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Comparative study of the effects of selenium nanoparticles and selenite on selenium content and nutrient quality in soybean sprouts

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ABSTRACT

Soybean (*Glycine max* L.) sprouts are a common vegetable with rich nutrients, such as protein, vitamin C and isoflavones. Soybean is also capable of accumulating selenium (Se). To study the effects of Se biofortification on the nutrient of this crop, soybean sprouts were treated with different concentrations of selenium nanoparticles (SeNPs) or selenite (i.e., 10 µM, 20 µM, 40 µM, 80 µM and 100 µM) in a hydroponic experiment. Results showed that SeNPs and selenite remarkably increased the total Se concentration in soybean sprouts. Five Se speciations, namely, selenocystine, selenomethionine, methyl selenocysteine, selenite and selenate were detected in soybean sprouts, but selenomethionine was found to be the dominant Se speciation. SeNPs and selenite increased the contents of chlorophyll, carotenoid, soluble sugar, soluble protein, vitamin C and isoflavones in soybean sprouts. SeNPs treatments led to less malondialdehyde content compared with selenite. SeNPs and selenite both enhanced the glutathione content. The modest dosage of exogenous Se stimulated the catalase activity, whereas the large amount reduced it. The peroxidase and ascorbate peroxidase activities were stimulated by SeNPs and selenite. SeNPs posed no significant influence on the superoxide dismutase activity. This study suggests that SeNPs are a good exogenous Se source for the production of Se-rich soybean sprouts.

Keywords: nutrient, selenite, selenium forms, selenium nanoparticles, soybean sprouts

INTRODUCTION

Soybean (*Glycine max* L.) is a crop that originated in China and is now grown all over the world. Soybean is primarily grown for its oil, protein and fodder value. Soybean sprout is a fragile plant that emerges from a soybean seed after germination and is eaten as a fresh vegetable. Soybean sprouts have not only a good taste for direct intake but also a variety of nutrient substances, such as vitamin C, isoflavones, soluble protein, chlorophyll and carotenoids (Kim and Kim, 2020). Furthermore, soybean sprouts have higher contents of several chemicals than soybean seeds. For example, a higher content of flavonoid metabolites was observed in soybean sprouts than soybean seeds (Bi et al., 2022). Selenium (Se) is a microelement that is required by both humans and animals. There are 25 selenoproteins in the human body, all of which play vital roles in human

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health (Santesmasses et al., 2020). Hypoimmunity, reduced fertility, cognitive decline and an increased risk of cancer are symptoms of Se deficiency in humans (Hatfield et al., 2014). However, around 1,000 million people live in Se-deficient areas throughout the world (Winkel et al., 2012; Schiavon et al., 2020), including 70 million people in China, where 70% of the regions are Se-deficient areas (Wu et al., 2015). As a result, Se supplementation is critical for these individuals.

Because vegetables can take up and transform Se, they are ideal Se carriers for daily dietary Se supplementation. Due to the chemical similarities of Se and sulphur, plants generally take up and assimilate it through the sulphur metabolism system (Lima et al., 2018). Se can be converted into seleno-amino acids, such as selenocysteine (SeCys) and selenomethionine (SeMet) (Lima et al., 2018). Methylated SeCys (MeSeCys) and methylated SeMet (MeSeMet) may be formed from SeCys and SeMet, respectively (Chen et al., 2019; Kolbert et al., 2019). These organic Se forms have bioactive properties that are helpful to human health, including anti-cancer (Radomska et al., 2021) and inflammatory regulation (Hariharan and Dharmaraj, 2020). As a result, they are crucial nutritional molecules for plants. Soybeans have a high capability for Se accumulation (Chan et al., 2010). SeMet, SeCys, MeSeCys and $SeCys_2$ are among the Se compounds identified in soybean, while SeMet is the most abundant (Lu et al., 2018; Deng et al., 2022). Exogenous Se treatments can also boost antioxidant defences and raise salt tolerance in soybean plants, according to a recent study (Rahman et al., 2021). Nonetheless, the majority of these findings came from soybean seeds or plants, few studies focussed on the effects of exogenous Se on soybean sprouts.

Studies have demonstrated that exogenous Se treatments exert influences on the nutrient of crops. Selenate treatments reduce overall glucosinolate levels while increasing flavonoid content in broccoli florets (Rao et al., 2021a). By contrast, Se yeast increased overall glucosinolate levels while decreasing flavonoid content (Gui et al., 2021). Se yeast and selenite both had opposing effects on vitamin C levels in broccoli florets, with the former increasing while the latter decreasing (Gui et al., 2021). Similarly, selenite has been found to reduce the amount of vitamin C in broccoli sprouts (Tian et al., 2016). These findings suggest that various Se sources and phases of development have varied effects on crop nutrient molecules.

