Correlations between Leaf and Soil Mineral Concentrations and Ginsenoside Contents in American Ginseng

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Abstract. Four-year-old American ginseng (*Panax quinquefolium* L.) plants and soil samples were collected from nine ginseng gardens. Soil and leaf mineral contents were determined and six major ginsenosides, Rb₁, Rb₂, Rc, Rd, Re, and Rg₁, were extracted from leaves and roots and quantified by high-performance liquid chromatography (HPLC). Correlation coefficients were more significant for soil nutrient levels vs. ginsenoside contents of leaves than of roots, suggesting that soil nutrient levels may play a major role in the synthesis of leaf ginsenosides. Minor elements in the leaf were also better correlated with ginsenoside contents of the root than that of the leaf. Iron content in the leaves exhibited highly significant correlations with the levels of Rb₁, Rb₂, Rc, and Rd, but calcium and copper contents were negatively correlated with Rg₁ in the roots.

American ginseng is one of the most widely used medicinal herbs in the world. It is native to North America, and was found in 1716 near Montreal, Quebec (Evans, 1985). Ginseng has been used as an energy booster and for a sense of well-being for thousands of years (Li, 1995). It may help maintain metabolic balance by eliciting a homeostatic effect (Lewis, 1988), and has been touted as an adaptogen (Xiao et al., 1987). It is harmless and ineffective in the absence of stress, but can normalize body processes when there is stress or damage, irrespective of the source (Fulder, 1977). The popularity of American ginseng among consumers has led to extensive cultivation in many parts of the world. In Canada, >3000 ha of ginseng are cultivated, mainly in the provinces of British Columbia and Ontario.

The active constituents of ginseng are dammarane saponins, commonly referred to as ginsenosides (Shibata et al., 1985), which are an important index of quality of ginseng. The major ginsenosides present in American ginseng are Rb₁, Rb₂, Rc, Rd, which possess 20(S)-protopanaxadiol as the aglycon; and Re and Rg₁, which possess 20(S)-protopanaxatriol as the aglycon (Shibata et al., 1985). Quantitative differences in ginsenosides vary depending on species (Lewis, 1988), plant organ (Li et al., 1996; Soldati and Sticher, 1980), age

(Soldati and Tanaka, 1984), growing conditions (Betz et al., 1984; Konsler et al., 1990), and harvest time (Xiao et al., 1987).

Many factors, including soil moisture level, fertility, texture, and pH, affect the growth of ginseng. Stolz (1982) demonstrated that N, P, Ca, and Mg were the major elements affecting root weight. Physiological stresses associated with differences in growth rate may also affect ginsenoside synthesis and accumulation (Konsler et al., 1990). Soil and plant mineral analyses are tools for understanding soil and plant nutrient levels, and interpretations can be used to decide amounts of fertilizer to apply. The literature regarding the adequate nutrient level and fertilization practices is limited. Khwaja and Roy (1995) used yield as a guide for determining suitable soil and plant nutrient levels for ginseng. The purpose of this project was to determine the relationships between mineral levels in soil and leaves and ginsenoside contents in root and leaf of American ginseng grown in British Columbia.

Materials and Methods

Six 4-year-old American ginseng plants and composite soil samples were collected from each of nine selected commercial ginseng gardens in British Columbia in early Sept. 1996. These producers were chosen because they had little disease incidence and above average yield per hectare (2800–3360 kg·ha⁻¹). Portions of each leaf sample were thoroughly mixed, and, together with soil samples, were subjected to mineral analyses conducted by a commercial laboratory (Griffin Laboratory Ltd., Kelowna, B.C.). For soil analysis, nitrate-N was extracted with 0.25 N HOAc + 0.015 N NH₄F and its concentration determined as nitrite using an automated copper-cadmium reduction procedure followed by color development by sulfanilamide and N-

(1 naphthyl) ethylenediamine dihydrochloride. Extractable Ca, Mg, P, S, and Na were determined by extraction with 0.25 N HOAc + 0.015 N NH₄F at a 1:10 v/v soil-extractant ratio. Their concentrations were measured on an inductively coupled argon plasma spectrophotometer (ARL 3400; Applied Research Laboratory, Valencia, Calif.) after 5 min of shaking. Levels of Z, Fe, Mn, and Cu were determined by extraction with DTPA-TEA-CaCl₂ solution at a 1:2 v/v soil-extractant ratio; TEA and CaCl₂ were used to buffer the extractant. The micronutrient concentrations were measured on the same argon plasma spectrophotometer. Boron was extracted from soil by refluxing in Taylor tubes at 1:2 v/v and 1:4 v/v (soil/distilled water), respectively. The concentration of B was measured on an inductively coupled spectrophotometer (ICP).

