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Effects of Soil Selenium Supplementation Level on Selenium Contents of Green Tea Leaves and Milk Vetch

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Abstract

This study was conducted to investigate the effects of soil selenium (Se) supplementation level on Se contents of green tea and milk vetch. Four different concentrations of sodium selenite (Na_2SeO_3) solutions (0.0, 3.3, 33.0 and 165.0 μg/mL) were prepared and one liter of each solution was well mixed with 10 kg of compost (cowpea soil) to give four different levels of Se-containing soil: T_1, 0; T_2, 33; T_3, 330; T_4, and 1,650 μg/100 g soil. Green tea plants and milk vetch were individually cultivated in those soils for 60 days. Se contents of freeze-dried green tea leaves were 6.87, 10.40, 12.04, and 20.19 μg/g, respectively; all of which were significantly different ($p<0.05$) from the others except for T_2 and T_3. The results showed that Se-contents of green tea leaves were increased 1.5~2.9 times as the Se level in the soil increased. Regression equation between Se contents in green tea (Y) and soil Se supplementation level (X) was: $Y=0.007X+8.857$. However, Se contents in the milk vetch were increased significantly ($p<0.05$) more with the same treatments T_3 (74 μg/g) and T_4 (187 μg/g) in comparison to those at T_1 (5.0 μg/g) and T_2 (12.0 μg/g). The increases ranged from approximately between 2.4 to 37.4 times that of the control group. Regression equation between Se contents in milk vetch (Y) and soil Se supplementation level (X) was: $Y=0.1063X+15.989$. The large difference of Se contents between green tea leaves and milk vetch would be attributed by the difference of protein contents between the 30% or higher protein-content of legumes and 15~20% protein of shrubs. The present study clearly indicates that green tea leaves and milk vetch can be enriched in selenium by supplementing the soil with Se. Therefore, Se-enriched green tea or milk vetch powder could be utilized as functional foods in Se-fortified green tea drinks or salads, or as food additives to enhance the daily intake of Se.

Key words: selenium, green tea leaves, milk vetch, sodium selenite

INTRODUCTION

Selenium (Se) is a trace element mainly distributed in those liver, kidney, heart, and spleen. It is a component of glutathione peroxidase, a strong antioxidant which helps remove harmful reactive oxygen species (ROS) from the body (1). The element offers protection against oxidative red blood cell breakdown, helps regeneration of the liver in liver cirrhosis, delays ageing, and prevents mercury toxicities. Selenium has also found to enhance immunity against viruses in hepatitis and AIDS, and relieve pains in arthritis (2). Clark et al. (3) found that people who took 200 μg/d of Se were shown to have 63, 58, and 46% less susceptibility against prostatic carcinoma, rectal cancer, and lung cancer, respectively, than people who did not. It has been suggested that 50 μg be set as Dietary Reference Intake for Koreans and that the maximum daily intake should be 400 μg (4). The average Se intake in Koreans is 43 μg/day, which is a less amount than the recommended daily allowance (5). As the Se content of plants generally depends on Se content of soil (6), Se contents of Korean crops are low because of low Se content of Korean soil. Therefore, Se intake of Koreans may not be sufficient to meet the daily needs, since plant foods are the predominant components of Korean dishes. Se availability in metabolic processes and biochemical functions and its toxicity can be affected by the types of Se sources. The most abundant biological forms of Se existing in nature are selenomethionine and selenocysteine and

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these organic forms of Se can be absorbed into body more readily than the inorganic forms (7). Plants synthesize selenomethionine from the absorbed inorganic Se in the soil and then incorporate it into protein (8). The selenomethionine uptake through vegetable food is first stored as part of amino acids in body proteins, Se can then be released during protein degradation and used for selenocysteine biosynthesis (9,10). Selenium plays important roles in animals and humans; thus Se-rich products or plants have been widely developed and used in various food and pharmaceutical products. For example, Se-enriched mushrooms were grown in a medium supplemented with inorganic Se. The absorbed inorganic Se was converted into organic Se (11). In addition to the Se-enriched mushrooms, various other Se-supplemented products such as high calcium dropwort (12), bean sprouts (13), and pumpkin (14) have been developed. The purpose of this study was to investigate the effects of soil selenium supplementation level on Se contents of green tea leaves and milk vetch.

