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Impact of Selenium Supplementation on Growth and Selenium Accumulation on Spinach (*Spinacia oleracea* L.) Plants

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Abstract

Selenium (Se) has been proved to be an essential element for humans and animals. However, less is known about its effects on plants. A hydroponic experiment was carried out to investigate the effects of selenium on growth, selenium accumulation and some physiological characteristics of spinach (*Spinacia oleracea* L. cv. 'Missouri') plants. Plants were grown in Hoagland nutrient solution amended with sodium selenite at 0 (control), 1, 2, 4, 6 and 10 mg.L^{-1} for 28 days. Growth parameters like shoot and root fresh weight, shoot and root dry weight, total dry weight, shoot and root length increased by 17, 15, 38, 19, 18 and 34 percent in response to the lowest concentration of Se (1 mg L^{-1}), respectively over control. However, application of higher Se concentrations reduced these parameters as compared to control. Selenium up to 1 mg L^{-1} enhanced the levels of chlorophyll a and chlorophyll b by 87 and 165 percent, respectively, while higher levels of Se exert toxic effects. Total phenolic compounds in leaves increased directly by increasing the level of Se and plants treated with 10 mg. L^{-1} Se had the highest values. Selenium, sodium and calcium content increased, while potassium content decreased, by increasing selenium treatments. The highest amounts of Se in shoots (3.89 mg g^{-1} DW) and roots (4.27 mg g^{-1} DW) were obtained for the highest concentration of Se (10 mg L^{-1}). The present results suggested the beneficial effects of Se on spinach growth and also its contribute ion to improving the nutritional value of spinach for livestock and human nutrition.

Keywords: human nutrition, hydroponic culture, phenolic compounds, selenite sodium, toxicity

Introduction

Selenium (Se) is an essential trace element for both people and plants, which has multiple biological functions. Increasing scientific evidence indicates that selenium has many beneficial effects for human beings and many other forms of life (Birringer *et al.*, 2002). There is evidence that less overt Se deficiency can affect human health in a number of ways, such as immune function, viral infection, reproduction (especially male fertility), thyroid function, asthma and inflammatory conditions (Rayman, 2000, 2002). Selenium also has its role in the prevention of cardiovascular disease (Rayman, 2000; Stranges *et al.*, 2006). Selenium is a constituent of selenoproteins, many of which have important functions, including antioxidant protection, energy metabolism and redox regulation during transcription and gene expression (Combs, 2001; Fordyce, 2005; Kong *et al.*, 2005; Rayman, 2002).

Higher plants are thought not to require Se and to have a low tolerance to it, but there are increasing indications that Se may also have beneficial biological functions in higher plants. Selenium was shown to affect several physiological and biochemical processes in plant species (Moussa *et al.,* 2010; Moya *et al.*, 1993; Pennanen *et al.*, 2002; van Assche *et al.*, 1988). In plants, Se may serve a role in antioxidative mechanisms and was indicated to be a component of glutathione peroxidase (Ekelund and Danilov, 2001). Among higher plants, the largest beneficial effects of Se on growth (up to 2.8-fold higher biomass with Se) have been observed in the Se hyper-accumulator plants, and Se has been suggested to be essential for these species (Shrift, 1969). In non-hyper-accumulator plants Se is toxic at high concentrations; it can exert beneficial effects on plants at low concentrations. Trace amounts of Se stimulated growth in a variety of plants e.g. sorgrass (Carlson *et al.*, 1991), ryegrass (Hartikainen *et al.*, 2000), tobacco (Yang and Ding, 2000) and lettuce (Xue *et al.*, 2001) as well as potato (Turakainen *et al.*, 2004). Simojoki (2003) reported that small Se addition, that increased Se contents in lettuce shoots up to 1.5 mg kg^{-1} dry matter, tend to enhance plant growth. Moreover, in potato plants, Se increased carbohydrate accumulation in leaves and tubers. Spraying leaves with Se increased seed yield in soybean, likely due to a better partitioning efficiency, as evidenced by a greater number of pods per plant, seeds per pod and seed weight (Djanaguiraman *et al.*, 2004). Selenium supplementation to plants also enhance the production and quality of edible plant products, by increasing antioxidant activity of the plants, as shown in tea leaves (Xu *et al.*, 2003) and in rice (Xu and Hu, 2004). More-

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over, Se treatment has been reported to improve growth in plants subjected to various abiotic stresses such as UV (Hartikainen and Xue, 1999), salt (Hawrylak-Nowak, 2009) and drought (Yao *et al*., 2009).

