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Effects of selenium on the growth and photosynthetic characteristics of flue-cured tobacco (*Nicotiana tabacum* L.)

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Abstract

The objective of this study was to investigate the effect of Selenium (Se) supply (0, 3, 6, 12, 24 mg kg^{-1}) on the growth, photosynthetic characteristics, Se accumulation and distribution of flue-cured tobacco (*Nicotiana tabacum* L.). Results showed that low-dose Se treatments (≤6 mg kg^{-1}) stimulated plant growth but high-dose Se treatments (≥12 mg kg^{-1}) hindered plant growth. Optimal Se dose (6 mg kg^{-1}) stimulated plant growth by reducing MDA content and improving photosynthetic capability. However, excess Se (24 mg kg^{-1}) increased MDA content by 28%, decreased net photosynthetic rate and carboxylation efficiency by 34% and 39%, respectively. The Se concentration in the roots, stems, and leaves of the tobacco plants significantly increased with increasing Se application. A linear correlation ($R = 0.95$, $P < 0.01$) was observed between Se level and tobacco plant tissue Se concentration. This correlation indicated that the tobacco plant tissues were not saturated within the concentration range tested. The pattern of total Se concentration in the tobacco plant tissues followed the order root > leaf > stem. The Se concentration in the roots was 3.17 and 7.57 times higher than that in the leaves and stems, respectively, after treatment with 24 mg kg^{-1} Se. In conclusion, the present study suggested that optimal Se dose (6 mg kg^{-1}) improved the plant growth mainly by enhancing photosynthesis, stomatal conductance, carboxylation efficiency and Rubisco content in the flue-cured tobacco leaves. However, the inhibition of excess Se on tobacco growth might be due to high accumulation of Se in roots and the damage of photosynthesis in leaves.

Keywords: flue-cured tobacco; selenium (Se); photosynthesis; carboxylation efficiency; Rubisco content

Introduction

Selenium (Se) is essential to animals and humans [1]. Recent research has shown that this trace element is also beneficial to plants [2]. However, high Se concentrations may elicit toxic effects on plants [3]. The difference between the deficiency and toxicity of Se, as in other essential trace elements, is narrow [4]. Plant species differ in Se uptake and accumulation in shoots and roots, as well as in tolerance to high Se concentrations in solution or soil [5]. For example, *Astragalus bisulcatus* and *Stanleya pinnata* exhibit high tolerance to Se in soil; these plants can hyperaccumulate Se up to 1% of their dry weights (DWs) [6]. By contrast, tobacco and soybean are sensitive to Se; these plants can be affected by low Se concentrations (e.g., 1 mg kg^{-1}) in culture media [7]. It's been well reported that the phytotoxicity of Se varies among agricultural crops [5].

Evidence to prove that nonaccumulator plants require Se remains lacking. However, numerous studies have reported that low Se concentrations benefit the growth of these plants. Turakainen et al. [8] showed that appropriate Se concentrations has positive effects also on potato carbohydrate accumulation and possibly on yield formation. Similarly, other studies revealed that Se promotes the growth of ryegrass [3], tea [9], rice [10], and soybean [11]. However, excess Se accumulation (>0.1% plant DW) is generally toxic to plants, except for rare Se-hyperaccumulating plants [4].

Plants subjected to Se stress exhibit different physiological changes, including stunted root growth, reduced biomass, chlorosis, reduced photosynthetic efficiency, and ultimately plant death [4]. Previous studies reported that Se improves the antioxidant capacity of plants [3,12]. Feng et al. [2] have recently discovered that Se elicits protective effects on plants against abiotic stresses. Soil treatment with Se has been highly recommended to produce Se-enriched food for human consumption. Se-enriched products, such as tea [9], rice [10,13], and vegetables [14] have been developed. Furthermore, various studies have associated the consumption of Se-enriched vegetables with reduced risk of developing cancer [14,15]. Broccoli can accumulate Se and convert it into a form that is chemoprotective against cancer [15]. These findings suggest that Se-enriched vegetables benefit human nutrition and health [14]. Therefore, understanding

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the effect and function of Se on the plant growth is important to the development of Se-enriched agricultural products.

