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Effect of Selenium on the Yield and Quality of Green Tea Leaves Harvested in Early Spring

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Foliar applications of a fertilizer of selenite or selenate were carried out to determine the influence of selenium on the yield and quality of green tea leaves harvested in early spring. Numbers of sprouts and the yield were significantly increased by the application of selenium. The sweetness and aroma of green tea leaves were also significantly enhanced, and bitterness was significantly decreased by the application of selenium. However, no significant differences were found in sweetness, bitterness, and aroma between tea leaves fertilized with selenite and selenate. Se concentration was significantly increased by selenium fertilization, and tea enriched by sodium selenate had a significantly higher selenium content than did tea enriched by sodium selenite. Total amino acid and vitamin C contents were significantly enhanced by the application of selenium. Tea polyphenol contents were significantly decreased by fertilization with selenium. The marked difference of tea polyphenols was also found between applications of selenite and selenate.

KEYWORDS: Green tea; selenium; yield; tea quality

INTRODUCTION

Selenium (Se) is important as an antioxidant in humans and animals (*1*). Selenium compounds inhibit tumorigenesis in experimental animals, and supplemental selenium reduces cancer risks in humans (*2*, *3*). It functions in the active site of a large number of selenium-dependent enzymes such as glutathione peroxidase (GSH-Px), which has antioxidant activity (*4*).

Little attention has been paid to selenium's role on the growth and yield of plants. Plants are generally considered not to require Se but to have a low tolerance for selenium. However, recently it has been demonstrated that Se is able to protect plants, such as ryegrass (*Lolium perenne* L.) and lettuce (*Lactuca sativa* L.), against UV-induced oxidative stress and even to promote the growth of plants subjected to high-energy light (*5*). The antioxidant capacity of Se was related to its inhibition of lipid peroxidation and promotion of GSH-Px and superoxide dismutase (SOD) activities (*6*, *7*). Se addition markedly promoted wheat seedling and seed germination and stimulated the growth of a senescing seedling, rice tiller (*8*−*10*). The effect was found to be dose-dependent. However, there were no significant effects of Se treatment on coriander (*Coriandrum sativum* L.) (*11*). Se fertilization also increased the yield of lettuce and rice (*6*, *10*). Selenium at <20 mg kg^{-1} can also enhance the contents of total amino acids and several elements, such as iron, copper, and calcium in *Spirulina platensis* and endive (*Cichorium endivia*) (*12*, *13*). The application of selenium increased Se accumulation, both total protein and amino acids, soluble sugar, and DNA and RNA concentrations in potatoes, onions, endive, and garlic (*14*−*16*).

Few papers have discussed the effect of Se on the tea tree (*17*, *18*). Recent attention has been focused on the antioxidative activity, antimutagenic activity, and anticancer effects of green tea, due to its high polyphenol compound content and microelements (*19*). Our previous studies have shown that foliar application of Se-enriched fertilizer could significantly increase not only Se, total amino acid, and vitamin C concentrations but also the sensory quality of green tea leaves harvested in summer (*20*−*22*). Tea leaves harvested in early spring before April 5 have a lower yield, better sensory quality, and higher contents of total amino acids and vitamin C and lower contents of tea polyphenols and caffeine (*23*). The tea leaves harvested in early spring provide much higher commercial and economic value (*24*). It was of interest to determine whether the yield and quality of tea leaves harvested in early spring are enhanced by foliar application of fertilizer of selenite and selenate compared to regular green tea.

MATERIALS AND METHODS

Selenium Fertilizer. Preparation of selenium fertilizer was detailed by Hu (*21*). Lobster waste, chicken excreta, silkworm excreta, pig excreta, and EM bacterium were mixed in an appropriate ratio to ferment for ∼2 weeks in a methane-generating pit. Se in the form of sodium selenite or sodium selenate and water were added to mixed fertilizer and well distributed, and the mixture continued to ferment for 4 weeks. The fermented solution was filtered and concentrated in

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Table 1. Effect of Application of Selenium on Number of Sprouts in Tea Tree and Yields of Tea Leaves Harvested in Early Spring[a]

treatment	sprout numbers of tea tree per m^2 after application of selenium				yields of tea leaves harvested during early spring ($g\ m^{-2}$)			total yield ($g\ m^{-2}$)
	14 days	16 days	17 days	18 days	first	second	third	
control	23 ± 4.0a	41 ± 4.5a	45 ± 6.5a	51 ± 9.5a	0.47	1.15	3.13	4.97
selenite fertilizer	45 ± 1.5b	71 ± 3.5b	72 ± 0.6b	81 ± 0.6b	1.37	2.29	5.83	8.83
selenate fertilizer	58 ± 5.0c	56 ± 5.7b	73 ± 8.1b	79 ± 10.8b	1.91	2.50	5.83	10.24

[a] Values are means of three determinations ± standard deviations. Values followed by different letters are significantly different ($P < 0.05$) from one another.

