16.20%, while the total ginsenoside content obtained by other treatments decreased in the order of T4, T5 (control), T2, and T3, at 13.21%, 12.30%, 14.84%, and 14.86%, respectively. The total ginsenoside content of the ginseng leaves was found to be significantly higher in the treatment of T1 in the lower layer of the nutrient bath, at 15.30%, while the content of the ginseng roots in the treatments of T3 and T4, at 1.27% and 1.23%, respectively, was significantly higher than in other treatments in the upper layer of the nutrient bath.

Keywords: *Panax ginseng*, Aeroponic system, Ginsenoside, Natural bioactive products

INTRODUCTION

Ginseng (*Panax ginseng* Meyer) is a perennial herbaceous plant with a variety of medicinal efficacies that have been recognized and documented throughout the long history of Asian countries such as Korea and China, the major producers and consumers of ginseng. The cultivation and production of American ginseng (*P. quinquefolium* L.), which is grown in North America (USA and Canada), have increased in recent times, to the extent that it has become another major ginseng species in the world, and consequently leading to an expansion in both interest and consumption. However, these two species of ginseng differ in terms of their components, which have been well documented in various reports, and their efficacies, which have rarely been documented, except in

Received 30 Aug. 2011, **Revised** 11 May. 2012, **Accepted** 29 May. 2012

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studies on ginseng.

The representative bioactivities of ginseng reported thus far include anti-diabetes [1,2], antioxidant [3,4], antitumor [5-7], neuro-protectiveness, neuro-protectiveness [8,9], liver-function promotion [10,11] and anti-stress [12,13] activities. Although these bioactivities have been frequently applied to remedy human diseases, ginseng products are popularly purchased for use as health-functional foods to prevent disease and promote health, as consumers are presently more interested in the prevention of diseases than their remedy. In the case of ginseng, however, only seven kinds of health claims have been approved by the Korea Food and Drug Administration for products currently available on the market, including two health claims, i.e., immunity boosting and fatigue recovery for white ginseng, and five health claims, i.e., immunity boosting, fatigue recovery, antioxidant efficacy, blood flow improvement by inhibiting platelet aggregation, and memory improvement for red ginseng. This is due to a lack of scientific evidence concerning ginseng's efficacy based on clinical testing on human subjects, despite the abundance of experimental reports on ginseng's presumed efficacies. It is predicted that ginseng production will be further expanded to meet the growing market for functional health foods, so continuous attempts will be made to develop a variety of ginseng products for this purpose. Public demand for diverse raw ginseng materials may increase given that the consumption of ginseng products is expanding in line with the increased interest in ginseng as a functional health food.

The hydroponics of ginseng is a representative example of raw ginseng production for use as a functional food material by culturing ginseng seedlings for a short period of 3 to 4 mo under a controlled environment (light, temperature, moisture, and carbon dioxide content) in a high-tech greenhouse, producing not only pesticide-free ginseng roots but also ginseng leaves with a high ginsenoside content. In our previous study [14], the quality of ginseng cultured by a hydroponic system was evaluated by analyzing the ginsenoside content in several parts of a ginseng plant. In addition, wood-cultivated and organically-cultured types of ginseng are further examples of raw ginseng production to meet the current situation in the ginseng market.

Currently, the interest in safe agricultural products of high quality is gradually increasing, leading to an increase in the cultivation acreage by hydroponics for the production of vegetables and flowers in high-tech cultural facilities. This study aims to develop an aeroponic system for the cultivation of ginseng plants for a short period of time (i.e., 4 mo) using ginseng seedlings, which can be utilized as a fresh cut functional vegetable or high-quality food material. Several natural bioactive products popularly used in hydroponic culture systems were examined for their effects on ginseng growth and ginsenoside content in order to provide basic information for the promotion of value-added of ginseng using an aeroponic culture system.

MATERIALS AND METHODS

Ginseng materials

Experiments were conducted using the ginseng variety known as Cheonpoong, which is known to be resistant to moisture stress, thus making it suitable for aeroponic and paddy culture. One-year-old ginseng seedling roots weighing 0.7 to 0.8 g were purchased from a ginseng market and stored in a chamber at low temperature (1°C to 2°C) before use. The ginseng seedling roots were transplanted to nutrient baths and cultured in an aeroponic system. After 4 mo of culture, the ginseng plants were pulled out for harvesting. The harvested ginseng plants were washed clean of dust with water and sorted into leaves and roots, which were then dried for 72 h in a freeze dryer (KR/PVTFD 30A; Ilshin Biobase Co., Yangju, Korea). The freeze-dried ginseng leaves and roots were ground into a powder and used as assay samples in this study.

Chemicals and tools

Ginsenoside components such as ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, and Rh1 were purchased from a chemical company (ChromaDex Inc., Santa Anna, CA, USA). MeOH (Merck & Co Inc,. Darmstadt, Germany) and other GR-grade solvents were used for the quantitative analysis of ginsenosides by an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA), using Sep-Pak Plus C18 cartridges (Waters Corp., Milford, MA, USA) for the solid phase extraction (SPE).

