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Research Article

Novel Cultivation of six-year-old Korean Ginseng (*Panax ginseng*) in pot: From Non-Agrochemical Management to Increased Ginsenoside

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ABSTRACT

Background: Ginseng (*Panax ginseng* Meyer) is a perennial plant belonging to the Araliaceae family that is known to have various beneficial effects including improving memory loss and spatial cognitive ability, and anti-cancer and anti-diabetes activity. Its functional benefits also include improving liver function, regulating blood pressure, stress, and providing antioxidant activity. Usually, various agrochemicals are used in cultivating ginseng preventing from many diseases.
Methods: FCGP (field cultivated ginseng in pot) was implemented by imitating MCWG (mountain cultivated wild ginseng). Pesticide analysis of pot cultivation was carried out and the contents of bioactive components such as ginsenoside were also analyzed.
Results: FCGP ginsenoside content was higher than that of FCG (field cultivated ginseng) and MCWG. FCGP has been shown to have a relatively high antioxidant effect compared with cultivated ginseng.
Conclusion: It was confirmed that ginseng can be grown for 6 years without resorting to use of pesticides. In addition, it was confirmed that effective accumulation of physiologically active ingredients such as ginsenoside is possible. Our result represents FCGP is a novel method of pesticide-free ginseng cultivation

1. Introduction

Ginseng (*Panax ginseng* Meyer) is from the Araliaceae genus, which grows for many years [1]. Ginseng is cultivated as a medicinal crop in many countries because of its medicinal value [2]. The ginseng plant is susceptible to high temperature, strong light, overhumidity, and disease and takes much time to grow [3]. Ginseng contains saponins (ginsenoside), acidic polysaccharides, polyacetylene, alkaloids, phenolic compounds, and various ingredients, such as gomisin-A, −N. Accordingly, various effects such as anticancer activity, anti-diabetic activity, strengthening liver function, control blood pressure, anti-stress, and antioxidant activity are associated with ginseng intake [4–9]. About 40 species of ginsenosides isolated from ginseng have been reported. Ginseng saponins that are mainly detected during ginseng extraction and analysis are six ginsenosides, including Rg1, Rb1, Rb2, Re, Rd, and Rc. Rb1, Rb2, Rc, and Rd are ProtoPanaxaDiol (PPD) saponin series with a central nervous system calming effect. Re and Rg1 are the ProtoPanaxaTriol (PPT) saponin series, which reduce cholesterol content [10]. Some ginsenosides, such as Rb1 and Rb2, are digested in the stomach and converted to Rg3 for absorption [11]. Ginsenoside contents accumulate differently depending on the cultivation location or cultivation method.

A great deal of damage is done to ginseng during cultivation by diseases, such as anthrax, spots, and gray mold disease, and the issue of residual pesticides has been raised as the use of pesticides has increased [12]. For this reason, ginseng is mainly cultivated with relatively short four-year roots, and six-year-old ginseng, the raw material of red ginseng, is challenging to cultivate. Ginseng can be divided into field cultivated ginseng (FCG) and mountain cultivated wild ginseng (MCWG) depending on growth conditions. MCWG is grown from ginseng seeds in their natural state in the mountains. Because of difficulty controlling moisture and space constraints, this method is challenging to manage and harvest. FCG

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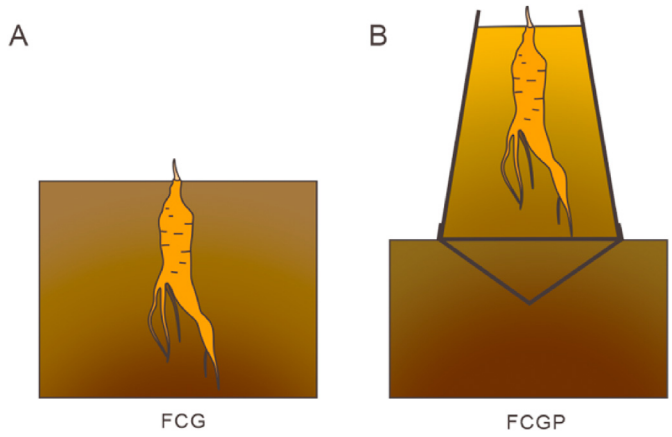

Fig. 1. The diagram of ginseng cultivation. A, FCG (Field Cultivated Ginseng); B, FCGP (Field Cultivated Ginseng in Pot).

is artificially cultivated ginseng. Growing FCG is easy compared with MCWG, but difficult without using pesticides.