Selenium nanoparticles (SeNPs) are a kind of elemental Se with bioactivity for plants. Bioactive SeNPs generally are produced by some Se-resistant microorganisms, such as *Azoarcus* sp. CIB (Fernandez-Llamosas et al., 2016) and *Bacillus subtills* (Ullah et al., 2021). Evidence revealed that rice plants can take up SeNPs, and aquaporin may be responsible for this process; however, SeNPs are not as efficient as selenite or selenate during the uptake by rice roots (Wang et al., 2020). Another study showed that SeNPs can decrease the translocation of arsenic from rice roots to shoots and ameliorate arsenic stress in rice plants (Wang et al., 2021). These findings suggest that SeNPs might be used as a Se source in the production of Se-enriched crops.

To reveal the differences between SeNPs and selenite in cultivating Se-enriched soybean sprouts, different concentrations of SeNPs and selenite were employed to treat hydroponically cultivated soybean sprouts in this study. Total Se content, Se speciation and several important nutritional indicators of soybean sprouts were compared. The findings of this study will contribute to a better understanding of the role of exogenous Se in the production of Se-enriched soybean sprouts.

MATERIALS AND METHODS

Plant materials

Soybean seeds (cultivar: 'Dongnong690') were purchased from Wuhan Fengyuan Seed Co. Ltd. (Wuhan, China). Sodium selenite was bought from Sigma-Aldrich (Shanghai) Trading Co. Ltd. (Shanghai, China). Bioactive SeNPs (suspension with a Se concentration of 5 mg · mL^{-1}) with a particle size of 100–600 nm were obtained from the Institute of Agricultural Economics and Technology, Hubei Academy of Agricultural Sciences (Wuhan, China). Soybean seeds were soaked in deionised water for 8 h. Sixty plump seeds were selected, put on a plastic mesh plate (mesh size: 0.3 cm length × 0.3 cm width) and then germinated in a plastic hydroponic box (34 cm length × 25 cm width × 4.5 cm depth). The plastic hydroponic box was added with 1 L of deionised water. Deionised water was replaced with SeNPs or selenite solutions after the plantule reached a length of 1 cm. A single factor experiment was carried out, and the test variable was Se concentration. The concentrations of the SeNPs or selenite solutions were set as follows: 10 μM, 20 μM, 40 μM, 80 μM and 100 μM. Deionised water was used as control. The solutions with SeNPs or selenite were renewed every 2 days. The plants were placed in an illumination incubator for growth with the following growth parameters: 24 °C and 16 h of illumination during the day, 20 °C and 8 h of darkness during the night, 75% of relative humidity and 300 μmol · m^{-2} · s^{-1} of optical density. Each treatment was set with three biological replications. Soybean sprouts were collected, weighed and separated into roots and shoots after 7 days of two exogenous Se treatments. Fresh samples were frozen in liquid nitrogen and stored at –80 °C. Samples for Se detection were dried at 60 °C, crushed and kept in an airtight container.

Total Se and Se speciation contents

The total Se of soybean sprouts was determined following the method of Rao et al. (2020). In brief, 0.2 g of samples was weighed, placed in a digestion tube and treated with 10 mL of guaranteed nitric acid and 2 mL of hydrogen peroxide. This solution combination was placed in a microwave digestion system and digested according to a set of instructions until it was clear. The clear solution was then mixed with water to make up 10 mL for total Se determination using hydride generation atomic fluorescence spectrometry (HG-AFS). Working conditions of HG-AFS (AFS8530, Beijing Haiguang Instrument, Beijing, China), were as follows: negative high voltage, 320 V; lamp current, 90 mA; atomisation temperature, 800 °C; carrier gas flow rate, 500 mL · min^{-1}; injection volume, 1 mL.

Determination of Se speciations in soybean sprouts was performed using liquid chromatography-HG-AFS (LC-AFS530, Beijing Haiguang Instrument, Beijing, China) following the method of Rao et al. (2021b). The five Se standard compounds ($SeCys_2$, SeMet, MeSeCys, Se^{4+} and Se^{6+}) were bought from the National Institute of Metrology, China, and used to establish the standard equation. Se speciations were extracted by enzymatically hydrolysing 0.1 g of samples with proteinase K and pronase E under ultrasonication at 37 °C for 1 h. The solution was then centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2 μm filtration membrane for detection. LC-HG-AFS working conditions were as follows: mobile phase, 40 mM KH_2PO_4 + 20 mM KCl; pH, 6.0; flow rate, 1.0 mL · min^{-1}, chromatographic column, Hamilton PRP-X100 (Hamilton Co., Reno, NV, USA); column temperature, 27 °C; injection volume, 150 μL; cathodic current, 80 mA; carrier gas flow rate, 600 mL · min^{-1} and negative high voltage, 320 V.

