Journal of Plant Biology, April 2007, 50(2) : 198-205

Physiological and Chemical Characteristics of Field- and Mountain-Cultivated Ginseng Roots

Yong Eui Choi[1]*, Yong Suk Kim[1], Myong Jong Yi[1], Wan Geun Park[1], Jae Seon Yi[1], Seong Ryeol Chun[1], Sang Sup Han[1], and Sung Jae Lee[2]

[1]Division of Forest Resources, College of Forest and Environmental Sciences, Kangwon National University, Chunchon 200-701, Korea
[2]Forest Development Research Institute, Chunchon 200-140, Korea

Demand is increasing for mountain-cultivated *Panax ginseng* (MCG) because its quality is considered superior to that of field-cultivated ginseng (FCG). However, MCG grows very slowly, and the factors that might affect this are unknown. In addition, little information is available about the physiological characteristics of its roots. Here, we investigated local soil environments and compared the histological and chemical properties of MCG and FCG roots. Average diameters, lengths, and fresh weights were much smaller in the former. Photosynthesis rates and root cambial activity also were reduced in the MCG tissues. Our analysis of soil from the mountain site revealed an extremely low phosphorus content, although those samples were richer in total nitrogen and organic matter than were the field soils. MCG roots also contained higher amounts of ginsenosides, and total accumulations increased with age. Moreover, ginsenoside Rh2, a red ginseng-specific compound, accumulated in the MCG roots but not in those from FCG plants. Interestingly, numerous calcium oxalate crystals were found in MCG roots, particularly in their rhizomes (i.e., short stems). Therefore, we can conclude from these results that low levels of the essential mineral phosphorus in mountain soils are a critical factor that retards the growth of mountain ginseng. Likewise, the high accumulation of calcium oxalate crystals in MCG roots might be an adaptation mechanism for survival in such a harsh local environment.

Keywords: calcium oxalate crystal accumulation, field-cultivated ginseng, ginsenoside analysis, mountain-cultivated ginseng, *Panax ginseng*, soil analysis

Panax ginseng, in the family Araliaceae, is a genus of five or six species of slow-growing perennial plants with fleshy roots. These live in northeastern Asia, typically in cooler climates. Ginseng is one of the most highly regarded herbal medicines in the Orient, where it has gained an almost magical reputation for promoting health and general body vigor, while also prolonging life (Ellis and Reddy, 2002; Coleman et al., 2003). Among its many active ingredients, the most important are the pharmacologically active ginsenosides, called triterpene saponins (Vogler et al., 1999; Shibata, 2001).

Ginseng grows naturally in the mountains, and can survive for several decades. Plants collected in the wild are regarded as having precious quality as a medicine. Ginseng is now being planted on mountain sites, where conditions mimic those of naturally grown plants. These are harvested after about 10 to 15 years. This mountain-cultivated ginseng (MCG) is considered far superior to field-cultivated ginseng (FCG) because it simulates the quality of wild ginseng. MCG roots are relatively rare but extremely expensive compared with FCG roots.

Growth of MCG is much slower than FCG, although survival can be far longer (several decades) for the former. Despite the increasing demand on farmers for its cultivation and by the consumer for medicinal utilization, the inherent slow growth rate and low levels of productivity are major limitations for users. Little information is available as to why MCG grows slowly and is so long-lived. Furthermore, variations in the physiological and chemical characteristics of FCG and MCG have not been clearly demonstrated except for an analysis of their polyacetylene contents (Chang et al., 2003).

In this project, we focused on how factors associated with a mountain-soil environment might affect the growth rate of MCG. We also evaluated the physiological and chemical differences between FCG and MCG roots.

*Corresponding author; fax +82-33-252-8310
e-mail yechoi@kangwon.ac.kr

MATERIALS AND METHODS

Plant Materials

Field-cultivated roots of *P. ginseng* (FCG) were collected from an agricultural field at Hongchon-kun in Kangwon Province of Korea. Mountain-cultivated roots (MCG) were gathered from a ginseng-cultivating mountain situated in Samcheok-kun and from the Kangwon National Research Forest at Hongchon-kun. After harvesting, we recorded the lengths, diameters, and fresh weights of the roots. At least three individual roots per sample were analyzed.

Histological Observations

For our histological observations, root samples were fixed at 4°C for 24 h in 1.5% glutaraldehyde and 1.6% paraformaldehyde, buffered with 0.05 M phosphate buffer (pH 6.8). They were then dehydrated in an ethanol series (30, 50, 60, 70, 80, 90, 95, and 100%), and embedded in Technovit 7100 (Kulzer, Germany) according to the protocol of Yeung (1999). The samples were semi-thin-sectioned (3 μm) with an autocut microtome (RM 2165; Leica, Germany) and mounted on glass slides. They were stained with 0.05%

toluidine blue O and examined under a light microscope (Olympus BX51).

Photosynthesis Analysis

Net photosynthesis in MCG and FCG leaves was measured at around 10 PM with a portable photosynthesis system (LCA-4, ADC; Hoddeson, UK). Three fully expanded leaves per plant were evaluated at light saturation (1200 μmol m^{-2} s^{-1}) that was provided by an LED module after determining the light-response curve (between 0 and 1200 μmol m^{-2} s^{-1}) and the steady-state photosynthetic rate. Calculations were performed at various levels of photosynthetic photon flux density (PPFD; 0, 100, 200, 400, 600, 800, 1000, and 1200 μmol m^{-2} s^{-1}). Conditions at the time of these measurements included 25°C, 400 μbar ambient CO_2 pressure, 500 μbar flow rate, and 60 to 70% RH. Net photosynthesis in the MCG and FCG leaves also was determined at different temperatures (15, 20, 25, 30, 35, and 40°C), under conditions of 1200 μmol CO_2 m^{-2} s^{-1}, 400 μbar ambient CO_2 pressure, 500 μbar flow rate, and 60 to 70% RH.