Various attempts have been made to control ginseng disease and pests naturally, but pesticide-free cultivation has not yet been reported [13]. In order to control pests, experiments were conducted using pots in in-vitro [14]. However, despite these various reports, no pesticide-free cultivation has been reported in the field yet. In this study, ginseng was cultivated in pot (FCGP) with non-pesticide management to compensate for the cultivation environmental disadvantages of FCG and MCWG. Although FCGP was cultivated in the field, it was morphologically similar to MCWG. Also, PPT and PPD were contained in high amounts in FCGP. Therefore, it can be proposed that FCGP can improve the problems of existing cultivation methods.

2. Material and methods

2.1. Cultivation method

This study was conducted in Jinan-gun, Jeollabuk-do (300 m above sea level) from March 2014 to March 2020 to investigate the effects of ginseng growth through conventional cultivation methods and bottle cultivation in ginseng cultivation. A connection-type iron sun cover with a height of 180 cm for the front pillar and 100 cm for the behind pillar was prepared. It was covered upper one sun-shielding net (75%). FCG and MCWG were purchased from farms in Jinan. The MCWG used was qualified as pesticide-free according to Korean special management forest product quality standards. The pot used comprised an upper panel and a lower cover. The upper panel had an upper diameter of 11 cm and a lower diameter of 16 cm, and its height was 25 cm. The lower cover was an inverted conical shape, 16 cm in diameter and 5 cm in

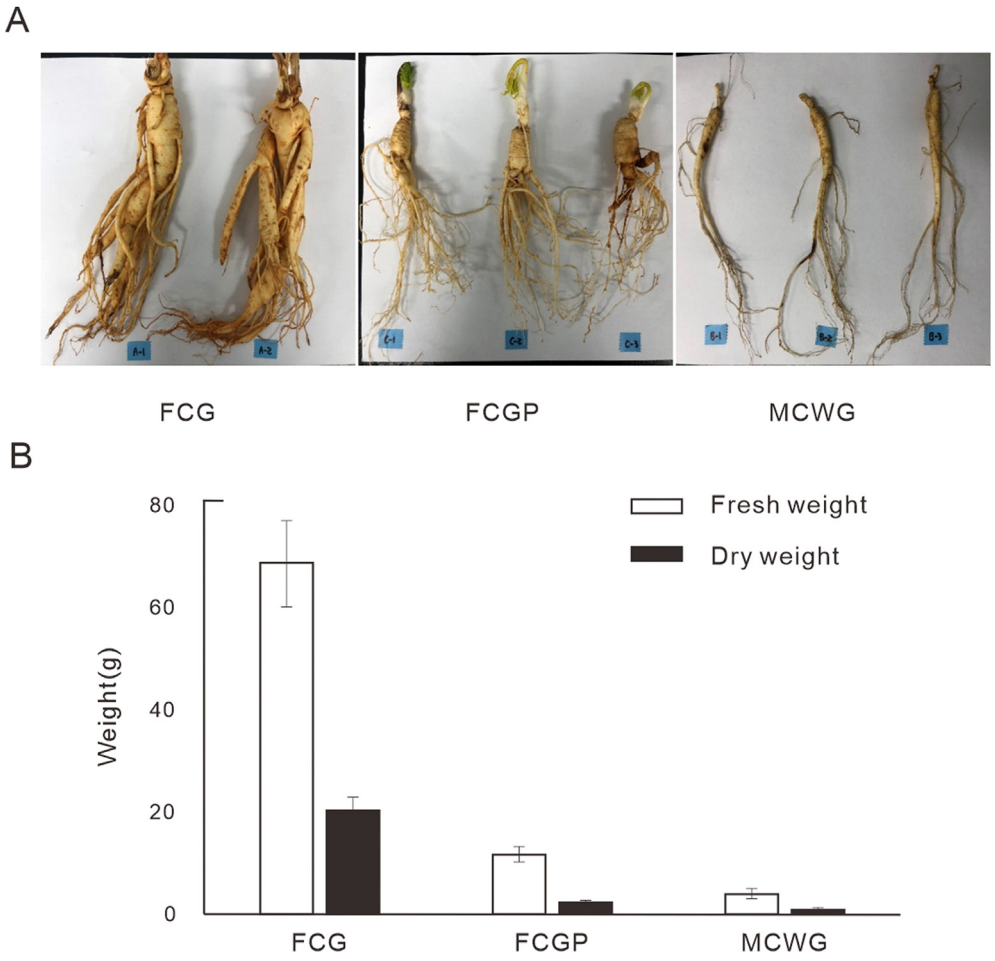

Fig. 2. Ginseng shape and weight by cultivation method. A, Photograph of the roots of ginseng after harvesting, from left to FCG, FCGP and MCWG; B, The weight of ginseng according to the cultivation method. The white and black square represent the fresh weight and the dry weight, respectively.