Samples of dried leaf tissue were ground and digested in HNO₃/HClO₄ (3:1) and Ca, K, Mg, P, Fe, Zn, Mn, Cu, B, and S were measured using ICP. Total nitrogen was measured colorimetrically with a Technicon Analyzer (Technicon Inc., Montreal) after organic N had been converted by Kjeldahl digestion with H₂SO₄, H₂O₂, and Se.

Remaining individual leaf and root samples were refrigerated and then freeze-dried at -65 °C within 1 week of sampling. Freeze-dried tissue was ground in a Wiley mill and stored at -35 °C until analyzed. Six major ginsenosides, Rb₁, Rb₂, Rc, Rd, Re, and Rg₁, in leaves and roots were extracted and quantified using reverse-phase HPLC (Li et al., 1996). Each ginsenoside was quantified using standard curves prepared by injecting measured volumes of stock solutions of authentic ginsenosides (Carl Roth GMBH, Karlsruhe, Germany). Correlation coefficients between mineral levels in leaf and soil samples and leaf and root ginsenoside contents were calculated with the PROC CORR procedures (SAS, 1990).

Results and Discussion

The analyses of soil and leaf samples collected from nine ginseng gardens showed a considerable variation, especially in concentrations of mineral elements (Table 1). Soil content of organic matter was negatively correlated with N and Ca ($r = -0.75$ and -0.74 , respectively) levels in the leaf. Salts and sulfur in soil were correlated with sulfur ($r = 0.67$ and 0.83 , respectively) level in the leaf, and phosphate and potassium in the soil were significantly correlated with potassium ($r = 0.68$ and 0.76 , respectively) level in the leaves. Calcium in the leaf was negatively associated with potassium and boron levels ($r = -0.77$ and -0.66 , respectively) in the soil. These results indicate that soil nutrient level may be one of the major factors affecting leaf nutrient level. Similar results were reported by Konsler and Shelton (1990). They found that foliar nutrient status of American ginseng was related to the growth, which may be directly affected by the nutrient level in the soil.

The level of total ginsenosides in the leaves was higher than in the roots (3.49% to 5.57%, vs. 2.45% to 3.89%, respectively) (Table 2).

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Table 1. Leaf and soil analysis results of samples collected from nine ginseng gardens.

Elements	Location								
	1	2	3	4	5	6	7	8	9
<i>Leaf</i>									
N ^z	1.90	2.20	2.00	2.10	2.50	2.10	2.30	2.20	2.40
P	0.19	0.18	0.13	0.16	0.19	0.17	0.15	0.14	0.13
K	1.70	1.63	2.00	0.69	1.44	0.48	1.77	1.97	1.34
Mg	0.46	0.14	0.11	0.33	0.32	0.45	0.30	0.19	0.15
Ca	1.34	1.55	1.23	1.81	1.85	1.80	1.61	1.56	1.66
S	0.70	0.62	0.46	0.77	1.00	0.92	1.14	0.49	0.90
Zn ^y	88	48	41	53	75	281	24	70	23
Mn	684	638	232	500	693	1814	682	432	161
Fe	238	212	230	235	299	224	244	567	296
B	71	44	36	23	56	31	35	62	35
Cu	6	4	4	7	10	9	7	6	7
<i>Soil</i>									
OM ^x (%)	15.9	4.1	9.3	2.6	2.4	2.2	2.5	2.2	0.8
pH	7.4	6.4	6.1	6.5	7.4	8.4	4.5	6.2	7.1
BD ^x (g·mL ⁻¹)	0.91	0.98	1.12	1.08	1.19	1.08	1.12	1.08	1.00
Salts (dS·m ⁻¹)	0.52	0.88	0.44	0.40	0.64	0.48	1.04	1.6	1.80
N ^w	22	40	23	23	20	22	24	109	25
P	19	127	126	21	96	16	118	80	26
K	201	197	472	117	177	103	205	333	123
Mg	676	151	187	139	179	257	92	268	111
Ca	2674	1734	1475	1347	1566	1755	266	1245	1418
S	47	67	37	27	36	53	68	45	108
B	0.57	0.44	1.05	0.11	0.28	0.58	0.39	0.94	0.35
Cu	1.3	0.8	2.0	2.5	1.7	1.7	2.3	2.5	1.4
Fe	31.7	66.5	94.6	49	27.4	8.6	177.8	31.5	38
Mn	15.3	12.0	24.1	15	5.1	5.0	146.2	15.8	4.9
Zn	3.1	5.3	4.6	2.2	3.0	0.8	3.3	1.8	2.0

^zAll major elements as %.