Physicochemical Soil Analysis

Mountain soils were collected either from the Research Forest of Kangwon National University, Hongchon-kun, Kangwon Province; or from Samchock-kun mountain in Kangwon Province. Field soils were sampled from a ginseng farm in Hongchon-kun. Prior to our physical and chemical analyses, all samples were air-dried at room temperature and passed through a 2-mm sieve. Soil pH was measured with an Orion 720^+ pH meter (Orion Research, USA) at a 1:5 ratio with distilled water for 1 h. Soil organic matter was determined according to the Tyurin method (Bel'chikova, 1954). Total N was analyzed on a micro Kjeldahl apparatus (B324; Buchi, Switzerland) following wet digestion in concentrated H_2SO_4 on a block digester (K438; Buchi) (Jackson, 1962). Available P was measured with a UV-spectrophotometer (UV-2501PC; Shimadzu, Japan) via the Lancaster method (Cox, 2001), in which the extracting solution is based on the reaction with ammonium molybdate. Exchangeable base cations were extracted with 1 N ammonium acetate (pH 7.0) and then measured with a pH meter (F-53; Horiba, Japan). Exchangeable K^+ and Na^+ were determined by Flame photometry (PFP7; Jenway, UK) while Ca^{2+} and Mg^{2+} levels were obtained by Atomic Absorption spectrophotometry (280FS; Varian, USA), using 1 N ammonium acetate as the extracting solution.

Ginsenoside Analyses by HPLC

MCG and FCG roots were sampled from Samcheok-kun. Their ginsenosides were extracted according to the method described by Ando et al. (1971). One gram of milled powder from freeze-dried roots was soaked in 80% MeOH at 60°C. After the liquid was evaporated, the residue was dissolved in H_2O and washed twice, followed by extraction with H_2O-saturated n-butanol. The butanol layer was evaporated to produce a saponin fraction. Each sample was dissolved in EtOH, then filtrated with a SepPak C-18 Cartridge (Waters, USA). The HPLC separation was performed on a NovaPak C18 column (4 μm, 3.9 X 150 mm; Waters, USA), applying the following gradient system: 0 min, 100% acetonitrile; 10 min, 75% acetonitrile and 25% water; 25 min, 67% acetonitrile and 33% water. Flow rate of the mobile phase was 1.2 mL min^{-1}, and ginsenosides were monitored at a wavelength of 202 nm. Each was then compared with authentic ginsenoside purchased from ChromaDex (USA). Quantitative analysis was performed via a one-point curve method, using external standards of those authentic ginsenosides.

Scanning Electron Microscope Observations and Energy-Dispersive X-Ray Analysis

Variable-pressure scanning electron microscopy (VP-SEM) was employed to observe the crystals per the method of Sarret et al. (2006). Excised roots from FCG and MCG samples were glued to aluminum stubs, then put on a chamber stage after cooling to -20°C. These explants were viewed with a low-vacuum scanning microscope (LV-SEM, S-3500N; Hitachi, Japan), at a chamber pressure of 30 Pa and an accelerating voltage of 15 KV. The elemental composition of crystals that accumulated in the roots was analyzed with an energy-dispersive X-ray (EDX) analysis system (EMAX-7000; Horiba, Japan).

RESULTS AND DISCUSSION

Slow Growth of MCG Roots

Lengths, diameters, and fresh weights were much smaller for roots of mountain-cultivated ginseng than from plants that were field-cultivated (Table 1). In fact, the fresh weights of 15-year-old MCG roots were less than those measured from 3-year-old FCG plants. This indicates that environmental conditions at the mountain site are not as suitable for the growth of *Panax ginseng*. Aged MCG also had characteristically long stems at the tops of their roots (Fig. 1A, B), and their ages could be determined only according to the number of notches (scars) on their rhizomes.

Photosynthesis Analysis by Portable Measurement System

As measured with a portable system, net photosynthesis

Table 1. Growth parameters for FCG and MCG roots over time.

Root parameter	FCG (yr)				MCG (yr)				
	1	3	5	6	1	3	5	10	15
Length (cm)	13.2 ± 1.26	22.5 ± 1.75	29.5 ± 3.73	32.4 ± 2.78	10.1 ± 2.23	12.6 ± 2.27	15.1 ± 2.12	19.9 ± 2.35	23.2 ± 2.89
Diameter (mm)	5.23 ± 4.36	11.2 ± 1.26	18.9 ± 3.42	31.2 ± 4.23	4.5 ± 3.62	6.3 ± 1.14	8.2 ± 1.18	9.7 ± 1.18	11.2 ± 2.24
Fresh weight (g)	1.1 ± 0.17	18.4 ± 2.73	42.4 ± 5.32	57.2 ± 6.72	0.3 ± 0.01	1.8 ± 1.93	5.2 ± 0.87	8.1 ± 0.96	9.3 ± 1.17

