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Changes in growth characteristics and ginsenoside contents of wild-simulated ginseng with different harvest times in South Korea

Yeong-Bae Yun[1], Hae-Yun Kwon[1] and Yurry Um[1*]

Abstract

Wild-simulated ginseng (WSG, *Panax ginseng* C.A. Meyer) is grown in mountainous forests, without the chemical treatment or installation of artificial facilities. This study aimed to investigate monthly changes in growth characteristics and ginsenoside contents in WSG to suggest the optimal harvest time. Four-year-old WSG were collected in the same area every month, and their growth characteristics and ginsenoside contents were measured. The growth characteristics of aerial and root parts were measured from May to July and from March to December, respectively. For the aerial part, most growth characteristics of WSG decreased over time, except for stem length. For the root part, rhizome length increased over time except for September, while the root diameter and weight of root part were mostly consistent. The root length increased by September, while the number of rootlets was the highest at May. At July, the total ginsenoside content of WSG was significantly the highest, while the total ginsenoside content at October was the lowest. This result was believed to be due to the F2, Rd, and Rg1 contents of the aerial part, rather than the root part. Also, based on these growths and the ginsenoside contents of WSG, the optimal harvest time for WSG is considered to be late spring–summer (May–July) when the aerial part can be identified.

Keywords Correlation analysis, High-performance liquid chromatography, Optimal harvest period, *Panax ginseng* C.A. Meyer, Spearman's coefficient analysis

Introduction

Medicinal plants have played a critical role in human health with their beneficial effects and their use to treat various types of diseases or ailments has been around since ancient times [36]. Ginseng (*Panax ginseng* C.A. Meyer) is one of the oldest medicinal plants and it belongs to family Araliaceae. Genus Panax contains 11 species, including *P. trifolius*, *P. notoginseng*, *P. pseudoginseng*, *P. ginseng*, *P. quinquefolius*, *P. stipuleanatus*, *P. zingiberensis*, *P. japonicus*, *P. japonicus* (var. *major*, var. *bipinnatifidus*, var. *angustifolius*) and they are distributed in the Eastern Asia and Northern America. Ginseng prospers in the mountain forest environments of the northern temperate area, therefore, it is cultivated or harvested in Korea, Japan, China, Russia, and America [38, 48]. With the need for cultivation arising from indiscriminate collection, there has been increasing research into cultivation techniques and sites for wild-simulated ginseng (WSG), which is grown under near-natural conditions in forests, and the eventual industrialization of WSG.

Ko and Leem [20] reported that the cultivation environments, such as soil properties, topography, and climate, affects the pharmacological activity of ginseng.

*Correspondence:
Yurry Um
urspower@korea.kr
[1] Forest Medicinal Resources Research Center, National Institute of Forest Science, Yeongju 36040, Republic of Korea

Recently, ginseng planted in forests is termed mountain-cultivated ginseng (MCG), while ginseng and MCG seeds grown under natural light and without chemically synthesized pesticides and fertilizers treatment are termed as WSG. WSG is “specially managed forest product” under the [Forestry and Mountain Villages Developments Promotion Act] established by Korea Forest Service (KFS). Therefore, the growth and active components of WSG may be affected by climate change and cultivation environments. The air temperature and solar radiation in shrub layer were significantly correlated with the growth of WSG [18]. Furthermore, soil properties and soil bacterial community can be affected to the growth of WSG in coniferous and mixed forests [17]. The main bioactive components of WSG and ginseng are ginsenosides and polyacetylenes, ginseng proteins, polysaccharides, and phenolic compounds [34, 37]. Many studies have focused on characterization of the ginsenosides and investigation of pharmacological activity [25, 48], since they have been reported to exhibit biochemical and pharmacological effects, and their chemical structures were elucidated by Shibata et al. [40]. Based on their chemical structure, they can be classified into protopanaxadiol (PD)-based, protopanaxatriol (PT)-based, and oleanane-based ginsenosides. On the other hand, since WSG is expected to have a higher ginsenoside content compared to the cultivated ginseng, WSG is known to have better pharmacological effects than cultivated ginseng and is considered to be highly effective in herbal prescriptions [30, 32].