Although Se is known to elicit detrimental effects on plants, the effects of Se stress on the photosynthetic characteristics of tobacco have yet to be elucidated. Tobacco (*Nicotiana tabacum* L.) is an economically important non-food crop worldwide; flue-cured tobacco accounts for approximately 80% of the world's tobacco production [16]. Therefore, the present study aims to determine the effects of treatments with different Se concentrations (0 mg kg^{-1} to 24 mg kg^{-1}) on the plant growth, gas exchange, chlorophyll concentration, Rubisco content, malondialdehyde (MDA) content, Se accumulation and distribution of flue-cured tobacco.

Material and methods

Experimental materials and growth conditions

A soil pot experiment was conducted in greenhouse conditions in major rice-growing areas of Anhui province, China. Tobacco (*N. tabacum* L., cv. Yunyan 87), a popular flue-cured tobacco cultivar in China, was used in this study. The seeds were provided by Chizhou Tobacco Corporation, Anhui province, China. Seeds were surfaced-sterilized with 2% (v/v) NaOCl for 10 min, rinsed with deionized water, and then sown in floating nursery. During the growing season, the plants were placed in a greenhouse with a daytime temperature of 25°C to 33°C, a nighttime temperature of 15°C to 23°C, and a relative humidity (RH) of 60% to 85%.

The soil was paddy soil, collected from the tobacco field in Chizhou, Anhui province, China. Main physical and chemical properties were as follows: $pH_{water\ 2.5:1}$ 5.4, organic matter 17.6 g kg^{-1}, available N 157.7 mg kg^{-1}, available P 16.6 mg kg^{-1}, available K 184.7 mg kg^{-1}, and total Se 0.16 mg kg^{-1}.

Experimental design

Five levels of Se (sodium selenite, Na_2SeO_3) treatment, i.e. 0 (CK), 3, 6, 12, and 24 mg kg^{-1} Se were performed in the experiment. Each pot (35 cm in diameter, 28 cm in height) was filled with 20 kg of air-dried and 2 mm-sieved soil. Therefore, each pot was added with 0, 60, 120, 240, or 480 mg of Na_2SeO_3 to produce the five treatments. The fertilizers for each pot were as follows: flue-cured tobacco special fertilizer (N:P:K = 9:13.5:22.5) 45.45 g, KNO_3 10.10 g, K_2SO_4 3.28 g, $Ca(H_2PO_4)_2$ 14.61 g. All the fertilizers and Na_2SeO_3 were mixed thoroughly and applied as basal dressing at 10 days before tobacco seedlings transplanting. Tobacco seedlings (approximately 12 cm in height) were transplanted into the pots on April 9, 2011, with one plant for each pot. The pots were arranged at 1.2 m inter-row spacing and 0.5 m intra-row spacing. Each treatment was replicated for four times, and each replicate included four plants.

Gas exchange measurements

At 40 d after the treatments, the gas exchange on the newly expanded leaves was measured from 8:30 to 12:00 using a Li-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NB, USA) as previously described [17]. During the measurements, leaf temperature was maintained at 25 ±1°C with a photosynthetic photon flux intensity of 1200 μmol m^{-2} s^{-1}. Ambient CO_2 concentration in the cuvette (C_{a-c}) was adjusted to Ca (380 μmol CO_2 mol^{-1}), and RH was maintained at 50% ±5%. After 10 min, C_{a-c} was controlled across the series of 1000, 800, 600, 400, 200, 100, and 50 μmol CO_2 mol^{-1}. Carboxylation efficiency (CE) was calculated as the initial slope of the A/Ci response curves. Data were recorded after equilibration to a steady state.

Leaf chlorophyll concentration determination

At 40 d after the treatments, the relative chlorophyll concentrations in the newly expanded leaves of the labeled leaf segments were determined using a SPAD-502 Chlorophyll Meter (Minolta, Mahwah, NJ, USA).

