

Effect of Selenium Enrichment on the Growth, Photosynthesis and Mineral Nutrition of Broccoli

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Abstract

Broccoli is placed in primary selenium (Se) accumulator group plants, which is considered as an important source of Se for providing human daily need. This experiment used an outdoor hydroponic system to evaluate the effects of Se foliar application at the rates of 0, 10, 50, and 100 $\mu\text{g Se/ml}$ concentrations fortnightly. Among yield parameters, the head weight of broccoli was significantly affected. Se treated broccoli plants produced heavier head than the control; however, head weight among three Se concentrations (Se_{10} , Se_{50} , Se_{100}) was not significantly different. Although most of the chlorophyll fluorescence parameters were not significant, Se treated broccolis maximal fluorescence yield (Fm) was higher than the control. Significant increase in chlorophyll content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) was observed as a result of Se treatments. Different Se concentrations did not have positive or negative effects on nitrogen, phosphorus and potassium uptake. Se treatment at 100 $\mu\text{g Se/ml}$ concentration however, contributed to the highest content of sulfur in broccoli head. Se content of broccoli head increased with the increase in sprayed Se concentrations. The highest concentration of Se (1.41 mg Se/kg dry matter) in broccoli head was recorded in Se_{100} , which showed significant difference compared with Se_0 and Se_{10} .

Keywords: chlorophyll, carotenoids, pigments, fluorescence, mineral elements

Introduction

Selenium (Se) is a trace element that is an important micronutrient for both animals and humans (Feng *et al.*, 2013). Se has been reported to reduce tumor growth in laboratory tests and may provide protection against specific cancers in humans (Ip, 1998). Based on the recommendations by the World Health Organization (WHO- FAO, 2002), adults need 26-34 $\mu\text{g Se/day}$. The health benefits of Se, which include cancer protection, shed light on the importance of Se enrichment foods (Finley *et al.*, 2005).

Higher plants have different capacities to accumulate and tolerate Se and some particular plant species are termed as Se hyperaccumulators. The Se hyperaccumulators are classified in two groups: primary and secondary Se accumulators. The primary Se accumulators are able to accumulate thousands of milligrams of Se kg^{-1} ($> 4000 \text{ mg kg}^{-1}$), while the secondary accumulators are able to accumulate hundreds of milligrams Se kg^{-1} (Turakainen, 2007). Brassicaceae species including Indian mustard (*Brassica juncea* L.), broccoli (*Brassica oleracea* var. *botrytis* L.) and canola (*Brassica napus* spp. *oleifera* L.) are placed in the primary accumulators group (Turakainen, 2007). Avila *et al.* (2014) found that *Brassica* genus including broccoli with

Se-biofortified sprouts, were able to synthesize significant amounts of Se-methylselenocystine (Se-MeSeCys).

Many studies showed that high-Se broccoli can reduce cancer risk. Se-MeSeCys, a main organic form of Se in broccoli, can be easily converted to methyl selenol that is required for cancer prevention (Foster *et al.*, 1986). Se enriched broccoli is considered as a vegetable with high anticancer effect that can be more effective than selenite, selenate or broccoli alone (Finley *et al.*, 2000). Se-enriched broccoli was also protective against chemically induced mammary, colon and intestinal cancer in rats (Davis *et al.*, 2002). According to Abdulah *et al.* (2009), Se-enriched broccolis had more anti-prostate cancer effect than normal broccolis. Thus, Se-enriched broccolis were suggested as an alternative Se source for prostate cancer for both prevention and therapy aims.

Se, applied at low concentrations, may enhance growth and strengthen antioxidant defense system of plants. Singh *et al.* (1980) showed that the application of 0.5 mg kg^{-1} Se stimulated growth and dry-matter yield of Indian mustard (*Brassica juncea* L.). Low concentrations of Se can alleviate the oxidative stress caused by ultraviolet (UV) irradiation in lettuce (Hartikainen and Xue, 1999). Numerous studies have shown that Se can enhance glutathione peroxidase (GSHPx) activity and decrease lipid peroxidation; consequently, this element can be considered as a protective role against the oxidative stress in higher plants

(e.g. Hartikainen *et al.*, 2000; Cartes *et al.*, 2005; Djanaguiraman *et al.*, 2005). Germ *et al.* (2007) demonstrated the high efficiency of energy conversion in PSII in Se treated potato under drought conditions. Hajiboland *et al.* (2014) reported the same results in two genotypes of durum wheat under drought stress.