Analysis of ginsenosides

The pretreatment of powdered ginseng samples and the analysis of ginsenosides followed the methods described in a previous study [15]. For each treatment, 2 g of freeze-dried powder sampled from 30 freeze-dried ginseng plants was used for the extraction of ginsenosides. Each sample was suspended in 40 mL of 50% MeOH in a 50 mL centrifuge tube with the lid closed, placed in an ultrasonic bath (Powersonic 410; Hwashin Tech., Seoul,

Korea), extracted by sonication for 15 min, and filtered through Whatman no.1 filter paper (Advantech, Tokyo, Japan). These processes were replicated twice and the resulting filtrates were combined and adjusted to the volume of 100 mL with MeOH in a 100 mL-scaled flask, of which 1 mL was used for pretreatment using SPE before the HPLC analysis. For SPE, a Sep-Pak Plus C18 cartridge was eluted slowly using 3 mL MeOH for the lower conditioning, and again using 3 mL dd-H_2O for the upper conditioning. One mL of the extracted solution (50% MeOH extract) was loaded into the cartridge and eluted slowly using 10 mL dd-H_2O to remove any sugar soluble materials, then eluted slowly using 2 mL MeOH to extract the ginsenoside components. The final eluent was adjusted to an exact volume of 2 mL and filtered through a membrane filter (with a pore size of 0.45 μm), and then analyzed by HPLC on a YMC-Pack ODS AM column (250×4.6 mm, 5 μm; YMC Inc., Wilmington, NC, USA) at 43°C with a flow rate of 0.8 mL/min. The gradient elution of the extracts was processed as follows: in 27% aq. acetonitrile for 10 min; in a gradient concentration of acetonitrile from 27% to 42% for 35 min, and from 42% to 95% for 2 min; and in 95% aq. acetonitrile for 15 min. The extracts were analyzed by a UV detector at a wavelength of 203 nm.

Ginseng culture in an aeroponic system and treatment with natural bioactive products

This study was conducted in the greenhouse of the National Institute of Horticultural & Herbal Science located at Eumseong, Chungbuk Province, Korea for 4 mo from April 20 to August 20, 2009, starting with the transplantation of the ginseng seedlings in a 1-2W type of greenhouse. Cultivation baths were constructed with the two-layer vertical type of benches made with 30 mm rectangular steel frames. Forty cm high first layer and 160 cm high second layer with 5.5 m length for both were welded for fabrication. To provide sufficient intensity of radiation to the ginseng plants by aeroponics, a space of 80 cm was maintained between the aeroponic systems on the top of the lower layer and bottom of the upper layer.

The aeroponic system was made by joining together polystyrene baths with dimensions of 85 cm (width)×35 cm (height)×4 cm (thickness), and overlaying the inside of the baths with black and white polyethylene (PE) film for waterproofing. The nutrient supply pipelines were made of PE tubes with an inner diameter of 25 mm, which were laid down on the baths in two rows and equipped with two nozzles with a spraying capacity of 2 L/min and a controlled spraying pressure of 1.5 to 2.0 kgf/cm^2. The ginseng seedlings were transplanted on April 20, 2009 by inserting them into a polyurethane sponge block (3×3×3 cm) with holes arranged at a distance of 7×9 cm from each other on the polystyrene baths. To ensure efficient transplantation, homogeneous naturalization, and timely foliation and plant growth, the plants were transplanted into the lower layer with a favorable environment for transplantation because of the low light intensity, moved to the upper layer after naturalization for 2 to 3 d, and then transplanted into the lower layer again.

Nutrient provision was performed with a nutrient solution (developed exclusively for ginseng by the Rural Development Administration) composed of NO_3-N 12.0, NH_4-N 1.0, K 8.0, Ca 4.0, Mg 2.0, PO_4-P 3.0, SO_4-S 2.0 me/L, Fe-EDTA 0.6, Mn 0.5, B 0.5, Cu 0.02, Mo 0.05, and Zn 0.05 mg/L, using the aeroponic (water spray) method. The nutrient solution was sprayed for 30 s every 10 min in the daytime and for 30 s every 30 min in the nighttime using a twin-timer, but for 30 s every 5 min in the daytime during the hot summer season.

The natural bioactive products used as treatments in this study were as follows: for treatment 1 (T1), a 10,000 x diluted solution of Manda enzyme (T1; Manda Fermentation Co., Hiroshima, Japan) made from 50 kinds of plant materials including fruits, citrus, root vegetables, grains, legumes, and seaweeds after microbial culture and fermentation for at least 3 yr for aging was used. Thus, this product is known to contain plant bioactive substances including 18 amino acids like arginine and lysine, nutrients and minerals, which enhance photosynthesis enabling plants to grow stronger. For treatment 2 (T2), an 8,000 x diluted solution of Yangmyeongwon (Dream AGRO, Nonsan, Korea) was used. This product was made from fermenting a mixture of about 70 plant materials such as fruits, vegetables, citrus, root vegetables, and fruit vegetables and 15 microbes such as *Mongolian lactobacilli* and yeasts at a low temperature in natural environments for more than 3 years. Treatment 3 (T3) was a 3000 × diluted solution of effective microorganisms (EM; EMcenter, Seogwipo, Korea) made from the culture of 80 microbial species such as photosynthetic bacteria, lactobacilli and yeasts, which are known to have remarkable antioxidant and revivifying properties. Treatment 4 (T4) was a 3,000 x diluted solution of Kelpak (T4; Agrichem Ltd., Queensland, Australia), which is a bioactive substance extracted from seaweed (*Ecklonia maxima*) grown to a giant size, i.e., with a shoot height of 12 to 13 m and a stem diameter of 7 to 8 cm, and which contains plant growth hormones such as cytokinins, gibberellins, auxins

and amino acids required for plant growth and cell division, as well as 66 active components. The application of an extremely small amount of this product is effective in promoting plant growth. Untreated control was used as treatment 5 (T5).

