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Characteristics of Absorption and Accumulation of Inorganic Germanium in *Panax ginseng* C. A. Meyer

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The characterstics of absorption and accumulation of inorganic germanium in *Panax ginseng* C. A. Meyer were examined. In 4-year-old *P. ginseng*, the germanium content of the field soil increased with increased amounts and frequencies of inorganic germanium application, while chemical components of the soil, such as available phosphate and exchangeable calcium, potassium, and magnesium, decreased with the increased inorganic germanium application. In the 4-year-old *P. ginseng*, the germanium content was highest in the rhizome and increased in the order of stem, leaf, lateral root, and main root, suggesting that inorganic germanium was absorbed from the root and translocated to the stem and leaf via the rhizome. As for changes in ginsenosides in 4-year-old *P. ginseng* rhizomes, the contents of ginsenosides Rb_1, Rb_2, Re, and Rf decreased as the germanium content in soil increased. Ginsenosides Rb_1, Rb_2, Rc, Re, and Rf in the main root also decreased with increasing germanium content in the main root. The results suggest that inorganic germanium treatment may increase organic germanium in harvested *P. ginseng*, thus enhancing the medicinal efficacy of ginseng products.

Keywords: *Panax ginseng*, Germanium, Absorption, Accumulation, Soil

INTRODUCTION

Germanium is a lustrous, hard, grayish-white metalloid element discovered in argyrodite by the German chemist Clemens Winkler in 1886. It has the symbol Ge, atomic number 32, atomic mass number 72.64, melting point 938.25°C, boiling point 2,833°C, and specific gravity of 5.35; it belongs to group 4A in the periodic table, which also contains C, Si, Sn, and Pb. It is classified into inorganic and organic germanium, the latter when combined with organic matter (organometallic compound).

The curative properties of inorganic germanium have received attention since high amounts were detected in the 1930s in chemical analyses of spring water considered to be curative at Lourdes, located on the border area between Spain and France [1,2]. Inorganic germanium is mainly used in industrial semiconductor production as well as in, e.g., cosmetics, clothes, and furniture.

The average soil content of inorganic germanium in Gyeongnam province, Korea, is 0.24 mg/kg in paddy field soils, reaching 0.30 mg/kg in Masan, Jinju, and Hadong-gun. The content varies depending on soil type: 0.27 mg/kg in common rice paddy and silty loam rice paddy soils, which is more than in sandy and wetted paddy soils [3].

The germanium content of ashed crop plants was found to be 20 mg/kg [4], ranging from 0.1 to 1.0 mg/kg, although medicinal plants contain higher amounts. Yang and Zhang [5] reported a germanium content of 82 μg/kg

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in ginseng, 88 μg/kg in fungi, 75 μg/kg in chrysanthemum, and 88 μg/kg in ginkgo leaf. The germanium content in *Angelica keiskei* growing in Korea differs by 3.8-fold in leaves and 5.0-fold in stems depending on the cultivation region, and leaves contain 13.3 times more germanium than stems, i.e., 386 mg/kg and 29 mg/kg, respectively [6].

Inorganic germanium occurs naturally in soils and is absorbed by and accumulates in animals, plants, and microorganisms, in which it is transformed into organic germanium compounds having increased physiological compatibility. Relatively large amounts of natural organic germanium compounds are found in medicinal plants with recuperative and tonic effects such as ginseng, *Ganoderma*, *Angelica keiskei*, and garlic [7,8].

Recently, several studies have examined the organic germanium content in rice [9], lettuce [10], soybean sprouts [11], Chinese cabbage [12], and ginseng [13]. However, the absorption and accumulation of inorganic soil germanium by ginseng plants had not yet been fully characterized. Thus, this study examined ginseng plants grown in soil supplemented with inorganic germanium to assess its absorption by the ginseng, accumulation in different plant parts, effects on chemical components of the soil, and content of ginsenosides. The findings of this study will provide useful information on the application of germanium to ginseng as a new bioactive substance.

MATERIALS AND METHODS

Germanium treatment

The experiment for examining the absorption and accumulation of soil germanium by ginseng was conducted in an experimental ginseng field of the KT&G Central Research Institute in Suwon, Korea. Four-year-old ginseng plants (violet-stem native variant, Jakyungjong) were planted in fields supplemented with inorganic germanium metallic compounds containing germanium dioxide and germanium chloride under shading with four layers (one black and three blue) of polyethylene net. The completely randomized experimental design comprised nine ginseng fields, each composed of three plots (kans) (replications) of about 1.6 m^2. The metallic germanium compounds were applied in different amounts (20 g, 40 g, and 80 g/plot applied to all experimental plots in June) at different times (one to three times), with a non-application control. The compounds were applied to all experimental plots on 14 June 2008, with second or third applications to selected plots on 23 July 2008 and 7 August 2008, respectively.

