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To cite this article: Seong Hyeon Yong , Yeong Rong Seo , Hak Gon Kim , DongJin Park , Yuwon Seol , Eunji Choi , Jeong Hee Hong & Myung Suk Choi (2020): Growth characteristics and saponin content of mountain-cultivated ginseng (*Panax ginseng* C. A. Meyer) according to seed-sowing method suitable for cultivation under forest, Forest Science and Technology, DOI: [10.1080/21580103.2020.1829098](https://doi.org/10.1080/21580103.2020.1829098)

To link to this article: <https://doi.org/10.1080/21580103.2020.1829098>



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Published online: 13 Oct 2020.



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Growth characteristics and saponin content of mountain-cultivated ginseng (*Panax ginseng* C. A. Meyer) according to seed-sowing method suitable for cultivation under forest

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ABSTRACT

Mountain-cultivated ginseng (MCG) is a very important health functional material in Korea and the USA. In this study, the growth and saponin of MCG change according to the sowing method were investigated. Six methods of sowing according to the natural cultivation method that grows in the Sancheong mountain ginseng farm were investigated. Germination rate and budding rate were different according to the sowing method. The growth characteristics of the MCG were also different in the sowing method. Among the six sowing methods, the length of leaves was the widest in BFS. The width of the leaves was wide in DFS and DS. The weight of the shoot was the heaviest in BFS, and the DF was the lightest. Root diameter was highest in BFS and DFS covering both fallen leaves and soil. Total fresh weight was the highest at 4.03 g in BFS, followed by DFS. The S/R ratio of DF was lower than that of the other methods. This suggests that the S/R ratio is lowered because the growth of the ground part is worse. The treatment group with the highest crude saponin content was BF and the lowest treatment group was BS. Shoot and root growth tended to increase with growing period, saponin content increased slightly within 1–3 years, but decreased slightly after that. In particular, the ratio of PD/PT, which has a great influence on the efficacy of ginseng, was also different according to the sowing method. The seed sowing method identified in this study will greatly contribute to the improvement of the survival rate decrease over the cultivation period, and it will also contribute to the production of high-quality MCG.

ARTICLE HISTORY

Received 25 June 2020
Accepted 22 September 2020

KEYWORDS

Mountain-cultivated ginseng; sowing method; germination and budding; saponin

Introduction

Panax ginseng C.A. Meyer (Family *Araliaceae*) is a slowly growing perennial herb that grows wild in South Korea, China, Eastern Siberia. *Panax ginseng* is an important medicinal herb plant in East Asia. In South Korea, *P. ginseng* that cultivated naturally without pesticides and chemical fertilizer in a forest is called “Mountain-cultivated ginseng” (MCG, SanYangSam; Lee 2011). There are direct sowing and transplanting methods for the cultivation of wild ginseng. The cultivation of wild ginseng is mainly used as a transplant cultivation while direct cultivation is used as an auxiliary means (Ko and Im 2009; Kwon et al. 2011).

In addition to the economic downturn in countries around the world, the World Trade Organization (WTO) and the Free Trade Agreement (FTA) have brought a crisis to Korean agriculture. Accordingly it is very urgent to find economical crops that can contribute to improving the income of farm households

and supply them to farm villages. The productivity of mountain farm is low compared to other regional farms in flatland, so it is necessary to develop highly economically efficient crops (Lee 2010).

MCG has become a social issue by the law on forest and was designated as a special product to promote forest management. MCG showed the high market value as a forest product and cultivated increased accordingly in the forest village. In 2018, the production of MCG was 1,30,191 kg, the production amount was 40.9 billion won, and it is increasing every year (Korea Forest Service (KFS) 2018).

The optimal cultivation techniques of MCG are demanded to enhance its quality. The latest studies conducted to investigate the biological activity of *P. ginseng* extracts (Kim and Kim 2005; Bae et al. 2009), genetic differentiation (Choi et al. 2009), and pest development characteristics (Kim et al. 2007). Seo et al. (2007) and Lee (2010) also examined the growth

and photosynthetic properties of *P. ginseng*. However, studies are insufficient yet on the growth and cultivation of wild ginseng grown in mountain areas. Park (1996) reported the importance of developing ginseng or MCG in the forest.

From the previous time, Korean ginseng was evaluated as superior ginseng as the shape of the main root was similar to the human from the brain head, and preferred the transplanting cultivation rather than the direct sowing that caused the loss of the main root (Lee et al. 2005; Li et al. 2010). It is said that cultivation of wild ginseng in Korea is mainly used for transplanting cultivation and direct seeding is used as an auxiliary method, but until now, studies on growth characteristics according to the type of transplantation of wild ginseng have been insufficient (Kwon et al. 2011). Lee (2010) was examined the cultivation of MCG with seed germination and budding rate using seeds and seedling. However, optimized conditions for seed germination in the forest have not been established. Also, Korean wild ginseng seedlings are re-transplanted by picking ginseng 3–5 or 7–8 years after sowing because direct seeding is too low growth index. The study of the cultivation of MCG was not studied much more than the study of pharmacological effects. Therefore, the purpose of this study is to investigate the germination, growth, and saponin content of seeds according to the sowing method of wild ginseng seeds.

Materials and methods

Plant material and experimental sites

Panax ginseng seed was collected from 6-year-old MCG at the Daesan Mountain Cultivated Ginseng Farm, Sancheong-gun, South Korea, August 2012

(Figure 1A). The research site was located in Sancheong-gun, the South Korea (GPS N37°54'28.87", E128°42'34.49"). The area is 300 m above sea level, and the dominant tree species is *Pinus densiflora*.

To reduce the genetic difference between MCG, seeds were collected in one place. After removing the flesh of the cultivated fruit, *P. ginseng* seeds were washed thoroughly, soaked in water for 1 day, and all the long-term ingredients attached to the seeds were extracted, and then shaded for 1 day or more. Seeds were stratified for 100 days for sowing. The stratification was mixed with seeds and sand (1:3), placed in a cage and left until November 29th. The moisture in the cage was always maintained at 15%.

This site was a slope of 15 degree in northern direction. According to Ministry of Agriculture and Forestry (1998a), northern and north eastern directions are appropriate for cultivating MCG. It is reported that the slope suitable for cultivation of MCG is in the range of 5–15 degrees. The measured temperatures in plantation sites were from -8 to 33 °C during the observation period. It showed that the temperatures in the plantation site continuously increased from March to August. The shoot (aerial part) of ginseng begun to appear when the temperature of the plantation site was higher than 15 °C. The measured humidity varied to respond to rainfall in the plantation site. When the plantation site temperature was over 30 °C, the phenomenon of dormancy was clearly shown in MCG. This is because the breeding and growth temperature of MCG is between 5 and 20 °C (Lee et al. 1985). The light intensity of the plantation site was 7500 lux, which was a similar environment than the optimal light intensity (Ministry of Agriculture & Forestry 1998b) when growing MCG.

