Applied Biochemistry and Microbiology, Vol. 37, No. 3, 2001, pp. 297–300. Translated from Prikladnaya Biokhimiya i Mikrobiologiya, Vol. 37, No. 3, 2001, pp. 344–348.

Mineral Composition of Cultured Ginseng Cells

T. M. Sovetkina*, T. K. Kalenik*, V. P. Bulgakov, E. G. Yasnetskaya**, and Yu. N. Zhuravlev****

** Far-Eastern State Academy of Economics and Management, Vladivostok, 690019 Russia*

*** Institute of Biology and Soil Science, Far-Eastern Division, Russian Academy of Sciences, Vladivostok, 690022 Russia*

Received September 14, 2000

Abstract—The contents of macroelements and microelements in ginseng roots and callus cultures was determined by atom absorption spectroscopy. Ginseng cells and tissues were shown to accumulate considerable amounts of microelements. The content of six of the eleven mineral components studied (K, Ca, Na, Mo, Mn, and Cr) in callus cultures was higher than that in roots of agricultural ginseng plants. We revealed good correlations between the contents of microelements (K, Ca, and Mg), as well as between the concentrations of macroelements (Mo, Li, Cu, and Cr), in ginseng cultures. The ability to accumulate elements varied between ginseng species, which was probably related to their genetic features. Our findings indicate that cultured ginseng cells hold much promise as a source of microelements.

Ginsenoids are the major biologically active substances of ginseng [1]. The medicinal properties of these plants are also related to the presence of other physiological components, such as oligopeptides, amino acids, microelements, vitamins, and enzymes [2]. The importance of many microelements is beyond doubt. As, B, Cr, Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, V, and Zn are believed to be essential to living organisms [3]. The mineral composition of ginseng roots has received little attention [2]. Ginseng calluses were shown to contain Ag, Cu, Fe, Ge, Mg, Ni, and Zn [4]. We found no data on the mineral composition of callus cultures of other plants (SCI database). Lovkova *et al.* showed that alkaloid-producing medicinal plants accumulate considerable amounts of microelements that determine their properties [5].

This work was designed to evaluate the importance of ginseng cell cultures as sources of mineral elements, to compare the mineral compositions of natural ginseng roots and callus cultures, and to study a culture of transgenic ginseng roots obtained in recent works.

MATERIALS AND METHODS

Materials. Roots of five-year-old *Panax ginseng* C. A. Mey plants were obtained from the Zhen'shen' state farm (Varvarovka, Primorskii krai). Callus cultures of *P. ginseng* maintained in the Collection of Cell Cultures since their isolation in 1988 (Institute of Biology and Soil Science) [6] and transgenic roots 1c-rolC-II obtained from the line 1c in 1993 [7] were used. The following cultures were studied: shoots of two-month-old ginseng plantlets (1c, Mimaki; 2c, Japanese population; 4c, Korean population; and 7c and 7l, Primorskaya populations), leaf plates of 2-year-old greenhouse plants, roots of two-month-old seedlings (7k, Primorskaya population), rhizome base of 17-year-old wild ginseng (10, Chuguevskii region, Primorskii krai), flower buds of eight-year-old wild ginseng (8ts, Ussuriiskii region, Primorskii krai), and seedlings of Primorskii ginseng obtained after transformation of *Agrobacterium rhizogenes* A4 (R-1) [8].

Culturing conditions. Callus cultures and the culture of transgenic roots 1c-rolC-II were grown in 250-ml Erlenmeyer's flasks with 60 ml of agar-treated W_{4CPA} and W_{IBA} media, respectively, in the dark at 25°C and 50–70% relative humidity. The nutrient medium contained 400 mg/l NH_4NO_3, 1900 mg/l KNO_3, 665 mg/l $CaCl_2 \cdot 6H_2O$, 170 mg/l $MgSO_4 \cdot 7H_2O$, 170 mg/l KH_2PO_4, 6.2 mg/l H_3BO_3, 22.3 mg/l $MnSO_4 \cdot 4H_2O$, 0.025 mg/l $CoCl_2 \cdot 6H_2O$, 0.025 mg/l $CuSO_4 \cdot 5H_2O$, 8.6 mg/l $ZnSO_4 \cdot 7H_2O$, 0.25 mg/l $Na_2MO_4 \cdot 2H_2O$, 0.83 mg/l KJ, 22.3 mg/l $FeSO_4 \cdot 7H_2O$, 38 mg/l Na–EDTA, 100 mg/l mesoinositol, 100 mg/l peptone, 0.2 mg/l thiamine hydrochloride, 0.5 mg/l pyridoxine hydrochloride, 0.5 mg/l nicotinic acid, 0.4 mg/l 4-chlorophenylacetic acid, 25 000 mg/l sucrose, and 6000 mg/l agar [6].