MDA content determination

At 40 d after the treatments, the MDA contents in the newly expanded leaves of the labeled leaf segments were measured as previously described [18]. The absorbance at 450, 532, and 600 nm was obtained with an ultraviolet spectrophotometer (UV-755B, Shanghai Precision and Scientific Instrument Co., Ltd., China). The concentration of MDA was calculated using the following formula: $C = 6.45(D_{532} - D_{600}) - 0.56D_{450}$.

Rubisco measurements

After the gas exchange measurements, the Rubisco contents in the newly expanded leaves were measured according to the method of Li et al. [19]. Briefly, about 0.50 g newly expanded leaves were ground with a cooled extraction buffer containing 50 mmol l^{-1} Tris-HCl (pH 8.0), 5 mmol l^{-1} β-mercaptoethanol and 12.5% (v/v) glycerol at 0–4°C. The homogenate was centrifuged at 1500 *g* for 15 min at 4°C. The supernatant solution was mixed with a dissolving solution containing 2% (w/v) SDS, 4% (v/v) β-mercaptoethanol and 10% (v/v) glycerol. Then the mixture was boiled in water for 5 min for gel electrophoresis. An electrophoretic buffer system was used for SDS-PAGE with a 12.5% (w/v) stacking gel and a 4% (w/v) separating gel. Afterwards, the gels were washed with deionized water several times then dyed in 0.25% Coomassie Blue for 12 h and detained. Large subunits and relevant small subunits were transferred to a 10 ml cuvette with 2 ml of formamide and washed in a 50°C water bath for 8 h. The washed solutions were measured at 595 nm using background gel as a blank and bovine serum albumin (BSA) as the protein standard.

Determination of plant biomass

After the above measurements were completed, the plants were harvested and analyzed for root, stem, and leaf fresh weights. All samples were oven-dried at 105°C for 30 min and then at 60°C until constant weight were reached.

Total Se analysis

The roots, stems, and leaves of the plants were milled into powder using a mixer mill (Shanghai Bilon Instrument Co., China). The total Se concentrations in the samples were determined as previously described [20,21]. The dried plant powders (0.5 g) were digested overnight with 10.0 ml of HNO_3:$HClO_4$ (9:1) in a polypropylene sample tube at

room temperature. The digested solution was heated on an electrical hot plate at 60°C for 2 h and then at 100°C for 1 h. The tube containing the solution was added with 10.0 ml of HNO_3:$HClO_4$ (9:1) and then stored at 170°C for 2 h until a white fume formed. After cooling to room temperature, the tube was added with 5 ml HCl (1:1) and then heated until the solution became colorless within at least 3 h. After cooling, the solution was filtered and diluted to 50 ml with deionized water. The total Se concentration in the solution was analyzed through inductively coupled plasma–mass spectrometry [X Series ICP–MS (Thermo Electron Corporation, United States)]. Four replicate determinations were performed for each material.

Statistical analysis

All measurements were carried out on replicate samples collected from four individual plants. Statistical analyses were performed using one-way ANOVA with SPSS statistical software. Data were presented as mean and *SE*. The results were verified via Duncan's multiple-range test.

Results

Effects of Se treatment on plant growth

Tobacco plants were grown in soil with fertilizers containing five Se concentrations (0, 3, 6, 12, and 24 mg kg^{-1}) under greenhouse conditions. As shown in Tab. 1, low-dose Se treatments (≤6 mg kg^{-1}) enhanced the growth of tobacco plants. Treatment with 6 mg kg^{-1} Se significantly enhanced root, stem, leaf, and whole-plant DWs by 11%, 29%, 18% and 19%, respectively, compared with CK. In contrast to low-dose Se treatments, high-dose Se treatments (≥12 mg kg^{-1}) reduced the growth of tobacco plants. For instance, treatment with 24 mg kg^{-1} Se decreased root, stem, leaf, and whole-plant DWs by 18%, 10%, 19%, and 16%, respectively, compared with CK. However, no significant difference in root/shoot ratio was detected between the CK- and Se-treated tobacco plants.