Soil sampling

Soil samples of 200 g to 300 g were taken from 5 cm to 20 cm below the surface in five areas of each experimental plot in a zigzag pattern. Debris was removed and soil samples were air-dried under shade for 2 weeks. The dried soil samples were sieved through a 10-mesh sieve prior to use in the experiments.

Analysis of soil germanium

To analyze the soil germanium content, 10 mL of ultrapure grade-HNO_3 was added to 0.5 g soil samples and digested in a MARS Microwave Digestion System (CEM Corporation, Matthews, NC, USA) under the conditions shown in Table 1, and then cooled to room temperature. The digested soil solutions were diluted with deionized water to a total volume of 50 mL with a specific resistance over 18.2 MΩ/cm, and then subjected to analysis of germanium content using a 7500 ICP-MS (Agilent, Vernon Hills, IL, USA) (Table 1).

Analyses of the physicochemical characteristics of soil

The experimental field soils supplemented with inorganic

Table 1. Microwave digestion parameters for dissolving the germanium in plant and soil

Step	Type	Temperature (°C)	Power (%)	Ramp (min)	Dwell[1)] (min)
First digestion program					
1	Time to temp	140	60	10	10
2	Time to temp	160	60	10	20
3	Cooling	-	-	-	30
Second digestion program					
1	Time to temp	140	60	10	10
2	Time to temp	180	60	10	20
3	Cooling	-	-	-	30

[1)]Regular halt along the step changing.

germanium were analyzed for pH, electric conductivity (EC), available phosphate, and exchangeable cations (K, Ca, and Mg) according to the methods described in National Institute of Agricultural Science and Technology (Korea) [14]. Soil pH and EC were measured using 5-g soil samples suspended in 25 mL of distilled water, after a 1 h agitation, with a D-12 pH meter (Horiba, Fukuoka, Japan) and a DS-15 conductivity meter (Horiba). The amount of available phosphates (average P_2O_5) was determined by the Lancaster method using 5 g of soil in a 125-mL flask with 20 mL of extraction solution, followed by 10 min agitation and passage through filter paper (No. 2; Toyo Roshi, Tokyo, Japan); the solution was then transferred to a test tube with 3 mL of the standard solution, 3 mL of the sample solution, and 6 mL of the manipulated solution with an additional supplementation of 0.4 mL 1-amino-2-naphthol-4-sulfonic acid. The pretreated samples were incubated at 30ºC for 30 min and analyzed with a Varian Cary IE UV/Visible spectrophotometer (Spectra Lab Scientific, Toronto, ON, Canada) at an absorption wavelength of 720 nm. Soluble cations (K^+, Ca^{2+}, and Mg^{2+}) were determined by photometry with a Varian Cary IE UV/Visible Spectrophotometer (Spectra Lab Scientific) using 5-g soil samples. Soluble cations (Ca^{2+}, Na^+, K^+, and Mg^{2+}) were determined using a flame photometer and 5 g of soil dissolved in extraction solution (pH 5), with 30 min shaking and passage through filter paper (No. 2, Toyo Roshi), and were measured in an Optima 5300DV inductively coupled plasma-optical emission spectrometer (ICP-OES; Perkin-Elmer Inc., University Park, PA, USA).

Ginseng sampling

Aboveground plant parts of 4-year-old ginseng plants grown in field soils supplemented with inorganic germanium compounds were harvested by cutting at the stem base on 28 September 2008, rinsed twice in distilled water, and dried at 80°C for 10 h in a hot-air circulation dryer (Eyela, Bohemia, NY, USA). The dried plant material was ground to a powder in a rotary mill and sieved through a 40-mesh sieve; it was then only used for chemical analyses. For analysis of belowground plant parts, 4-year-old ginseng roots (including rhizomes) were carefully pulled out without damaging the fine roots using a ginseng-digging hoe on 29 September 2008, washed with distilled water, and dried at 60°C for 3 days in a hot-air circulation dryer (Eyela). The dried ginseng roots (main roots and lateral roots) and rhizomes were separated and ground to a powder in a rotary mill, sieved through a 40-mesh sieve, and then used for chemical analyses.