Spinach is a medicinal edible plant bearing vitamins, antioxidant compounds (e.g. flavonoids, acid ascorbic) and essential elements (e.g. Fe and Se) (Dehkharghanian *et al*., 2010). Spinach is capable of accumulating large amounts of Se mostly in shoot and root tissues (Zhu *et al*., 2004). Moreover, the majority of research has reported numerous beneficial effects of supplemental Se on plant growth and performance (Turakainen *et al*., 2004; Xue *et al*., 2001; Yang and Ding, 2000). However, Se is not a very abundant element in soil nor in greenhouse production, most plants being cultivated using soilless substrates where Se availability is limited (Pilon-Smits *et al*., 2009). On the other hand cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits (Finley *et al*., 2001; Lyons *et al*., 2005). This study aimed to investigate the effects of selenium on growth, selenium accumulation and some physiological characteristics on spinach (*Spinacia oleracea* L.) plants.

Materials and methods

The hydroponic experiment was carried out in a growth room with a 14-h light period (PAR of 300-350 μmol m^{-2} s^{-1}). Temperature was set at 28°C/- day and 20°C/- night. Seeds of spinach (*Spinacia oleracea* L., cv. 'Missouri') were disinfected by soaking in 5% sodium hypochlorite (NaOCl) for 1 min and subsequently rinsed thoroughly with distilled water. Seeds were then germinated on moist filter paper for 2-3 days. After 10 days (two-leaf stage) the uniform seedlings were then transferred to a plastic pot (7.5 cm in diameter and 15 cm in height) containing 1500 ml Hoagland solution with continuous aeration.

After spinach seedlings were grown for one week in the nutrient solution, selenium (as sodium selenite (Na_2SeO_3) was used at five concentrations (0, 1, 2, 4, 6 and 10 mg L^{-1}) and each treatment had four replicates. Plants were allowed to grow for 28 days in the nutrient solution. Nutrient solution was changed once a week, and solution pH was adjusted to 6.8 using dilute HCl or NaOH solutions.

Measurements and data collection

Growth parameters

After 4 weeks of selenium exposure, the plants were harvested and growth parameters like shoot and root fresh weight, shoot and root dry weight, total dry weight, shoot and root length and leaf number per plant were determined.

Photosynthetic pigments

0.02 g of leaves were grounded in 80% acetone for the determination of chlorophyll a, chlorophyll b and total chlorophyll contents, according to Lichtenthaler (1987).

Total phenolic compounds

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method (Singelton *et al*., 1999). The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 60 min. Using galic acid as standard total phenolic content (standard curve was prepared using concentrations,5-50 mg/L) was expressed as mg GA equivalent/L of extract.

Extraction and analysis of minerals

Na^+, K^+ and Ca^{2+} in leaves and roots were determined by the methods described by Allen *et al*. (1986). Grounded dry plant samples (100 mg each) were digested in 2 mL of sulfuric-peroxide digestion mixture until a clear and almost colorless solution was obtained. After digestion, the volume of each sample was set to 100 mL, with distilled de-ionized water. Ions, i.e. Na^+, K^+ and Ca^{2+} were determined with a flame photometer (Jenway, PFP7). Selenium in plant samples was analysed by electrothermal atomic absorption spectrometry method (Kumpulainen *et al*., 1983). Briefly, the lyophilised samples were digested in an acid mixture HNO_3, $HClO_4$ and H_2SO_4. Se concentration was determined by atomic absorption spectrophotometer (Perkin Elmer Model 5100) at 196.1 nm, equipped with HGA-600 graphite furnace. Three in-house reference samples were included in each sample batch to check the accuracy of the analytical method.