MATERIALS AND METHODS

Cultivation of green tea & milk vetch

Green tea seedlings that were seeded in October, 2002 and grown for 8 months were obtained on 24th June 2003. Sodium selenite (Na_2SeO_3) solutions of four different concentrations (0.0, 3.3, 33.0 and 165.0 μg/mL) were prepared and one liter of each solution was mixed well with 10 kg compost (cowpea soil) to give four supplemental levels of Se supplementation in the soil: T_1, 0; T_2, 33; T_3, 330; T_4, 1650 μg/100 g soil (Table 1). The mixed soil was placed in PVC pots (43 cm × 57 cm × 22 cm) and eighteen green tea seedlings were placed in each pot, 4 cm apart from each other. Each of the four treatments was set up in triplicates. The milk vetch was planted in the same manner as the green tea. Cultivation temperature was monitored and maintained at 25±2.4°C (Table 2) in an experimental greenhouse system. Soil moisture content was maintained between 85 and 90%. Green tea and milk vetch were harvested 60 days after the planting. Green tea plants were separated into leaves, stems and roots to determine the percentage yield of each part.

Selenium analysis

Green tea and milk vetch were freeze-dried and ground with a 0.5 mm grinder. A precise 3 g of ground sample was put into a flask containing a 10 mL of equal volumes of nitric acid (HNO_3) and distilled water, and digested by heating the flask for 3～4 hours until the solution become clear. Once the sample was completely digested, H_2O_2 solution was added into the flask and the sample was redigested. The digested sample was reconstituted to 5 mL, and Se content analyzed. Se contents in both green tea and milk vetch were measured by ICP-MS (PerkinElmer 6100 USA).

Table 1. Selenium supplementation in the soil

Treatment	Se supplementation (μg/100 g)	Cowpea soil[1)] (kg)
T_1	0	10
T_2	33	10
T_3	330	10
T_4	1650	10

[1)]pH (1:5, v/v), 5.5～6.5; CC (ds/m, 1:5 v/v) 0.7～0.9; NH_4^+-N (mg/L), 60～90; NO_3^--N (mg/L), 180～230; Ava.-P_2O_5 (mg/L), 230～280; K_2O (mg/L), 60～100; CEC (cmol$^+$/L), 8～10; bulk density, 0.2～0.25 (g/mL).

Table 2. Maximum and minimum cultivation temperatures (°C)

Date	Max	Min	Date	Max	Min	Date	Max	Min
7.13	24	22	8.2	27	23	9.1	25	23
15	24	23	5	30	23	3	25	23
17	24	23	7	30	23	5	28	23
22	27	24	9	30	23	8	30	24
23	27	25	12	28	24	15	28	23
24	27	24	14	28	24			
29	28	24	19	28	24			
31	28	26	27	26	24			
			29	28	24			

Statistical analysis

All data were analyzed by Microsoft Excel (Microsoft Office 2003) to test the effects of soil Se supplementation level on Se contents of green tea leaves and milk vetch. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

Yields of green tea leaves, stems and roots

Yields in dry matter (DM, g/18 plants) of leaves, stems and roots of green tea after 60 days are shown in Table 3. Yields of leaves were 9.50, 9.17, 10.9, and 8.27 for T_1, T_2, T_3 and T_4, respectively. The yield of the T_3 group was significantly ($p<0.05$) higher than that of T_4. However, no significant differences in the yield of green tea leaves were found among T_1, T_2, and T_3 or among T_1, T_2, and T_4. Yields of both stems and roots for T_1, T_2, and T_3 were significantly ($p<0.05$) higher than those of T_4 with no significant differences among T_1, T_2, and T_3. The development of roots for T_4, which