Effects of Se treatment on gas exchange

Tab. 2 presents the changes in the gas exchange parameters of newly expanded tobacco leaves after 40 d of Se treatment. Compared with CK, low-dose Se treatments (≤6 mg kg^{-1}) significantly increased the net photosynthetic rate (Pn) in the leaves, whereas high-dose Se treatments (≥12 mg kg^{-1}) significantly decreased this parameter. The Pn values of the tobacco leaves under 3, 6, 12, and 24 mg kg^{-1} Se treatments were 1.17-, 1.26-, 0.96-, and 0.66-fold higher than those of the tobacco leaves under CK treatment, respectively. Similar changes in stomatal conductance (gs) were observed under the different Se treatments. Compared with CK, low-dose Se treatments (≤6 mg kg^{-1}) increased the CE, whereas high-dose Se treatments (≥12 mg kg^{-1}) decreased

Tab. 1 Effects of different levels of Se treatment on biomass of tobacco plants.

Se treatments (mg kg^{-1})	Root DW (g $plant^{-1}$)	Stem DW (g $plant^{-1}$)	Leaf DW (g $plant^{-1}$)	Whole-plant DW (g $plant^{-1}$)	Root/shoot ration
CK	9.95 ±0.85 b	13.18 ±0.55 c	29.38 ±0.59 b	52.51 ±1.66 b	0.234 ±0.016 a
3	9.81 ±0.35 b	14.92 ±0.66 b	31.86 ±1.76 b	56.59 ±2.43 b	0.210 ±0.013 a
6	11.05 ±0.54 a	16.98 ±0.65 a	34.61 ±1.35 a	62.64 ±1.06 a	0.214 ±0.017 a
12	9.42 ±0.40 b	12.90 ±0.40 b	28.30 ±1.56 b	50.95 ±1.61 b	0.229 ±0.009 a
24	8.12 ±0.75 c	11.91 ±1.06 c	23.84 ±1.71 c	43.88 ±3.38 c	0.227 ±0.013 a

Tobacco plants were supplied with different concentrations of selenite (Na_2SeO_3) [0 (CK), 3, 6, 12, or 24 mg kg^{-1})] through soil application. After 40 d, the plants were harvested and analyzed for root, stem, and leaf dry weights, as well as root/shoot ratio. The values are presented as mean and *SE* ($n = 4$). Different letters indicate a significant difference in the same column at $P < 0.05$.

Tab. 2 Effects of different levels of Se treatment on net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and carboxylation efficiency (CE) measured from A/Ci curves of newly expanded tobacco leaves.

Se treatments (mg kg^{-1})	Pn (μmol CO_2 m^{-2} s^{-1})	gs (μmol H_2O m^{-2} s^{-1})	Ci (μmol CO_2 mol^{-1})	CE
CK	9.30 ±0.34 b	0.098 ±0.004 b	224.31 ±4.04 c	0.0558 ±0.0024 bc
3	10.89 ±0.61 a	0.118 ±0.010 ab	223.29 ±3.10 c	0.0593 ±0.0011 ab
6	11.69 ±1.11 a	0.129 ±0.020 a	227.12 ±11.99 bc	0.0629 ±0.0012 a
12	8.97 ±0.24 b	0.108 ±0.017 b	239.27 ±9.58 b	0.0521 ±0.0025 c
24	6.13 ±0.50 c	0.077 ±0.003 c	252.07 ±8.95 a	0.0339 ±0.0025 d

The values are presented as mean and *SE* ($n = 4$). Different letters indicate a significant difference in the same column at $P < 0.05$.

this parameter. The CEs of the plants under 3 and 6 mg kg^{-1} Se treatments increased by 3% and 13% compared with those of the plants under CK treatment, respectively. However, the CEs of the plants under 12 and 24 mg kg^{-1} Se treatments were only 93% and 61% those of the plants under CK treatment, respectively. No significant differences in intercellular CO_2 concentration (C_i) was observed between the plants treated with CK and low-dose Se concentrations (≤6 mg kg^{-1}). By contrast, high-dose Se treatments (≥12 mg kg^{-1}) significantly increased the C_i compared with CK ($P < 0.05$).