Analysis of germanium content in ginseng

To analyze the germanium content, 0.5-g plant samples were added to 10 mL of ultrapure grade HNO_3 and digested in a MARS Microwave Digestion System (CEM Corporation) as described in Table 1 and then cooled to room temperature. The digested soil solutions were diluted with deionized water to a total volume of 50 mL with a specific resistance over 18.2 MΩ/cm and subjected to analysis of germanium content using an Agilent 7500 ICP-MS (Table 2).

Analysis of ginsenosides in ginseng

To analyze ginsenosides, 1-g plant samples were dissolved in 50% aqueous methanol in a 250-mL reflux flask, with reflux extraction at 75°C for 1 h in a water bath; the solution was then cooled to room temperature and centrifuged, after which the supernatant was collected. The supernatant was combined with the first extracted supernatant and condensed in reduced pressure at 60°C in a rotary vacuum evaporator. The condensed samples were dissolved in 2 mL of water/acetonitrile (80:20, v/v) and filtered through a 0.45-μm membrane filter. The samples were then analyzed by HPLC (Alliance 2695; Waters Corporation, Milford, MA, USA) on

Table 2. Optimum instrumental conditions for ICP-MS[1)] operation for the germanium analysis

Parameters	Conditions
Plasma parameters	
Rf power	1,500 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	1 L/min
Carrier gas flow rate	1 L/min
Sampler and skimmer	Nickel, 1 and 0.4 mm id
Ion lens setting	Optimised for best sensitivity of 10 g/Li, Co, Y and TI 1% (v/v) HNO_3 solution
Data acquisition parameters	
Points per peak	3
Integration time per point	0.3 sec
Replicates	3

[1)] Inductively coupled plasma-mass spectrometer, Agilent 7500 C.

Table 3. The composition of mobile phase in HPLC for the ginsenoside analysis

Time (min)	Mobile phase[1)]	
	Solvent A (%)	Solvent B (%)
0	20	80
10	20	80
40	32	68
48	42	58
50	100	0
60	100	0
62	20	80
70	20	80

[1)] Solvent A, 100% acetonitrile; Solvent B, 100% distilled water.

a 250×4.6-mm Hyersil ODS 5-μm column in a mixture of acetonitrile and distilled water (Table 3) and flow velocity of 1.6 mL/min, then measured at 203 nm UV [15].

RESULTS AND DISCUSSION

Germanium content in soil

In fields containing 4-year-old ginseng plants, the germanium content in soil with no added germanium was 0.20 mg/kg, which was close to the average germanium content in paddy field soil in Geongnam province [9]. The germanium content in soil treated with inorganic germanium compounds was 4.05, 4.46, or 4.52 mg/kg after one, two, or three applications of 20 g/plot, respectively; 4.98, 5.98, or 7.09 mg/kg after one, two, or three applications of 40 g/plot, respectively; and 6.73, 7.99, or 8.65 mg/kg after one, two, or three applications of 80 g/plot, respectively. These results suggest that the germanium content in soil increased significantly ($p \leq 0.05$) with increases in the amount and frequency of application. The germanium content in soil was divided into five groups (levels) according to analysis with Duncan's multiple range test (DMRT) at $p \leq 0.05$: group 1 was the nontreatment control, group II had one to three applications of 20 g/plot and one application of 40 g/plot, group III had two applications of 40 g/plot, group IV had three applications of 40 g/plot and one with 80 g/plot, and group V had two or three applications of 80 g/plot (Table 4).

The germanium content of the inorganic germanium metallic compounds was 48.88 g/kg; therefore, 20 g, 40 g, and 80 g of germanium compounds applied in each plot contained 0.98, 1.96, and 3.92 g of inorganic germanium, respectively. The content of germanium in a plot ([plot area, 180 cm×90 cm]×[effective plot depth,10 cm]×[soil density, 1.2 g/cm^3]=194.4 kg) was theoretically 5.03, 10.01, and 20.11 mg/kg for the three treatments, respectively. These values are far greater than the actual germanium content examined in the plots, probably because the amount of germanium metallic compounds had decreased beyond the top soil layer of 0 cm to 5 cm depth. However, the germanium content in soil increased with the increase in application frequency, except in the 20 g/plot treatment, suggesting that artificial fertilization with germanium metallic compounds increases the germanium content in soil.