Table 1
The analysis of pesticide residue

Method	Agrochemicals	Detected amount (mg/kg)/(permissible standard amount of residual agrochemicals)[1]
FCG	Azoxystrobin	1.214/(0.1)
	Indoxacarb	0.036/(0.05)
	Kresoxim-methyl	0.780/(0.2)
	Pyraclostrobin	0.167/(2.0)
	Tebuconazole	0.077/(0.5)
	Tolclofos-methyl	0.092/(1.0)
FCGP	Not detected	-

[1] Permissible standard for pesticide residues implemented in Korea, if there is no standard for use, expressed as 0.01 mg/kg.

height (Figure S1). In addition, cultivation was carried out using soil in which FCG is growing to exclude changes due to soil. FCG and MCWG were cultivated during the same period as FCGP.

2.2. The analysis of agrochemicals residues in FCG, FCGP

In accordance with the permission criteria of 'Ministry of Food and Drug Safety' in Korea, agrochemical residues (245 agrochemicals) in ginseng were analyzed. In this process, the inspection was conducted through the 'SGS (Société Générale de Surveillance)', an inspector specializing in the inspection.

2.3. The ginsenoside content of FCG, FCGP, and MCWG

The analysis of the ginsenoside content was conducted by requesting the 'Institute of Jinan Red Ginseng'. 2 g of a freeze-dried sample was shaken in 70% methanol for 15 minutes, and then used. After that, it was used by filtration through a 0.45μm membrane filter. High-performance liquid chromatography-ultraviolet absorption detector (HPLC-DAD) was used for the analysis, and Eclipse Plus C18 (4.6 × 150 mm, 3.5 μm) was applied at 203 nm as the detection condition, and acetonitrile (A) was applied as the solvent for the mobile phase. And distilled water (B) had a gradient from 82% to 10% during 0 minutes to 71 minutes, and from 10% to 82% during 71 minutes to 75 minutes. Statistical significance was determined using Student's t-test (**P < 0.01; *P < 0.05).

2.4. Analysis of useful substances measurements

Ginseng used in the experiment was crushed after freeze drying. The reagent purchases Sigma Chemical Co. (St. Louis, Missouri, USA). The samples were extracted to be 2 g in 20 ml of 50% ethanol. The extraction time was immersed for 16 hours and filtered (membrane filter, size 0.45 mm, Hyundai Micro, Korea). The total phenol content was analyzed using the Dewanto et al [15] method using Garlic acid as a standard material. A calibration curve was made and quantified. In 100 μL of each extract, 2 mL of a 2% Na2CO3 solution was added, allowed to stand for 3 minutes, and 100 μL of 50% Folin-Ciocalteu reagent was added, and the absorbance of the reaction solution was measured at 700 nm after 30 minutes. The total flavonoid was analyzed using the Choi et al [16] method using Catechin as a standard material. A calibration curve was made and quantified. 1 mL of distilled water and 75 μL of 5% NaNO2 were added to 250 μL of methanol extract according to the method of Choi et al [16]. After 11 minutes, the absorbance was measured at 500 nm. UV/Vis-spectrometer (UV-1601, Japan's Shimadaz) was used for absorbance measurement. Statistical significance was determined using Student's t-test (**P < 0.01; *P < 0.05).

2.5. Antioxidant activity

The electron donating abilities (EDA) of the extract was measured by the Blois [17] method, and the reducing power for each extract exhibited by the EDA effect was measured. That is, 1.8 mL of 0.1 mM 1-1-diphenyl-2-picrylhydrazyl (DPPH) and 100 μL of extraction solvent were added to 100 μL of the extract, so that the total volume of the extract was 2 mL. The reaction solution is mixed for about 10 seconds and left at room temperature for 30 minutes in dark conditions. The reaction solution was measured absorbance in a spectrophotometer UV/Vis-spectrometer (UV-1601, Japan's Shimadaz) 517 nm. The EDA was expressed as a percentage through the absorbance of the group with and without the extract. Antioxidant activity was measured using Kang's method [18] and 2,2′-Azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical using ABTS cationic bleaching assay. 2.45 mM potassium persulfate and 7 mM ABTS were dissolved in distilled water, left in the dark for 16 hours to form ABTS radicals, absorbance was measured at 700 nm and diluted with ethanol to reach 0.70 ± 0.02. The absorbance was measured by mixing 100 μL of each extract in 2 mL of the diluted solution. The control was measured using an extraction solvent, and was calculated and expressed according to the removal rate calculation formula