Kapolna *et al.* (2009) used foliar application for Se enrichment of carrot. They found out selenate-enriched carrot leaves accumulated more Se than selenite-enriched leaves. Foliar applied Se was efficiently absorbed by plant leaves and transported to the tubers of potato (Germ *et al.* 2007).

Flower head is the main part of broccoli, which accumulates Se, even more than other parts of the plant (Sindelarova *et al.* 2015) and foliar application of Se can be a proper method for broccoli enrichment.

Although many researches have been conducted about anticancer effect of high-Se broccoli, a few studies deal with physiological effect of Se treatment on broccoli plant. Since commercial production of enriched broccoli needs to have more information about Se impacts on both plant growth and physiology, the present study was undertaken to investigate the possible effects of Se enrichment on growth, yield, photosynthesis and mineral nutrition uptake in broccoli.

Materials and Methods

Plant growth conditions and treatments application

The experimental field is located in Sari Agricultural Sciences and Natural Resources University, Mazandaran province, Iran, which has a humid climate, according to the de Martonne climate classification system. The experimental site was geographically located at 36°39' latitude, 53°04' longitude and -15 m altitude. Broccoli was grown in outdoor hydroponic system in October 2014. The medium used in this experiment was sand covered by a combined layer of perlite and cocopeat. Nutrient solution providing all essential elements was used to reach optimal growth of broccoli (Table 1). When the broccoli heads appeared (Mid December 2014), sodium selenate (Na_2SeO_4) solution was sprayed at concentrations of 0, 10, 50 and 100 μg Se/ml (treatments S_0 , S_{10} , S_{50} and S_{100} , respectively), about 25 ml for each plant, every two weeks.

Determination of yield parameters

Plant height, head weight, head diameter and dry matter were determined as growth and yield parameters at the end of the experiment. Three plants per treatment were measured from the line of culture medium to the highest point of the plant. Main heads of broccolis, as consumable parts, were weighted and reported as head fresh weight (g). The largest head diameter was determined as the horizontal by calipers and was recorded in mm to two decimal places. Thereafter, harvested broccoli heads were dried at a temperature of 75 °C to calculate dry matter percentage.

Determination of photosynthetic pigments

Pigments extraction with methanol solvent was carried out according to Carter and Knapp (2001). The absorbance of the

extraction was recorded at 470, 652.4 and 665.2 nm. The concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids were calculated using Equations 1 to 3 (Lichtenthaler and Buschmann, 2001):

Methanol pure solvent

$$C_a (\mu\text{g Se/mL}) = 16.72 A_{652.4} - 9.16 A_{665.2} \text{ [Equation 1]}$$

$$C_b (\mu\text{g Se/mL}) = 34.09 A_{652.4} - 15.28 A_{665.2} \text{ [Equation 2]}$$

$$C_{(x+c)} (\mu\text{g Se/mL}) = (1000 A_{470} - 1.63 c_a - 104.96 c_b) / 221 \text{ [Equation 3]}$$

Chlorophyll fluorescence analysis

To study the possible stress effect of Se treatment on chloroplast activity, chlorophyll fluorescence parameters comprising minimal fluorescence yield (F_0), maximal fluorescence yield (F_m), variable fluorescence of dark-adapted sample $F_m - F_0$ (F_v), maximal quantum yield of PSII (F_v/F_m), quantum yield of photochemical energy conversion in PSII ($Y(II)$), and quantum yield of regulated non-photochemical energy loss in PSII ($Y(NPQ)$), were recorded by a fluorometer (Portable Chlorophyll Fluorometer PAM-2500, Walz Company, Germany), after 30-min dark adaptation to serve as a measure of photosynthetic efficiency (Kraus and Weis, 1991).

Determination of mineral elements

Chemical analysis was performed on dried samples of broccoli heads. The concentration of total nitrogen (N) was determined by Kjeldahl method. Phosphorus (P) was analyzed by a vanadate-molybdate method using a spectrophotometer and potassium (K) was analyzed using a flame photometer after samples digestion (Waling *et al.*, 1989).