Chlorophyll, carotenoid, soluble sugar and soluble protein contents

Chlorophyll, carotenoid, soluble sugar and soluble protein contents were determined according to the method recorded by Zhang and Li (2006). Briefly, 0.2 g of shoots of soybean sprouts were homogenised with 95% ethanol and centrifuged at 4,000 rpm for 10 min. Chlorophyll and carotenoid were detected at 665 nm, 649 nm and 470 nm by an ultraviolet-visible (UV-Vis) spectrophotometer. Soluble sugar content was determined via anthrone colorimetry with a UV-Vis spectrophotometer at 630 nm. Soluble protein was evaluated by coomassie brilliant blue G-250 colour rendering at 595 nm.

Vitamin C and total isoflavone contents

Vitamin C content was measured using a vitamin C colorimetric assay kit (A009-1-1, Nanjing Jiancheng Bioengineering Co. Ltd., Nanjing, China) according to the operation manual. Total isoflavone content was measured following the method described by He et al. (2013). Briefly, 0.2 g of soybean shoots was added with 4 mL of 80% ethanol, ultrasonically extracted at 60 °C for 30 min and centrifuged at 8,000 rpm for 10 min. The supernatant was collected and measured via a UV-Vis spectrophotometer at 260 nm. Genistein was used to plot the standard curve.

Malonaldehyde (MDA) and antioxidant indexes

The content of MDA was measured using thiobarbituric acid (TBA) colorimetry following the method of Siddiqui et al. (2012). In brief, 0.5 g of shoots was ground with 5% of trichloroacetic acid and centrifuged at 3,000 rpm for 10 min. Two milliliters of the supernatant were transferred and added with 2 mL of 0.67% TBA. The solution was water bathed at 100 °C for 10 min and centrifuged at 3,000 rpm for 10 min. The supernatant was collected and detected at 450 nm, 532 nm and 600 nm. MDA content was calculated according to the formula. Glutathione (GSH) content was determined using a GSH assay kit (A006-2-1, Nanjing Jiancheng Bioengineering Co. Ltd., Nanjing, China) following the procedure recorded in the specification. Activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) were measured by using CAT (A007-1-1), POD (A084-3-1), SOD (A001-3-2) and APX (A123-1-1) assay kits produced by Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, China).

Statistical analysis

Data were analysed using SPSS (version 19, SPSS Inc., Chicago, IL, USA) with one-way ANOVA followed by Duncan's new complex range test. All data were expressed as mean ± standard errors from three biological replicates. The significant difference was set at $p \leq 0.05$. Column diagrams were plotted by Sigma plot (version 14.0, Systat Software Inc., San Jose, CA, USA).

RESULTS

Biomass of soybean sprouts

Similar changing trends in the biomass of soybean sprouts were observed between SeNPs and selenite treatments. As shown in Figure 1, 10 μM, 20 μM and 40 μM of SeNPs and 10 μM of selenite significantly increased the fresh weight of soybean sprouts compared with the control. Notably, the fresh weight of soybean sprouts was remarkably lower in the 100 μM of SeNPs treatment than in the control. Different concentrations of selenite treatments, except for 10 μM, dramatically reduced soybean sprout biomass.

Total Se concentration

The total Se concentration of soybean sprout shoots and roots was dramatically increased by both SeNPs and selenite. In the shoots, total Se concentration significantly increased following the increase of exogenous Se dosage (Figure 2A). For example, the total Se concentration in soybean shoots treated with 100 μM of SeNPs or selenite was 31-fold or 44-fold higher than that under control. However, compared with 80 M of

SeNPs or selenite, 100 M of SeNPs or selenite resulted in a significant reduction in the total Se concentration of the shoots.

The application of exogenous Se substantially increased the total Se concentration in the roots of soybean sprouts. The highest total Se concentrations were 518 μg · g^{-1} DW and 587 μg · g^{-1} DW under 100 μM of SeNPs and 100 μM of selenite treatment, respectively (Figure 2B). The total Se concentration in the roots was considerably greater than that in the shoots under the same conditions. For instance, the total Se concentration was 425 μg · g^{-1} DW and 456 μg · g^{-1} DW in the roots treated with 100 μM of SeNPs and 100 μM of selenite, respectively, whereas the total Se concentration was 46 μg · g^{-1} DW and 66 μg · g^{-1} DW in the corresponding shoots.