The nutrient solution was managed to maintain electric conductivity (EC) at 0.6-0.8 dS/m and pH 6.0±0.5; EC of 0.6±0.1 dS/m initially, and EC 0.8±0.1 dS/m from one month after transplantation to the end. The temperature of the nutrient solution around the rhizosphere was managed at around 21±1°C; and the temperature of the aeroponic system was maintained around 23°C to 25±2°C in May and June and around 28°C to 30±2°C in July and August.

Light intensity on the baths was maintained at 8,000-12,000 lx using an illumination sensor, which was controlled by a double-layered curtain metalized with 55% aluminum operating under the horizontal towing method. A tunnel made of white felt (55 g) was established in the upper portion of the two-layer baths to prevent the leaves from being burnt by the high temperature and strong sunlight at midday.

Statistical analyses

The values of the growth measurements and ginsenoside content were expressed as mean±standard deviations, and conducted in a split plot design. ANOVA were carried out using the SAS (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was employed to test for significant differences between the treatments at $p<0.05$.

RESULTS AND DISCUSSION

Ginseng growth in an aeroponic system influenced by treatment with natural bioactive products

The ginseng's growth characteristics were examined in 10 plants with 3 replications for each treatment on August 20, 2009 over a harvesting time of 120 d after transplanting the ginseng seedling roots into the aeroponic system (Table 1). Of the 5 treatments, growths generally improved in T2 and T4. Growth in the aerial part of the ginseng plants was better in those grown in the lower layer baths at a light intensity of 4,000 to 6,000 lx compared with those grown in the upper layer, showing an average shoot height of 20.3 and 18.0 cm and an average leaf area of 42.7 and 37.8 cm^2 for the lower and upper layer plants, respectively. The size of the leaf area increased significantly in T2 (46.70 cm^2), T4 (46.70 cm^2) and T5 (46.20 cm^2) compared to other treatments (Table 1). It was reported in previous studies [16,17] that ginseng growth is significantly influenced by temperature and light intensity; while stem height, leaf length and chlorophyll contents decrease with an increase of light intensity. In our study, too, growth in the aerial part of the ginseng plants was poorer in the upper layer baths with higher light transmissibility than those in the lower layer, which is assumed to be better for ginseng growth in the lower layer of the aeroponic system owing to the relatively lower decrease in the chlorophyll content of the ginseng leaves than in the upper layer illuminated with higher light intensity. Considering the fact that these

Table 1. The growth characteristics of ginseng plants cultured by an aeroponic system using a two-layer vertical type of nutrient bath with different natural bioactive products

Natural bioactive products[1)]		Plant height (cm)	No. of leaves (each)	Leaf length (cm)	Leaf area (cm^2/plant)	Stem length (cm)	Stem diameter (mm)	Root length (cm)	Root diameter (mm)	Root weight (g)
Lower layer	T1	20.30a[2)]	2.20a	6.53ab	35.30c	8.06a	1.98ab	10.30a	9.57a	5.27b
	T2	19.50ab	2.13a	6.84ab	46.70a	7.49a	1.87b	11.90a	9.62a	6.18a
	T3	19.50bc	2.37a	7.00c	38.70b	8.33a	2.04a	12.80a	9.23a	4.71c
	T4	20.70c	2.30a	7.19bc	46.70a	8.77a	1.98ab	12.30a	9.16a	5.33b
	T5	21.30bc	2.13a	6.90bc	46.20a	8.19a	2.02ab	10.90a	8.19b	4.47c
	Mean	20.30	2.23	6.89	42.70	8.17	1.98	11.60	9.16	5.19
Upper layer	T1	16.50d	2.27a	6.04b	31.50a	6.10a	2.15a	10.30a	9.71ab	5.23d
	T2	17.30cd	2.10a	6.31b	35.90ab	6.20ab	2.00ab	11.40a	9.53ab	5.84bc
	T3	18.00bc	2.30a	6.36b	40.40b	6.90ab	2.04ab	11.50a	10.01a	6.26ab
	T4	18.40b	2.37a	6.39b	38.30ab	7.30c	2.04ab	12.60a	9.97a	6.46a
	T5.	19.80a	2.10a	6.98a	42.70ab	7.80bc	2.04b	10.50a	9.21b	5.49cd
	Mean	18.00	2.21	6.42	37.80	6.84	2.03	11.30	9.70	5.90

[1)] T1,Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control.
[2)] Mean values±SD from separate experiments conducted in triplicate are shown. Mean with different letters (a-d) within the same growth characteristics are significantly different at $p<0.05$ by Duncan's multiple range test.

Fig. 1. Pictures of the aeroponic system using a two-layer vertical type of nutrient bath under natural light condition for ginseng culture (A,B) and harvested ginseng plants (C). The total growth period was 120 d. Ginseng plants are shown before harvesting (B) and after harvesting (C) on August 20, 2009.