3. Results and discussion

Ginseng, which is vulnerable to strong light and cultivated by blocking sunlight, is cultivated at a place for at least 4–6 years. FCG is naturally exposed to diseases and pests during the growing season, so various pesticides must be used [19]. MCWG was grown at high altitude and without artificial moisture or pesticide supply. To mimic MCWG, FCGP formed the ground and then was cultivated without artificial moisture or pesticide supply (Fig. 1A and B).

Ginseng is damaged if the soil moisture content is high, and growth is inhibited if it is low [20]. However, moisture is a critical factor in plant growth. Ginseng growth may be hampered by excessive as well as deficient moisture, so both continuous drainage and adequate moisture supply are important. Therefore, we confirmed the moisture levels and distribution of FCG and FCGP. In both methods, the aim was to see that moisture was transferred to the underside. According to the cultivation method, the moisture of the soil was measured for 10 d, the average was confirmed (Figure. S2). In both methods, the aim was to see that moisture was transferred to the underside. However, FCGP was found to have lower moisture content in the pot cultivation. The moisture content of the portion where ginseng grows in the pot contained at least 2% and up to 4% less moisture than that of the field. In addition, it can be seen that moisture is stored in the ground under the pot. Moisture is quickly drained inside the pot, and the drained moisture is absorbed into the adjacent soil, forming a high moisture. It is possible that FCGP grew by drawing moisture under the pot.