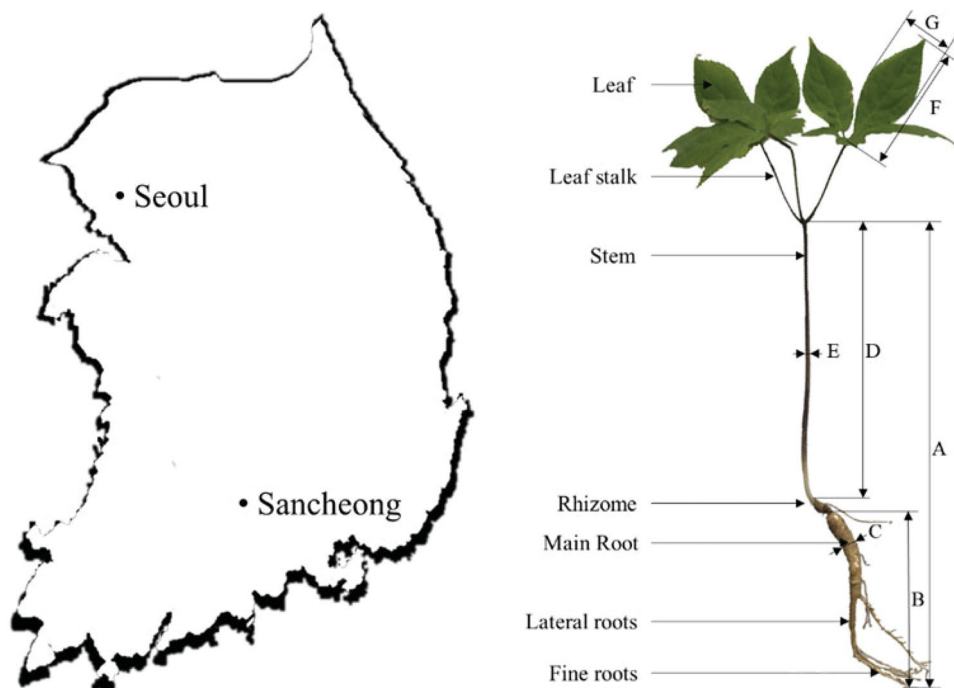


Figure 1. Map showing the research sites and structure MCG and investigate of growth characteristics. (A) Length of total ginseng, (B) length of root, (C) diameter of root, (D) length of shoot, (E) diameter of shoot, (F) length of leaf, (G) width of leaf.

Temperature and humidity data were obtained by installing an automatic weather observation instrument (Datalogger, SELCO HE-170). The temperature of the research site reached 30 degrees from the end of July to the middle of August. The humidity showed a large variation. Especially, the variation of daytime humidity in the winter season was larger than that during the wet season (Figure 2).

The soil characteristics in the plantation site were similar with common characteristics of forests soil of Korea (Table 1). The soil in the plantation site was silt loam soil, and it has shown sub-acidity (pH 4.9). The total nitrogen content in the soil was 0.332%, and the organic content in the soil was 9.85%. Each content of phosphate and potassium in the soil were $9 \text{ mg}\cdot\text{kg}^{-1}$ and $0.33 \text{ cmol}_c\cdot\text{kg}^{-1}$, respectively. Overall, this study site was judged to be a suitable place for sowing experiments of MCG seed.

Experimental design and sowing of MCG

The plot size was $10 \times 20 \text{ m}$ (W \times H). The plots were divided into six equal sections of 3 m for seed sowing experiments, and each test section was spaced at 50 cm. Experimental plot placement was done with randomized block design.

For sowing, the ridge and furrow were prepared for each section. The direction of the ridge was made from the foot of a mountain to the top of the mountain. Ridge raised the soil to both sides of the ridge so that it was 100 cm wide and 30 cm high, and furrow was made 30 cm deep on both sides of the ridge. Sowing was sown on November 30 for seeds that had been opened in open air for 100 days. About 15,000 seeds were used for each treatment.

The method of sowing MCG seeds was the drill method and the broadcast method. The first is a method of drilling a hole in the soil and sowing seeds

at intervals of 10 cm back, forth, left and right. And the broadcast sowing method is to spread 10 g seeds evenly per m^2 . The test site was located in an area with similar soil, slope, light, etc., to reduce the environmental differences as much as possible.

Method 1 is the spreading of seeds all over the prepared field followed by covering with fallen leaves (Broadcast sowing—fallen leaves, BF method). Method 2 is the dropping of seeds on drill and then covered by fallen leaves (Drill sowing—fallen leaves, DF method). Method 3 is spreading of seeds and then cover them with soil. (Broadcast sowing—soil, BS method). Method 4 is the drill sowing and then covered with soil (Drill sowing—soil, DS method). Method 5 is the spreading of seeds and then covered by fallen leaves and soil (Broadcast sowing—fallen leaves and soil, BFS method). Method 6 was covered with fallen leaves and soil after drill sowing (Drill sowing—fallen leaves and soil, DFS method). Seeds sown in two ways were covered with 3 cm thick soil or fallen leaves. After sowing, germination rates, budding rates, and plant growth rates were compared in June.

Measurement of growth appearance

The dormancy of the ginseng cultivated on the method was the beginning of August to the end of February. And, bud emerged at the beginning of March. Seeds were considered to be germinated when both stem and root were 2 mm or more in length. Cumulative germination percentage (%) was recorded every day for 30 days. Germination rates were calculated according to the *Association of Official Seed Analysts Handbook* (AOSA 1983).

Germination percentage (GP)