Effects of Se treatment on leaf chlorophyll concentration and Rubisco content

As shown in Fig. 1, the chlorophyll concentration (SPAD value) slightly increased in the newly expanded leaves under low-dose Se treatments (≤6 mg kg^{-1}). However, the chlorophyll concentration declined with increasing Se concentration. Compared with CK treatment, 24 mg kg^{-1} Se treatment significantly decreased the chlorophyll concentration in the leaves. Similarly, compared with CK treatment, low-dose Se treatments (≤6 mg kg^{-1}) increased the Rubisco content, whereas 24 mg kg^{-1} Se treatment decreased this parameter by 18% (Fig. 2).

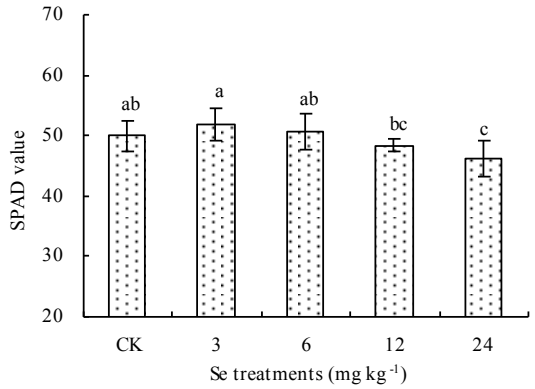

Fig. 1 Effects of different levels of Se treatment on the chlorophyll concentration of tobacco leaves. The results are presented as mean and *SE* ($n = 4$). Different letters indicate a significant difference at $P < 0.05$.

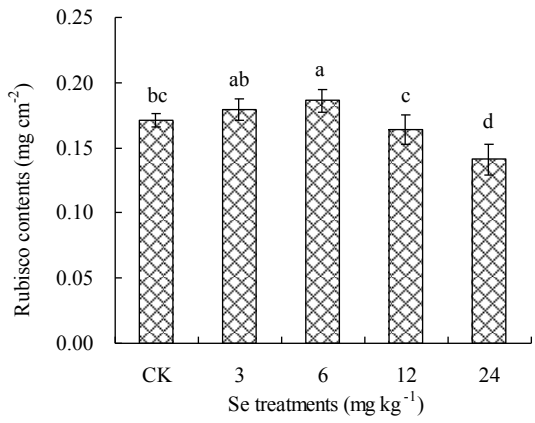

Fig. 2 Effects of different levels of Se treatment on Rubisco content of tobacco leaves The results are presented as mean and *SE* ($n = 4$). Different letters indicate a significant difference at $P < 0.05$.

Effects of Se treatment on MDA content

Compared with CK treatment, low-dose Se treatments (≤6 mg kg^{-1}) significantly reduced the MDA contents in the tobacco leaves, whereas high-dose Se treatments (≥12 mg kg^{-1}) increased this parameter (Fig. 3). The MDA contents in the leaves under 3 and 6 mg kg^{-1} Se treatments were only 86% and 82% those in the leaves under CK treatment, respectively. The MDA contents in the leaves under 12 and 24 mg kg^{-1} Se treatments were 1.05 and 1.28 times those in the leaves under CK treatment, respectively.

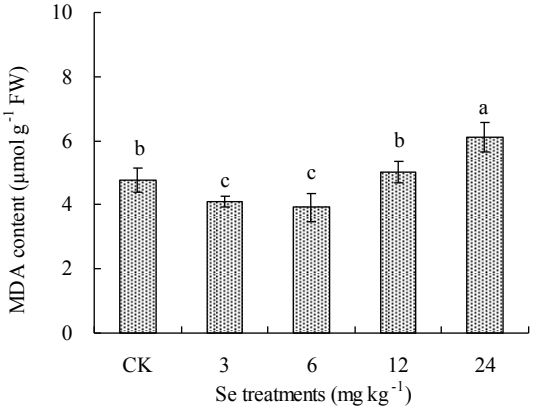

Fig. 3 Effects of different levels of Se treatment on MDA content of tobacco leaves. The results are presented as mean and *SE* ($n = 4$). Different letters indicate a significant difference at $P < 0.05$.