Although the content of ginsenosides was lower in cultivated plants that were aggregated from field soil, it is reported that ginsenosides treat many chronic diseases and modulate various physiological activities [24]. One of the various advantages of ginsenosides is their ability to interact with target molecules in the cells resulting in combined pharmacologically beneficial effects [13, 45]. WSG naturally grown in China and Korea showed greater health benefits [29]. WSG has been reported to have pharmacologically active effects, such as anticancer [8, 29], blood pressure control [33], antioxidant activity [12], liver function improvement [22], and blood lipid reduction [46]. Recently, various studies have been conducted on the ginsenoside composition of WSG and cultivated ginseng according to the age and cultivation region [6, 9, 23]. The composition and quality of ginsenosides can be influenced by various factors such as age, species, cultivation method, preservation method, part of the plant, and harvesting season [29, 39]. Furthermore, Park et al. [35] reported that metabolic pathways affected by different cultivation age were involved in amino acid metabolic pathways. However, although the appropriate harvest time for WSG must be determined in order to increase profits for foresters and medicinal efficacy of WSG, no studies have been conducted on WSG according to harvest time. Therefore, this study aimed to investigate and compare the growth characteristics and ginsenoside contents of 4-year-old WSG collected monthly to identify the optimal harvest time.

Materials and methods

Collection of WSG samples

In this study, four-year-old WSG samples were collected from the Bonghwa (GPS information N 36°51′08.3″, E 128°55′42.6″) managed by the National Institute of Forest Science (NIFoS) from March to December 2022, and 30 samples were collected each month. The collected samples were washed with distilled water, and naturally dried at room temperature until the surface moisture was removed. After measuring the growth characteristics of the WSG samples, all samples were stored at −70℃. The samples were dried in a freeze-dryer, ground using a mortar and pestle, and the powder that passed through an 80-mesh standard sieve was stored at −70℃, and used as a sample for component analysis.

Investigation of the growth characteristics of WSG

The growth characteristics of 4-year-old WSG collected at different harvest times was carried out according to the test guidelines (ginseng), including stem length, stem diameter, number of leaflets per stem, petiole length, leaflet length, leaflet width, aerial weight, rhizome length, root length, root diameter, number of rootlets, and root weight were measured [21].

WSG sample extraction and reagents

For the analysis of ginsenosides in 4-year-old WSG collected at different harvest times, 0.2 g of powder sample was subjected to 10 mL of 70% methanol, followed by ultrasonic extraction (JAC-5020, KODO, Korea) for 30 min. The extract was centrifuged (Labogene, BMS, Korea) for 10 min, the supernatant was filtered through a 0.2 μm membrane filter (Whatman Syringe Filter, Maidstone, United Kingdom), and the filtrate was diluted tenfold with distilled water for analysis. Ginsenoside standards used in the analysis were purchased from Chemfaces (China). Methanol, acetonitrile, and sterile distilled water used in the extraction and HPLC analysis were purchased from J.T. Baker (Easton, PA, USA).

Analyzing the ginsenoside content of WSG

The quantitation of ginsenosides was conducted by a LC–ESI–MS/MS system (LCMS-8050 system, Shimadzu, Japan) in the negative mode electrospray ionization. LC condition was follows; LC separation was on a C18 column (Cortecs®UPLC®T3 1.6um, 2.1 × 150 mm, Waters, USA) using gradient elution with solvent A (0.1%, v/v, formic acid in water) and solvent B (0.1%, v/v, formic acid in acetonitrile including 10% methanol). The gradient elution was conducted 35% solvent B (0.5 min), 40% solvent B(6.0 min), 45% solvent B (7.0 min), 70% solvent B (14.0 min), 75% solvent B (16.0 min) and 95% solvent B (16.5 min). The elution flow rate was 0.45 mL/min and sample injection volume was 1.0 μL. The temperature conditions in the mass spectrometer were interface temperature 300 ℃, the desolvation temperature 250 ℃, heating block 400 ℃. Gas conditions were nebulizing nitrogen gas 3.0 mL/min, heating nitrogen gas 10.0 mL/min, drying nitrogen gas 10.0 mL/min. Precursors and products ions of ginsenoside standards in mass spectrometry were determine by an automated process in LC/MS spectrometer, and also MRM (multiple reaction monitoring) conditions of ginsenoside standards were determined such as Q1 pre-bias voltage, dwell time, collision energy and Q3 pre-bias voltage, and then these conditions collected were applied to ginsenoside analyses of samples. Quantitation of ginsenoside was conducted by an internal (digoxin) linear regression method to peak area. Both information peak area at a unique retention time and products ions information were used for each ginsenoside identification and quantitation.

Statistical analysis

The analyzed data values of growth characteristics and ginsenoside contents of WSG were expressed as the means ± standard error (S.E.). The mean of each experimental value was tested by one-way ANOVA and Duncan's Multiple Range Test (DMRT) using IBM SPSS statistics (version 25, IBM Corp., Armonk, NY, USA) at a significance level of 5% ($p < 0.05$). Correlation analyses between growth characteristics and ginsenoside content of 4-year-old WSG by harvest time were conducted for significance ($p > 0.05$) with Spearman's coefficient (r) using IBM SPSS statistics.