Table 3. Yields in green tea leaves, stems and roots (DM, g/18 plants)

Sample[1)]	Leaves	Stem	Root	Total plant
T_1	$9.50\pm1.93^{ab2)}$	6.10 ± 0.46^{a}	28.0 ± 7.35^{a}	43.6 ± 8.83^{a}
T_2	9.17 ± 5.77^{ab}	6.30 ± 0.85^{a}	32.6 ± 6.92^{a}	48.1 ± 12.51^{a}
T_3	10.9 ± 1.12^{a}	5.83 ± 0.65^{a}	29.8 ± 4.68^{a}	46.5 ± 4.94^{a}
T_4	8.27 ± 1.86^{b}	4.17 ± 0.81^{b}	15.7 ± 1.91^{b}	28.1 ± 1.69^{b}

[1)]Refer to table 1.
[2)]Mean±SD of triplicate groups of 18 plants, where values in the same column with different superscripts are significantly different at $p<0.05$.

was supplemented with high concentrations of Se, was severely retarded. The results suggested that Se soil supplementation should be less than 165 μg/g (T_4) for the optimal production of Se-enriched green tea leaves. A possible cause of the low yield at the highest level of Se in the soil could be the competitive absorption between Se and sulfur (S) into the plant, resulting in disruption of disulfide bond formation in proteins. This might have led to the formation of selenocystein instead of cystein, ultimately leading to structural changes of proteins that alter normal activities of sulfur-containing proteins (15).

Selenium contents of green tea leaves

Selenium contents of green tea leaves after 60 days of cultivation are shown in Table 4. They were 6.87, 10.40, 12.04, and 20.19 μg/g for T_1, T_2, T_3, and T_4, respectively. It was significantly higher in T4 ($p<0.05$) than other treatment groups. T_2 and T_3 also had significantly higher ($p<0.05$) Se content than T_1 group, but no difference was found between T_2 and T_3. The results showed that Se concentration in green tea leaves consistently increased with increased Se supplementation in the soil. The range of increase was from 1.5 to 2.9 fold. This indicates that only 2.48 g of green tea leaves from T_4 group would be sufficient to meet the recommended daily requirement of 50 μg Se (4), as compared to 7.28 g of green tea from T_1 group. As shown in Fig. 1, regression equation between the Se content of green tea (Y) and the supplementation level in the soil (X) was

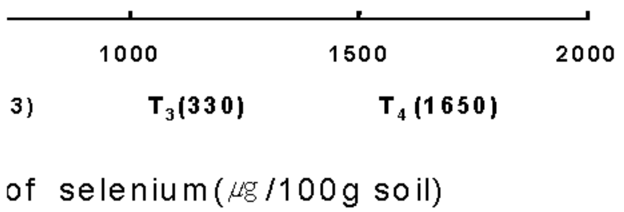

Fig. 1. Selenium contents of green tea leaves (μg/g).

$Y=0.007X+8.857$ ($r^2=0.9321$, $p<0.05$).

Selenium is volatile, very soluble in water and unstable with heating (16). Foster et al. (17) reported that Se content of Se enriched milk decreased by 6.2 to 7.8% with pasteurization at 71.7°C for 15 seconds. Therefore, more Se may be available from green tea in the form of powder mixed with foods than from pasteurized beverages.

Selenium contents of milk vetch

Se contents of milk vetch after cultivation for 60 days dramatically increased with higher Se supplementation levels in the soil as shown in Table 5. T_4 had significantly ($p<0.05$) higher content than other treatment groups and T_3 also had significantly ($p<0.05$) higher content than T_1 or T_2. No difference was found between T_1 and T_2. The increase of Se content in milk vetch ranged from 2.4 fold at low supplementation level to 37.4 fold at high supplementation level as compared to the control.