^yAll minor elements as µg·g⁻¹.

^xOM = organic matter; BD = bulk density.

^wAll elements ug·mL⁻¹.

There were more significant correlations between soil nutrient levels and ginsenoside contents in leaves (12 out of 17, or 70.6%) than in roots (5 out of 17, or 29.4%) (Table 3), indicating that soil nutrient levels may affect the synthesis of leaf ginsenosides. This finding is in agreement with the results of Konsler et al. (1990), who reported that root and leaf ginsenoside production tends to vary with soil fertility. Soil fertility was more often correlated with levels of ginsenosides in leaves than in roots. Zito et al. (1984) reported that differences in root and leaf ginsenoside content of 4-year-old American ginseng was associated with soil pH and phosphate level. In our study, soil pH, which ranged from 4.5 (garden 7) to 8.4 (garden 6) (Table 1), was negatively correlated with contents of Rb₁, Rb₂, and Rd in the leaves, but not in the roots (Table 3), indicating that soil pH may be an important contributing factor to differences between leaf and root ginsenosides. Phosphate level in the soil, which ranged from 16 to 127 µg·mL⁻¹, was not correlated with ginsenoside contents of either leaves or roots (Table 3).

Only a few correlations between leaf mineral contents and leaf and root ginsenoside contents were significant (Table 4). Minor element levels in the leaf were better correlated than those in the root. Iron content of the leaves (212–567 µg·g⁻¹) was highly correlated with the levels of Rb₁, Rb₂, Rc, and Rd in the roots, while Ca (1.34% to 1.85%) and Cu (4–6 µg·g⁻¹) levels were negatively correlated with Rg₁ in the roots.

Correlation coefficients between leaf and

Table 2. Contents of individual and total ginsenosides (g/100 g DW) in leaf and root samples of American ginseng collected from nine ginseng gardens.

Ginsenoside							
Garden	Rg ₁	Re	Rb ₁	Rc	Rb ₂	Rd	Total
<i>Leaf</i>							
1	0.27	1.57	0.09	0.17	0.43	1.58	4.11
2	0.33	1.78	0.22	0.39	0.83	2.02	5.57
3	0.22	1.63	0.26	0.28	0.59	1.43	4.41
4	0.24	1.26	0.31	0.33	0.70	2.29	5.13
5	0.22	1.38	0.22	0.36	0.93	2.02	5.13
6	0.35	1.76	0.19	0.33	0.50	1.00	4.13
7	0.25	1.71	0.29	0.32	0.50	1.81	4.88
8	0.26	1.54	0.23	0.30	0.42	1.63	4.38
9	0.26	1.56	0.17	0.27	0.35	0.88	3.49
<i>Root</i>							
1	0.16	1.04	0.87	0.21	0.02	0.22	2.52
2	0.16	1.35	0.84	0.15	0.02	0.27	2.79
3	0.19	1.03	0.77	0.17	0.02	0.27	2.45
4	0.16	1.26	1.46	0.17	0.02	0.23	3.30
5	0.14	1.23	1.86	0.23	0.03	0.40	3.89
6	0.19	1.38	1.44	0.21	0.03	0.31	3.56
7	0.12	1.00	1.09	0.17	0.02	0.25	2.65
8	0.11	0.84	1.13	0.14	0.02	0.29	2.53
9	0.11	1.14	1.53	0.17	0.02	0.33	3.30

root ginsenosides were low and nonsignificant, confirming previous reports by Konsler et al. (1990) and Li et al. (1996). Few correlation coefficients among individual ginsenosides within leaf or root were significant. Within the leaf, levels of Re and Rg₁ ($r = 0.66$), Rb₂ and Rc, and Rd ($r = 0.69$ and 0.71 , respectively) were significantly correlated; in the root, the correlations between content of Rb₁ and Rd ($r = 0.72$), contents of Rb₂ and Rc ($r = 0.76$), and Rb₂ and Rd ($r = 0.71$) were

significant. The consistent positive correlations between Rb₂ and Rc and Rd in both leaf and root suggest that Rb₂ could be considered as indicative of total ginsenoside content.

In summary, the results of this investigation point out the importance of soil nutrient levels in the accumulation of the biologically active ginsenosides in leaves and roots of American ginseng. Soils with slightly acidic pH and relatively high levels of Fe and S may produce ginseng with higher ginsenosides.

Table 3. Correlation coefficients (*r*) between soil analysis and leaf (L) and root (R) ginsenosides of ginseng.