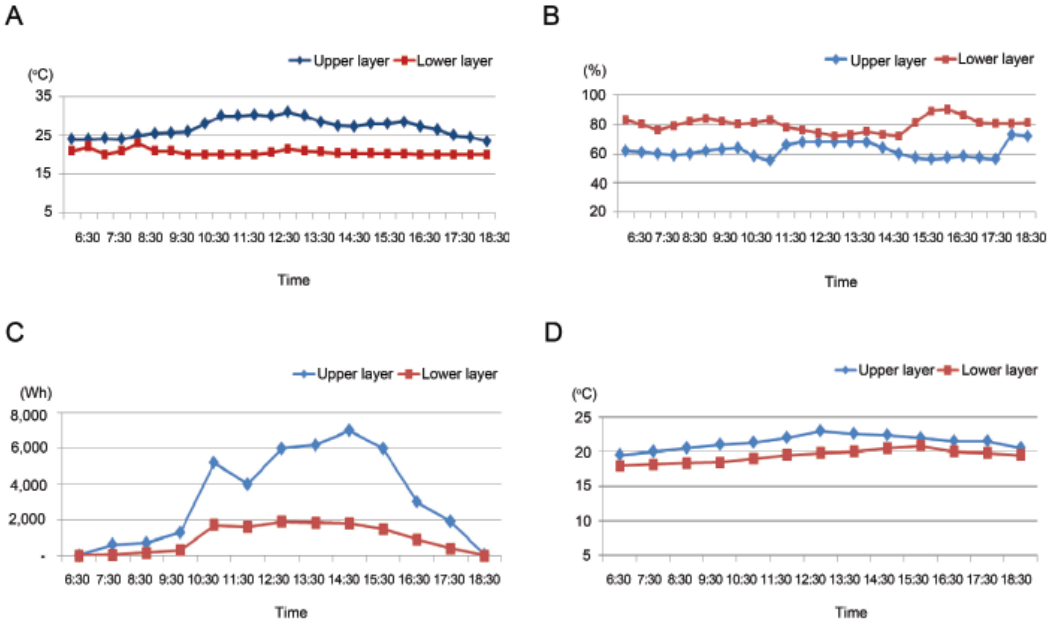

Fig. 2. Changes in the weather conditions such as temperature (A), humidity (B), solar radiation (C) and root zone temperature (D) in the daytime in the lower and upper layers of the greenhouse.

decreases in stem height, leaf length and chlorophyll content are more severe in one-year-old ginseng than 2-, 3-, or 4-year-old ginseng [16], light transmissibility should be one of the important environmental factors that influence the growth of ginseng in the aeroponic system with a short period of growing stages for one-year-old ginseng seedlings.

Unlike the growth of the aerial part of the ginseng, root growth, such as root weight and root diameter, was better in the upper layer with light intensity of 12,000 to 15,000 lx than in the lower layer of the aeroponic system, which coincides with the findings of the report that the weight of individual ginseng roots increases with an increase of light intensity [18], suggesting that the optimum light transmissibility for ginseng root yield should be 18.5% of natural light. In all treatments, the average root weight of the ginseng grown in the upper layer of the aeroponic system was 5.9 g, i.e., 13% more than ginseng grown in the lower layer, which showed an average root weight of 5.2 g. Especially, the weight of the ginseng roots increased significantly in T3 (6.26 g) and T4 (6.46 g) compared to the other treatments and the untreated control. The average root length of the ginseng plants grown in the lower layer was 11.6 cm, showing a slight increase compared to those in the upper layer with an average root length of 11.3 cm; however, the average root diameter was comparably larger for the plants grown in the upper layer (9.7 mm) than those in the lower layer (9.2 mm), indicating that root diameter may be one of the major parameters contributing to the increase in the roots' weight. The plant growth of the aerial part and root differed depending on the upper and lower baths in which the ginseng seedlings were planted, regardless of the treatments. Examination of the indoor

environmental conditions during the aeroponic culture of the ginseng seedlings showed that the natural light intensity was higher in the upper layer than in the lower layer (Figs. 1 and 2). Also, the indoor temperature was 2°C to 3°C higher in the upper layer (25°C to 28°C) than in the lower layer (23°C to 26°C). This suggests that ginseng root growth should be influenced more by light intensity and temperature than by treatment with natural bioactive products. However, the growth of the aerial part of the ginseng increased more in the lower layer baths of the aeroponic system, where there was a relatively low light intensity, which is unfavorable for ginseng root growth. All of the results suggest that proper management of light intensity and temperature would promote the production of specific parts of a ginseng plant to be used for specific purposes.

Effects of natural bioactive products on the total ginsenoside content in ginseng plants cultured in a two-layer aeroponic system

The results of the analysis of the total ginsenoside content of the ginseng roots and leaves cultured in the two-layer aeroponic system are shown in Fig. 3. In the untreated control (T5), the total ginsenoside content was higher in the ginseng roots cultured in the lower layer baths (1.24%) than those cultivated in the upper layer, which does not coincide with the report that the crude saponin content in ginseng roots increases with an increase of light intensity [19]. On the other hand, the total ginsenoside content was significantly higher in the ginseng leaves cultured in the upper layer baths (16.57%) than in the lower layer ones (13.62%). Also, in the treatments with natural bioactive products (T1-T4), the total ginsenoside content in the ginseng roots and leaves was found to be the opposite of each other depending on whether they were cultured in the lower or the upper layer baths; i.e., higher in the leaves, lower in the roots, and vice versa. The total ginsenoside content of the ginseng roots in T1 and T2 came to 1.10% in the upper layer and 1.17% in the lower layer, respectively, i.e., higher than in the other layer in the same treatment; however, the content in T3 and T4 ranged from 1.23% to 1.30%, showing no significant difference between the lower and upper layer baths.