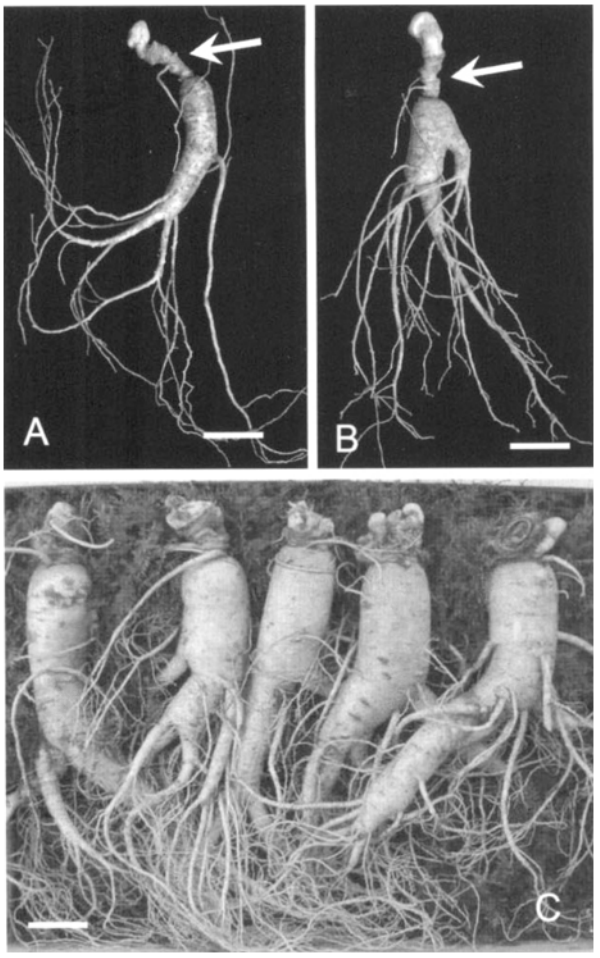

Figure 1. Mountain- and field-cultivated roots of ginseng. (**A**, **B**) 15-year-old MCG roots (arrows indicates rhizomes). Bars = 10 mm. (**C**) 5-year-old FCG roots. Bar = 25 mm.

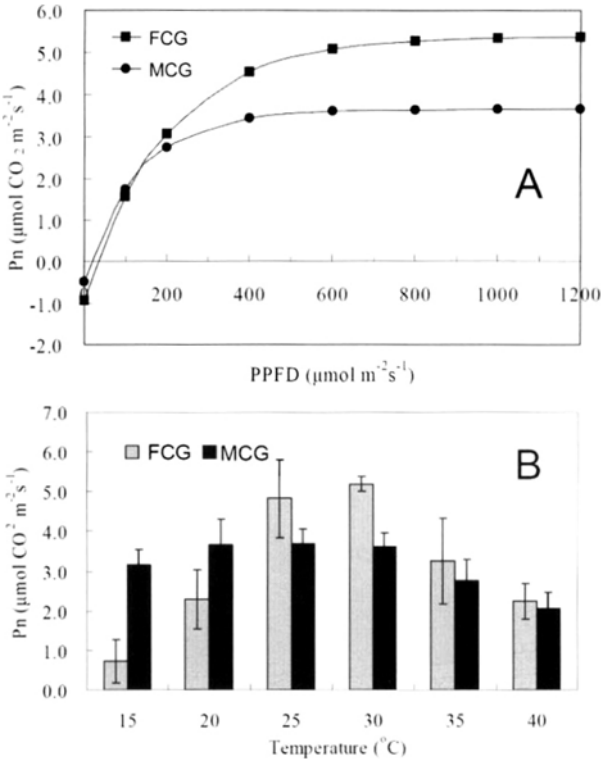

Figure 2. Difference in net photosynthesis rate between MCG and FCG leaves. (**A**) Light-response curve of photosynthesis. Measurements were made at 25°C, 400 μbar ambient CO_2 pressures, 500 μbar flow rate, and 60-70% RH. (**B**) Temperature-response curve of photosynthesis. Measurements were made at 1200 μmol CO_2 m^{-2} s^{-1}, 400 μbar ambient CO_2 pressure, 500 μbar flow rate, and 60-70% RH.

rates were significantly lower in the leaves of MCG plants compared with those of FCG (Fig. 2). In the latter type, the photosynthesis light-response curve increased continuously to 600 μmol m^{-2} s^{-1} photosynthetic photon flux density (Fig. 2A), whereas, for MCG, that rate was saturated at 400 μmol m^{-2} s^{-1} PPFD (Fig. 2A). At different temperatures, rates also were generally decreased in the MCG leaves (Fig. 2B). The primary factors affecting photosynthesis rates are temperature, light, and CO_2 levels (Urban, 2004). In the current study, the low rate for MCG may have been caused by unsuitable environmental conditions, including a deficiency of phosphorus in the soil (Rao and Terry, 1989; Kirschbaum and Tompkins, 1990; Jacob and Lawlor, 1991).

Histological Analysis of Ginseng Roots

Histological observations of the roots revealed that cambial activity was less active in MCG than in FCG (Fig. 3A, B), with approx. three to five cambial layers being detected in the FCG roots (Fig. 3A), compared with only two to three in MCG roots (Fig. 3B). In addition to slower lateral growth in the latter, cross sections of the MCG fine roots showed that their cells were smaller and more homogenous, with smaller-diameter vessel elements (Fig. 3E, F). However, more starch grains were accumulated in the MCG roots than in FCG roots (Fig. 3A-F). Because MCG roots are known to be more elastic and harder than FCG roots, this higher accumulation of grains and smaller cells might be related to those physical properties of MCG roots.

Physicochemical Soil Analysis

It is unclear why the growth of mountain ginseng roots is slow. Here, we compared soil properties and found that total organic matter was richer in the mountain soil, probably due to the accumulation of fallen leaves (Table 2). Although total nitrogen content also was greater in those samples, the levels of exchangeable cations, such as Na^+, K^+ and Mg^{2+}, were slightly lower, and only Ca^{2+} content was greater than the field soil (Table 2). As suggested earlier, the amount of phosphorus (P_3O_5) in the mountain soil was at least ten-fold lower than in the field samples (Table 2). Because plants generally require 60 mg kg^{-1} for optimal growth, our results indicate that this decrease in phosphorus ions might be a major limiting factor for the production of ginseng in mountainous habitats. This situation is often encountered in many types of environments, such that the availability of phosphorus governs the growth rates for many organisms (Constant and Sheldrick, 1991). Its deficiency is manifested by a reduction in leaf size (Cromer et al., 1993; Rodríguez et al., 1998), less leaf expansion (Cromer et al., 1993), and a lower light-saturated rate of photosynthesis per