Changes in physicochemical soil characteristics

The physicochemical changes (pH, EC, average phosphates, soluble cations of K, Ca, and Mg) of the experimental field soils supplemented with different amounts of germanium metallic compounds at different frequencies are shown in Tables 5 and 6. The pH was 4.63 to 4.96 in the soil with germanium treatment, which is not significantly different from pH 4.87 in soils of the nontreatment control, suggesting that the ginseng growth may not

Table 4. Changes of chemical properties in soil treated with inorganic germanium (Ge)

Inorganic Ge amounts (g/plot[1)])	Ge input times	pH	EC (dS/m)	Av P_2O_5 (mg/kg)	Ex cation (cmol/kg)		
					Ca	K	Mg
0	-	4.87	0.84	71.81	3.87	1.03	1.81
	1	4.63	0.83	52.94	3.38	0.72	1.48
20	2	4.79	0.62	65.31	3.55	0.89	1.42
	3	4.81	1.09	67.42	3.87	0.99	1.67
	1	4.73	0.64	63.57	3.43	0.85	1.48
40	2	4.79	0.66	60.18	3.31	0.97	1.41
	3	4.96	0.51	67.14	4.03	0.94	1.72
	1	4.69	0.87	60.18	3.94	0.89	1.97
80	2	4.91	0.41	61.46	3.40	0.86	1.56
	3	4.92	0.59	59.36	3.79	0.94	1.63

EC, electric conductivity; Av, available; Ex, exchangeable.
[1)] Plantation unit area (kan) (180 cm×90 cm) of *Panax ginseng* C. A. Meyer.

Table 5. Correlation coefficients for germanium (Ge) content with physico-chemical properties in soil treated with inorganic Ge

Ge	pH	EC	Av P_2O_5	Ca	K	Mg
Ge	0.293	-0.544	-0.456	-0.026	-0.191	-0.051
pH		-0.509	0.533	0.390	0.602	0.138
EC			0.118	0.309	0.179	0.359
Av P_2O_5				0.534	0.770**	0.327
Ca					0.529	0.836**
K						0.365

EC, electric conductivity; Av, available.
**Significantly differ, $p<0.01$.

be adversely affected by pH changes due to the germanium treatment. However, the pH was slightly lower than the pH 5.0 to 6.0 optimum for ginseng growth. The EC of soil treated with germanium metallic compounds was 0.41 to 1.09 dS/m, a wider range than that of the non-treatment control (0.84 dS/m); however, ginseng growth is unlikely to be adversely affected, as the EC value of ginseng field soils allowable for ginseng cultivation is 0.5 to 1.0 dS/m. A correlation appeared to exist between increased germanium and decreased EC, but it was not significant. Also, the salt toxicity of soils with a high EC may be reduced by inorganic germanium amendment, leading to improved growth of ginseng plants, which are vulnerable to high soil salt content.

The available phosphate (average P_2O_5) in plots without germanium treatment was 71.81 mg/kg, which is lower than in most ginseng fields in Korea, probably because the experimental ginseng field is on reclaimed land in the mountainous area. However, the average P_2O_5 content tended to decrease proportionally to 52.94 to 67.42 mg/kg with increasing amounts of germanium, although this change was not significant at $p\leq0.05$. Further studies on the effect of germanium treatment on the average P_2O_5 content of soil are needed as phosphates are immobilized in soils with low pH and ginseng growth is adversely influenced by high phosphate content derived from overuse of chemical and livestock manure fertilizers.

For exchangeable cations, Ca^{2+} content was 3.31 to 4.03 $cmol^+$/kg after germanium treatment and 3.87 $cmol^+$/kg in the non-treated control plots, tending to increase with increased germanium, but without significance. These values were in the range of Ca^{2+} content of 2.0 to 4.5 $cmol^+$/kg for optimum ginseng growth. The content of exchangeable K^+ was 1.03 $cmol^+$/kg in the non-treated control, which is above the allowable content of 0.8 $cmol^+$/kg for ginseng growth; however, the value decreased to 0.72 to 0.97 $cmol^+$/kg after germanium treatment and tended to decrease proportionally with increases in the amount of germanium, but without significance at $p\leq0.05$. The correlation coefficient for K^+ with average P_2O_5 was 0.770, indicating a highly significant correlation ($p\leq0.01$). The content of exchangeable Mg^{2+} was 1.81 $cmol^+$/kg in the non-treated control and 1.41 to 1.91 $cmol^+$/kg in germanium treatments, but was not significant at p=0.05, and was within the range of Mg^{2+} allowable for ginseng growth. The correlation coefficient for Mg^{2+} and Ca^{2+} was 0.836, indicating a highly significant (p=0.01) correlation.