Soil analysis	Rg ₁		Re		Rb ₁		Rc		Rb ₂		Rd	
	L	R	L	R	L	R	L	R	L	R	L	R
OM ² (%)	-0.17	0.32	0.05	-0.32	-0.54	-0.63	-0.77*	-0.19	-0.19	-0.37	-0.01	-0.51
pH	0.29	-0.41	0.48	-0.29	-0.72*	-0.04	-0.44	0.01	-0.66*	-0.01	-0.79**	0.26
BD ² (g·mL ⁻¹)	-0.56	-0.40	-0.41	-0.46	0.69*	0.24	0.28	-0.48	-0.04	-0.15	0.12	0.22
Salts (dS·m)	0.37	0.39	-0.14	0.71*	0.01	0.64	0.51	0.59	0.51	0.91**	0.06	0.54
N ³	-0.27	-0.05	-0.34	0.31	0.01	0.58	0.44	0.57	0.77*	0.65	0.38	0.75*
P	-0.20	0.28	-0.27	0.09	0.54	-0.23	0.48	-0.43	0.61	-0.19	0.59	-0.07
K	-0.55	0.47	-0.23	-0.11	0.20	-0.20	0.01	0.15	0.46	0.07	0.24	0.14
Mg	-0.12	-0.02	-0.07	-0.28	-0.82**	-0.22	-0.80**	0.43	-0.25	-0.13	-0.12	-0.18
Ca	0.26	-0.06	0.52	-0.29	-0.86**	-0.47	-0.62	0.14	-0.41	-0.15	-0.44	-0.10
S	0.76*	0.55	0.18	0.81**	-0.17	0.27	0.28	0.27	0.09	0.52	-0.26	0.08
B	-0.47	0.37	-0.26	0.07	-0.16	0.08	-0.12	0.40	0.37	0.23	-0.06	0.42
Cu	-0.78*	-0.26	-0.58	-0.27	0.59	0.48	0.11	0.21	0.16	0.15	0.30	0.23
Fe	-0.23	0.43	-0.47	0.25	0.66*	-0.10	0.24	-0.21	0.37	-0.26	0.59	-0.49
Mn	-0.30	0.15	-0.70	0.23	0.54	0.18	0.13	-0.10	0.28	-0.21	0.58	-0.40
Zn	0.13	0.50	0.21	0.05	0.22	-0.75**	0.17	-0.43	0.38	-0.40	0.46	-0.50

²OM = organic matter; BD = bulk density.³All elements as µg·mL⁻¹.*,**Correlation coefficient values significant at *P* ≤ 0.05 or 0.01, respectively.Table 4. Correlation coefficients (*r*) between leaf analysis and leaf (L) and root (R) ginsenosides of ginseng.

Leaf analysis	Rg ₁		Re		Rb ₁		Rc		Rb ₂		Rd	
	L	R	L	R	L	R	L	R	L	R	L	R
N ²	0.33	-0.15	-0.11	0.13	0.35	0.39	0.56	-0.26	0.07	0.33	0.07	0.27
P	0.14	-0.57	0.09	-0.43	-0.42	-0.36	-0.35	-0.43	-0.33	-0.59	0.10	-0.35
K	-0.19	0.62	-0.36	0.16	0.06	-0.14	0.09	0.26	0.62	0.22	0.51	-0.02
Mg	-0.20	-0.63	-0.25	-0.45	-0.42	0.07	-0.68*	-0.03	-0.63	-0.44	-0.18	-0.27
Ca	0.16	-0.77**	0.02	-0.11	0.18	0.46	0.28	-0.33	-0.33	-0.01	-0.19	0.26
S	0.69*	0.48	0.38	0.50	-0.16	0.23	0.14	0.36	-0.17	0.65	-0.50	0.15
Zn ³	-0.13	-0.60	-0.02	-0.17	-0.43	0.26	-0.34	-0.08	-0.47	-0.23	-0.56	0.31
Mn	-0.10	-0.65	-0.15	-0.12	-0.28	0.22	-0.26	-0.28	-0.41	-0.42	-0.32	0.11
Fe	-0.30	-0.10	-0.43	0.13	-0.01	0.69*	0.31	0.83**	0.55	0.76**	0.23	0.79**
B	-0.09	-0.01	-0.28	-0.18	-0.60	-0.02	-0.35	0.38	0.18	0.17	0.22	0.13
Cu	-0.06	-0.71*	-0.24	-0.41	-0.06	0.43	-0.18	-0.21	-0.60	-0.05	-0.32	0.16

²All major elements as %.³All minor elements as µg·g⁻¹.*,**Correlation coefficient values significant at *P* ≤ 0.05 or 0.01, respectively.

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