Se concentration and accumulation in tobacco plant tissues

The Se concentration in the different tobacco plant parts significantly increased ($P < 0.01$) with increasing Se application in soil (Tab. 3). For example, the Se concentrations in the roots, stems, and leaves under 24 mg kg^{-1} Se treatment were 6.59, 5.91 and 5.43 times higher than those in the same plant parts under 3 mg kg^{-1} Se treatment, respectively. Under the same Se treatment, the Se concentration in the roots was evidently higher than those in the stems and leaves. In general, the pattern of total Se concentration in tobacco plant tissues followed the order root > leaf > stem. Under the same treatment of 24 mg kg^{-1} Se, the Se concentration in the roots (31.36 mg kg^{-1}) was 3.17 and 7.57 times higher than those in leaves and stems, respectively (Tab. 3).

The accumulation of Se in the tobacco roots, stems, and leaves significantly increased with increasing Se application (Tab. 4). For example, the roots, stems, and leaves under 24 mg kg^{-1} Se treatment accumulated 254.64, 43.60, and 179.30 μg plant^{-1} of Se; these values were 6.28-, 5.51-, and 4.81-fold higher than those accumulated by the roots, stems, and leaves under 3 mg kg^{-1} Se treatment, respectively. Under high-dose Se treatments (24 mg kg^{-1}), the roots accumulated up to 254.64 μg plant^{-1} of Se, which was 1.42 and 5.84 times higher than those accumulated by the leaves and stems, respectively.

Relationship between Se concentration and tobacco plants

The relationship of the Se concentrations in the different treatments with those in the different tobacco plant parts

Tab. 3 Se concentration in the roots, stems, and leaves of tobacco plants under different levels of Se treatment.

Se treatments (mg kg^{-1})	Root (mg kg^{-1})	Stem (mg kg^{-1})	Leaf (mg kg^{-1})
CK	0.15 ±0.02 e	0.04 ±0.00 e	0.06 ±0.00 e
3	4.13 ±0.44 d	0.53 ±0.10 d	1.17 ±0.20 d
6	6.65 ±0.74 c	1.04 ±0.08 c	1.59 ±0.08 c
12	13.81 ±1.28 b	1.69 ±0.15 b	3.54 ±0.35 b
24	31.36 ±2.84 a	3.66 ±0.33 a	7.52 ±0.71 a

The values are presented as mean and *SE* (n = 4). Different letters indicate a significant difference in the same column at $P < 0.05$.

Tab. 4 Se accumulation in roots, stems, and leaves of tobacco plants under different levels of Se treatment.

Se treatments (mg kg^{-1})	Root (μg plant^{-1})	Stem (μg plant^{-1})	Leaf (μg plant^{-1})
CK	1.49 ±0.13 e	0.53 ±0.02 e	1.76 ±0.04 e
3	40.52 ±1.43 d	7.91 ±0.35 d	37.27 ±2.06 d
6	73.46 ±3.56 c	18.00 ±0.69 c	55.03 ±2.14 c
12	134.65 ±2.59 b	24.61 ±1.08 b	103.73 ±4.53 b
24	254.64 ±23.58 a	43.60 ±3.89 a	179.30 ±12.84 a

The values are presented as mean and *SE* (n = 4). Different letters indicate a significant difference in the same column at $P < 0.05$.

(root, stem and leaf) was analyzed and compared after 40 d of treatment (Fig. 4). The Se concentrations in the roots, stems, and leaves significantly correlated with those in the different treatments. The amounts of absorbed Se in the roots, stems, and leaves were closely related to the Se concentrations in the different treatments.