Results

Growth characteristics of WSG by harvest time

We analyzed the growth characteristics of 4-year-old WSG collected monthly from March to December 2022 in the Bonghwa managed by the National Institute of Forest Science. Aerial parts were collected from May to July of the same year, with stem length (10.17 ~ 12.99 cm), stem diameter (1.38 ~ 1.46 mm), number of leaflets per stem (5.36 ~ 6.3 ea), petiole length (3.74 ~ 5.2 cm), leaflet length (4.54 ~ 4.62 cm), leaflet width (1.88 ~ 1.97 cm), and aerial weight (0.48 ~ 0.54 g). In particular, petiole length and stem length were significantly higher in WSG collected in May and July, respectively. The number of leaflets per stem (6.3 ea), leaflet length (4.62 cm), and aerial weight (0.54 g) were the highest for the May-collected WSG, and as the collection date approached July, tended to decrease, but did not show significant differences. In the root part, growth characteristics were varied, including rhizome length (2.94 ~ 10.21 mm), root diameter (3.32 ~ 4.43 mm), root length (5.8 ~ 10.51 cm), number of rootlets (1.77 ~ 5.83 ea), and weight of root part (0.28 ~ 0.45 g) (Fig. 1 and Fig. 2). As the harvest time progressed, the rhizome length tended to gradually increase, with the greatest increase in December, but the lowest increase in September. Also, root length also tended to increase as the harvest time got later, but it was significantly highest in September, and was significantly lower. The root diameter and weight of root part did not show significant differences among harvest times, but they were significantly higher in November and December, respectively. On the other hand, the number of rootlets

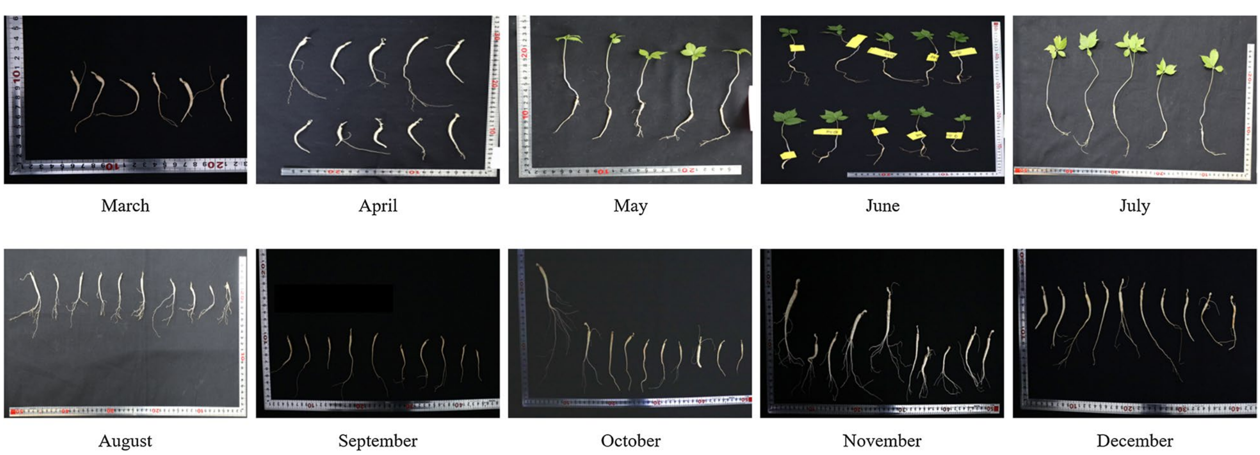

Fig. 1 Photographs of the seasonal 4-year-old wild-simulated ginseng harvested at different times