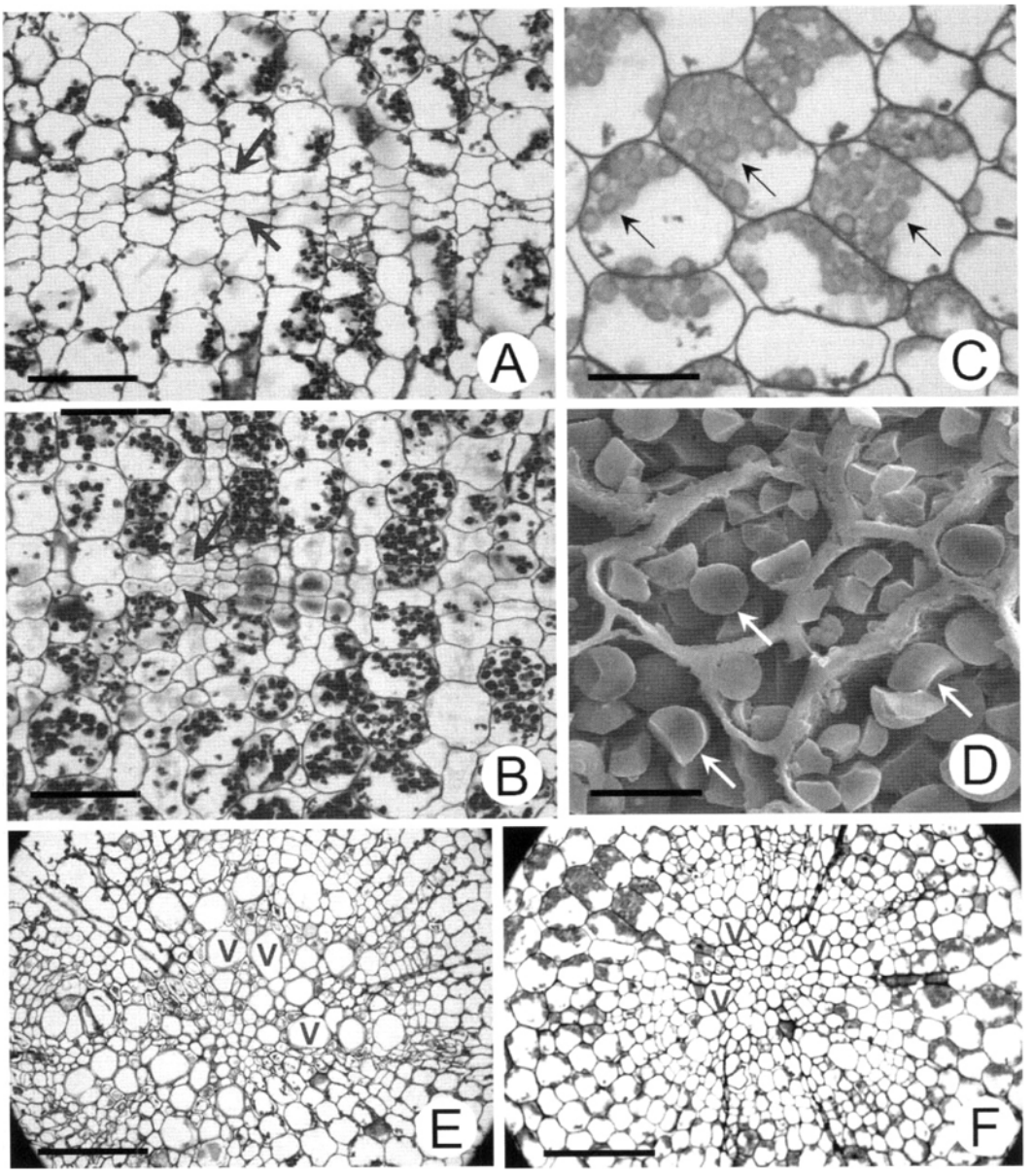

Figure 3. Histological observations from ginseng roots. (**A**) Cross section of vascular cambial region of FCG root (arrows indicate cambial layer). Bar = 86 μm. (**B**) Cross section of vascular cambial region of MCG root (arrows indicate cambial layer). Bar = 86 μm. (**C**) Starch grain accumulation in root parenchyma cells. Grains were colored red by Periodic Acid Schiff Staining (arrows indicate grains). Bar = 35 μm. (**D**) Scanning electron microscope observation of starch grains accumulated in roots (arrows indicate grains). Bar = 15 μm. (**E**) Cross section of newly formed 1-mm-diam. fine FCG roots with enlarged vessel elements (V). Bar = 350 μm. (**F**) Cross section of newly formed 1-mm-diam. fine MCG roots with small vessel elements (V) and numerous starch grains. Bar = 350 μm.

Table 2. Physicochemical properties of non-rhizosphere soil samples from mountain and field sites.

Plant type	pH	Organic matter (mg kg^{-1})	Total nitrogen (mg kg^{-1})	Phosphorus (mg kg^{-1})	CEC ($cmol^{+}$ kg^{-1})	Exchangeable cation ($cmol^{+}$ kg^{-1})			
						K^{+}	Na^{+}	Ca^{2+}	Mg^{2+}
MCG	4.84 ± 0.11	51.4 ± 12.9	2.63 ± 0.80	17.8 ± 4.04	18.4 ± 0.82	0.19 ± 0.02	0.09 ± 0.01	1.36 ± 0.57	0.31 ± 0.13
FCG	4.54 ± 0.14	15.8 ± 2.93	1.43 ± 0.20	302.6 ± 62.6	11.02 ± 1.14	0.47 ± 0.17	0.20 ± 0.09	1.31 ± 0.67	0.76 ± 0.64

MSG and FCG soils were sampled from Honchun-kun of Kangwon province.
Soils were sampled 20 cm from ginseng plants, in the non-rhizosphere zone.

unit leaf area (Rao and Terry, 1989; Kirschbaum and Tompkins, 1990; Jacob and Lawlor, 1991). Low phosphorus content in mountain soil is mainly caused by erosion and water runoff into streams, wetlands, and lakes (Sharpley et al., 1992; Catt et al., 1998).

Cation exchange capacity (CEC), which quantifies the

Table 3. Comparison of physicochemical properties between non-rhizosphere and rhizosphere samples of MCG soil.