Total sulphur (S) from broccoli heads' material was measured in the digest as described by Quin and Wood (1976). Broccoli samples (0.1 g) were analyzed for S after magnesium nitrate and perchloric digestion. Barium chlorate was added to the mixture and it was left overnight, following which the absorbance of the final reaction mixture was measured at 420 nm. Results were expressed as percentage of total S in dry matter.

For the Se analysis, 1 g of homogenized sample of broccoli head was mineralized by 10 ml of concentrated HNO_3 and then 2 ml of HClO_4 . Final step of digestion was performed by 1 ml of HCl and extracted by filter paper (ASTM, 1992). Total Se content from mineralized plant material was determined by using an atomic absorption spectroscopy. The absorbance of final extract was recorded at 196 nm (International Standard Organization, 1993).

Statistical analysis

The experiment was arranged in a randomized complete block design. A statistical analysis was performed using analysis of variance in the Statistical Analysis System (SAS) software (version 9.1) and means were compared using Duncan's multiple range tests.

Results and Discussion

Growth parameters and yield

According to the results, broccoli height, head diameter and dry matter were not significantly affected by Se application

Table 1. Nutrient solution formulation used for broccoli production in outdoor hydroponic system

Element	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn
Concentration (mg L^{-1})	153.7	70	144	170	61	82	0.7	0.08	5	2	0.05	0.25

Table 2. Yield parameters of broccoli as affected by Se treatments

Treatment	Height (cm) ^{ns}	Head weight (g) [*]	Head diameter (mm) ^{ns}	Dry matter (%) ^{ns}
Se ₀	36.00±0.33 ^a	1106.7±81.4 ^b	125.80±13.32 ^a	11.55±3.92 ^a
Se ₁₀	40.22±3.47 ^a	1540.0±144.2 ^a	156.93±17.59 ^a	11.06±1.00 ^a
Se ₅₀	38.33±4.58 ^a	1199.3±289.6 ^b	145.37±26.62 ^a	10.64±6.50 ^a
Se ₁₀₀	36.86±0.75 ^a	1390.0±85.4 ^{ab}	160.86±6.57 ^a	11.05±6.97 ^a

Values represent means ± SD. Figures followed by different letter within a column are significantly different (*: $p \geq 0.90$, **: $p \geq 0.95$, ns: non-significant).

(Table 2). Among yield parameters, just head weight of broccoli significantly responded to Se treatment. Se treated broccolis produced heavier heads than the control; however, head weight among the three Se concentrations (Se₁₀, Se₅₀, Se₁₀₀) was not significantly different. These results showed that Se treatment in the studied concentrations had positive effect on consumable yield of broccoli. The growth-promoting response to Se was demonstrated in different plants including ryegrass (Hartikainen and Xue, 1999), soybean (Djanaguiraman *et al.*, 2005) and potato (Turakainen, 2007). Nevertheless, Alsina *et al.* (2012) pointed out that Se treatment had no positive effect on lettuce growth. According to the research on Se treatment in potato, the results were so different. Se stimulated the starch accumulation in upper leaves and potato yield were increased, because of the larger tuber size (Turakainen, 2007). In tea plants, when selenite or selenate was applied foliar, the numbers of sprouts and the yield were significantly increased (Qihui *et al.*, 2003). Numerous studies have shown that the appropriate concentration of Se reinforced the antioxidant defense system of plants (Hartikainen and Xue, 1999; Pennanen *et al.*, 2002; Cartes *et al.*, 2005). It seems that growth promoting in some parameters like head weight may be related to the enhancement of protective ability of the plants against oxidative stress, which has been demonstrated.

Photosynthetic pigments and chlorophyll fluorescence

The effect of Se on plant pigments was studied and it showed significant effects on these parameters. Significant increase in chlorophyll content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) was observed as a result of Se treatments (Table 3). Similarly, Alsina *et al.* (2012) reported an increase in lettuce pigments as a result of Se application.

In outdoor hydroponic culture, some environmental stresses are natural. It is confirmed that Se has a protective role against radical species produced in stressful conditions and may alleviate environmental stress in Se treated plants. It is hypothesized that appropriate concentration of Se can increase chlorophyll contents following environmental stress alleviation.

However, most of the chlorophyll fluorescence parameters were not significant; in Se treated broccolis, maximal fluorescence yield (F_m) was higher than control (Table 4). Active fluorescence parameters including F_m can be interpreted for baseline data, as a comparative tool or as an early sign of system change. High amount of F_m in Se treated broccolis can show environmental stress alleviation of these plants resulting from Se antioxidant effect. Mateja *et al.* (2007) reported higher efficiency of energy conversion in PSII, expressed by a higher effective quantum yield in Se treated plants within two weeks after treatments.