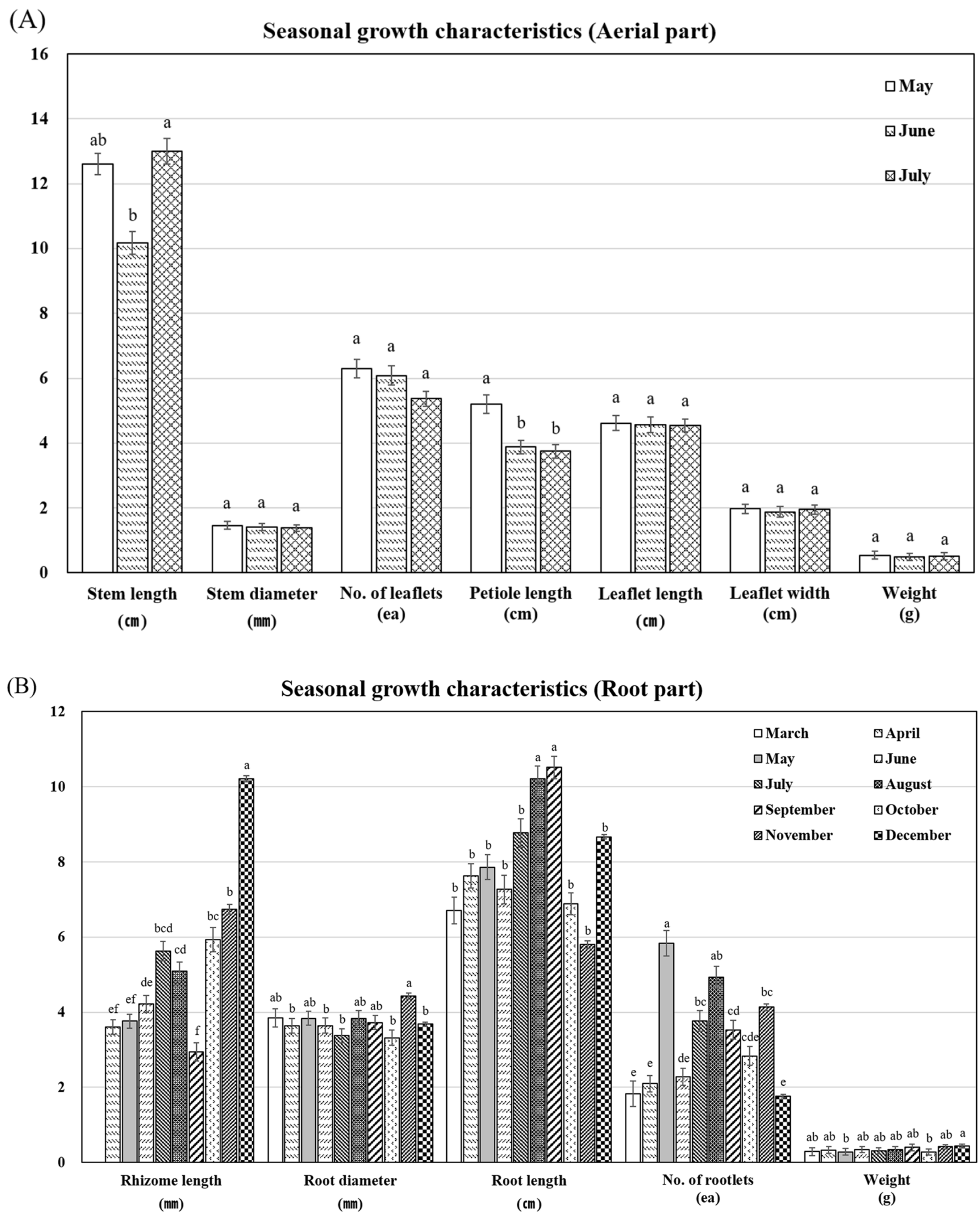

Fig. 2 Growth characteristics of 4-year-old wild-simulated ginseng with different harvest times. **A** aerial parts, **B** root parts. Results are mean ± standard error. Different letters above the bars indicate significant differences between the groups based on the Duncan's multiple range test (DMRT)

was significantly higher in May, and the lowest in March, April, and December.

Ginsenoside content of WSG by harvest time

This study was conducted to provide basic information on the composition of ginsenosides, and to compare the changes in the content of each ginsenoside at different harvest times. The content of 10 ginsenosides (F2, Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Ro) were analyzed. The highest total ginsenoside content was found in July (41 g/kg) among the WSG collected at each harvest time (Fig. 3). From May to July, the ginsenoside content of the whole plant, including the aerial part, was 33, 31, and 41 g/kg in May, June, and July, respectively, showing a content level of more than 30 g/kg. However, the total ginsenoside content in March–April, and August-December, when only the root part was present without the aerial part, ranged from 15 g/kg to 23 g/kg. These results suggest that the ginsenoside content in the aerial part of the WSG was largely contributed to total ginsenoside content. In the ginsenoside contents of the aerial part, the contents of F2, Rb2, Rb3, Re, and Rg1 in July were significantly higher than those in May and June (Table 1). In the root part, the overall ginsenoside content maintained a high level from March to May, but showed a significant decrease in June and July (Table 2). Comparing the ginsenoside content of the aerial and root parts, it was found that the F2 content of the aerial part (9 ~ 24 g/kg), was much higher than that of the root part (0 ~ 44.9 mg/kg). Furthermore, it was also similar for in the aerial part, with Rd, Re, and Rg1 (Rd 6.1 ~ 8.9 g/kg, Re 14 ~ 19 g/kg, Rg1 6.9 ~ 10 g/kg) and root part (Rd 0.4 ~ 1.2 g/kg, Re 3.3 ~ 6.1 g/kg, Rg1 1.4 ~ 2.3 g/kg). In contrast, the content of Rb1 was about 10 times higher in the root part (3.7 ~ 6.4 g/kg) than in the aerial part (0.3 ~ 0.6 g/kg).