In ginseng leaves, the total ginsenoside content in T1 was similar in the lower (15.33%) and upper (14.23%) layer baths of the aeroponic system; however, the content in T2-T4 came to 14.32, 15.61, and 15.11% in the ginseng leaves cultured in the upper layer baths, i.e., significantly higher than those cultured in the lower layer baths (Fig. 3). This seems to be due to the differential influences of the environmental conditions between the lower and upper layer baths. In particular, the total ginsenoside content of the leaves came to 15.61% and 16.57% in the upper layer baths treated with T3 and T5, respectively (with no significant difference between the two treatments), being significantly higher than in T1 and T2, but not between T3 and T4 (15.11%). These results suggest that the treatments of T3 and T4 may be advantageous for the production of ginsenoside in the roots and leaves together in the upper-layer aeroponic system, while T1 may be advantageous for it in the lower-layer aeroponic system. On the other hand, the total ginsenoside content per unit weight of the ginseng roots and leaves in T1 was highest at 16.20% in the lower- layer baths of the aeroponic system among all the treatments, and was observed to decrease in the order of T5, T4, T2, and T3, at 14.86%,

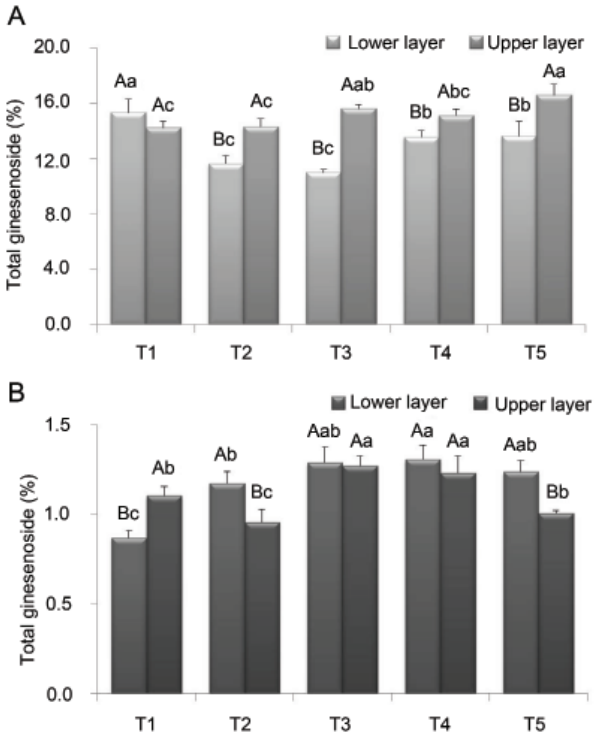

Fig. 3. The total ginsenoside content of the roots and leaves from *Panax ginseng* cultured using an aeroponic system with a two-layer vertical type of nutrient bath. (A) Ginsenoside content of leaves from *P. ginseng* cultured by hydroponics in the lower and upper layers of the greenhouse. (B) Ginsenoside content of roots from *P. ginseng* cultured by hydroponics in the lower and the upper layers of the greenhouse. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. The mean values±SD obtained from triplicate separate experiments are shown. Means with different letters (A,B) within the same natural bioactive products are significantly different at $p<0.05$, while means with different letters (a-c) within the same layer are significantly different at $p<0.05$ by Duncan's multiple range test.

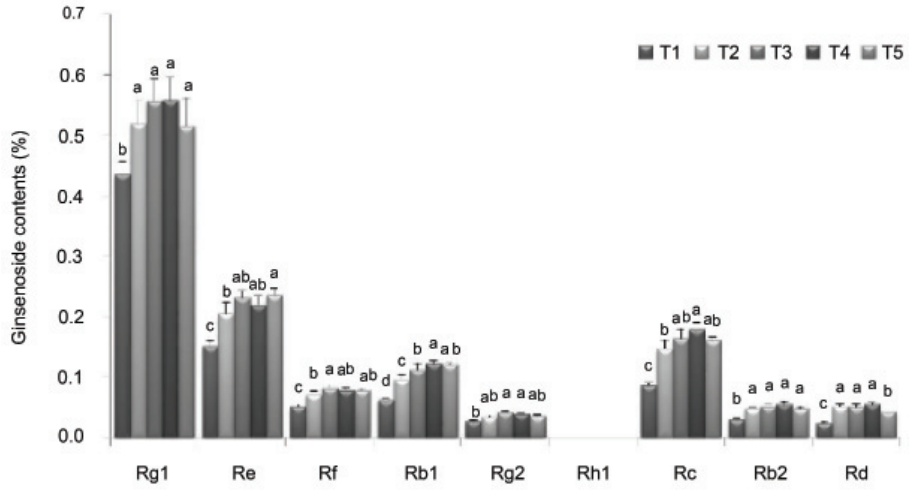

Fig. 4. Ginsenoside content of roots from *Panax ginseng* cultured in the lower layer of the aeroponic system using a two-layer vertical type of nutrient bath. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. Mean values±SD from triplicate separate experiments are shown. Means with different letters (a-c) within the same bioactive products are significantly different at $p<0.05$ by Duncan's multiple range test.