Age of root (yrs)	Sampling zone	pH	Organic matter (mg kg^{-1})	Total nitrogen (mg kg^{-1})		Phosphorus (mg kg^{-1})	CEC ($cmol^+$ Kg^{-1})	Exchangeable cation ($cmol^+$ Kg^{-1})			
				NO_3^-	NH_4^+			K^+	Na^+	Ca^{2+}	Mg^{2+}
5	Non-rhizosphere	5.91	51.26	8.75	3.32	7.6	19.2	0.28	0.22	7.28	1.16
	Rhizosphere	5.84	33.23	4.02	3.76	3.5	12.3	0.25	0.24	5.16	1.01
7	Non-rhizosphere	6.28	70.49	43.57	7.35	17.2	60.4	1.09	0.24	12.15	1.75
	Rhizosphere	5.9	41.78	5.25	7.52	2.7	14.5	0.34	0.22	6.02	0.99
15	Non-rhizosphere	7.16	70.89	4.72	4.37	7.4	29.3	1.40	0.19	13.45	1.56
	Rhizosphere	6.91	37.38	4.02	2.97	2.3	13.8	0.36	0.23	10.11	1.23

MCG soil was sampled from mountain of Samcheok-kun of Kangwon province.
Non-rhizosphere soils were sampled 20 cm from ginseng plants.
Rhizosphere soils were sampled within the root zone.

ability of soil to hold cations by electrical attraction, is a useful indicator of fertility because it demonstrates the capacity of soil to supply three important plant nutrients: calcium, magnesium, and potassium. The five most abundant exchangeable cations in the soil are calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), sodium (Na^+), and aluminum (Al^{3+}). Cations are held by negatively charged particles of clay and humus called colloids. Concentrations of cations are expressed in centimoles of positive charge per kilogram of soil (cmol kg^{-1}). Values >10 cmol kg^{-1} are preferred for plant production (Huffaker and Wallace, 1959). Soils with high amounts of swelling clay and organic matter can have CECs of at least 30 cmol kg^{-1}. In this study, CEC was at suitable levels in both mountain and field soils (Table 2).

The most important soil environmental factor for plant growth is pH. On mountain slopes, rainfall leaches out the basic minerals in soils, and tends to speed this acidulation process (Sharpley et al., 1992; Catt et al., 1998). Plant nutrients, such as calcium, magnesium, and potassium, are often deficient in acidic soils (Jönsson et al., 2003). In Korean mountains, the soil pH is approximately 5.1, which is much lower than in soils normally used for agricultural plant production. Here, however, the growth of ginseng in the fields was not severely affected by such an acidic pH because values were similar for mountain and field soils. This suggests that, for mountain ginseng, soil pH is not a major limiting factor.

Physicochemical Analysis of Rhizosphere and Non-Rhizosphere Soils

Soils were sampled from the root zone (rhizosphere) and from the non-root zone, 20 cm away from the roots. Regardless of the root age, organic matter contents were lower adjacent to the plants than farther away (Table 3). The amount of NO_3^- was reduced in the root zone while NH_4^+ levels were similar for the rhizosphere and non-rhizosphere (Table 3). Phosphorus levels were markedly decreased in the root zone. Except for Na, which was constant between root and non-root zones, the CEC and amounts of exchangeable cations were reduced in the rhizosphere (Table 3). These results indicate that the quality of soil properties declines around the root zone, causing the growth rate of ginseng to decrease due to a loss of minerals. Historically, when ginseng is cultivated on mountains, farmers tend to transfer plants to new sites at three-year intervals.

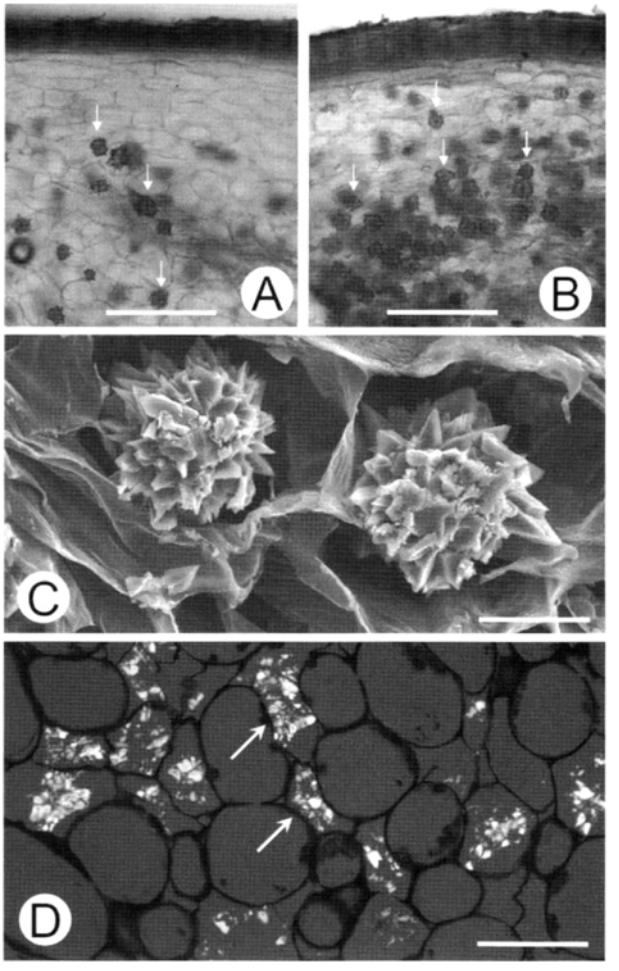

Figure 4. Calcium oxalate crystal accumulation in parenchyma cells. (**A**) Cross section of FCG rhizome (arrows indicate crystals). Bar = 180 μm. (**B**) Cross section of MCG rhizome (arrows indicate crystals). Bar = 180 μm. (**C**) Scanning electron microscope observation of crystals accumulated in roots. Bar = 25 μm. (**D**) Nomarski DIC prism fixed on light-microscopic observation of crystals after semi-thin-sectioning of roots (arrows indicate crystals). Bar = 60 μm.