Table 2. Effect of Application of Selenium on Sensory Quality of Tea Leaves Plucked in Early Spring[a]

sample	sweetness[b]	bitterness	astringency	aroma
control	7.2 ± 0.8a	8.7 ± 0.5a	9.0 ± 0.0a	7.0 ± 1.2a
selenite fertilizer	8.9 ± 0.4b	7.9 ± 0.3b	8.7 ± 0.3a	9.1 ± 0.8b
selenate fertilizer	8.8 ± 0.8b	7.9 ± 0.2b	7.7 ± 0.5b	8.8 ± 0.5b

[a] Values are means of three determinations ± standard deviations. Values followed by different letters are different ($P < 0.05$) from one another. [b] Sweetness and aroma were assessed on a scale with 10 as the highest intensity and 0 as the lowest intensity; astringent taste and bitterness were assessed on a scale with 0 as the best test and 10 as the worst taste.

a vacuum condition below 50 °C. The final organically bound Se solution contained Se at 50 g L^{-1}.

Experiment. The experiment was conducted on March 6, 2002, at the Institute of New Century Horticulture Science of Nanjing, Jiangsu Province. The tea garden area of the test district amounted to 460 m^2. Eight rows of tea trees under similar growth conditions were randomly selected, and the tea garden area of the test district was 20 m^2 in triplicate. The control was sprayed with water. Treatment 1 was a fertilizer of selenite, and treatment 2 was a fertilizer of selenate. Fertilizers of selenite and selenate were dissolved in water and applied as a foliar spray at the same Se content and volume. Se at a concentration of 60 mg of Se L^{-1} and a rate of 75 g ha^{-1} was sprayed onto old leaves of the tea trees. Sprouts in square meters of each plot were counted 13 days after foliar application up to 18 days. Then, all of the fresh sprouts of each treatment were collected and weighed every 5 days. The harvested sprouts were washed with distilled water three times and immediately processed into commercial roasted green tea (*21*). The moisture content of the final roasted green tea was <5%.

Assessment of Sensory Quality. Aqueous extraction of tea leaves was carried out under controlled conditions to give the extracts the optimum aroma, taste, and color characteristics. One gram of tea leaves was extracted with 100 mL of boiling distilled water in a glass for 3 min. The extract solution was filtered and then used for sensory evaluation in in the Food Analysis Laboratory. Ten qualified panelists were selected from 20 volunteers by a triplicated pretest in three tea infusions. Questionnaires, based on the method of magnitude estimation of descriptive analysis, were composed of 10 records—the highest intensity was expressed as 10 and the lowest as 0. The results of triplication were statistically analyzed for multiple comparisons using SPSS software.

Assay of the Chemical Quality. The contents of Se, vitamin C, amino acid, and tea polyphenol (TPP) were determined according to the method of Hu et al. (*21*). The tea sample was ground manually to a fine powder in a mortar. Two 1-g subsamples of each were taken for analysis. The fine powder samples were digested with 10 mL of a 4:1 mixture of HNO_3 and $HClO_4$ at 170−175 °C for 45 min. After cooling, 5 mL of concentrated HCl was added to the sample for reduction of Se^{6+} to Se^{4+} and continued for an additional 30 min until the sample was completely mineralized. Atomic fluorescence spectroscopy was used for the determination of Se in solution.

Vitamin C contents were analyzed by 2,6-dichlorophenolindophenol titration methods.

TPP contents were measured according to the Folin−Ciocalteu spectrophotometer assay.

Total amino acids were determined by using the ninhydrin spectrophotometer method.

Statistics Analysis. The data were presented as mean ± standard deviation of three determinations. Statistical analyses were performed using Student's *t* test and one-way analysis of variance. Multiple comparisons of means were done by least significant difference (LSD) test. A probability value of <0.05 was considered to be significant. All computations were made by employing the statistical software (SPSS, version 11.0).

RESULTS AND DISSCUSSION

Effect of Selenium on Growth and Yields of Tea Trees. The numbers of sprouts were significantly ($P < 0.05$) increased by foliar application of Se fertilizer (**Table 1**). Except on the 14th day after spraying, no significant difference was present between fertilizers of selenite and selenate. Sprouts were plucked from tea trees and the weights of harvestable tea per plot recorded. The total yield of tea leaves collected three times was ∼2-fold by application of Se compared with no selenium treatment. These data indicated that both selenite and selenate promoted tea tree growth. This result is in agreement with previously published work that sodium selenite can promote the growth of plants cultured in medium at modest concentrations, which are 78.9 mg L^{-1} for gallic and 12 mg L^{-1} for *Spirulina maxima* (*15*, *25*, *26*). In our study, the selenium concentration in fertilizer was 60 mg L^{-1}, and no toxic effect was observed during the growth of tea trees.