$$= (\text{Germination seed} / \text{Total seeds}) \times 100$$

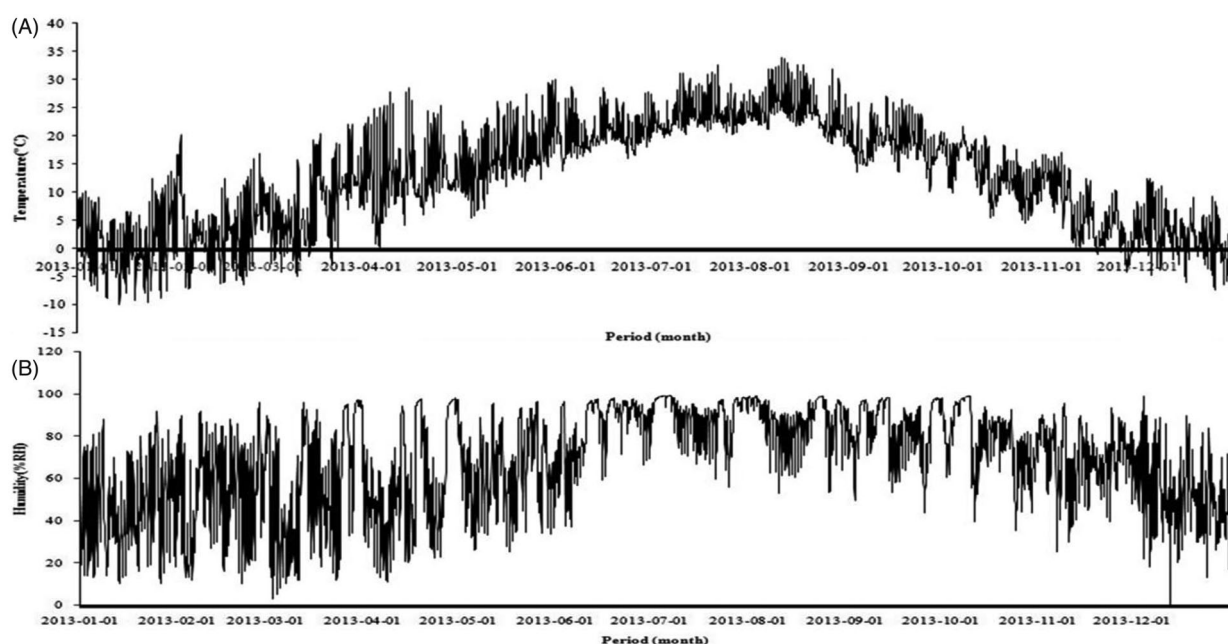


Figure 2. Temperature and humidity of Sancheong MCG site, A: Temperature, B: Humidity. The numbers below indicate the measurement period.ap showing the research sites and structure MCG and investigate of growth characteristics.

Table 1. Soil characteristics of mountainous cultivated ginseng experimental site in Sancheong.

Soil texture		Exchangeable cation (cmol/kg-1)												
Sand (%)	Silt (%)	Clay (%)	Soil texture	pH	Organic matter (%)	Total nitrogen (%)	Available phosphate (mg kg ⁻¹)	CEC (cmol c kg ⁻¹)	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	EC (dS m ⁻¹)	NaCl (%)
50.9	37.9	11.2	L (Loam)	4.9	9.85	0.332	9	16.17	0.33	0.07	0.68	0.28	0.37	0.013

MCG seeds were germinated 2 years after sowing. Therefore, in this study, the germination rate of mountain-grown ginseng was investigated until 2 years after seed sowing. The budding rate was investigated by counting the plants that appeared on the ground in the last week of April every year. The budding rate was calculated as the percentage of the number of shoots sprouted from the above-ground portion.

MCG was measured every year after sticking a stick and marking, and the age of MCG was measured by counting the number of rhizomes. The plant growth indicators are as follows shoot growth (leaf length, leaf width, number of leaves, and fresh weight) and root growth (root length, root diameter, rhizome width, and a number of the root) (Figure 1B). Growth measurement was carried out in August every year by collecting MCG for each experimental site and measured using a vernier caliper (Mitutoyo 500-181-30, Japan). In the case of root measurement, from the lower part of the rhizome to the far end of the main root, the root diameter was measured at the midpoint of the main root. All experiments were carried out in five repetitions every august per year.

Analysis of crude saponin and ginsenoside

The crude saponin extraction and content analysis were carried out using the method of Brain and Turner (1975). Extraction and content analysis of crude saponin was carried out on June 30th every year. 100 mg each was extracted with aqueous ethanol (70%). The obtained extract was concentrated individually under reduced pressure, suspended in distilled water, filtered, and partitioned into ethyl acetate and then *n*-butanol. The butanol fraction was further partitioned with 1% potassium hydroxide to remove the polyphenol compound. The butanol fraction was then concentrated under reduced pressure, dissolved in methanol, precipitated with diethyl ether and the saponin residue was weighed. The total saponin content was determined by the Vanillin Sulfate method to determine the total saponin content of the MCG according to the sowing method. The crude saponin extract obtained above is dissolved in 1.25 ml of methanol and filtered through a 0.45 µm membrane filter. After adding 0.3 ml of 8% vanillin solution and 4 ml of 75% sulfuric acid solution to 100 µl of the filtered saponin extract, the mixture was heated at 60 °C for 10 min to develop color. The absorbance at 545 nm was measured and the total saponin content was determined from the standard curve.

Ginsenosides (Rb2, Rc, Rd, Re, Rf, Rg1, and Rh1) content was extracted by collecting and extracting MCG grown for 5 years for each seeding method, and then analyzed by HPLC. The investigated saponins were examined for the content of Ginsenoside Rb2, Rc, Rd, Re, Rf, Rg1, Rh1 in PD (protopanaxadiol, diol) system, and content of Ginsenoside Rb2, Rc, Rd, Re, Rf, Rg1, and Rh1 in PT (protopanaxatriol, triol) were examined.

Ginsenoside content was expressed as the weight of ginsenoside relative to the root and leaf weight. Extraction methods were based on the protocol of Kim et al. (2015) but were simplified and modified for smaller samples. An accurately weighed sample (100 mg) was transferred to a 15-ml falcon tube. Ginsenosides were extracted in 50% MeOH. Samples were extracted 15 min from the Ultrasonic bath. This process was repeated two times. Then the passage of extraction on Sep-Pak C18 cartridge (Milford, MA). The extract was filtered in 0.2 µm membrane filters

The HPLC separation was carried out on Alliance 2695 HPLC system (waters, USA), equipped with an autosampler and an UV detector using a C18 column (5.0 µm, 4.6–250 mm). Gradient elution was used as solvent A; water and solvent B; acetonitrile at 35 °C using the following gradient program: 0–10 min, 20% B; 10–40 min, 20–32% B; 40–48 min, 32–42% B; 48–50 min, 42–100% B; 50–60 min, 100–20% B; 60–62 min, 20% B. The flow rate was kept at 1 ml/min, the sample injection volume was 20 µl, and UV absorption was measured at 203 nm. Quantitative analysis was performed using a one-point curve method using an external standard of authentic ginsenosides.

Ginsenosides in ginseng have different efficacy depending on the ratio of panaxadiol/panaxtriol (PD/PT). Therefore, the PD/PT ratio according to the seeding method was investigated. Ginsenosides in ginseng have different efficacy depending on the ratio of panaxadiol/panaxtriol (PD/PT). Therefore, the PD/PT ratio according to the seeding method was investigated. PD was calculated by summing all the contents of Ginsenoside Rb2, Rc, Rd, Re, Rf, Rg1, and Rh1, and PT was calculated by summing all of Ginsenoside Rb2, Rc, Rd, Re, Rf, Rg1, and Rh1. The ratio of PD/PT was determined by summing the ginsenosides of the PD and the ginsenosides of the PT based on the results of HPLC content analysis, and dividing the PT content by PD.