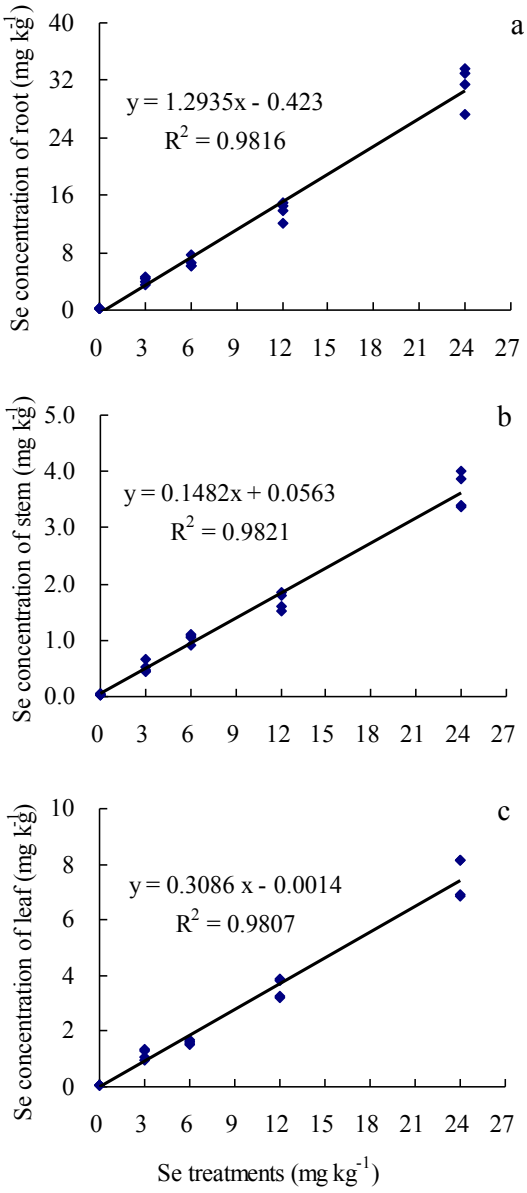

Fig. 4 Relationship between soil Se treatments and Se concentration in different parts of tobacco plants

Discussion

Research has revealed that Se exerts a dual effect on the growth of different plant species [1,5,22]. At low doses, Se stimulates plant growth; at high doses, this element hinders plant growth [2]. In the present study, the exposure of plants to 6 mg kg^{-1} Se increased the yield of roots, shoots and leaves by 11%, 18% and 29%, respectively (Tab. 1). These findings indicate that the growth of flue-cured tobacco plant increased at low-dose Se treatments (≤6 mg kg^{-1}), which agreed with earlier observations of Yao et al. [22], who found that treatment with 1 mg kg^{-1} to 3 mg kg^{-1} Se promotes biomass accumulation in wheat seedlings. However, several reports have provided evidence that high Se addition levels decrease the biomass of non-accumulator plants like wheat [5] and rice [23]. Our results showed that selenite inhibited tobacco plant growth at concentrations up to 12 mg kg^{-1}. The decrease in the root, stem, and leaf DWs was much more apparent at concentrations up to 24 mg kg^{-1} (Tab. 1).

The improvement of plant growth at low-dose Se treatments (Tab. 1) may be due to the significant increase in photosynthesis of tobacco plants (Tab. 2). In the present study, low-dose Se treatments (≤6 mg kg^{-1}) increased the photosynthetic rate, stomatal conductance and carboxylation efficiency in tobacco leaves (Tab. 2). A similar pattern was observed in rice [23] and sorghum leaves [11]. Wang et al. [23] revealed that in rice seedlings, low doses of Se enhanced photosynthesis. In addition, in sorghum, Se application significantly increased the photosynthetic rate and stomatal conductance [11]. In contrast, at high Se supply levels, the photosynthetic rate in tobacco leaves decreased significantly. High-dose Se treatments (24 mg kg^{-1}) reduced the Pn, CE and Rubisco content by 34%, 39%, and 18%, respectively (Tab. 2 and Fig. 2). This fact suggests that, growth inhibition of tobacco plants under high-dose Se treatments may result from impaired photosynthesis.