The transformed roots were grown on a shaker at 80 rpm with an amplitude of 20–30 mm for 30 days.

Elemental composition. The elemental composition was estimated, after incinerating with nitric acid vapors, by atom absorption spectroscopy on an ASH device (Nippon–Jarrel, Germany) [9]. The content of elements was expressed in μg/g dry weight.

Data processing. The results were analyzed by using Statistica software (Windows 98).

RESULTS AND DISCUSSION

Importance of Ginseng callus cultures as a source of mineral elements. Table 1 shows the elemental compositions of natural and transgenic ginseng roots and various callus cultures. The cultured ginseng cells differed in their ability to accumulate mineral elements. The content of six of the eleven mineral components

Table 1. Contents of macroelements and microelements in natural ginseng roots, cultured cells, and tissues, and their daily consumption rates

Sample*	Origin	Elemental composition, μg/g dry weight										
		K	Ca	Na	Mg	Fe	Al	Mo	Li	Mn	Cu	Cr
Root	Zhen'shen' state farm	10000	2000	1500	1800	465	550	1	14	30	14	4
1c-rol-II	Culture 1c transformed with rolC gene	62000	4500	2500	1850	470	350	30	10	310	11	5
R-1	Root cultures	32000	4000	1500	1250	200	175	10	9	150	13	7
7k		50000	8250	2300	2650	195	175	15	11	270	6	4
10		70000	5830	2500	2000	283	125	42	6	258	38	8
1c	Stem cultures	70000	10250	2100	2750	178	350	40	3	335	9	6
2c		62000	7000	1800	3000	550	475	20	3	280	7	9
4c		34000	4250	2100	1350	235	875	10	7	175	20	5
7cc		38000	4000	3600	2300	400	500	40	10	180	1	8
7l	Leaf cultures	85000	7750	2750	3850	360	975	10	7	345	11	10
8ts	Flower cultures	75000	13250	3000	3500	345	175	25	15	490	15	8
Recommended daily rates, μg		$6–8 \times 10^6$	$0.5–1 \times 10^6$	$4–5 \times 10^6$	$0.3–0.4 \times 10^6$	10000–18000	2000–3000	30–500	2000	500–5000	$2–5 \times 10^6$	50–200

* The sample included roots of five plants or callus tissues taken from five culture flasks.

Table 2. Correlation matrix of mineral elements in ginseng cultures

Element	K	Ca	Na	Mg	Mo	Li	Mn	Cu	Cr
K	1.00	0.81*	0.66*	0.83*	–0.29	–0.62*	0.89*	–0.51	–0.54
Ca	0.81*	1.00	0.56	0.83*	–0.30	–0.51	0.91*	–0.50	–0.49
Na	0.66*	0.56	1.00	0.69*	–0.42	–0.70	0.57	–0.72*	–0.71*
Mg	0.83*	0.83*	0.69*	1.00	–0.49	–0.63*	0.76*	–0.67*	–0.69*
Mo	–0.29	–0.30	–0.42	–0.49	1.00	0.82*	–0.11	0.82*	0.86*
Li	–0.62*	–0.51	–0.70*	–0.63*	0.82*	1.00	0.37	0.92*	0.98*
Mn	0.89*	0.91	0.57	0.76*	–0.11	–0.37	1.00	–0.34	–0.33
Cu	–0.51	–0.50	–0.72*	–0.67*	0.82*	0.92*	–0.34	1.00	0.94*
Cr	–0.54	–0.49	–0.71*	–0.60*	0.86*	0.98*	–0.33	0.94*	1.00

* Statistically significant correlation ($p < 0.05$, Statistica software).

studied (K, Ca, Na, Mo, Mn, and Cr) in the callus cultures was higher than in the roots of natural ginseng plants. Some ginseng lines displayed especially high capacities of accumulating these minerals. The content of potassium in line 7 l was 8.5 times higher than in natural ginseng roots. In lines 1c, 8ts, and 10, the concentration of potassium was sevenfold higher than that in natural ginseng roots. The content of calcium in lines 8ts and 1c was five times higher than that in natural ginseng roots. In contrast to ginseng roots that contain no molybdenum, cultured cells accumulate considerable amounts of this element. The content of manganese in lines 8ts, 1c, and 7l was one order of magnitude higher than that in ginseng roots. The content of chromium in lines 7l, 2c, 7c, 8ts, and 10 was 2–2.5 times higher than that in natural ginseng roots.