Statistical analysis

The experiments were conducted for three times with repetitive results. Data were subjected to statistical analysis by using the SPSS software. One-way analysis of variance (ANOVA) was conducted, and means were compared using Duncan's multiple-range test (DMRT) at 0.05 level of probability. Values were represented as mean ± standard deviation (SD).

Results

Effect of sowing methods on germination and budding

Among MCG seeding methods, DF had the highest germination rate at 18.3%, followed by BF and DFS (Table 2). On the other hand, the germination rate of the DS method was 3.9%, which was the worst for germination.

Table 2. Effects of sowing methods to germination of mountainous cultivated ginseng.

Sowing method	Germination rate (%)
BF	14.9 ± 1.41 ^{b*}
DF	18.3 ± 1.81 ^a
BS	7.5 ± 0.72 ^d
DS	3.9 ± 0.31 ^e
BFS	11.5 ± 1.12 ^c
DFS	10.52 ± 1.11 ^c

BF: Broadcast sowing-fallen leaves; BFS: Broadcast sowing-fallen leaves, soil; BS: Broadcast sowing soil; DF: drill sowing-fallen leaves; DS: drill sowing soil; DFS: Drill sowing-fallen leaves, soil; NS: not significant.

*Values are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$).

$p > 0.05$.

Budding rate also showed different patterns accord-

Table 3. Effects of sowing methods to a budding rate of mountainous cultivated ginseng.

Sowing method	Budding rate (%)			
	2 years	3 years	4 years	5 years
BF	73.6 ± 7.41 ^{a*}	49.6 ± 5.03 ^a	16.1 ± 1.60 ^b	1.6 ± 1.02 ^c
DF	42.1 ± 4.21 ^b	36.8 ± 3.71 ^b	29.9 ± 3.01 ^a	1.6 ± 0.11 ^c
BS	21.3 ± 2.12 ^c	11.2 ± 1.12 ^d	12.8 ± 1.30 ^b	2.1 ± 2.03 ^c
DS	19.2 ± 7.91 ^c	16.5 ± 1.72 ^d	5.9 ± 0.51 ^c	8 ± 0.81 ^b
BFS	42.7 ± 4.21 ^b	35.7 ± 3.61 ^b	33.1 ± 3.32 ^a	11.2 ± 1.13 ^a
DFS	40.52 ± 4.01 ^b	25.08 ± 2.51 ^c	34.12 ± 3.41 ^a	12.8 ± 1.32 ^a

BF: broadcast sowing-fallen leaves; BFS: Broadcast sowing-fallen leaves, soil; BS: broadcast sowing soil; DF: drill sowing-fallen leaves; DS: drill sowing soil; DFS: drill sowing-fallen leaves, soil; NS: not significant.

*Values are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$).

$p > 0.05$.

ing to the seeding method (Table 3). The treatment with the highest budding rate at 2 years of sowing was BF (73.6%), followed by BFS and DF. At 3 years after sowing, the budding rate showed a difference. DFS was the highest. Instead, the BF, which had the highest budding rate in the previous year, was 16.1%, which was low. The budding rate at 5 years was the highest in DFS.

Effect of sowing methods on shoot growth

The growth of MCG was different according to various sowing methods and cultivation period (Table 4). Growth of MCG increased in the second year but did not increase until the fourth year. Growth of MCG was slightly decreased in 4th year and then increased greatly in 5th year. According to the sowing method, the germination rate did not agree with the growth at the shoot. BFS, which had a high initial growth rate, grew well in the third year. However, DFS grew well in the first year, but has not grown well since then.

Effect of sowing methods on root growth

The seedling's sowing method greatly influenced the growth of the mountainous cultivated ginseng root (Table 5). Growth of mountainous cultivated ginseng root was increased with increasing cultivation. The length of ginseng roots increased gradually from 1 to 3 years, but rapidly increased from 4 years. The DF

Table 4. Effects of sowing methods on shoot growth per year of mountainous cultivated ginseng.

Sowing method	Shoot growth (mm)				
	1 year	2 years	3 years	4 years	5 years
BF	33.22 ± 4.76 ^{c*}	68.91 ± 16.61 ^b	78.26 ± 4.97	67.51 ± 12.72 ^b	187.03 ± 9.53 ^a
DF	34.26 ± 1.4 ^c	92.09 ± 14.62 ^a	69.46 ± 8.79	43.37 ± 16.57 ^c	113.76 ± 11.97 ^c
BS	33.99 ± 1.6 ^c	71.22 ± 4.45 ^b	66.84 ± 22	44.25 ± 8.43 ^c	134.46 ± 10.84 ^b
DS	37.82 ± 1.41 ^{bc}	67.68 ± 7.29 ^b	73.79 ± 6.62	51.63 ± 16.69 ^{bc}	182.67 ± 15.66 ^a
BFS	40.56 ± 4.87 ^b	83.04 ± 9.69 ^{ab}	74.41 ± 6.57	93.13 ± 21.33 ^a	183.34 ± 12.77 ^a
DFS	65.73 ± 5.03 ^a	81.56 ± 18.34 ^{ab}	63.47 ± 11.6	69.89 ± 15.35 ^b	138.21 ± 14.87 ^b

BF: broadcast sowing-fallen leaves; BFS: broadcast sowing-fallen leaves, soil; DF: drill sowing-fallen leaves; BS: broadcast sowing soil; DS: drill sowing soil; DFS: Drill sowing-fallen leaves, soil; NS: not significant.

*Values are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$). $p > 0.05$.

Table 5. Effects of sowing methods on root growth per year of mountainous cultivated ginseng.

Sowing method	Root growth (mm)				
	1 year	2 years	3 years	4 years	5 years
BF	30.85 ± 3.30 ^a	34.07 ± 9.16 ^b	37.75 ± 10.47	55.02 ± 8.69 ^b	58.65 ± 5.60 ^c
DF	29.75 ± 2.12 ^a	51.32 ± 8.20 ^a	29.89 ± 7.41	56.01 ± 7.35 ^b	49.44 ± 12.01 ^c
BS	31.14 ± 1.98 ^a	27.47 ± 4.19 ^{bc}	29.57 ± 5.53	50.95 ± 18.31 ^{bc}	72.57 ± 9.30 ^b
DS	31.93 ± 1.93 ^a	20.30 ± 3.95 ^c	30.73 ± 7.22	36.31 ± 14.43 ^c	58.16 ± 12.00 ^c
BFS	32.24 ± 1.98 ^a	23.63 ± 5.11 ^c	32.81 ± 9.07	56.65 ± 13.98 ^b	79.08 ± 8.82 ^{ab}
DFS	23.51 ± 1.90 ^b	32.85 ± 7.39 ^b	29.98 ± 3.76	74.5 ± 12.52 ^a	86.81 ± 11.11 ^a

BF: broadcast sowing-fallen leaves; BFS: broadcast sowing-fallen leaves, soil; BS: broadcast sowing soil; DF: drill sowing-fallen leaves; DS: drill sowing soil; DFS: drill sowing-fallen leaves, soil; NS: not significant.