Excess Se was toxic to tobacco plants, leading to reduction of chlorophyll concentration (SPAD value; Fig. 1) that may cause photosynthesis suppression. In our experiment, the chlorophyll concentration in the leaves decreased significantly under 24 mg kg^{-1} Se treatments (Fig. 1). Similarly, Chen et al. [24] reported that *Chlorella vulgaris* has lower total chlorophyll content under high Se treatments than under low Se treatments, suggesting that Se affects chlorophyll synthesis or chlorophyllase activity. A decrease in pigment concentrations could also decrease photosynthetic functioning and elicit negative effects on the levels [24]. However, to acquire detailed regulatory mechanisms underlying these effects, further studies should focus on the nature of PSII photochemistry and photosynthetic apparatus under excess Se.

The inhibition of photosynthesis in tobacco plants under high Se application may be closely related to the increassed MDA levels. As a product of lipid peroxidation, MDA is an indicator of oxidative damage [25]. Reports have shown that proper doses of Se can reduce MDA accumulation in various plants [2,26]. In the present study, Se application of 6 mg kg^{-1} significantly decreased the MDA content in tobacco leaves; however, 24 mg kg^{-1} Se remarkably increased this parameter (Fig. 3). Similarly, Cartes et al. [26] found that low-dose Se treatments (≤6.0 mg kg^{-1}) reduce the MDA content in ryegrass, and vice versa. The MDA content reflected the extent of lipid peroxidation and indirectly reflected the degree of cell damage. Therefore, our results suggested that the optimal Se dose (≤6 mg kg^{-1}) enhanced antioxidant capacity and reduced lipid peroxidation in flue-cured tobacco leaves, whereas excess Se accelerated lipid peroxidation.

Plant growth responses were closely related with the concentrations of Se in the plant tissues (Tab. 3). The Se concentrations in the different parts of tobacco plant increased as those in the different treatments increased (Tab. 3). A linear correlation ($R = 0.95$, $P < 0.01$) was found between soil and tobacco plant tissue Se concentrations (Fig. 4), indicating that the tobacco plant tissues were not saturated within the tested concentration range. Moreover, Se concentration was much more higher in the tobacco roots than in the leaves and stems (Tab. 3). Therefore, a significantly higher amount of Se was accumulated in the roots than in the leaves and stems (Tab. 4). The pattern of total Se concentration in tobacco plant tissues (root > leaf > stem) was similar to that previously observed in ryegrass [27], wherein Se was principally accumulated in the roots. Previous studies have demonstrated that hyperaccumulators were characterized by a high leaf Se concentration, and a higher shoot:root Se concentration ratio [28,29]. Valdez et al. [30] reported that as a Se hyperaccumulator, the pattern of total Se concentration in *Astragalus bisulcatus* follows the order root < leaf < stem. Under 24 mg kg^{-1} Se, the Se concentration in the roots (31.36 mg kg^{-1}) was 3.17 and 7.57 times higher than those in leaves and stems, respectively (Tab. 3). It suggested that high accumulation of Se in roots (31 mg kg^{-1}) caused tobacco plant toxicity. Generally, most cultivated plants contain less than 25 mg Se kg^{-1} DWs and are considered to be non-accumulators [31]. More recently, it was revealed that most plant species growing on seleniferous soils contain <10 mg Se kg^{-1} DWs, and experience toxicity at levels above ~100 mg Se kg^{-1} DWs [32]. Therefore, from our results it can be suggested that the flue-cured tobacco had a low tolerance to high Se levels, an should be classified as a Se non-accumulator.

Conclusions

The present results showed that Se stimulated tobacco plant growth at low-dose application (≤6 mg kg^{-1}) but inhibited the plant growth at high-dose (≥12 mg kg^{-1}), and the optimal dose of Se application was 6 mg kg^{-1}. Optimal Se dose (6 mg kg^{-1}) improved the plant growth mainly by enhancing photosynthesis, stomatal conductance, carboxylation efficiency and Rubisco content in the flue-cured tobacco leaves. However, the inhibition of excess Se on tobacco growth might be due to high accumulation of Se in roots and the damage of photosynthesis in leaves.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: designed the research: JC, ZC, SF; performed the experiments: JC, SJ; analyzed the data: JC, LT; wrote the paper: JC.

Competing interests

No competing interests have been declared.

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