The content of Mg, Fe, Al, Li, and Cu in the cultured ginseng cells was lower than in the roots. In some ginseng lines, the contents of Mg and Al (line 7l), Fe (line 2c), Li (line 8ts), and Cu (lines 10) were higher than in the natural roots (Table 1).

The elemental composition of transgenic ginseng roots was studied. Transgenic roots were shown to be a good source of essential elements. These roots accumulated greater amounts of K, Ca, Na, Mg, Fe, Mo, Cr, and Mn (compared to natural roots). In addition to this, the content of Al, Li, and Cu in transgenic roots approached that in natural roots.

Features of accumulation of individual metals. The relationship between the processes of accumulation of various metals in cultured ginseng cells was studied. We revealed good correlations between the contents of

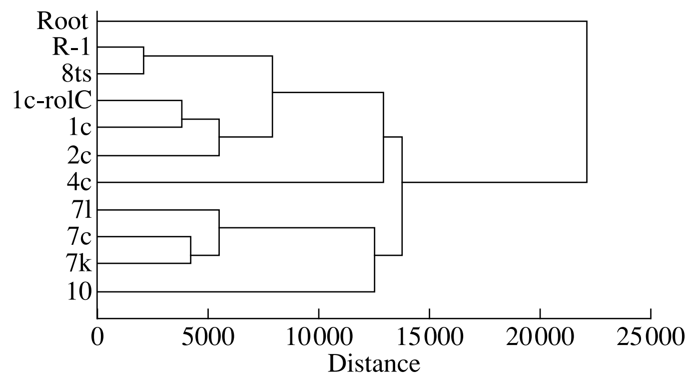

Fig. 1. Cluster analysis of elemental compositions of natural ginseng roots, callus cultures, and transgenic roots.

microelements (K, Ca, and Mg; Table 2), as well as between the concentrations of Mn and other macroelements in cultured cells. These data suggest the same mechanisms of their accumulation (although Mn is believed to belong to microelements). The analysis of correlation coefficients for sodium showed that the accumulation of Li, Cu, and Cr should be expected to be inhibited in Na-rich cultures (Table 2).

Correlations between the accumulation of such microelements as Mo, Li, Cu, and Cr were high. Cluster analysis by the method of Ward with the percentage of variance taken into account showed minimal variances in the contents of these elements in callus cultures (data not shown).

Grouping of ginseng lines by their ability to accumulate metals. Cluster analysis was performed and a dendrogram was constructed to estimate the contents of metals in ginseng cultures originating from various sources (Fig. 1). Roots of natural ginseng plants differed from ginseng cultures in the content of their elements. The natural and transgenic roots could be expected to form a separate cluster in the dendrogram. However, transgenic roots and line 1c occurred in the same cluster. Transgenic roots 1c-rolC were obtained from 1c calluses by their direct transformation [7], which indicated the close relationships between these cultures. This phylogenetic similarity of transgenic roots and 1c calluses appeared to be the major factor determining the content of elements. The accumulation of metals in ginseng cultures appeared to depend on their origin. All Primorskaya populations (7l, 7c, 7k, and 10) obtained from various plant organs formed an individual cluster. Despite long-term *in vitro* culturing, ginseng cells retained their genetically determined peculiarities of metal accumulation. The accumulation of elements also depended on the source of calluses (stems, leaves, and roots). For example, ginseng stem cultures of non-Primorskii origin (1c, 2c, and 4c) were similar with respect to this parameter.

Thus, the accumulation of metals in ginseng cultures depended on their phylogenetic similarity rather than on the source from which they were obtained. Studies of the distribution of ginsenoids in 22 ginseng cultures [6] showed that their contents were determined by the origin of the plants and (to a lesser extent) the source of the calluses. We calculated the coefficients of correlation between the contents of metals and ginsenoids in ginseng roots and cultures (such a relationship was demonstrated for alkaloids [5]). In our experiments, this dependence was not found for the majority of metals (correlation analysis, data not shown). The one exception was the negative correlation between the accumulation of ginsenoids and chromium in ginseng tissues (a correlation coefficient of –0.67).

Contents of heavy metals. We measured the contents of toxic elements (heavy metals), because the data discussed above showed that ginseng calluses can accumulate relatively large amounts of metals (Table 3). The ginseng calluses contained no arsenic, cadmium, or mercury. The ginseng roots and calluses contained insignificant amounts of lead, not exceeding the maximum permissible concentrations (0.05 and 0.88 μg/g dry weight, respectively) [10].