A greater concentration of Se in milk vetch than in green tea could be attributed to a higher protein content in leguminous plants such as milk vetch. The regression equation between Se contents of milk vetch (Y) and Se supplementation level in the soil (X) was $Y=0.1063X+15.989$ ($r^2=0.9661$, $p<0.05$) as presented in Fig. 2.

Table 4. Selenium contents of leaves of green tea (μg/g)

	Sample[1)]			
	T_1	T_2	T_3	T_4
Se cont.	$6.87\pm0.57^{a2)}$	10.40 ± 0.75^{b}	12.04 ± 1.35^{b}	20.19 ± 3.45^{c}
Index[3)]	100	151	175	293

[1)]Refer to Table 1.
[2)]Mean±SD, where values in the same row with different superscripts are significantly different at $p<0.05$.
[3)]Represented as the increased percentage value relative to the control.

Table 5. Selenium contents of milk vetch (μg/g)

	Sample[1)]			
	T_1	T_2	T_3	T_4
Se cont.	$5.0\pm0.02^{d2)}$	12.0 ± 0.04^{c}	74.0 ± 0.31^{b}	187 ± 0.40^{a}
Index[3)]	100	240	1480	3740

[1)]Refer to Table 1.
[2)]Mean±SD, where values in the same row with different superscripts are significantly different at $p<0.05$.
[3)]Represented as the increased percentage value relative to the control.

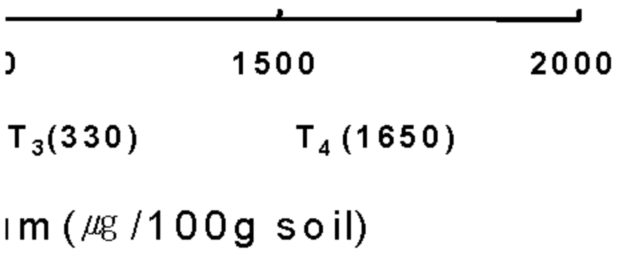

Fig. 2. Selenium contents of milk vetch (μg/g).

Comparison of Se in green tea and milk vetch

Selenium has similar properties as sulfur and is more readily absorbed by cruciferae and legumes (18). Rhee (19) reported that Se contents of Korean leguminous plants were 86, 40 and 25 μg/g for sweet clover, lespedeza and ladino clover, respectively; whereas Se content of acacia leaves, a shrub, was 14 μg/g. In this study, Se contents in green tea leaves, a shrub in camellia family, and milk vetch, a leguminous plant, grown in control soil were lower than the above-reported values, primarily because of shorter cultivation period.

Although no large difference was found in Se contents between green tea and milk vetch in unsupplemented control soil, the accumulation of Se in milk vetch was much higher than that of green tea at high levels of Se supplementation in the soil (T_3 and T_4). This could be partially attributed to high protein content in legumes (30%) as compared to shrubs (15～20%). However, a drastically higher Se content in milk vetch (187.0 μg/g) of T_4 as compared to green tea (20.19 μg/g) grown in the same soil (T_4) cannot be explained by the difference in protein content alone, so further study is need for investigating the mechanism. In a study with *Flammulina velutipes* cultivated in 2 mg Se-enriched medium, Lee and their colleagues (11) increased Se content of mushroom by approximately 4.51 μg of Se per gram dry weight (DM) by adding Se to the media, but a larger amount of Se was not absorbed into the mushrooms and remained in the medium. This may suggest that the production of Se-enriched green tea or milk vetch by addition of Se into the compost would be more effective than cultivating mushroom in a Se-supplemented medium. The accumulation of Se in the proteins of plants has been found to increase with increased Se-supplementation in the soil (20). Therefore, the production of Se-enriched plants through Se-supplementation in the soil would be an effective way of enhancing dietary intake of Se and improving Se nutriture in humans and animals. From the regression equations shown in Figures 1 and 2, the contents of Se in green tea and milk vetch can be regulated by controlling the Se supplementation level in the soil and thus, Se-enriched plants containing proper amount of Se can be used as food additives to provide optimum level of dietary selenium.

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