14.84%, 13.21%, and 12.30%, respectively. Ginseng leaf growth is better in the lower than in the upper layer because the lower layer is advantageous for chlorophyll production and photosynthesis in ginseng leaves due to low light transmission [16,17]. In the lower layer culture conditions, plant photosynthesis might be enhanced by T1 (Manda enzyme), which contains 18 kinds of amino acids, minerals, and nutrients, consequently leading to accelerated plant growth presumably related to ginsenoside production. Manda enzyme, which is made from the 3-year-long fermentation of 50 kinds of fruits and vegetables, is a fermentation product with a variety of uses including agronomic and animal husbandry applications and as a health functional food. It was reported that it contains useful materials for humans owing to its antioxidant, antitumor and immune enhancement efficacies [20,21]. Its efficacies with regard to its immunostimulatory and growth stimulatory effects were also reported in the aquaculture of Japanese halibut [22]. On the other hand, the total ginsenoside content in ginseng plants cultured in the upper layer baths of the aeroponic system by the treatments (T1-T5) came to 15.33%, 15.28%, 16.88%, 16.34%, and 17.58%, respectively, showing a decrease in ginsenoside production in the order of T5, T3, T4, T1, and T2. This suggests that natural bioactive products have different effects on ginsenoside production in ginseng roots and leaves depending on the upper and lower locations of the aeroponic system, or that the influence of T1 may not be very high in cultural conditions including high light transmission and high temperature.

Production of ginsenoside components in ginseng plants cultured in the upper and lower layer baths of the aeroponic system and treated with natural bioactive products

Fig. 4 shows the contents of ginsenoside components in ginseng roots cultured in the lower layer baths of the aeroponic system; the contents of the major ginsenoside components on average in T1-T5 were Rg1>Re>Rc>Rb1>Rf>Rb2>Rd>Rg2. The pattern of ginsenoside composition in the ginseng roots in the upper layer baths was the same as that in the lower layer baths of the aeroponic system (Fig. 5). However, the ginsenoside composition of the ginseng roots used in this study differs slightly from that produced in a hydroponic culture system with different nutrient media, in which the contents of the ginsenoside components were Rg1>Rc>Rb1>Re>Rb2>Rf>Rd>Rh1 [14]. The fact that the ginsenoside composition differs between aeroponic and hydroponic systems even in the untreated control suggests that the production level of individual ginsenoside components may differ depending on the system of cultivation. Given that the ginsenoside composition of 4-year-old ginseng roots cultivated in open fields is similar to those cultivated in a hydroponic system [15], ginseng cultivation in an aeroponic system may have a different influence on ginseng's efficacies compared to those grown in hydroponic or open field systems, altering the ginsenoside composition and especially decreasing the PD/PT ratios due to the increased content of ginsenoside Re.

It is widely known that the expression of ginseng

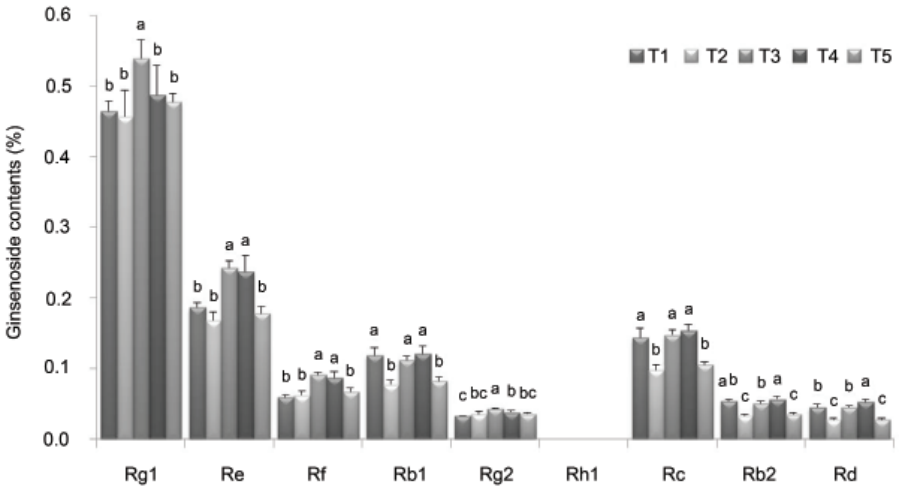

Fig. 5. Ginsenoside content of roots from *Panax ginseng* cultured in the upper layer of the aeroponic system using a two-layer vertical type of nutrient bath. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. Mean values±SD from triplicate separate experiments are shown. Means with different letters (a-c) within the same bioactive products are significantly different at $p<0.05$ by Duncan's multiple range test.

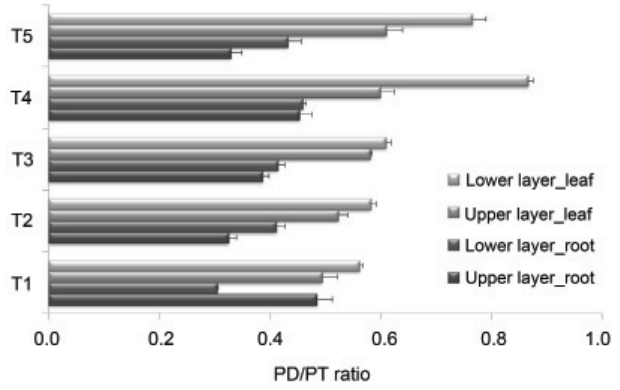

Fig. 6. Protopanaxadiol (PD)/ protopanaxatriol (PT) ratio of *Panax ginseng* cultured with the aeroponic system using a two-layer vertical type of nutrient bath. *P. ginseng* was cultured by the aeroponic system in the lower and the upper layers in a two-layer type of nutrient bath. PD/PT means the ratio of PD ginsenosides and PT ginsenosides. PD: Rb1, Rb2, Rc, Rd; PT: Re, Rf, Rg2, Rh1. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. The mean values±SD obtained from triplicate separate-experiments are shown.