Effect of Se on Sensory and Chemical Qualities of Green Tea Leaves. The quality of green tea leaves is reflected in the four major characteristics of bitterness, astringency, sweetness, and aroma. The sweetness and aroma of green tea were significantly ($P < 0.05$) enhanced and the bitterness was significantly decreased by Se fertilization. However, except for astringency, there were no statistical ($P < 0.05$) differences in the bitterness, sweetness, and aroma of tea extracts between fertilizers of selenite and selenate (**Table 2**).

The Se content of green tea was significantly ($P < 0.05$) increased by foliar application of Se (**Table 3**). A marked

Table 3. Effect of Application of Selenium on Chemical Quality of Tea Leaves Plucked in Early Spring[a]

sample	selenium (mg kg^{-1})	tea polyphenol (g kg^{-1})	amino acid (g kg^{-1})	vitamin C (g kg^{-1})
control	0.033 ± 0.002a	240.92 ± 4.71a	34.77 ± 0.34a	2.45 ± 1.51a
selenite fertilizer	7.555 ± 0.823b	231.02 ± 3.30b	36.20 ± 0.56b	2.89 ± 0.93b
selenate fertilizer	10.610 ± 0.371c	209.35 ± 0.64c	35.11 ± 0.58b	2.58 ± 0.45b

[a] Values are means of three determinations ± standard deviations. Values followed by different letters are different ($P < 0.05$) from one another.

difference was found between selenate and selenite. Selenate has been reported to accumulate in concentrations exceeding selenite in plants (*27*). Phenolic compounds are key elements that determine both the color and taste of green tea (*28*). The content of tea polyphenol was significantly ($P < 0.05$) decreased by the application of Se as compared to the control. Fertilization with selenate had a significant effect on decreasing TPP content compared with selenite (**Table 3**).

Amino acids play a vital role in the aroma of green tea, especially free amino acid such as theanine, which accounted for 40% of free amino acid and the content of which correlated to sweetness and aroma of green tea (*29*). The concentration of total amino acids in green tea was significantly enhanced by the application of Se (**Table 3**). Tea sprayed with selenium fertilizer had significantly ($P < 0.05$) higher amino acid content than regular tea without selenium treatment, and no significant difference was found between fertilizers of selenite and selenate. The vitamin C concentration was also significantly increased by the application of selenium. These data are consistent with the results of green tea leaves harvested in the typical summer tea-producing season reported by Hu (*21*). Lee et al. reported that essential oil and vitamin C contents were increased by selenate fertilization of coriander and that the vitamin C content of endive leaf tissues increased in response to increasing sodium selenate concentration (*11*, *13*). However, vitamin C content decreased linearly with increasing sodium selenite concentrations. Therefore, further studies were guaranteed for the efficacy of different chemical forms of Se in promoting the growth and enhancing the yield of tea leaves.

In conclusion, Se promoted tea tree sprouting in advance and increased yields of green tea. However, different chemical forms of Se have provided unequal effects on the growth and qualities of green tea.

LITERATURE CITED

(1) Ursini, F.; Bindoli, A. The role of Se peroxidases in the protection against oxidative damage of membranes. *Chem. Phys. Lipids* **1989**, *44*, 255−276.

(2) Ip, C. Lessons from basic research in Se and cancer prevention. *J. Nutr.* **1998**, *128*, 1845−1854.

(3) Ei-Bayoumy, K. The protective role of Se on genetic damage and on cancer. *Mutat. Res.* **2001**, *475*, 123−139.

(4) Rotruck, J.; Pope, A.; Gtanther, A.; Swanson, A.; Hafeman, D.; Hoekstra, W. Se: Biochemical role as a component of glutathione peroxidase. *Science* **1973**, *179*, 588−590.

(5) Hartikainen, H.; Xue, T. L. The promotive effect of selenium on plant growth as triggered by ultraviolet irradiation. *J. Environ. Qual.* **1999**, *28*, 1372−1375.

(6) Hartikainen, H.; Ekholm, P.; Piirongen, V.; Xue, T. L.; Koivu, T.; Yli-Halla, M. Quality of the ryegrass and lettuce yields as affected by selenium fertilization. *Agric. Food Sci. Finland* **1997**, *6*, 381−387.

(7) Xue, T. L.; Hartikainen, H. Association of antioxidative enzymes with the synergistic effect of selenium and UV irradiation in enhancing plant growth. *Agric. Food Sci. Finland* **2000**, *9*, 177−186.