*Values are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$). $p > 0.05$.

Table 6. Growth characteristics after 5 years of mountainous cultivated ginseng sown by six methods^a.

Sowing method	Leaf length (mm)	Leaf width (mm)	Number of leaf (no.)	Shoot fresh weight (g)
BF	58.41 ± 2.36 ^{b*}	23.48 ± 0.95 ^b	13.80 ± 0.97	1.16 ± 0.01 ^d
DF	51.97 ± 1.72 ^{bc}	27.34 ± 0.86 ^a	14.40 ± 0.4	0.79 ± 0.05 ^e
BS	56.30 ± 1.86 ^{bc}	27.70 ± 1.17 ^a	14.20 ± 0.37	1.12 ± 0.02 ^d
DS	50.46 ± 3.77 ^c	28.40 ± 1.05 ^a	14.40 ± 0.40	1.31 ± 0.06 ^c
BFS	71.02 ± 1.99 ^a	19.83 ± 1.61 ^c	13.60 ± 0.98	2.03 ± 0.07 ^a
DFS	67.85 ± 1.55 ^a	28.39 ± 1.34 ^a	13.80 ± 0.97	1.64 ± 0.06 ^b

BF: broadcast sowing-fallen leaves; BFS: broadcast sowing-fallen leaves, soil; BS: broadcast sowing soil; DF: drill sowing-fallen leaves; DS: drill sowing soil; DFS: drill sowing-fallen leaves, soil; NS: not significant.

*Values are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$). $p > 0.05$.

method showed the best growth in the first and second year, but after that the growth was gentle. On the other hand, DFS showed very strong growth from the third year on, although root growth was not good until the third year.

Growth characteristics by sowing methods

The growth characteristics of the MCG were also different in the sowing method (Tables 6 and 7). Among the six sowing methods, the length of leaves was the widest in BFS and the narrowest in DS. The width of leaves was wide in DFS and DS and narrow in BFS. The number of leaves was the highest in DF and BS, and the lowest in BFS. The weight of the shoot was the heaviest of the BFS, and the DF was the lightest.

Root diameter was highest in BFS and DFS covering both fallen leaves and soil. Rhizome height was higher in DS treatments. In the case of underground biomass, BFS was the highest at the same time. In the undergrowth, the weight of the DS treatments was lower than that of the top shoots. Total fresh weight was the highest at 4.03 g in BFS, followed by DFS. The S/R ratio, which is the weight ratio between the top and bottom of the plant, shows the balance between growth

at the top and bottom. Among the six sowing methods, the S/R ratio of DF was lower than that of the other methods. This suggests that the S/R ratio is lowered because the growth of the ground part is worse.

The length of the stem, the size of the leaves, and the color of the leaves were slightly different.

Profiling of MCG saponin by sowing methods

Contents of the major ginsenosides, Rb2, Rc, Rd, Re, Rf, Rg1, and Rh1 were quantified using HPLC for MCG grown through six types different sowing methods. Additionally, the chromatogram is shown for each of the ginseng ginsenoside according to sowing methods (Figure 3). The profiling of ginsenosides did not show any significant difference by sowing method. However, the peak height of ginsenoside was slightly different.

Total saponin, PD/PT ratio by sowing methods treatment

There was no significant difference in the total saponin content of the MCG according to the sowing method (Table 8). Total saponin content of MCG was not significantly different from that of cultivation studies.

Table 7. Root growth of mountainous cultivated ginseng to the different sowing methods during experimental period^a.

Sowing method	Root diameter (mm)	Rhizome height (mm)	Root fresh weight (g)	Total fresh weight (g)	S/R ratio
BF	7.51 ± 0.17 ^{bc*}	7.93 ± 0.15 ^b	1.16 ± 0.02 ^c	2.31 ± 0.03 ^c	1.00
DF	7.34 ± 0.36 ^{bc}	8.24 ± 1.3 ^b	1.02 ± 0.04 ^d	1.8 ± 0.06 ^d	0.78
BS	6.53 ± 0.28 ^c	7.83 ± 0.15 ^b	1.17 ± 0.02 ^c	2.29 ± 0.03 ^c	0.96
DS	8.36 ± 0.17 ^{ab}	11.15 ± 1.14 ^a	1.09 ± 0.04 ^{cd}	2.41 ± 0.07 ^c	1.21
BFS	8.98 ± 0.24 ^a	6.19 ± 0.13 ^{bc}	2.01 ± 0.05 ^a	4.03 ± 0.06 ^a	1.02
DFS	8.82 ± 0.61 ^a	4.22 ± 0.18 ^c	1.29 ± 0.06 ^b	2.93 ± 0.06 ^b	1.29

NS: not significant.