Table 1 Ginsenoside contents of 4-year-old WSG aerial part (μg/g)

	May	June	July
F2	9043.1 ± 409.3 b	13032.7 ± 1479.2 b	24776.8 ± 1994.6 a
Rb1	560.7 ± 37.1 a	286.6 ± 21.3 b	495.3 ± 62.9 a
Rb2	2266.2 ± 358.2 ab	1652.2 ± 125.7 b	2659.0 ± 195.1 a
Rb3	329.9 ± 54.3 ab	269.6 ± 21.2 b	410.5 ± 28.1 a
Rc	1491.5 ± 253.9 a	1164.7 ± 86.6 a	1580.8 ± 115.6 a
Rd	8938.3 ± 1557.0 a	6138 ± 174.2 a	8383.2 ± 790.1 a
Re	14153.5 ± 756.7 b	16186.6 ± 1195.2 ab	19106.5 ± 1069.3 a
Rf	229.1 ± 13.5 a	231.4 ± 15.5 a	147.6 ± 14.3 b
Rg1	6894.8 ± 374.1 b	6977.9 ± 309.4 b	10435.2 ± 864.5 a
Ro	665.9 ± 32.9 a	371.8 ± 38.2 b	307.5 ± 42.7 b

Results are mean ± standard error. Different letters indicate significant differences between the groups based on the Duncan's multiple range test (DMRT)

Correlation between growth characteristics and ginsenoside content of 4-year-old WSG by harvest time

Spearman's coefficient analysis was performed to analyze the correlation between growth characteristics and ginsenoside content of WSG with different harvest times (Table 3). Among the various growth characteristics of WSG, the number of leaflets per stem correlated with

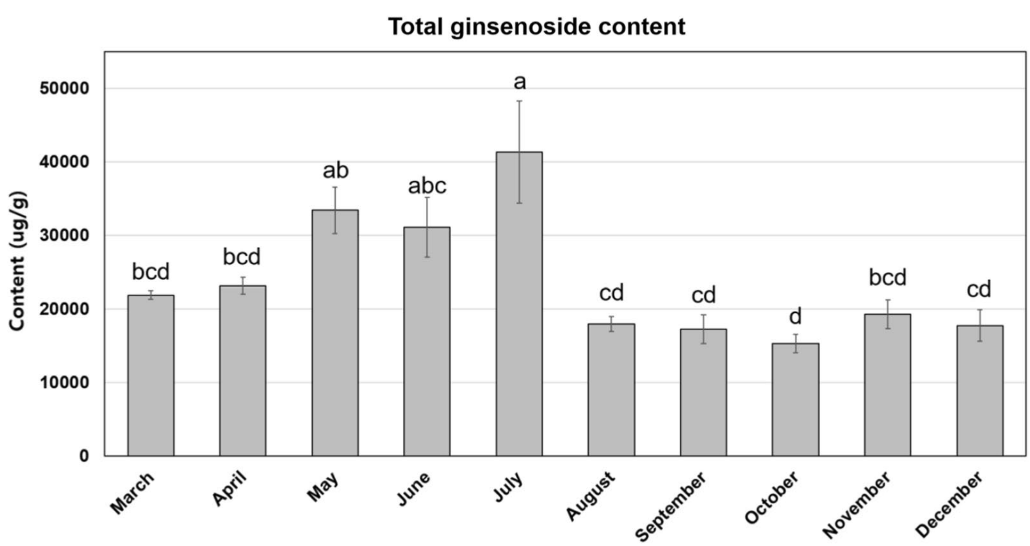

Fig. 3 Seasonal total ginsenoside contents of 4-year-old wild-simulated ginseng. Results are mean ± standard error. Different letters above the bars indicate significant differences between the groups based on the Duncan's multiple range test (DMRT)