Mineral elements concentration

Se spray in applied concentrations within the current experiment did not show any effect on rising or falling nitrogen, phosphorus and potassium uptakes (Table 5). Se treatment at 100 µg Se/m concentration, however, contributed to the highest content of sulfur in broccoli heads. According to

Table 3. Photosynthetic pigments of broccoli as affected by Se treatments

Treatment	SPAD ^{ns}	Chlorophyll <i>a</i> (mg g ⁻¹) [*]	Chlorophyll <i>b</i> (mg g ⁻¹) ^{**}	Total chlorophyll (mg g ⁻¹) ^{**}	Carotenoids (mg g ⁻¹) ^{ns}
Se ₀	69.89 ± 2.89 ^a	0.962 ± 0.13 ^b	0.474 ± 0.05 ^c	1.436 ± 0.17 ^c	0.182 ± 0.12 ^a
Se ₁₀	72.47 ± 4.19 ^a	1.025 ± 0.19 ^{ab}	0.528 ± 0.04 ^{bc}	1.553 ± 0.22 ^{bc}	0.186 ± 0.26 ^a
Se ₅₀	74.00 ± 1.91 ^a	1.251 ± 0.07 ^a	0.610 ± 0.04 ^{ab}	1.860 ± 0.10 ^{ab}	0.215 ± 0.17 ^a
Se ₁₀₀	73.61 ± 0.89 ^a	1.285 ± 0.04 ^a	0.628 ± 0.04 ^a	1.913 ± 0.06 ^a	0.237 ± 0.18 ^a

Values are the mean ± SD. Figures followed by different letter within a column are significantly different (*: $p \geq 0.90$, **: $p \geq 0.95$, ns: non-significant).

Table 4. Chlorophyll fluorescence parameters of broccoli as affected by Se treatments

Treatment	F ₀ ^{ns}	F _m ^{***}	F _v ^{ns}	F _v /F _m ^{ns}	Y(II) ^{ns}	Y(NPQ) ^{ns}
Se ₀	1.596 ± 0.069 ^a	6.905 ± 0.002 ^c	5.309 ± 0.070 ^a	0.769 ± 0.010 ^a	0.670 ± 0.038 ^a	0.150 ± 0.039 ^a
Se ₁₀	1.654 ± 0.290 ^a	6.907 ± 0.001 ^b	5.253 ± 0.290 ^a	0.761 ± 0.042 ^a	0.674 ± 0.009 ^a	0.182 ± 0.017 ^a
Se ₅₀	1.629 ± 0.046 ^a	6.909 ± 0.001 ^a	5.280 ± 0.047 ^a	0.764 ± 0.007 ^a	0.651 ± 0.003 ^a	0.195 ± 0.007 ^a
Se ₁₀₀	1.733 ± 0.162 ^a	6.909 ± 0.001 ^a	5.176 ± 0.162 ^a	0.749 ± 0.023 ^a	0.658 ± 0.012 ^a	0.194 ± 0.008 ^a

Values are the mean ± SD. Figures followed by different letter within a column are significantly different (***: $p \geq 0.99$, ns: non-significant).

Table 5. Nutrient elements of broccoli as affected by Se treatments

Treatment	Nitrogen (%) ^{ns}	Phosphorus (%) ^{ns}	Potassium (%) ^{ns}	Sulphur* (%)	Selenium** (mg Se/kg dry matter)
Se ₀	4.00 ± 0.38 ^a	0.50 ± 0.05 ^a	4.27 ± 0.70 ^a	0.34 ± 0.17 ^{ab}	0.07 ± 0.08 ^b
Se ₁₀	4.15 ± 0.00 ^a	0.53 ± 0.01 ^a	3.93 ± 0.11 ^a	0.26 ± 0.07 ^b	0.28 ± 0.11 ^b
Se ₅₀	4.90 ± 0.66 ^a	0.71 ± 0.15 ^a	3.85 ± 0.16 ^a	0.19 ± 0.12 ^b	0.76 ± 0.49 ^{ab}
Se ₁₀₀	5.02 ± 0.64 ^a	0.61 ± 0.06 ^a	3.73 ± 0.14 ^a	0.67 ± 0.02 ^a	1.41 ± 0.00 ^a