efficacies is especially governed not only by the total ginsenoside content but also by the composition of each individual ginsenoside components. Fig. 6 shows the protopanaxadiol (PD)/protopanaxatriol (PT) ratios in ginseng roots cultured in the aeroponic system and treated with natural bioactive products. In the untreated control, the ginsenoside PD/PT ratios in the lower and upper layer baths were 0.44 and 0.33, respectively, suggesting that the ginseng roots cultured in the lower layer baths with low light transmission contain more PD-type ginsenoside components than those cultured in the upper layer with high light transmission (in the aeroponic system). This is contrary to the report that the PD/PT ratios increase with an increase of light transmission [19], therefore suggesting that the PD/PT ratios of the ginsenoside components may be influenced not only by light intensity but also by other minor cultural conditions. A further study is needed to understand the detailed relationships between ginsenoside production and composition with cultural conditions such as temperature and light transmission in the aeroponic system. On the other hand, the ginsenoside Rc and Rb1 contents were found to be higher in ginseng samples taken from the lower layer culture than the upper layer culture in the aeroponic system, with the former showing a higher percentage of ginsenoside Rc (13.0%) among the total ginsenoside contents compared to a relatively low percentage (10.4%) in those cultured in the latter.

The PD/PT ratios of the ginseng roots treated with T2-T4 were higher in the lower layer baths than in the upper ones, showing a similar tendency to the untreated control. However, the PD/PT ratios in the ginseng roots treated with T1 were 0.31 and 0.49 for the lower and upper layer baths, respectively, showing higher PD/PT ratios in the upper layer than in the lower one, i.e., contrary to the T2-T4 and the untreated control. Moreover, for T1, the PD/PT ratios in the upper layer were highest among all the treatments in either the upper or lower layer baths. Unlike other treatments, the total ginsenoside content of ginseng plants treated with T1 was higher in the upper layer, as shown in Fig. 3, which contained much higher

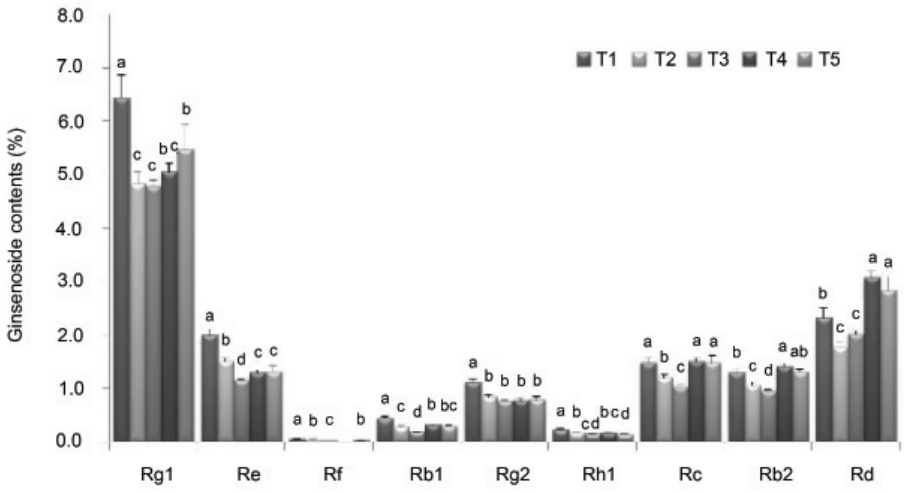

Fig. 7. Ginsenoside contents of leaves from *Panax ginseng* cultured in the lower layer of the aeroponic system using a two-layer vertical type of nutrient bath. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. The mean values±SD obtained from triplicate separate experiments are shown. Means with different letters (a-c) within the same bioactive products are significantly different at $p<0.05$ by Duncan's multiple range test.

ginsenoside Rb1 and Rc contents than in the lower layer, accounting for 10.8% and 13.0% of the total ginsenoside content, respectively.

The PD/PT ratios in the hydroponically grown ginseng plants were around 0.6, i.e., lower than those grown in open fields [14]. In our study examining the effects of natural bioactive products on ginsenoside production, T1 induced greater production of ginsenoside Rb1 and Rc than the other treatments, thus increasing the PD/PT ratios. Ginsenoside Rb1 and Rc are known to have immunostimulatory effects and antianxiety efficacies [12,23,24]. Further detailed studies will be needed to examine the influence of T1 on ginsenoside production and composition in the upper and lower locations of an aeroponic system with different levels of light transmission.

Investigation of the individual ginsenoside components produced in the ginseng plants cultured in the lower layer of the aeroponic system showed the content of ginsenoside Rg1, the most significant ginsenoside component of a ginseng root, to be 0.52% to 0.56% in T2-T5, except in T1, which contained 0.44%. T3 and T4, which similarly had the most prominent effect on the production of ginsenoside, had a similar effect, showing no significant difference in terms of the production of the 8 individual compeonents of ginsenoside. In the upper-layer aeroponic culture of the ginseng plants, there was no significant difference between T3 and T4 in terms of the total ginsenoside content; however, T3 contained 0.54% and 0.04% of ginsenoside Rg1 and Rg2, respectively, while T4 contained 0.06% and 0.05% of ginsenoside Rb2 and Rd, respectively (Fig. 5). There were no significant differences in the amounts of the other ginsenoside components in the ginseng plants cultured by T3 and T4 in the upper layer of the aeroponic system.