Table 2 Ginsenoside contents of 4-year-old WSG root part (μg/g)

	March	April	May	June	July	August	September	October	November	December
F2	N/A	29.8 ± 4.3 a	44.9 ± 13.9 a	N/A	N/A	N/A	N/A	8.1 ± 4.3 a	N/A	N/A
Rb1	5748.3 ± 301.3 ab	6387.6 ± 458.2 a	5863.7 ± 384.9 ab	4641.7 ± 895.2 bcde	4026.4 ± 474.1 cdef	5459.8 ± 275.2 abc	5092.5 ± 732.8 abc	3677.8 ± 440.7 def	3319.4 ± 34.6 ef	2873.2 ± 88.3 f
Rb2	2505.1 ± 106.3 a	2586.2 ± 7 a	2881.3 ± 168.9 a	1877.2 ± 204.9 b	1699.2 ± 149.9 b	1987.3 ± 77.2 b	1755.7 ± 208.1 b	1233.2 ± 166.3 c	1147.2 ± 98.4 c	1101.7 ± 5 c
Rb3	592.6 ± 19.5 bc	635.1 ± 16.3 ab	717.1 ± 47.5 a	489.8 ± 64.5 cd	423.2 ± 39.6 def	487.0 ± 16.6 cd	450.6 ± 51.1 de	371.9 ± 43.8 def	339.3 ± 24.8 ef	315.7 ± 15.8 f
Rc	2599.2 ± 60.1 a	2659.2 ± 80.6 a	2800.1 ± 178.8 a	1935.2 ± 238.2 bc	1568.1 ± 146.5 bcd	1999.4 ± 110.5 b	1718.4 ± 221.1 bcd	1483.6 ± 151.9 cd	1414.1 ± 173.3 d	1292.3 ± 26.0 d
Rd	994.4 ± 25.1 b	1175.5 ± 8 a	1210.5 ± 57.8 a	907.5 ± 77.1 b	679.8 ± 32.4 c	730.3 ± 53.9 c	431.4 ± 51.2 d	412.9 ± 24.9 d	397.1 ± 20.4 d	277.9 ± 3.5 d
Re	6080.2 ± 85.9 a	5897.1 ± 320.9 a	5648.4 ± 424.5 ab	3642.7 ± 620.2 de	3320 ± 345.7e	4368.8 ± 392.7 cde	4597.9 ± 466.1 bcd	5573.7 ± 318.8 abc	5551.1 ± 124.0 abc	4532.2 ± 133.9 bcd
Rf	897.5 ± 21.2 a	885.8 ± 40.1 a	842.6 ± 47.4 a	559.4 ± 61.6 c	592.2 ± 54.6 bc	735.1 ± 57.4 ab	742.1 ± 75.4 ab	767.2 ± 50.1 a	867.7 ± 37.0 a	775.3 ± 14.6 a
Rg1	1836.1 ± 49.3 bc	2275.1 ± 112.2 a	1740.3 ± 129.8 bcd	1435.9 ± 69.7 d	1628.4 ± 122.0 cd	1923.2 ± 61.5 bc	1998.8 ± 174.6 ab	1581.2 ± 66.8 cd	1846.1 ± 66.7 bc	1642.5 ± 87.3 cd
Ro	652.8 ± 15.4 a	610.9 ± 45.1 ab	503.9 ± 47.1 c	340.7 ± 43.9 d	380.9 ± 28.1 d	300.2 ± 28.3 de	495.3 ± 14.2 c	203.1 ± 2 e	524.8 ± 52.1 bc	631.1 ± 26.1 a

Results are mean ± standard error. Different letters indicate significant differences between the groups based on the Duncan's multiple range test (DMRT). *N/A* not applicable

Table 3 Relationships between ginsenosides and growth characteristics of 4-year-old WSG with different harvest period using Spearman's rank correlation analysis