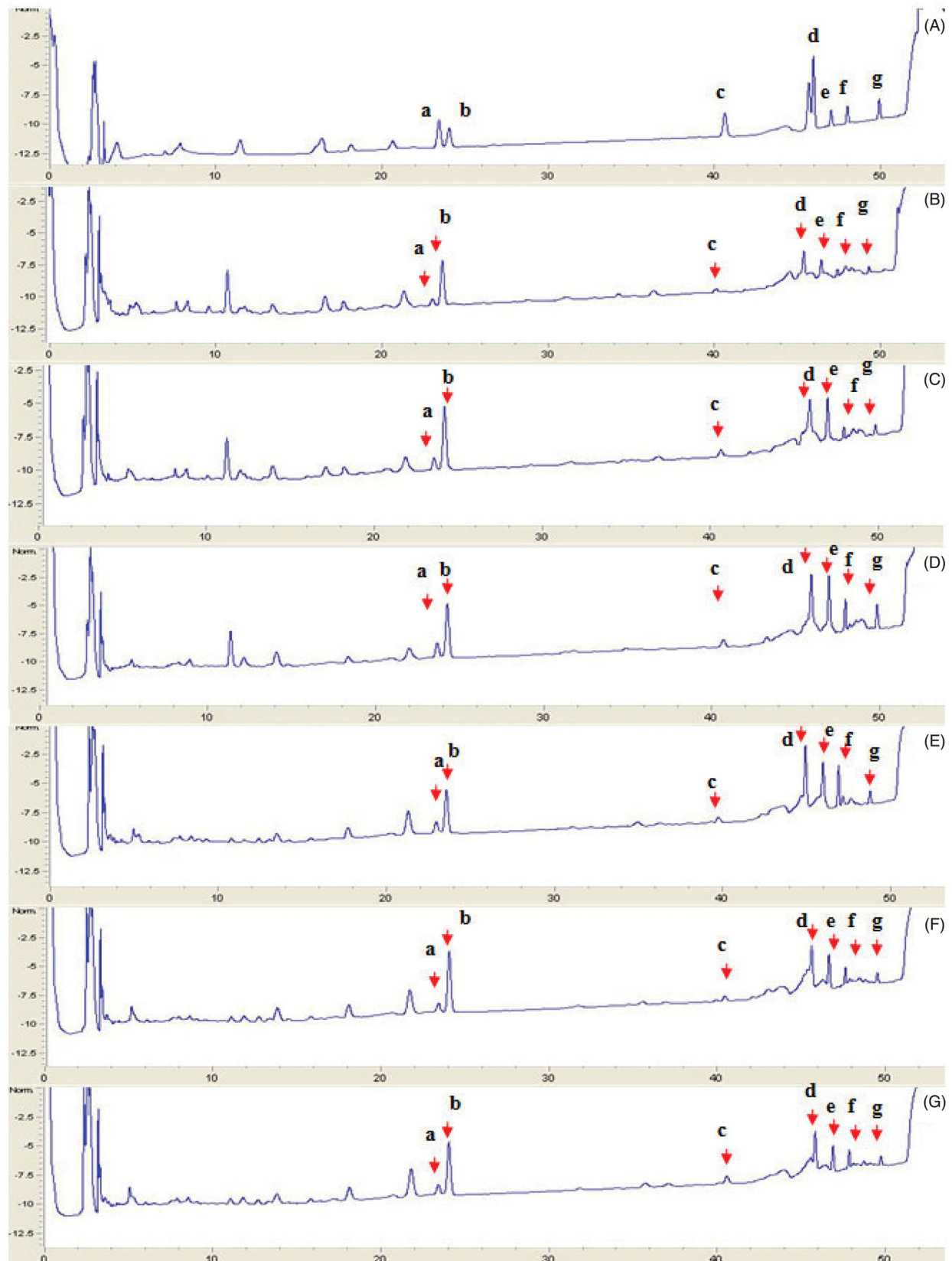
^aValues are means of five replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$). $p > 0.05$.**Figure 3.** HPLC chromatograph profiles of ginsenosides detected from the MCG on various seeding methods. a: Rg; b: Re; c: Rf; d: Rh1; e: Rg; f: Rb2; g: Rd; A: standard; B: BF; C: DF; D: BS; E: DS; F: BFS; G: DFS.

Table 8. Change in total saponin content of mountainous cultivated ginseng grown by different sowing methods for 5 years.

Sowing method	Total saponin content (mg/g DW)				
	1 year	2 years	3 years	4 years	5 years
BF	1.35 ± 0.07 ^{d*}	1.91 ± 0.13 ^a	1.81 ± 0.09 ^b	1.48 ± 0.07 ^a	1.98 ± 0.11 ^a
DF	2.34 ± 0.12 ^b	1.4 ± 0.07 ^{c,d}	1.25 ± 0.06 ^c	1.48 ± 0.07 ^a	1.68 ± 0.08 ^b
BS	2.93 ± 0.15 ^a	1.46 ± 0.08 ^c	1.04 ± 0.05 ^d	1.12 ± 0.06 ^c	1.31 ± 0.07 ^c
DS	1.64 ± 0.09 ^c	1.82 ± 0.09 ^a	1.79 ± 0.09 ^b	1.55 ± 0.08 ^a	1.35 ± 0.07 ^c
BFS	1.33 ± 0.07 ^d	1.27 ± 0.07 ^d	1.97 ± 0.12 ^a	1.20 ± 0.06 ^{bc}	1.44 ± 0.07 ^c
DFS	1.33 ± 0.07 ^d	1.62 ± 0.08 ^b	1.81 ± 0.09 ^b	1.31 ± 0.07 ^b	1.72 ± 0.09 ^b

NS: not significant.

*Values are means of three replicates. Means followed by the same letters are not significantly different by Duncan's test ($p < 0.05$). $p > 0.05$.

The treatment group with the highest total saponin content was BF and the lowest treatment group was BS.

This result is consistent with the report that the total saponin content increases with increasing number of years of growth even in cultivation of ginseng (Kim et al. 1995). However, it was reported that the content of saponin did not increase in 5 years or older, but it is unknown in MCG. This will have to be studied later.

The content of major ginsenosides also varied depending on the sowing method (Table 9). The content of total ginsenosides in the BS and DF methods (0.293%, 0.234%) is much higher than that in other ginseng of sowing methods. But the content of ginsenoside in BFS and DFS methods (0.133%, 0.133) is relatively low. In MCG, ginsenoside gradually increased with the growth period (Table 9). Among the first-year MCG, Rc was the most ginsenoside, followed by Rb2, Rf, and Rd. The most ginsenosides contained in 5-year-old MCG were Rd, followed by Rc and Re. Rd was the most weighted ginsenoside, but Rh1 did not change over time.

The PD/PT ratio also appeared to vary depending on the sowing method (Table 9). PD/PT was 0.98–2.18 in the first year and 1.99–4.16 in the fifth year. The proportion of these was higher in the fifth year than in the first year. Total contents of seven ginsenosides were 0.133–0.23 in first year and 1.2–1.55 in fifth year. However, the PD/PT ratio can vary depending on the type and number of standard substances used in the analysis, so it is difficult to see them as absolute values. It is clear, however, that their proportions vary depending on the sowing method.

The relationship between growth of production of saponin

The relationship between stem and root growth and total saponin content was examined (Figure 4). Stem growth tended to increase with the cultivation period. Stem growth increased very slowly until 1–4 years but increased rapidly between 4 and 5 years.

The growth of roots gradually increased as the cultivation study period increased. In particular, root growth was slightly different according to the sowing method except DFS, but there was no increase of 4–5 years as the stem.

Total saponin content did not change significantly with increasing cultivation studies. The total content of

saponin was slightly increased in 1–3 years, but a slight decrease was observed after that. However, there was no change in stem and root growth.

Discussion

Germination and budding rate of MCG were different according to the sowing method. The high germination and budding rate in the second year after the first year seem to be the place where the dormancy of the seeds of the mountainous cultivated ginseng has been destroyed. The results of this study are consistent with the results of Seo (2010) that the germination rate continues to increase until the second year after the first year after seed sowing of MCG.