Tab. 1. Effect of selenium supplementation on some growth parameters of spinach plants

Se Concentrations (mg L^{-1})	Root fresh Weight (mg/plant)	Shoot Fresh Weight (mg/plant)	Root Dry Weight (mg/plant)	Shoot Dry Weight (mg/plant)	Total dry weight (mg/plant)
0	0.195 ab	0.730 b	0.013 ab	0.059 ab	0.072 ab
1	0.225 a	0.868 a	0.018 a	0.068 a	0.086 a
2	0.120 ab	0.608 c	0.017 a	0.061 ab	0.078 ab
4	0.085 ab	0.248 d	0.007 ab	0.052 ab	0.059 ab
6	0.055 b	0.113 e	0.007 b	0.018 b	0.025 b
10	0.022 b	0.080 e	0.004 b	0.015 b	0.019 b

Means in each columns followed by at least one letter in common are not significantly different statistically using Duncans Multiple Range Test ($p \leq 0.05$)

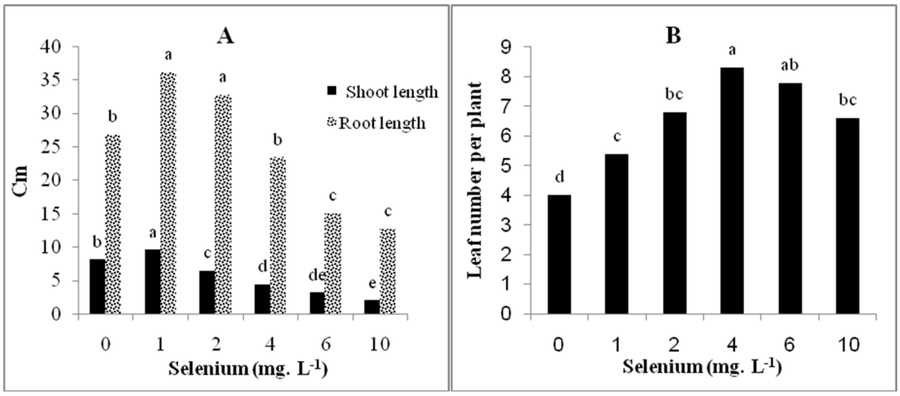

Fig. 1. Effect of selenium supplementation on shoot and root length (A) and leaf number per plant (B) of spinach plants

Statistical analysis

Analysis of variance (ANOVA) for all the variables was carried out using the MSTAT-C software. The significant differences between treatments were compared by Duncan's multiple range tests at 5% probability level.

Results

Growth parameters

Shoot fresh and dry weight, root fresh and dry weight and total dry weight of spinach plants were recorded as influenced by the different levels of Se supplementation. As shown in Tab. 1, these growth parameters were increased in response to the lowest concentration of Se (1 mg L^{-1}). However, application of higher Se concentrations (2, 4, 6, and 10 mg L^{-1}) reduced the shoot fresh and dry weight, root fresh and dry weight and total dry weight of spinach plants.

Similar results were obtained for shoot and root length of spinach plants (Fig. 1A). Se supplementation (1 mg L^{-1}) significantly increased shoot and root length by 18 and 34 percent as compared to control, respectively. The higher Se concentrations were toxic to the spinach plants and reduced shoot and root length (Fig. 1A). For plants treated with Se, leaf number per plant increased significantly when the concentration of Se in the nutrient solution were 4 and 6 mg L^{-1}, but leaf number would be inhibited by increasing Se concentrations (Fig. 1B).

Tab. 2. Effect of selenium supplementation on chlorophyll a, chlorophyll b and total chlorophyll contents in leaves of spinach plants

Se concentrations (mg L^{-1})	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)
0	0.48 b	0.29 b	1.20 b
1	0.90 a	0.77 a	1.73 a
2	0.67 ab	0.65 a	1.38 ab
4	0.45 b	0.18 c	0.58 c
6	0.38 bc	0.16 c	0.51 c
10	0.27 c	0.15 b	0.42 c

Means in each columns followed by at least one letter in common are not significantly different statistically using Duncans Multiple Range Test ($p \le 0.05$)

Photosynthetic pigments

Exposure of spinach plants to the lowest concentration of Se (1 mg L^{-1}) induced a significant increase in chlorophyll a, chlorophyll b and total chlorophyll contents (Tab. 2). However, increasing concentrations of Se significantly reduced the amount of chlorophyll a, chlorophyll b and total chlorophyll contents. With 1 mg L^{-1} Se level, chlorophyll a and b were stimulated by 87 and 165 percent as compared to control, respectively (Tab. 2).