Our experiments showed that food additives and medicines prepared from ginseng callus cultures contain considerable amounts of microelements but are poor sources of macroelements. The addition of ginseng at concentrations recommended for medical uses [11] or in the food industry [12] can compensate microelement deficiency and even cover the daily needs of the human body (Table 1). The biological accessibility of these elements and the form, in which they are accumulated by cells, are unknown. The data suggest that microelements are responsible for some physiological effects of the Biozhen'shen' tincture produced by several Russian manufacturers.

We showed for the first time that ginseng callus cultures differ in their ability to accumulate macroelements and microelements. These differences are significant and vary from 2.5 (for K, Na, and Cr) to 38 times (for Cu). We found no correlations between the ability to accumulate elements and any culture characteristic (morphological peculiarities, growth rate, and synthesis of ginsenoids). However, phylogenetically similar

Table 3. Content of heavy metals in ginseng plants and calluses

Sample	Heavy metals, μg/g dry weight					
	Zn	Cd	Pb	Cu	As	Hg
Natural root (Zhen'shen' state farm)	22.01	0.03	0.05	13.50	not found	not found
Strain R-1	124.15	not found	0.88	13.50	not found	not found

cultures accumulated comparable amounts of metals (e.g., 1c and 1c-rolC-II; and calluses of the Primorskaya population, lines 10, 7k, 7l, and 7c; Fig. 1). There were also good correlations between the contents of microelements and macroelements (Table 2). These data indicate a highly selective accumulation of elements by cultured ginseng cells.

We found no correlation between the contents of metals and ginsenoids in ginseng calluses (although such a interrelation was demonstrated for natural plant alkaloids [5]). This was probably related to the considerable variability in the content of ginsenoids in cultured ginseng cells [7].

The samples studied contained lead (Table 3). Since cultured ginseng cells can accumulate both useful and toxic metals, it is necessary to test the chemical reagents added to the culture medium for the presence of lead (especially, during culturing of plant cells).

REFERENCES

1. Tanaka, O. and Kasai, R., *Progress in the Chemistry of Organic Natural Products*, Vienna: Springer, 1984, vol. 46, pp. 1–76.
2. Domeu, S. and Bergmann, H., *Proc. 1st Europ. Ginseng Cong. on Ginseng in Europe*, Marburg: Philipps-Universität, 1998, pp. 199–205.
3. House, W.A., *Field Crops Res.*, 1999, vol. 60, pp. 115–141.
4. Slepyan, L., *Proc. VII Int. Symp. on Ginseng*, Seoul, Korea, 1998, pp. 71–82.
5. Lovkova, M.Ya., Sokolova, S.M., Ponomareva, S.M., Buzuk, G.N., and Kliment'eva, N.I., *Prikl. Biokhim. Mikrobiol.*, 1999, vol. 35, no. 1, pp. 80–86.
6. Bulgakov, V.P., Zhuravlev, Yu.N., Kozyrenko, M.M., *et al.*, *Rast. Res.*, 1991, vol. 27, no. 3, pp. 94–100.
7. Bulgakov, V.P., Khodakovskaya, M.V., Labetskaya, N.V., Chernoded, G.K., and Zhuravlev, Y.N., *Phytochemistry*, 1998, vol. 49, no. 7, pp. 1929–1934.
8. Zhuravlev, Yu.N., Bulgakov, V.P., and Moroz, L.A., *Dokl. Akad. Nauk SSSR*, 1990, vol. 311, no. 4, pp. 1017–1019.
9. *Bol'shoi praktikum po fiziologii rastenii* (Big Practical Guide on Plant Physiology), Rubina, B.A., Ed., Moscow: Vysshaya shkola, 1978.
10. *Gosudarstvennaya farmakopeya SSSR: Obshchie metody analiza. Lekarstvennoe rastitel'noe syr'e* (USSR State Pharmacopoeia: General methods of Analysis. Medicinal Plant Raw Material), Moscow: Meditsina, 1989, p. 400.
11. Mashkovskii, M.D., *Lekarstvennye sredstva* (Medicinal Preparations), Khar'eov: Torsin, 1998, vol. 1.
12. Kalenik, T.K., Sovetkina, T.M., and Bulgakov, V.P., *Vestn. Dal'nevost. Gos. Akad. Ekon. Upravl.*, 1999, vol. 3, no. 11, pp. 14–19.