All of the results in our study suggest that the content of chemical components of soil generally decreased with increasing germanium treatment. However, another study on the production of watermelon after germanium treatment [16] showed that application of 1, 2, and 3 tons/ha of biotite containing 38.5 mg/kg germanium increased the pH, organic matter, available phosphates, total nitrogen, and NO_3-N, but decreased the exchangeable cations K^+, Ca^{2+}, Mg^{2+}, and EC. Thus, the correlations between chemical characteristics of the soil and germanium treatment require further study.

Germanium content in ginseng

Responses of 4-year-old ginseng plants to different amounts and frequencies of germanium treatment were examined in relation to plant absorption or exclusion of inorganic germanium. The germanium content in ginseng stems was 2.29 mg/kg, about 9.5 times higher than in the control (0.24 mg/kg), and increased significantly ($p\leq0.05$) with increasing amount and frequency of treatment. The germanium content in soil could be divided into six groups (levels) according to DMRT at $p\leq0.05$: group 1 was the nontreatment control, group II had one or two applications of 20 g/plot, group III had three application with 20 g/plot and one or two applications with 40 g/plot, group IV had three applications of 40 g/plot, group V had one application of 80 g/plot, and group VI had one or two applications of 80 g/plot (Table 6).

The germanium content in ginseng leaves was 2.20

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Table 6. Comparison of germanium contents in the 4-year-old *Panax ginseng* grown in fields treated with inorganic germanium (Ge) (mg/kg)

Inorganic Ge amounts (g/kan[1)])	Ge input times	Germanium contents of aerial parts		Germanium contents of under parts		
		Stem	Leaf	Rhizome	Main root	Lateral root
0	-	0.24±0.02[a2)]	0.31±0.01[a]	0.20±0.01[a]	0.09±0.01[a]	0.12±0.01[a]
	1	1.43±0.09[b]	0.56±0.01[a]	1.21±0.14[b]	0.13±0.01[b]	0.22±0.02[b]
20	2	1.44±0.05[b]	0.54±0.47[a]	2.32±0.09[d]	0.31±0.02[b]	0.25±0.02[bc]
	3	1.75±0.09[c]	1.14±0.06[b]	2.06±0.06[c]	0.21±0.01[cd]	0.29±0.01[c]
	1	1.79±0.10[c]	1.28±0.03[b]	2.42±0.07[d]	0.20±0.01[c]	0.26±0.02[cd]
40	2	1.80±0.14[c]	1.38±0.12[b]	3.07±0.16[e]	0.21±0.01[cd]	0.34±0.02[d]
	3	2.07±0.07[d]	2.63±0.10[d]	3.63±0.10[f]	0.23±0.02[d]	0.40±0.01[e]
	1	2.45±0.15[e]	1.97±0.19[c]	3.87±0.15[g]	0.30±0.02[e]	0.47±0.02[f]
80	2	3.55±0.06[f]	4.17±0.23[e]	4.27±0.13[h]	0.39±0.01[f]	0.68±0.01[g]
	3	4.34±0.10[g]	6.11±0.18[f]	5.34±0.26[i]	0.53±0.03[g]	0.88±0.05[h]

Numeric values are mean±standard error.
[1)] Plantation unit area (180 cm×90 cm) of *Panax ginseng* C. A. Meyer.
[2)] Means in a column with superscripts without a common letter differ, $p<0.05$.

mg/kg in the germanium treatments, about 7.1 times higher than in the control (0.31 mg/kg). The germanium content in stems increased significantly ($p \leq 0.05$) with increasing amount and frequency of treatment. The germanium content could be divided into six groups (levels) according to DMRT at $p \leq 0.05$: group 1 was the control with one or two applications of 20 g/plot, group II had three applications of 20 g/plot and one or two of 40 g/plot, group III had one application of 80 g/plot, group IV had three applications of 40 g/plot, group V had two applications of 80 g/plot, and group VI had three applications of 80 g/plot (Table 6).

The germanium content in the ginseng rhizomes was 3.13 mg/kg in the germanium treatments, about 15.7 times higher than in the control (0.20 mg/kg), and it increased significantly ($p \leq 0.05$) with increasing amount and frequency of the treatment. The results could be divided into 10 groups according to DMRT at $p \leq 0.05$: group 1 was the control, group II had one application of 20 g/plot, group III had three applications of 20 g/plot, group IV had two applications of 20 g/plot, and groups V–X had one to three applications of 40 and 80 g/plot (Table 6 and Fig. 1).