Accumulation of Calcium Oxalate Crystals in MCG Roots

Our ginseng plants contained numerous calcium oxalate crystals in their roots and rhizomes. These accumulated mainly in the parenchyma cells around the cortex (Fig. 4A, B), and were rarely seen in the epidermal tissues, vascular cambium, phloem, or vessel elements. Their average size was about 118 μm and they appeared as clusters of tetrahedral-like tiny crystals (Fig. 4C). Accumulations were much higher in MCG roots than in FCG roots (Fig. 5), but for either type, these crystals were more abundant in their rhizomes (Fig. 4A, B, and 5). For MCG, the number of crystals in the rhizomes increased with root age (Fig. 5B) because rhizomes that formed earlier were situated in the basal region, which contained a higher number of crystals than did rhizomes that developed more recently in the upper portion (Fig. 5C). Crystals appeared in rainbow colors, as observed with a Nomarski DIC prism fitted on a light microscope (Fig. 4D). EDX analysis revealed that, as is commonly found, these calcium oxalate crystals contained Ca, C, and O as their major components (Fig. 6).

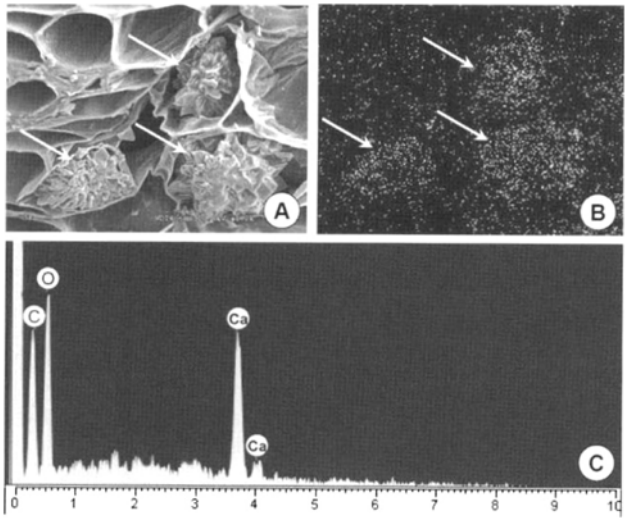

Figure 6. Energy dispersive X-ray analysis of crystals accumulated in MCG roots. (**A**) Scanning electron microscope observation of crystals. (**B**) Elemental mapping of Ca in crystals (arrows indicate Ca deposition). (**C**) EDX fraction of elements in crystals.

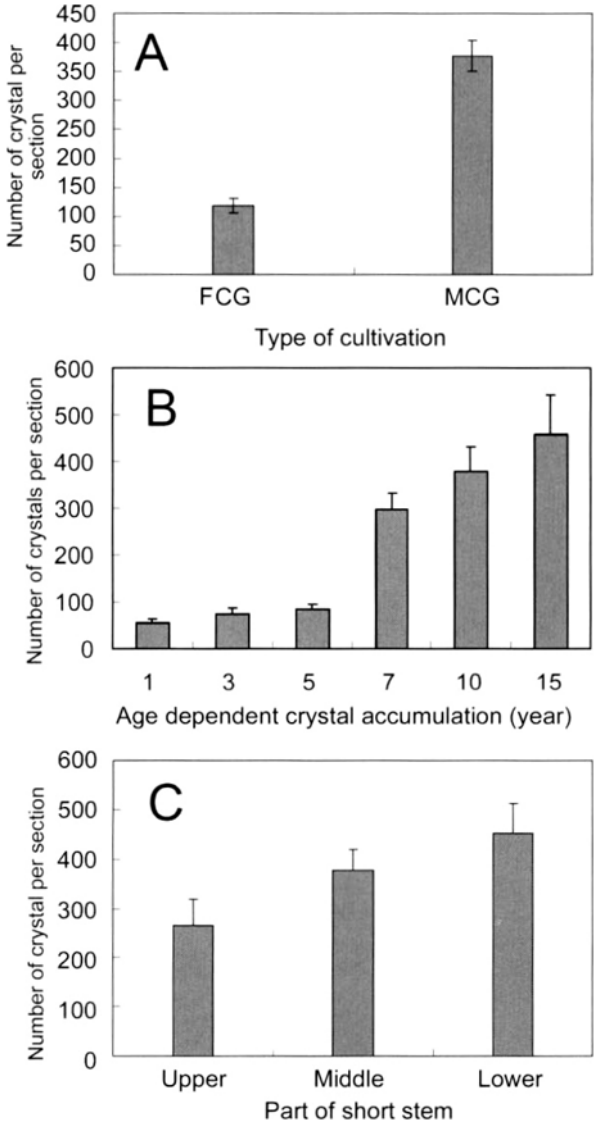

Figure 5. (**A**) Number of crystals per section of MCG and FCG rhizomes. (**B**) Increased number of chrystals per section of MCG rhizome. (**C**) Crystal numbers at different sites from MCG rhizome section.

In higher plants, calcium oxalate crystals can accumulate in the roots, stems, leaves, flowers, fruits, and seeds; their morphologies vary by species (Franceschi and Horner, 1980). These crystals mainly develop within the intra-vacuolar membrane chambers of specialized cells, i.e., crystal idioblasts (Arnott, 1983; Webb et al., 1995). Numerous functional roles have been hypothesized for such formations, including calcium regulation, plant defenses, and detoxification (Frank, 1972; Franceschi and Horner, 1980; Borchert, 1985). Accumulations in leaves are greatly enhanced when high concentrations of Ca are added to the culture media (Choi and Harada, 2005), although crystals can be deposited even when the calcium supply is limited (Frank, 1972). Leaves from seedlings subjected to herbivory also have greater crystal densities than those from protected seedlings (Molano-Flores, 2001). Furthermore, the mechanical wounding of leaves can sometimes lead to increased crystal densities in some species (Molano-Flores, 2001). In the desert lily (*Pancratium sickenbergeri*), three types of herbivores (mammalian, insect, and snail) all avoid feeding on tissues that contain raphide crystals (Ward et al., 1997; Ruiz et al., 2002). Calcium oxalate also has been suggested as a constitutive form of defense against bark borers in conifers (Hudgins et al., 2003). Based on those reports, we might conclude that the heavy accumulation of crystals in MCG rhizomes might be advantageous as a survival mechanism following herbivory attack, thereby explaining their decades-long growth, especially because the rhizomes are exposed year-round at the soil surface to promote future sprouting from those organs.