	Stem length	Stem diameter	No. of leaflets	Petiole length	Leaflet length	Leaflet width	Aerial weight	Rhizome length	Root diameter	Root length	No. of rootlets	Root weight
F2	0.332 (0.348)	0.001 (0.999)	− 0.028 (0.938)	− 0.052 (0.894)	− 0.088 (0.808)	0.108 (0.766)	0.017 (0.962)	− 0.043 (0.913)	− 0.317 (0.373)	0.059 (0.871)	0.295 (0.409)	− 0.082 (0.823)
Rb1	− 0.433 (0.211)	0.239 (0.506)	0.370 (0.293)	0.080 (0.837)	0.345 (0.329)	0.075 (0.837)	0.264 (0.461)	− 0.221 (0.568)	0.370 (0.293)	0.165 (0.649)	− 0.344 (0.331)	0.222 (0.538)
Rb2	0.246 (0.493)	0.594 (0.070)	0.607 (0.063)	− 0.168 (0.665)	**0.635* (0.048)**	**0.670* (0.034)**	**0.675* (0.032)**	− 0.254 (0.510)	0.243 (0.499)	− 0.075 (0.837)	0.179 (0.622)	− 0.338 (0.339)
Rb3	− 0.253 (0.481)	0.573 (0.084)	**0.651* (0.041)**	− 0.021 (0.958)	0.577 (0.081)	0.377 (0.283)	0.535 (0.111)	− 0.290 (0.450)	0.355 (0.314)	0.016 (0.965)	− 0.289 (0.418)	− 0.011 (0.975)
Rc	− 0.268 (0.454)	0.597 (0.068)	**0.649* (0.042)**	− 0.005 (0.990)	0.568 (0.087)	0.363 (0.303)	0.524 (0.120)	− 0.328 (0.388)	0.439 (0.204)	− 0.084 (0.817)	− 0.301 (0.398)	− 0.038 (0.916)
Rd	0.483 (0.157)	− 0.017 (0.963)	− 0.119 (0.743)	− 0.198 (0.610)	− 0.013 (0.971)	0.222 (0.538)	0.065 (0.859)	0.088 (0.823)	− 0.161 (0.657)	− 0.147 (0.685)	0.470 (0.170)	− 0.327 (0.356)
Re	0.414 (0.235)	− 0.051 (0.889)	− 0.091 (0.803)	− 0.067 (0.864)	− 0.143 (0.693)	0.102 (0.779)	− 0.057 (0.875)	0.155 (0.691)	− 0.352 (0.319)	− 0.190 (0.600)	0.239 (0.506)	− 0.269 (0.453)
Rf	− 0.486 (0.154)	0.152 (0.676)	0.263 (0.462)	0.121 (0.756)	0.188 (0.603)	− 0.078 (0.829)	0.105 (0.774)	− 0.173 (0.656)	0.313 (0.378)	0.051 (0.890)	− 0.479 (0.162)	0.313 (0.379)
Rg1	0.427 (0.218)	− 0.038 (0.917)	− 0.015 (0.968)	− 0.146 (0.709)	− 0.021 (0.955)	0.207 (0.567)	0.061 (0.866)	0.137 (0.725)	− 0.329 (0.354)	− 0.072 (0.844)	0.331 (0.350)	− 0.251 (0.484)
Ro	0.092 (0.801)	0.034 (0.925)	0.252 (0.482)	0.227 (0.557)	0.549 (0.101)	0.467 (0.174)	0.525 (0.119)	− 0.164 (0.674)	**0.718* (0.019)**	− 0.108 (0.765)	− 0.099 (0.786)	0.196 (0.586)

Bold values mean significant correlation between variables

Spearman's rho values (*r*) written are significantly correlated between the variables compared. Negative values denote negative correlation and positive values denote positive correlation. $^{*}p < 0.05$, $^{**}p < 0.01$

most ginsenoside content. The number of leaflets per stem showed significantly positive correlation with the ginsenosides Rb3 and Rc, indicating that as the number of leaflets per stem increases, their content tends to increase. Among the 10 ginsenosides, Rb2 was the most abundant, and correlated with three different growth characteristics. The ginsenoside Rb2 showed a significant positive correlation with leaflet length, leaflet width, and weight of aerial part. The ginsenoside Ro showed a significant positive correlation with root diameter. Rb1, Re, and Rc showed significantly negative correlation with most growth characteristics, including stem diameter, number of leaflets per stem, leaflet length, leaflet width, root diameter, root length, total weight, and dry weight [47], which is different from the results in this study. The difference between the results of the previous study and those of this study may be due to the geographical environment of WSG cultivation site, such as climate, topography, and soil properties between WSG cultivation sites, as well as the bacterial community living in the rhizospheric soil of cultivation site [15], the cultivation method, such as sowing time of seeds and the type of seeds.