The germanium content in the main root of ginseng was less than in the stem and leaf: 0.09 mg/kg in the control, increasing 2.9-fold to 0.26 mg/kg after the germanium treatment. The germanium content in the main root also increased significantly ($p \leq 0.05$) with increasing amount and frequency of germanium treatment, which could be divided into six groups according to DMRT at $p \leq 0.05$: group I was the control, group II had one and two applications of 20 g/plot, group III had three applications of 20 g/plot and one or two applications of 40 g/plot, and groups IV–VI had one to three applications of

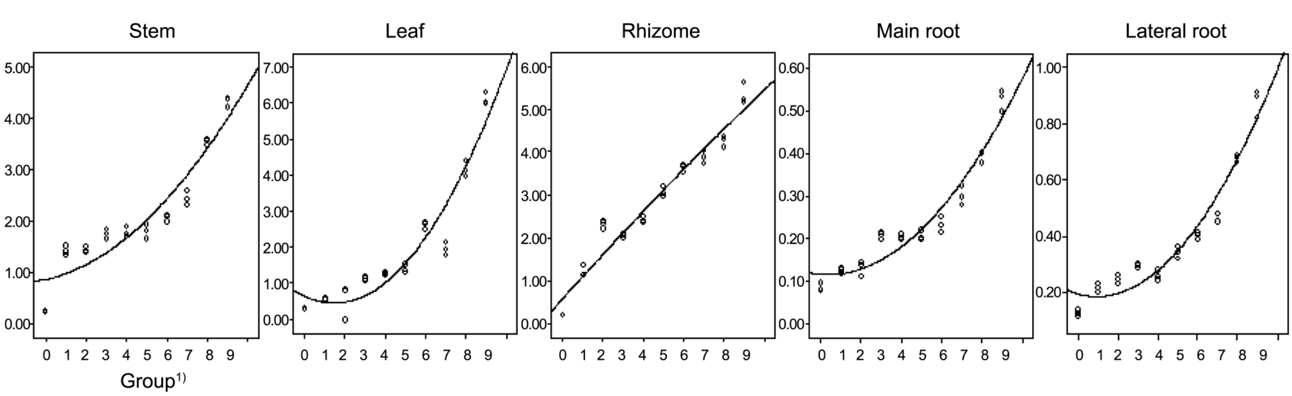

Fig. 1. Regression curves of germanium (Ge) content in 4-year-old *Panax ginseng* grown in fields treated with Ge. [1)]Treatments: 0, control; 1–3, inorganic Ge 20 g/plot, 1–3 input times; 4–6, inorganic Ge 40 g/plot, 1–3 input times; 7–9, inorganic Ge 80 g/plot, 1–3 input times (plot: plantation unit area [kan] [180×90 cm] of *P. ginseng* C. A. Meyer).

80 g/plot (Table 6).

The germanium content in lateral roots was 0.42 mg/kg after treatment, which was 3.5 times higher than in the control (0.12 mg/kg). As in other parts of the ginseng plant, especially the main root, the germanium content in lateral roots increased significantly ($p \leq 0.05$) with increasing amount and frequency of germanium treatments. From DMRT at $p \leq 0.05$, the germanium content in lateral roots could be divided into six groups: group I was the control, group II had one to three applications of 20 g/plot and one or two applications of 40 g/plot, group III had three applications of 40 g/plot, and groups IV–VI had one to three applications of 80 g/kg (Table 6).

Survival strategy of ginseng for inorganic germanium

The germanium content in ginseng increased significantly with increasing germanium content in soil, from a minimum of 2.9 times to a maximum 9.5 times, depending on the plant part, in the order of rhizome, stem, leaf, lateral root, and main root. The maximum germanium content was 6.11 mg/kg in the leaf. Regression analysis in relation to the germanium content of soil gave high coefficients of determination (R^2) of 0.914 for the leaf, 0.884 for the stem, 0.950 for the rhizome, 0.931 for the main root, and 0.945 for the lateral root (Fig. 1).

Among medicinal plants, the germanium content is reportedly over 100 μg/kg in *Artemisia capillaris*, *Angelica polymorpha*, ginseng, *Atractylodes japonica*, *Angelica dahurica*, *Liriope platyphylla*, and *Platycodon grandiflorum* [3]. The germanium content is 22 μg/kg in ginseng, 539 μg/kg in rice, 29 μg/kg in soybean leaves, and 49 μg/kg in perilla leaves in Guemsan, Chungnam province, Korea, while it is 3 μg/kg in ginseng, 1 μg/kg in rice, and 19 μg/kg in chili pepper [17]. Considering the germanium content of ginseng (22 μg/kg) and the report of Yang and Zhang [5] stating that the germanium content in ginseng was 82 μg/kg, the germanium content in ginseng in our study was very high.