Table 4. Compositions and contents of ginsenosides from FCG and MCG roots.

Root age (yrs)		Ginsenoside content (mg g^{-1} DW)										
		Protopanaxatriol				Protopanaxadiol						Total
		Rg1	Re	Rf	Total	Rb1	Rb2	Rc	Rd	Rh2	Total	
FCG	5	7.76	7.68	2.90	18.34	6.80	2.58	5.04	0.52	-	14.94	32.28
MCG	5	7.70	7.04	2.48	17.22	7.24	3.60	6.74	0.96	0.60	19.14	36.36
	8	8.92	7.16	2.66	18.74	7.94	4.64	6.10	1.24	1.18	21.10	39.84
	10	11.74	7.84	2.80	22.38	8.82	6.16	7.34	1.70	1.70	25.72	48.10
	15	9.36	10.22	2.26	21.84	15.98	10.62	8.08	0.50	0.50	35.68	57.52

Ginseng roots were collected from Samcheok-kun of Kangwon province.

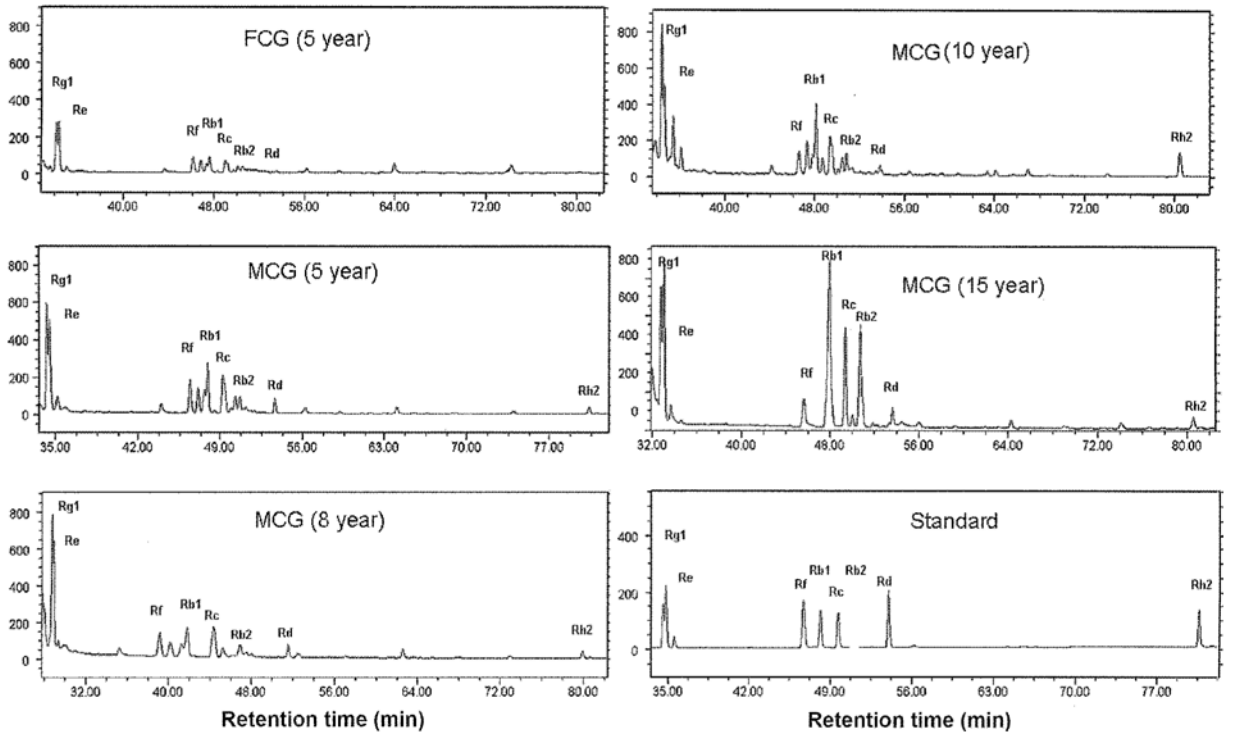

Figure 7. HPLC chromatogram of ginsenosides accumulated in MCG and FCG roots.

Ginsenoside Accumulation

HPLC analysis revealed that MCG roots contained higher amounts of ginsenosides compared with the FCG roots (Table 4). Total contents also slightly increased with root age (Table 4; Fig. 7). Interestingly, ginsenoside Rh2 was accumulated specifically in the MCG roots but not in those of FCG (Table 3; Fig. 7). That compound has been proposed to have a growth-suppressive effect on various cancer cells (Fujikawa-Yamamoto et al., 1987; Ota et al., 1997). When ginseng roots are treated with heat-steaming, the glucosyl moiety at C (20)-OH is partly lost to yield ginsenoside Rh2 as the artifact (Shibata, 2001). Ginsenoside Rh2 also can be produced by acid hydrolysis (Shibata, 2001). Nevertheless, it is still unclear why ginsenoside Rh2 accumulates only in mountain-cultivated ginseng.

To summarize, we have now demonstrated that, compared with field-cultivated ginseng roots, those grown on mountain sites have unique morphological and physiological characteristics, perhaps as a consequence of those particular environmental conditions. The heavy accumulation of Ca crystals in their rhizomes might be an adaptive mechanism for survival against herbivory attack. Furthermore, the age-dependent increase in MCG ginsenoside content can contribute to greater medicinal value from older roots.