Total phenolic compounds

Selenium significantly affected total phenolic compounds in the shoots and roots of spinach plants (Fig. 2). The shoots of Se-treated plants showed greater total phenolic compounds than the roots. The lowest level of Se significantly enhanced total phenolic compounds, by 66% as compared to control, while application of higher Se concentrations reduced total phenolic compounds in the roots (Fig. 2). In contrast, total phenolic compounds in the shoots manifested an increasing tendency along with an increase in the selenium concentration in the nutrient solution and the highest total phenolic compounds were

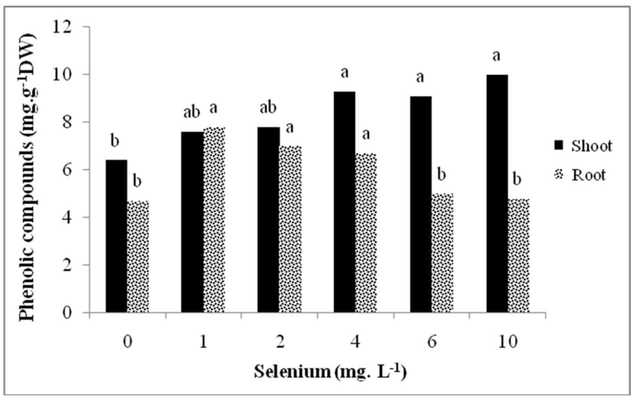

Fig. 2. Effect of selenium supplementation on total phenolic compounds in shoots and roots of spinach plants

observed in response to highest concentration of Se (10 mg L^{-1}) (Fig. 2).

Nutrient content

Introduction of selenium into the medium significantly interacted with the nutrient content of spinach plants (Tab. 3). Se concentration in roots and shoots increased by increasing the procent of Se added to the nutrition solution (Tab. 3). The highest amounts of Se in leaves (3.89 mg g^{-1} DW) and roots (4.27 mg g^{-1} DW) were obtained by the highest concentration of Se (10 mg L^{-1}) (Tab. 3). Sodium content in roots and shoots manifested an increasing tendency along with an increase in the selenium concentrations in the nutrient solution. Amounts of calcium in the roots increased by increasing Se concentrations added to the nutrition solutions, and the highest level of Se (10 mg L^{-1}) caused about 4-fold increase of calcium content in the roots as compared to control. Lower concentrations of Se (1, 2 and 4 mg L^{-1}) induced a significant increase in calcium content in shoots, while increasing concentrations of Se significantly reduced the amount of calcium. Potassium content in roots dramatically reduced by Se supplementation and the lowest amounts of potassium were obtained with 10 mg L^{-1} Se. At lower Se levels (1 and 2 mg L^{-1}), potassium content in shoots increased slightly without any significant difference, while higher levels of Se reduced potassium content (Tab. 3).

Discussion

Based on the present results, the growth of spinach plant increased at lowest concentration of Se (1 mg L^{-1} Se), but showed decrease with application of higher Se concentrations. These responses are similar to those obtained by Hartikainen *et al*. (2000) with ryegrass, Yang and Ding (2000) with tobacco and Xue *et al*. (2000) with lettuce as well as potato (Turakainen *et al*., 2004). Selenium interaction with plants depends on its concentration. At lower concentrations, selenium stimulated growth, while at high doses it acted as pro-oxidant, reducing yields and inducing metabolic disturbances. The growth stimulating effect of Se may be related to its antioxidative function, as demonstrated by the decreased lipid peroxidation, H_2O_2 and superoxide radical production and increased antioxidants enzymes (peroxidase and polyphenol oxidase) and higher contents of chlorophyll than control. However, effect of Se on phytohormones balance and/or polyamine content could not be excluded. Selenium treated potato plants had higher putrescine content (Turakainen *et al*., 2008). Polyamines have been implicated in various plant growth and developmental processes, including stimulation of cell division, embryogenesis and floral development (Kakkar and Sawhney, 2002). High Se levels may inhibit photosynthesis, impair nutrient uptake and transport (Kahle, 1988). Hawrylak-Nowak (2008) revealed that disturbances of growth and reduction of plant's biomass at the presence of high selenium concentrations in the nutrient solution may have resulted from the disturbance of mineral balance of plants, namely accumulation of large amounts of phosphorus in shoot tissues of maize.