Many plants can survive in areas with a high content of heavy metals such as nickel, chromium, cadmium, and arsenic either through exclusion or absorption of such metals. For the exclusion of heavy metals, absorption is prevented through differential permeability of the plasma membrane of root cells. Other plants, however, absorb and accumulate large amounts of heavy metals in cells, which can be fatal. Some accumulator species have real tolerance against heavy metal toxicity. Germanium was reported to accumulate in cells mainly as water-soluble fractions bound with nucleic acids and proteins [18]. Germanium in DNA of eukaryotic cells is involved in the control of genetic expression at the electron level by maintaining the level and direction of free-sliding electrons in DNA and modulating the electron conductivity and conductivity of DNA [19].

In our study, ginseng did not appear to exclude, but absorb and accumulate, germanium proportionally to its content in soil. Little germanium was accumulated in roots that translocate germanium via the rhizome to the shoot (stem and leaf), which had the highest germanium content. Ginseng shoots containing high amounts of germanium are lost at the end of the growing season, resulting in removal of the germanium from the main plant parts (roots), which are replaced by new shoots at the beginning of the growing season in the next year. This is a sophisticated strategy of ginseng for removing germanium that prevents continuous germanium accumulation yearly that could threaten its survival. Further detailed examination of the production and trafficking of proteins in relation to nonmetallic and metallic compounds such as germanium are necessary to understand the survival strategy and medicinal efficacy of ginseng. Also, ginseng that has been grown in fields with a high germanium content may be utilized strategically for the production of specialized ginseng products.

Changes in the ginsenoside content in ginseng

The content of ginsenosides was examined in the rhizome, main root, and lateral root of 4-year-old ginseng treated with inorganic germanium compounds and showed a correlation between germanium content in ginseng and that in the soil (Table 7). The total ginsenoside content in the rhizome was 15.23 mg/g after germanium treatment, which was lower than in the control (26.30 mg/g). The correlation coefficients for ginsenoside content with soil germanium content were -0.710 for Rb_1, -0.666 for Rb_2, -0.672 for Re, and -0.669 for Rf, which were all negative at $p \leq 0.05$, suggesting that the content of ginsenosides in ginseng decreases with increased germanium in the soil. Ginsenoside content in the main root was 4.05 mg/g after treatment, which is lower than in the control (4.97 mg/g), although the difference was not as high as in the rhizome. No significant correlations for ginsenoside content of the main root with that of the rhizome were noted; however, the main root ginsenoside content decreased with increasing soil germanium, showing significant negative correlations at $p \leq 0.05$ and correlation coefficients of –0.641 for Rb_1, -0.691 for Rb_2, -0.642 for Rc, -0.728 for Re, and -0.635 for Rf. Total ginsenosides in the lateral root were also lower in the germanium

Table 7. Comparison of ginsenoside content in 4-year-old *Panax ginseng* grown in fields treated with inorganic germanium (Ge)