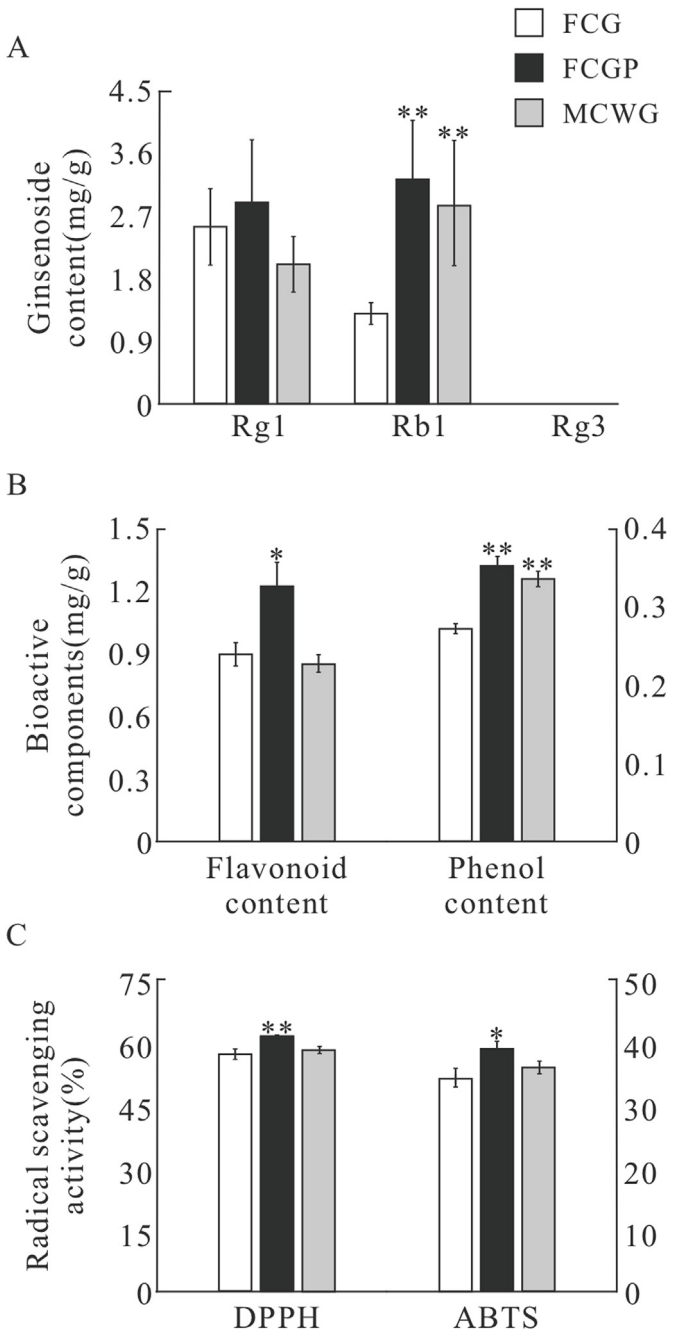

Fig. 3. Analysis of substances in ginseng. A, In terms of ginsenoside content, Rg1 is a PPT-based component, and Rb1 is a PPD-based component. In the case of Rg3, it is a substance that occurs when Rb1 is decomposed, and is not detected in the results (Student's *t*-test, $^{**}P < 0.01$; $^{*}P < 0.05$); B, Bioactive substance content analysis. Analysis of flavonoid and phenol content among bioactive substances (Student's *t*-test, $^{**}P < 0.01$; $^{*}P < 0.05$); C, DPPH radical scavenging activity (%); D, ABTS radical scavenging activity (%). The white, black and gray square represent the FCG, FCGP and MCWG, respectively.

When cultivated in pot, a water shortage can be induced to suppress diseases and pests and induce useful substances through water stress [21]. The primary root of FCGP is small compared with FCG and large compared with MCWG. The length of ginseng growth was similar in all cultivation methods. The prominent morphological feature of FCGP is that it has many lateral roots compared with other cultivation methods (Fig. 2A).

Consistent with ginseng morphology, the weight of FCG was the heaviest for both fresh weight (68.5 ± 8.5 g) and dry weight (20.2 ± 2.6 g), followed by FCGP (fresh weight 11.6 ± 1.5 g and dry weight 2.3 ± 0.3 g) and MCWG (fresh weight 3.9 ± 1 g and dry weight 0.9 ± 0.3 g) (Fig. 2B).

Ginseng takes much time to gain its medicinal value. Furthermore, FCG grows many years and causes yellowing and root disease due to poor drainage [22]. It is difficult to control the disease through environmental factors, so it is controlled with agrochemicals. The general harvesting period of ginseng is autumn, and pesticides are applied from spring to summer. Even though they decrease through the half-life, residual pesticides remain a problem. Interestingly, FCGP can also be grown for a long time without pesticide application, despite being grown in one place for a long time (Table 1). Plant root development has a negative correlation with light intensity and a positive correlation with soil moisture. These results suggest that FCGP developed a lateral root to supply insufficient water inside the pot.

Ginsenoside, the primary substance of ginseng, has different forms of accumulation depending on the cultivation method. Rg1 (PPT) content was high in FCG, whereas Rb1 (PPD) was high in MCWG, which is consistent with the previous studies [23]. Rg3, which produces ginsenosides by digestion, was undetected in FCG and MCWG [24], and FCGP was not detected. The content of Rg1 and Rb1 in FCGP was accumulated more than FCG and MCWG, suggesting that FCGP improves the cultivation method of FCG and MCWG (Fig. 3A).

Phenolic compound was higher in MCWG than FCG, but flavonoid compound was similar in both cultivation methods (Fig. 3B). FCGP produced more bioactive compounds than FCG and MCWG. Flavonoids and phenolic compounds accumulate during the initial growth of ginseng, and the accumulation of ginsenosides depends on the growth period in MCWG [25]. Therefore, FCGP accumulates more useful substances than FCG and MCWG during the growth period of ginseng. Antioxidant activity analysis is shown in Fig. 3C. When the same amount was treated, FCG showed the lowest activity, followed by MCWG, whereas FCGP showed the highest activity.

4. Conclusions

FCGP can manage ginseng for a long time as a non-pesticide method in the field. FCGP formed more lateral roots than FCG and MCWG. More interestingly, FCGP can accumulate a large amount of ginsenoside and bioactive substances. This is a novel method to grow ginseng. Conventional ginseng cultivation has also used containers for experimental purposes and in vitro. Our results differ in that we used pots to create the environment in the open field. In addition, existing cultivation methods utilize artificial moisture supply and pesticides, but our technique managed moisture naturally and pests were consequently suppressed. The pots are structured to draw moisture from the soil at the bottom of the container. Our research demonstrates that if ordinary farmers use such containers, they can reduce the use of manpower by avoiding separate pesticide spraying and moisture management. In addition, there is an advantage that additional costs are not incurred because agrichemicals need not be purchased. The most important point is that the soil is not contaminated, because the container is used, so that continuous cultivation is possible.

Authors' contributions

K.H.H., H.G.K., K.J. and Y.J.K. designed experiments. K.H.H. performed to cultivate FCGP. H.G.K. performed the bioactivity. All authors performed analysis of data and wrote the manuscript.

Declaration of competing interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2021.05.002.

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