As regards the ginseng plants cultured in the lower layer of the aeroponic system, the ginsenoside Rg1 content was lowest (0.44%) in the ginseng roots in T1, but it was significantly higher in the ginseng leaves in T1 than in the other treatments (Fig. 7). The contents of the major ginsenosides components produced in the ginseng leaves cultured in the lower layer of the aeroponic system were observed in the order of Rg1>Rd>Re>Rc>Rb2>Rg2>Rb1>Rh1>Rf (Fig. 7). The ginsenoside composition of the ginseng leaves cultured in the upper layer of the aeroponic system showed the same pattern as those cultured in the lower layer in our study (Fig. 5), and also in the hydroponic culture system in a previous study [14], suggesting that the composition of ginsenoside may not be greatly influenced by the aquiculture type (i.e., spray and substrate cultures) or by treatments with natural bioactive products.

The PD/PT ratios were found to be 0.57 to 0.87 and 0.50 to 0.61 in the ginseng leaves cultured in the lower- and upper-layer baths of the aeroponic system, respectively, showing that they were higher in the lower layer than in the upper layer regardless of the treatments (Fig. 6). This tendency is similar to that observed in the ginseng roots, which suggests that the biosynthesis of PD-group ginsenosides in ginseng plants may be higher in the lower layer baths with relatively low light intensity rather than in the upper layer of the aeroponic system. Especially high PD/PT ratios were shown in T4 and

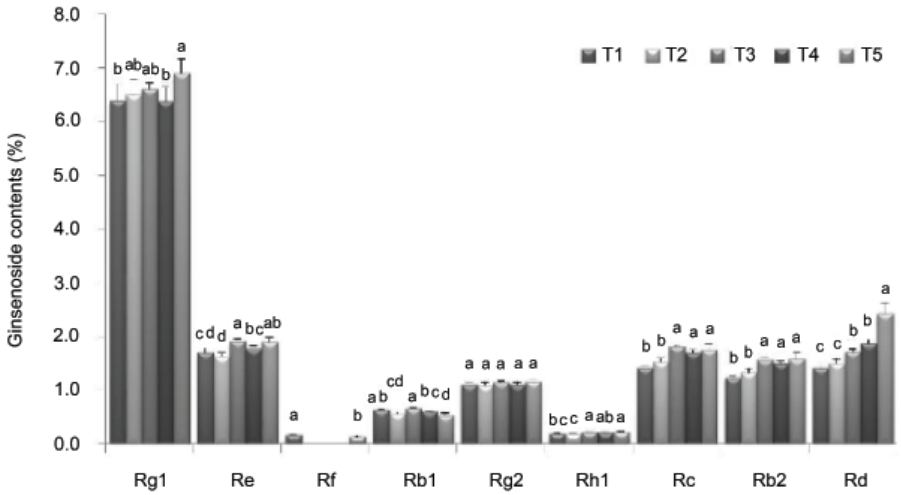

Fig. 8. Ginsenoside contents of leaves from *Panax ginseng* cultured in the upper layer of the aeroponic system using a two-layer vertical type of nutrient bath. T1-T5, natural bioactive products. T1, Manda enzyme (×10,000); T2, Yangmyeongwon (×8,000); T3, effective microorganisms (×3,000); T4, Kelpak (×3,000); T5, control. The mean values±SD obtained from triplicate separate experiments are shown. Means with different letters (a-c) within the same bioactive products are significantly different at $p<0.05$ by Duncan's multiple range test.

T5 (untreated control), indicating that their differences between the lower and upper layer baths were reduced in T1-T3, while they were increased in T4 compared to the untreated control (T5). This could be explained by the fact that the high PD-group ginsenoside Rd in the ginseng leaves (3.07%) cultured in the lower layer baths was 1.6 times higher than those cultured in the upper layer (1.87%) (Figs. 7 and 8). Ginsenoside Rd is a component known to be effective in enhancing antioxidant enzyme activities [25], cancer prevention [26], and brain cell protection [27]. T4 (Kelpak) is a bioactive substance extracted from brown algae (*E. maxima*), which has efficacies of plant growth promotion by enhancing rooting, flowering and fruiting [28-30] with the help of the plant hormones such as cytokinins and auxins it contains [31]. It is also useful for the mass production of commercial seaweed [32]. In our study, treatment with Kelpak might promote the growth of ginseng plants, which may have a positive influence on the production of PD-group ginsenosides, especially ginsenoside Rd.

Presently several natural bioactive products are used for growth promotion and pest control in open-field cultivation of ginseng. However, there are few cases of experiments being conducted on the use of these natural bioactive products in the hydroponics and aeroponics of ginseng. In this respect, this is the first report on such experimentation to have been conducted in detail. Few studies have been conducted on the effect of natural bioactive products on ginsenoside production in open-field ginseng cultivation. Therefore, this study may provide basic information that can profitably be used in the application of natural bioactive products in open-field cultivation of ginseng as well as in hydroponic and aeroponic systems for ginseng production.

ACKNOWLEDGEMENTS

This work was supported in part by a grant (PJ006499) provided by the Rural Development Administration, Republic of Korea for projects involving the development of agricultural farming techniques for practical usage from the Rural Development Administration, Republic of Korea.

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