Part	Ge[1)]	Ginsenosides (mg/g, w/w)											
		Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Rg_2	Rg_3	Rh_1	Total	PD/PT[2)]
Rhizome	0	7.94	2.87	3.37	2.00	4.39	1.71	2.25	1.09	0.51	0.17	26.30	1.74
	1	4.60	2.19	2.60	1.38	2.70	0.93	1.43	0.68	0.34	0.11	16.96	1.90
	2	4.36	1.81	2.17	1.15	2.57	0.63	1.23	0.67	0.33	0.10	15.02	1.88
	3	4.07	1.73	2.05	0.96	2.34	0.70	1.24	0.63	0.31	0.11	14.14	1.82
	4	3.89	1.60	1.95	1.05	2.11	0.68	1.28	0.60	0.28	0.10	13.54	1.83
	5	3.79	1.67	2.03	0.79	2.17	0.67	1.18	0.63	0.30	0.10	13.33	1.81
	6	3.88	1.82	2.18	1.02	2.31	0.72	1.40	0.67	0.31	0.12	14.43	1.76
	7	5.21	2.16	2.53	1.35	2.92	0.95	1.86	0.86	0.39	0.15	18.38	1.73
	8	4.70	1.80	2.32	1.33	2.60	0.96	1.54	0.76	0.35	0.12	16.48	1.75
	9	3.88	1.85	2.29	1.11	2.52	0.64	1.34	0.70	0.34	0.11	14.78	1.78
	Cor	-0.71#	-0.67#	-	-	-0.67#	-0.67#	-	-	-	-	-	-
Main root	0	1.29	0.57	0.69	0.13	0.72	0.42	0.95	0.08	0.12	0.00	4.97	1.28
	1	1.21	0.60	0.72	0.17	0.57	0.44	1.07	0.00	0.11	0.08	4.97	1.31
	2	0.81	0.35	0.45	0.11	0.45	0.25	0.63	0.00	0.10	0.00	3.15	1.36
	3	1.15	0.46	0.61	0.13	0.60	0.35	0.75	0.00	0.11	0.00	4.16	1.44
	4	1.48	0.56	0.72	0.16	0.58	0.47	1.20	0.10	0.13	0.10	5.50	1.25
	5	1.15	0.50	0.65	0.12	0.58	0.39	0.88	0.00	0.11	0.00	4.38	1.37
	6	0.96	0.46	0.59	0.15	0.55	0.32	0.77	0.00	0.10	0.00	3.90	1.37
	7	1.27	0.56	0.69	0.17	0.62	0.39	0.99	0.09	0.12	0.07	4.97	1.31
	8	0.72	0.27	0.38	0.09	0.37	0.28	0.65	0.00	0.09	0.00	2.85	1.19
	9	0.60	0.27	0.40	0.08	0.35	0.20	0.53	0.00	0.09	0.00	2.52	1.33
	Cor	-0.64*	-0.69*	-0.64*	-	-0.73*	-0.64* -0.74#	-	-	-	-	-	-
Lateral root	0	5.02	3.05	3.81	1.43	3.60	0.89	1.27	0.98	0.32	0.09	20.46	2.00
	1	3.22	2.41	2.96	1.23	2.34	0.65	1.01	0.64	0.26	0.07	14.79	2.14
	2	3.10	2.01	2.50	1.13	2.31	0.52	0.80	0.61	0.20	0.00	13.18	2.11
	3	3.39	1.96	2.57	1.06	2.50	0.56	0.64	0.69	0.25	0.00	13.62	2.10
	4	3.09	1.94	2.54	1.01	2.13	0.60	0.86	0.61	0.23	0.00	13.01	2.10
	5	3.31	2.06	2.63	0.90	2.35	0.59	0.76	0.67	0.25	0.00	13.52	2.09
	6	3.26	2.35	2.98	1.23	2.63	0.60	0.76	0.74	0.26	0.00	14.81	2.13
	7	4.85	3.46	4.33	1.88	3.68	0.79	1.28	1.02	0.34	0.09	21.72	2.17
	8	2.47	1.27	1.87	0.81	1.77	0.46	0.63	0.54	0.18	0.00	10.00	1.94
	9	3.82	2.44	3.42	1.31	3.34	0.73	1.00	0.93	0.31	0.07	17.37	1.86
	Cor	-	-	-	-	-	-	-	-	-	-	-	-0.67*

Cor, correlation significance probability.
1) 0, control; 1-3, inorganic germanium 20 g/kan, 1-3 input times; 4-6, inorganic germanium 40 g/kan, 1-3 input times; 7-9, inorganic germanium 20 g/kan, 1-3 input times (kan, plantation unit area [180x90 cm] of *Panax ginseng* C. A. Meyer).
2) Sum of protopanaxadiol saponins (Rb_1, Rb_2, Rc, Rd, Rg_3)/sum of protopanaxatriol saponins (Re, Rf, Rg_1, Rg_2, Rh_1).
*,# p>0.05 correlation with germanium contents of plant and soil, respectively.

treatment (14.67 mg/g) than in the control (20.46 mg/g). The ratio of protopanaxadiol ginsenoside to protopanaxatriol ginsenoside in the lateral root decreased significantly with increasing germanium content in the lateral root, showing a negative correlation and correlation coefficient of -0.673.

In soil containing increased nonmetallic and metallic compounds such as inorganic germanium, plants usually perceive this as stressful and increase production of secondary metabolites. In contrast, the content of ginsenosides, which are secondary metabolites in ginseng plants, decreased with increasing amount and frequency of germanium application in our study. Considering that ginsenosides are the most important component for the medicinal efficacy of ginseng, a decrease in their content in ginseng after soil germanium treatment may decrease its medicinal efficacy. Therefore, more detailed studies on the phenomena and mechanisms of changes in the ginsenoside content of ginseng after soil germanium treatment are required.

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