Chlorophyll content increased at the lowest Se concentration (1 mg L^{-1} Se), while decreased at higher Se concentrations. It was observed that the chlorophyll content increased significantly with Se application in the present study, which is in agreement with the positive effects of Se treatment in delaying the loss of chlorophyll in senescing *Vicia faba* plants (Moussa *et al*., 2010) and drought-stressed wheat plants (Yao *et al*., 2009). In contrast, Se treatment at its higher levels caused inhibition in chlorophyll content in mungbean seedlings (Padmaja *et al*., 1989). The increase in chlorophyll a and chlorophyll b contents of spinach leaves may be attributed to Se effect over protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments (Pennanen *et al*., 2002). Higher Se concentrations induced reduction in chlorophyll content observed in this study is consistent with the result of Panmaja *et al*. (1989) and Padmaja *et al*. (1995). High Se has an adverse effect on the production of porphobilinogen synthetase required for chlorophyll biosynthesis and also inhibits biosynthetic enzymes through lipid peroxidation.

Selenium significantly affected total phenolic compounds in the shoots and roots of spinach plants. The content of phenolic compounds was greatly increased by fo-

Tab. 3. Effect of selenium supplementation on selenium, sodium, calcium and potassium contents in roots and shoots of spinach plants

Se (mg L^{-1})	Se^{2+} Concentration (mg g^{-1} DW)		Na^{+} Concentration (mg g^{-1} DW)		Ca^{2+} Concentration (mg g^{-1} DW)		K^{+} Concentration (mg g^{-1} DW)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	0.00 c	0.00 ab	0.09 c	0.13 c	3.61 c	8.01 b	7.90 a	11.83 ab
1	1.81 bc	1.71 ab	0.32 c	0.40 c	7.84 b	13.54 a	5.84 b	11.95 a
2	2.42 ab	2.05ab	0.98 b	1.51 b	7.97 b	14.52 a	5.77 b	12.04 a
4	3.40 ab	2.84 ab	1.22 b	1.74 b	9.12 b	13.95 a	4.71 bc	9.91 bc
6	3.44 ab	3.83 a	1.32 b	1.86 b	13.31 a	13.29 ab	3.83 c	8.55 c
10	4.27 a	3.89 a	2.00 a	2.52 a	16.49 a	12.67 ab	3.58 c	8.12 c

Means in each columns followed by at least one letter in common are not significantly different statistically using Duncans Multiple Range Test ($p \leq 0.05$)

liar application of Se-enriched fertilizer in green tea leaves (Xu and Hu, 2004). Walaa *et al*. (2010) indicated that Se treatment caused a significant increase in Phenylalanine ammonialyase activity in cucumber seedlings grown in the presence or absence of NaCl stress.

Se concentration in roots and shoots increased with increasing the amounts of Se added to the nutrition solutions. Selenium hyper accumulators have been defined as those plants that can accumulate >1000 mg of Se kg^{-1} dry weight (Berken *et al*., 2002). Spinach accumulated high amounts of Se and it is an accumulator of Se. Increased Se fertilization enhanced Se concentration in potato (Poggi *et al*., 2000; Turakainen *et al*., 2004). In tea plants, foliar application with selenate significantly increased Se content in the leaves (Hu *et al*., 2003). Wu and Huang (1992), in their experiments on selenium affecting accumulation of macro- and micronutrients in fescue and clover, recorded similar dependencies regarding calcium concentrations. Calcium in particular, plays many physiological roles such as signal transduction and is involved in the maintenance of cell wall and plasma membrane structural integrity. Also, it plays a role in activating antioxidant enzymes (Kinraide, 2001). Positive effects of selenium in the form of selenite on potassium accumulation were also observed by Pazurkiewicz-kocot *et al*. (2003), who found that the content of potassium in maize leaves significantly increased when introducing 10 µmol Se dm^{-3} into the medium, but a contrary dependence was recorded in roots.

Conclusion

Selenium at the lowest level (1 mg L^{-1}) stimulated spinach growth parameters like shoot and root fresh weight, shoot and root dry weight, total dry weight, shoot and root length, while higher Se levels dramatically reduced these growth parameters. Low Se level may be required by this accumulator plant as a micronutrient and may increase its antioxidative capacity. Moreover, selenium enhanced nutrients and Se uptake in spinach leaves which is more useful for human health.

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