However, in all the sowing methods, the sharply lowered germination rate or budding rate after 4 years was closely related to the survival rate of wild ginseng seedlings. This is the biggest problem of cultivation of wild ginseng, and it means that cultivation of MCG is difficult. When grown through sowing, wild ginseng is said to have a very low survival rate in its natural state, with a 5-year survival rate of less than 5% (personal communication) and a survival rate of 0% after 12–13 years (Ministry of Agriculture and Forestry 1998b). In our study, the survival rate reached 20% after 5 years when sowing by DFS method. This is a much higher survival rate than the results of the aforementioned study.

The reason why the sowing rate of seeds sown by DFS method remained high after 4 years seems to be that the maintenance of moisture in soil, the maintenance of physical properties of soil, and the supply of appropriate nutrients were well done. In other words, it is considered that leaves and soil covered after seed sowing play a very important role in seed germination rate and sowing rate. Soil litter reduces wind erosion by preventing soil from losing moisture and providing cover preventing soil transportation (Chanasyk et al. 2003). Especially, when the recalcitrant or unorthodox seeds are dried below 20–40% moisture content, the seed germination has a moisture content such that it cannot germinate (Hay et al. 2000). Greenfield and Davis (2003) reported that it is advisable to spray solid bark, aged leaves, or compost sawdust in the presence of 1–2 inches immediately after planting to maintain soil moisture and winter protection in American ginseng cultivation.

Table 9. The ginsenoside contents in mountainous cultivated ginseng on various sowing methods.

Ginsenoside	Sowing methods												Mean ^a						
	BF			DF			BS			DS			BFS			DFS			
	1st	5th	Mean	1st	5th	Mean	1st	5th	Mean	1st	5th	Mean	1st	5th	Mean	1st	5th	Mean	
Rb1	0.014 ± 0.04 ^b	0.145 ± 0.01	0.034 ± 0.08	0.147 ± 0.01	0.041 ± 0.01	0.105 ± 0.01	0.028 ± 0.03	0.131 ± 0.01	0.020 ± 0.01	0.125 ± 0.01	0.020 ± 0.01	0.028 ± 0.03	0.131 ± 0.01	0.020 ± 0.01	0.125 ± 0.01	0.020 ± 0.01	0.117 ± 0.01	0.03	0.13
Rc	0.051 ± 0.24	0.217 ± 0.01	0.098 ± 0.27	0.29 ± 0.01	0.118 ± 0.04	0.195 ± 0.01	0.075 ± 0.32	0.24 ± 0.01	0.039 ± 0.01	0.222 ± 0.01	0.039 ± 0.01	0.075 ± 0.32	0.24 ± 0.01	0.039 ± 0.01	0.222 ± 0.01	0.039 ± 0.01	0.235 ± 0.01	0.07	0.23
Rd	0.009 ± 0.02	0.698 ± 0.01	0.019 ± 0.08	0.711 ± 0.01	0.027 ± 0.01	0.6 ± 0.01	0.100 ± 0.03	0.878 ± 0.01	0.011 ± 0.01	0.469 ± 0.01	0.011 ± 0.01	0.100 ± 0.03	0.878 ± 0.01	0.011 ± 0.01	0.469 ± 0.01	0.011 ± 0.01	0.522 ± 0.01	0.03	0.65
Re	0.046 ± 0.05	0.268 ± 0.01	0.065 ± 0.06	0.174 ± 0.01	0.007 ± 0.04	0.112 ± 0.01	0.004 ± 0.02	0.149 ± 0.01	0.006 ± 0.08	0.233 ± 0.01	0.006 ± 0.01	0.004 ± 0.02	0.149 ± 0.01	0.006 ± 0.01	0.233 ± 0.01	0.006 ± 0.01	0.265 ± 0.01	0.02	0.20
Rf	0.005 ± 0.01	0.052 ± 0.01	0.006 ± 0.01	0.066 ± 0.01	0.060 ± 0.01	0.028 ± 0.01	0.038 ± 0.01	0.046 ± 0.01	0.048 ± 0.02	0.041 ± 0.01	0.048 ± 0.02	0.038 ± 0.01	0.046 ± 0.01	0.048 ± 0.02	0.041 ± 0.01	0.048 ± 0.02	0.049 ± 0.01	0.03	0.05
Rg	0.010 ± 0.04	0.094 ± 0.01	0.100 ± 0.01	0.08 ± 0.01	0.010 ± 0.01	0.068 ± 0.01	0.007 ± 0.01	0.095 ± 0.01	0.007 ± 0.01	0.096 ± 0.01	0.007 ± 0.01	0.007 ± 0.01	0.095 ± 0.01	0.007 ± 0.01	0.096 ± 0.01	0.007 ± 0.01	0.112 ± 0.01	0.02	0.09
Rh1	0.001 ± 0.01	0.013 ± 0.01	0.003 ± 0.01	0.018 ± 0.01	0.029 ± 0.20	0.013 ± 0.01	0.002 ± 0.01	0.011 ± 0.01	0.001 ± 0.01	0.015 ± 0.01	0.001 ± 0.01	0.002 ± 0.01	0.011 ± 0.01	0.001 ± 0.01	0.015 ± 0.01	0.001 ± 0.01	0.011 ± 0.01	0.01	0.01
PD/PT ^c	1.216	2.478	1.817	3.402	1.745	4.062	2.182	4.154	1.147	2.124	1.147	2.182	4.154	1.147	2.124	1.147	1.999	2.16	3.04
T.S ^d	0.135	1.480	0.234	1.480	0.293	1.120	0.164	1.550	0.133	1.200	0.133	0.164	1.550	0.133	1.200	0.133	1.310	0.55	1.36

BF: broadcast sowing-fallen leaves; BFS: broadcast sowing-fallen leaves; DF: drill sowing soil; DS: drill sowing soil; DFS: drill sowing-fallen leaves; soil.

^aAccording to each sowing method, the average content of ginsenosides of MCG is determined after 1 and 5 years.

^bValues represent the mean ± S.D (n = 3).

^cPD: Ginsenoside Rb2 + Rc + Rd/Re + Rf + Rg1 + Rh1, PT: Ginsenoside Rb2 + Rc + Rd + Re + Rf + Rg1 + Rh1.

^dT.S. means total saponin.

The growth of germinated ginseng was also different depending on the sowing method. The growth of the stems of MCG was better to cover the leaves than the soil alone. Layers of fallen leaves create a compost that enriches the soil by providing a nutrient source for the microorganisms in the soil, and in turn provides nutrients (fertilizers) for the plants. This process builds the topsoil layer and soil's ecosystem, both of which are essential for healthy plants. The leaf layer also prevents rain drops from eroding the topsoil, regulates soil temperature, shades plant roots, and preserves moisture by preventing the sun from drying out the soil. Plant litter is the main source of carbon, energy and nutrients for heterotrophic microbial communities in the litter layer in terrestrial ecosystems (Li et al. 2014; Vivanco et al. 2018). Among the various sowing methods to MCG, fallen leaves covered after sowing was found to be very much beneficial in enhancing shoot.