ACKNOWLEDGEMENT

This work was supported by a grant from the Korea Forest Service, Korea.

Received January 2, 2007; accepted March 19, 2007.

LITERATURE CITED

Ando T, Tanaka O, Shibata S (1971) Chemical studies on the orien-

tal plant drugs: XXV. Comparative studies on the saponins and sapogenins of ginseng and related crude drugs. Syoyakugaku Zasshi 25: 28-32

Arnott HJ (1983) Three systems of biomineralization in plants with comments on the associated organic matrix, *In* GH Nancollas, ed, Biological Mineralization and Demineralization. Springer Verlag, Berlin, pp 199-218

Bel'chikova NP (1954) Determination of soil humus content by Tyurin's method, *In* Agrochemical Methods of Soil Analysis, Ed 2. Russian Academy of Science, Moscow, pp 35-42

Borchert R (1985) Calcium-induced pattern of calcium oxalate crystals in isolated leaflets of *Gleditsia triacanthos* L. and *Albizia julibrissin* Durazz. Planta 168: 571-578

Catt JA, Chambers BJ, Farina R, Harris GL, Hodgkinson R, Howse KR, Quinton JN (1998) Phosphorus losses from arable land in England. Soil Use Manage 14: 168-174

Chang MS, Yoo BS, Byun SY (2003) Characterization of polyacetylene contents in wild mountain ginseng and cultured ginseng. Kor J Biotechnol Bioengr 18: 440-442

Choi YE, Harada E (2005) Roles of calcium and cadmium on Cd-containing intra- and extracellular formation of Ca crystals in tobacco. J Plant Biol 48: 113-119

Coleman CI, Hebert JH, Reddy P (2003) The effects of *Panax ginseng* on quality of life. J Clin Pharm Ther 28: 5-15

Constant KM, Sheldrick WF (1991) An outlook for fertilizer demand, supply, and trade, 1988/89-1993/94, World Bank Technical Paper No. 137. World Bank, Washington DC

Cox MS (2001) The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci 166: 484-489

Cromer RN, Kriedemann PE, Sands PJ, Stewart LG (1993) Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of *Gamelia arborea*. Aust J Plant Physiol 20: 83-98

Ellis JM, Reddy P (2002) Effects of *Panax ginseng* on quality of life. Ann Pharmacother 36: 375-379

Franceschi VR, Horner HT (1980) Calcium oxalate crystals in plants. Bot Rev 46: 361-427

Frank E (1972) The formation of crystal idioblasts in *Canavalia ensiformis* DC at different levels of calcium supply. Z Pflanzenphysiol 67: 350-358

Fujikawa-Yamamoto K, Ota T, Odashima S, Abe H, Arichi S (1987) Different responses in the cell cycle of tumor cells to ginsenoside Rh2. Cancer J 1: 349-352

Hudgins JW, Krekling T, Franceschi VR (2003) Distribution of calcium oxalate crystals in the secondary phloem of conifers: A constitutive mechanism? New Phytol 159: 677-690

Huffaker RC, Wallace A (1959) Variation in root cation-exchange capacity within plant species. Agronom J 51: 118

Jackson ML (1962) Soil Chemical Analysis. Constable, London

Jacob J, Lawlor DW (1991) Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. J Exp Bot 42: 1003-1011

Jönsson U, Rosengren U, Thelin G, Nihlgård B (2003) Acidification-induced chemical changes in coniferous forest soils in southern Sweden 1988-1999. Environ Poll 123: 75-83

Kirschbaum MUF, Tompkins D (1990) Photosynthetic responses to phosphorus nutrition in *Eucalyptus grandis* seedlings. Aust J Plant Physiol 17: 527-535

Molano-Flores B (2001) Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Ann Bot 88: 387-391

Ota T, Maeda M, Odashima S, Ninomiya-Tsuji J, Tatsuka M (1997) G1 phase-specific suppression of the Cdk2 activity by ginsenoside Rh2 in cultured murine cells. Life Sci 60: PL39-PL44

Rao IM, Terry N (1989) Leaf phosphate status and photosynthesis *in vivo* in sugar beet: I. Changes in growth, photosynthesis and Calvin cycle enzymes. Plant Physiol 90: 814-819

Rodríguez D, Zubillaga MM, Ploschuk EL, Keltjens WG, Goudriaan J, Lavado RS (1998) Leaf area expansion and assimilate production in sunflower (*Helianthus annuus* L.) growing under low phosphorus conditions. Plant Soil 202: 133-147

Ruiz N, Ward D, Saltz D (2002) Calcium oxalate crystals in leaves of *Pancratium sickenbergeri*: Constitutive or induced defence? Funct Ecol 16: 99-105

Sarret G, Harada E, Choi YE, Isaure M, Geoffroy N, Fakra S, Marcus MA, Birschwilks M, Clemens S, Manceau A (2006) Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. Plant Physiol 141: 1021-1034

Sharpley AN, Smith SJ, Jones OR, Berg WA, Coleman GA (1992) The transport of bioavailable phosphorus in agricultural runoff. J Environ Qual 21: 30-35

Shibata S (2001) Chemistry and cancer preventing activity of ginseng saponins and some related terpenoid compounds. J Kor Med Sci 16: S28-37

Urban O (2004) Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses. Photosynthetica 41: 9-20

Vogler BK, Pittler MH, Ernst E (1999) The efficacy of ginseng: A systematic review of randomized clinical trials. Eur J Clin Pharmacol 55: 567-575

Ward D, Spiegel M, Saltz D (1997) Gazelle herbivory and interpopulation differences in calcium oxalate content of leaves of a desert lily. J Chem Ecol 23: 333-346

Webb MR, Cavaletto JM, Carpita NC, Lopez LE, Arnott HJ (1995) The intravacuolar organic matrix associated with calcium oxalate crystals in leaves of *Vitis*. Plant J 7: 633-648

Yeung EC (1999) The use of histology in the study of plant tissue culture systems-some practical comments. In Vitro Cell Dev Biol 35: 137-143