Discussion

Comparing the growth characteristics of WSG according to the harvesting time, as the harvesting time got later, stem length, rhizome length, and weight of root part were significantly increased (Fig. 2). Comparing the content of ginsenosides from March to December, the highest ginsenoside content was found in WSG harvested in July (Fig. 3). Since both aerial and root parts were present at this time, the results confirmed that July would be the optimal harvesting time for WSG based on ginsenoside content alone. A few studies have been conducted on the changes in ginsenoside content depending on the harvesting time. Wu et al. [42] and Kim et al. [19] reported that the highest ginsenoside content in 3-year-old American ginseng (*P. quinquefolius)* and hydroponically grown ginseng was at the leaf-development stage. Also, in 5-year-old ginseng collected by harvest time (January-May), the ginsenoside content increased significantly with the later harvest time [11]. In China, researchers found that the total ginsenoside content significantly decreased during ginseng growth from May to September [2]. However, in this study, the content of ginsenosides was particularly high in May and July, when the aerial part of 4-year-old WSG was present, and there was no significant difference in other periods except for October, which results differed from those of previous studies. These results may be due to changes in the soil chemical properties, soil physical texture, and soil bacterial communities, which were varied depending on the topography and climate of the cultivation site, and cultivation method [14, 15]. In addition, the level of nutrients in the soil can affect ginsenoside synthesis in leaves and roots [27]. A recent study was conducted to improve the quality of poor-quality American ginseng by altering the rhizosphere soil microbial community through biochar treatment. It enhanced growth, net photosynthetic rate, stomatal conductance, chlorophyll content, and ginsenoside content of American ginseng [44].

The ginsenoside content in the aerial part of WSG was mainly dominated by F2, Re, and Rg1, with the proportions of Re and Rg1, the representative protopanaxatriol (PT) type, in May, June and July accounting for 47.2%, 50.1%, and 43.2%, respectively. The ginsenoside Rb1 and Re were the highest in the root part, while the ginsenoside Re is abundant in both aerial and root parts, and is found in high concentrations in commercially available ginseng extracts [7]. The ginsenoside Re is known as a water-soluble compound that accounts for about 23% of the total saponins found in the plant's leaves, stems, seeds, and roots [1, 10, 43]. It has been found to have neuroprotective effects against Parkinson's disease (which is caused by damage to nerve cells), and to improve kidney function [3, 41, 49]. Furthermore, fermentation of the leaves of WSG using *Rhizopus oligosporus* increased the content of various ginsenosides, including squalene, phenol, L-carnitine, and Re, which showed high antioxidant activity [28]. The ginsenosides Rg1 and Rb1 also have neuroprotective effects against Alzheimer's disease caused by neurodegeneration [26], and Rg1 has the effect of hepatocyte function improvement and anti-inflammatory effect [4, 5]. Furthermore, since this study analyzed the growth and ginsenoside content of WSG collected monthly from the same environment, there is no difference in the topography and climate of the cultivation sites, and the results can be regarded as purely the result of the harvest time. It has been proposed to use the ratio of Rg1 to Ro (Rg1/Ro) as a specific marker to distinguish the harvest time of ginseng [31], and when comparing the contents of Rg1 and Ro investigated in this study, the highest ratio of Rg1/Ro was found in July (0.49), and the lowest in October (0.28). The highest ratio in July accords with the optimal harvest time suggested in this study. Based on these results, it is believed that the ratio of Rg1 and Ro content can be applied to establish the harvesting time in actual WSG.

As shown in the correlation analysis between the growth characteristics and ginsenoside content of WSG, the number of leaflets per stem showed significantly positive correlation with Rb3 ($r=0.651$, $p=0.041$) and Rc ($r=0.649$, $p=0.042$), while Rb2 was correlated with leaflet length ($r=0.635$, $p=0.048$), leaflet width ($r=0.670$, $p=0.034$), and aerial weight ($r=0.675$, $p=0.032$), and Ro showed a significant positive correlation with root diameter ($r = 0.718$, $p = 0.019$). It indicated that the content of ginsenosides increases as the growth of WSG increases (Table 3). Previously reported studies on 7- and 13-year-old WSG showed that the content of ginsenosides Rb1, Rb2, Rc, and Rd (PPD type) were significantly correlated with root length and cross-sectional area, surface area, volume, and root weight [16, 17].

Based on the results of this study, it is believed that the harvesting in May–July, when the total ginsenoside content is the highest, is suitable for use as a raw material for industrialization of WSG. Therefore, the raw materialization of the whole plant, including both the aerial and root parts, will require a revision of the current law on food ingredients of WSG.

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Author contributions
YU contributed to the study conception and design. Material preparation, data collection and analysis were performed by YYB and YU. The first draft of the manuscript was written by YYB. HYK and YU critically reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author (Y. Um) upon reasonable request.

Declarations

Ethics approval and consent to participate
The manuscript does not have potential conflicts of interest. The research does not involve human participants or animals.

Competing interests
The authors have no relevant to financial and non-financial interests to disclose.

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