(8) Peng, A.; Xu, Y.; Wang, Z. J. The effect of fulvic acid on the dose effect of selenite on the growth of wheat. *Biol. Trace Elem. Res.* **2001**, *83*, 275−279.

(9) Xue, T. L.; Hartikainen, H.; Piironen, V. Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil* **2001**, *237*, 55−61

(10) Wu, Y. Y.; Luo, Z. M.; Peng, Z. K. Research on the influence of Se provided at different levels upon the growth of rice and its accumulation of Se. *J. Hunan Agric. Univ.* **1998**, *24*, 176−179.

(11) Lee, M. J.; Kang, H. M.; Park, K. W. Effects of Se on growth, storage life, and internal quality of coriander (*Coriandrum sativum* L.) during storage. *J. Korean Soc. Horti. Sci.* **2000**, *41*, 490−494.

(12) Qiao, Y. H.; Shang, S. T. Effects of selenium (Se) on quality of *spirulina plate*ns (Sp.-D). *J. China Agric. Univ.* **2000**, *5*, 31−34.

(13) Lee, G. P.; Park K. W. Effect of Se concentration in the nutrient solution on the growth and internal quality of endive. *J. Korean Soc. Hortic. Sci.* **1998**, *39*, 391−396.

(14) Munshi, C. B.; Combs, G. F.; Mondy, N. I. Effects of Se on the nitrogenous constituents of the potato. *J. Agric. Food Chem.* **1990**, *38*, 2000−2002.

(15) Duan, Y, X.; Fu, T. Z. The absorption of Se by garlic and the effect of Se on the growth of garlic. *Guangdong Trace Elem. Sci.* **1997**, *4*, 52−55.

(16) Kopsell, D. A.; Randle, W. M. Selenate concentration affects selenium and sulfur uptake and accumulation by ‘Granex33’ onions. *J. Am. Soc. Hortic. Sci.* **1997**, *122*, 721−726.

(17) Xu C. X.; Li X. M.; Xiao, Y. S. Effect of spraying sodium selenite on Se content of tea. *J. Tea Sci.* **1996**, *16*, 19−23.

(18) Wang, M. Z. Se enrichment of tea from some areas of China. *J. Plant Nutr.* **1998**, *21*, 2557−2564.

(19) Amantana, A.; Santana-Rios, G.; Butler, J. A.; Xu, M. R.; Whanger, P. D.; Dashwood, R. Antimutagenic activity of Se-enriched green tea towards the heterocyclic amine 2-amino-3-methylimidazo[4,5]quinoline. *Biol. Trace Elem. Res.* **2002**, *86*, 177−191.

(20) Hu, Q. H.; Pan, G. X.; Zhu, J. C. Effect of fertilization on Se content of tea and the nutritional function of Se-enriched tea in rats. *Plant Soil* **2002**, *238*, 91−95.

(21) Hu, Q. H.; Xu, J.; Pan, G. X. The effect of Se sprays on green tea quality. *J. Sci. Food Agric.* **2001**, *81*, 1387−1390.

(22) Hu, Q. H.; Pan, G. X.; Zhu, J. C. Effect of Se on green tea preservation quality and amino acid composition of tea protein. *J. Hortic. Sci. Biotechnol.* **2001**, *76*, 344−346.

(23) Liang, Y. R.; Lu, J. L.; Shang, S. L. Effect of Tea Cuifasu on chemical composition and quality of tea. *J. Zhejiang For. Coll.* **1997**, *14*, 155−158.

(24) Wang, D. F.; Wang, C. H.; Shi, H. P.; Qi, Z. X. Applied study on foliar fertilizer for tea plants. *Acta Agron. Sinica* **1996**, *22* (1), 78−83.

(25) Guo J. C.; Yin S. P. The effect on glutathione peroxidase activity and glutathione content of higher plants by Se. *Acta Bot. Boreali-Occidentalia Sinica* **1998**, *18*, 533−537.

(26) Zhou, Z. H.; Zhong, G.; Liu, Z. L. Effects of Se on the growth and Se contents of *Spirulina maxima. Acta Oceanol. Sinica* **1997**, *19*, 42−45.

(27) Brown, T. A.; Shrift, A. Se: Toxicity and tolerance in higher plant. *Biol. Rev.* **1982**, *57*, 59−84.

(28) Wang, L. F.; Kim, D. M.; Lee, C. Y. Effects of wheat processing and storage on flavanols and sensory qualities of green tea beverage. *J. Agric. Food Chem.* **2000**, *48*, 4227−4232.

(29) Zhou, J. S. Chemical changes during green tea processing. In *Tea Biochemistry, 1*; Wang, Z. N., Ed.; Beijing, China, 1980; pp 259−263.

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