Total saponin content of MCG was not significantly different from that of sowing method. It was reported that the medical effects of various ginsengs should be evaluated using the ratio of panaxadiol/panaxatriol (PD/PT) and the absolute saponin content (Han 1974). It is known that the protopanaxadiol (diol) system, which acts as an inhibitor of the central nervous system, and the protopanaxatriol, which acts as a central nervous system, have different pharmacological effects in the body. Korean ginseng, which is known to have the best medicinal effect, had a PD/PT ratio of about 1.5, and American ginseng reported about 2.0 and *Panax notoginseng* (Burk) F.H.Chen. Kim et al. (1987) stated that the PD/PT ratio of the ginseng is higher than that of the main root of ginseng, and the PD/PT ratio increases as the ginseng's age increases. In this study, the PD/PT ratio was significantly different from those previously reported.

Our research shows that the saponin in the roots increases rapidly in the PD saponin. PD is known to be widely distributed in the fine roots and cortex of ginseng (Park et al. 1986) and is consistent with our research results.

According to the sowing method, the production of medicinal plants showed a great difference. Han et al. (2000) also found that the drill sowing increased by 19% compared to the broadcast sowing. Yang et al. (2014) reported the difference between direct ginseng seeds and transplanted ginseng as a result of profiling of metabolites of ginseng. Also, the growth of medicinal pumpkin and the yield of oil varies according to the sowing method (Bahlgerdi et al. 2014). In the broadcast sowing of seeds, the seeds were not sown in the entire plantation area, resulting in a dense plant partly, and the growth was not constant. And drill sowing seems to be more advantageous than broadcast sowing in management of plantation area.

Saponin content was highest in BF treatment. The BF treatment was a treatment where shoot and root growth were not so good. It is presumed that the biosynthesis of plant secondary metabolites is inversely related to growth. Rühmann et al. (2002) reported that there is a reverse correlation between phenolic compound

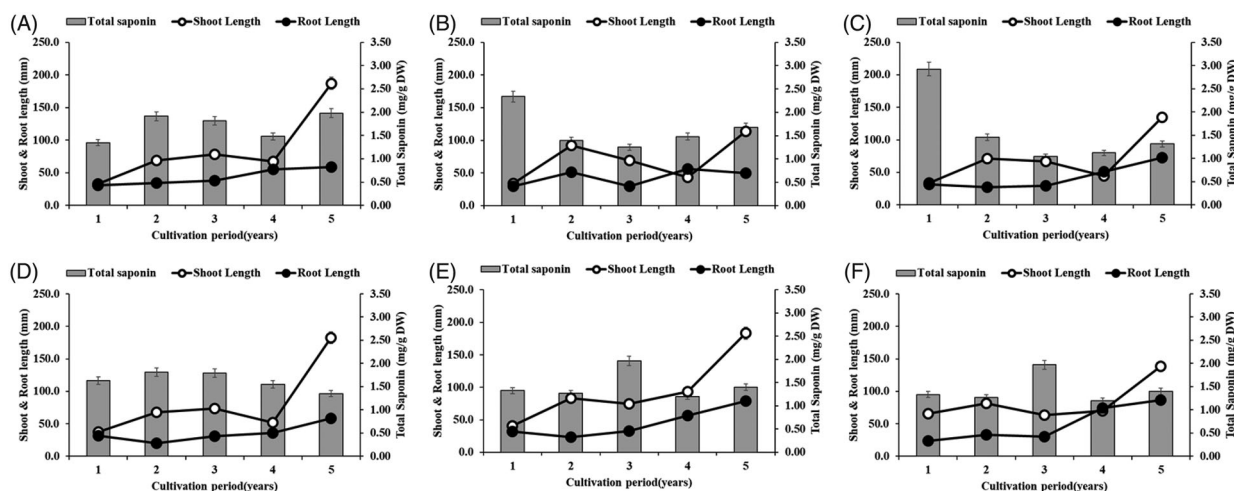


Figure 4. Growth and saponin content change according to sowing method. A: BF; B: DF; C: BS; D: DS; E: BFS; F: DFS.

biosynthesis and stem growth in apple tree young leaves. Perhaps the early growth inhibition of MCG may have affected stem and root growth and saponin biosynthesis. However, further study is required.

The reason for the difference in the growth of the root and the root of the MCG according to the seed sowing method may be the influence of the inorganic nutrients of the soil. Nutrients affect plant growth and survival of plants (Albert and Kinzel 1973; Chae et al. 2006). Especially, the presence and variations in the amount of inorganic salts in the form of macro-elements and micro-elements are known to affect the productivity of plants and the content of useful substances (Orians et al. 2003).

In our study, it was also found that the aforementioned inorganic salts influenced the growth of MCG depending on the seed sowing method. When comparing the soil environment analysis data of this research site (Table 1) with the cultivation site data of MCG (Ministry of Agriculture and Forestry 1998a), the pH of the research site is as low as 4.9, but the organic matter content is high as 9.85%. Also, the effective phosphoric acid is 9 mg/kg, which is much lower than the 100–200 mg/kg content of the area called the cultivation site. Ca is 0.68 mg/kg and Mg is very low, 0.28 mg/kg. Therefore, it seems that such changes in growth, saponin content and PD/PT ratio occurred. However, these results require more detailed investigation of each factor.

As a result, it was confirmed that the growth and saponin content of MCG were greatly influenced by the sowing method. MCG is mainly used for roots, and DF method and DFS method are good for root growth. In other words, drilled sowing method is better than broadcast sowing method. Saponin content was higher in the broadcast sowing method, which was not good for growth. MCG, which has a climate similar to that of Korea, germinates in March and has a unique feature that the above ground tissue dies in July–August, in summer. Therefore, little research has been conducted on the growth characteristics and saponin biosynthesis of MCG.

This study could contribute to understanding the MCG by observing the entire cycle from seed germination of the cultivated ginseng to the disappearance of the ground for 5 years. But above all, this study will contribute to increase productivity by direct sowing of MCG. Above all, the seed sowing method identified in this study is a significant improvement in the previous method of replanting seedlings, as the survival rate has decreased too much after 4 years of direct sowing. In addition, the sowing method also changed the PD/PT ratio. However, the biosynthesis of ginsenosides according to the sowing method requires further study. Therefore, this method will greatly contribute to overcoming the problems of agricultural power when growing wild ginseng. This result will contribute to the mass production of high-quality MCG in a short time.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was supported by the support of the Forestry Science and Technology Research and Development Project (Forest Convergence Specialist Training Project, Project No. 2020186A00-2022-AA02) in 2020.

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