Values are the mean ± SD. Figures followed by different letter within a column are significantly different (*: $p \geq 0.90$, **: $p \geq 0.95$, ns: non-significant).

different studies, sulfate uptake increased in *Brassica oleracea* with the increase in Se concentrations in nutrient solutions (Kopsell and Randle, 1999; Charron et al., 2001; Toler et al., 2007). Toler et al. (2007) demonstrated that S concentration of plants exposed to Se closely resembled those exposed to elevated S. On the other hand, selenate absorbs via sulfate carriers and then assimilates via the same enzymes used in S assimilation and can be incorporated into amino acids (De Souza et al., 1998). S can be replaced by Se in different organic compounds, especially in sulfuric amino acids, including methionine and cysteine (Terry et al., 2000). Poldma et al. (2011) showed a significant negative correlation between Se and S concentration in Se treated garlic. Similarly, Poldma et al. (2013) reported significant reducing S content in onion bulbs as affected by different Se treatments.

The contradiction between different studies can be related to either foliar spray of Se that impede uptake competition between Se and S, or associated with applied appropriate doses of Se in some studies, which prevented S assimilation disorder. Based on interpretation of Toler et al. (2007), Se either upregulates or prevents the downregulation of S uptake by the plant's roots. One possible way for this effect of Se is related to its ability to enhance S acquisition. According to recent study released by Liu et al. (2015), although sulfate inhibited the absorption of selenate by *Brassica napus*, it promoted the translocation of Se. These results showed that S had a stimulatory role for Se translocation. Spraying high concentration of Se in the current study may need more S for appropriate translocation, thus broccoli take up more S to meet this inner demand.

Broccoli has a good potential to accumulate Se, so as the content of Se in the head of the plants were increased by elevating the Se treatments' concentration. Sindelarova et al. (2015) reported that broccoli accumulates Se mainly in the flower heads and slightly less in the leaves, stems and roots. Accordingly, Se content of broccolis head increased by increasing sprayed Se concentration. The highest concentration of Se (1.41 mg Se/kg dry matter) was recorded in Se₁₀₀ which showed a significant difference with both Se₀ and Se₁₀ (Fig. 1). These results were consistent with the findings of some previous researches. Fangmei et al. (2003) reported the Se contents of soybean and okra were significantly increased by the application of sodium selenite and selenium-enriched

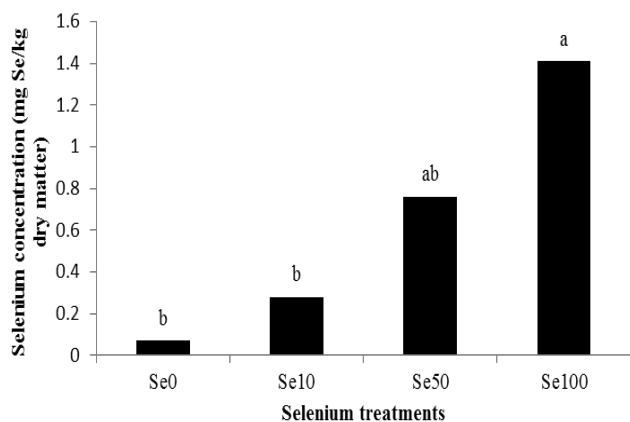


Fig. 1. Se concentration in broccoli heads as affected by different Se treatments

fertilizer. Also, foliar application of Se-enriched fertilizers increased Se content in green tea (Juan et al., 2003). Li et al. (2015) reported that Se concentration in *Brassica chinensis* L. shoots significantly increased as selenate and selenite rates increased within their experiments. Sindelarova et al. (2015) could produce Se enriched broccoli by foliar application of sodium selenate (Na₂SeO₄).

Conclusions

Foliar application of Se can have different physiological effects on broccoli, including increment of chlorophyll, S and Se content. No negative or antagonistic effects on mineral absorption were observed in broccoli treated with Se. These results emphasize that foliar spray can be an appropriate method to produce Se-enriched broccolis. Other approaches to produce high-Se broccolis, including seeds immersion in Se solution, soil fertilization and fertigation may be efficient as well, thus more investigation are needed and these methods can be the subject of the future researches.

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