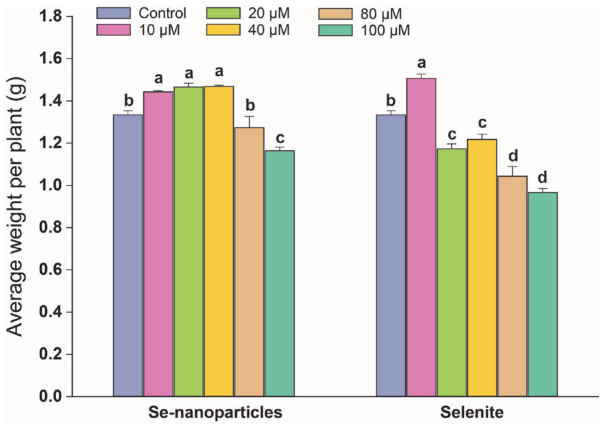

Figure 1. Biomass statistics of soybean sprouts. Different letters above the columns indicate significant differences at $p < 0.05$.

Analysis of Se speciation

Samples treated with 20 μM and 100 μM exogenous Se were utilised for Se speciation determination to demonstrate variations in Se speciation of soybean sprouts treated with SeNPs and selenite (Table 1). The five Se speciations can be well detected through LC-HG-AFS (Figure S1 in Supplementary Material). In soybean sprouts treated with SeNPs, five different Se species were found. Se^{6+}, on the other hand, was not detected under selenite treatments. In the shoots, the concentrations of $SeCys_2$ and MeSeCys were higher under SeNPs than those under selenite, whereas the

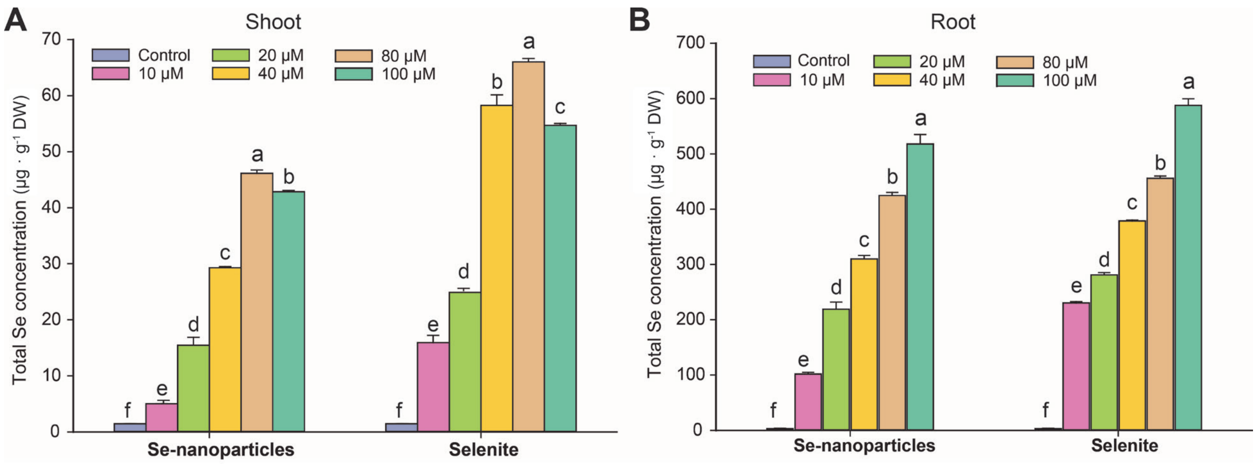

Figure 2. Total Se concentrations in the shoots (A) and roots (B) of soybean sprouts treated with SeNPs and selenite. Different letters above the columns indicate significant differences at $p < 0.05$.

Table 1. Selenium speciation in soybean shoots and roots.

Tissue	Treatment	Selenium speciation (μg · g^{-1} DW)				
		$SeCys_2$	MeSeCys	SeMet	Se^{4+}	Se^{6+}
Shoot	0	nd	nd	0.193 ± 0.021 d	0.035 ± 0.008 c	nd
	N20	0.188 ± 0.022 b	0.185 ± 0.048 c	2.182 ± 0.258 c	0.071 ± 0.023 c	0.453 ± 0.001 b
	N100	1.192 ± 0.199 a	0.953 ± 0.097 a	3.789 ± 0.611 b	0.147 ± 0.025 b	0.792 ± 0.092 a
	S20	0.093 ± 0.019 b	0.087 ± 0.008 d	1.857 ± 0.189 c	0.048 ± 0.005 c	nd
	S100	0.969 ± 0.041 a	0.696 ± 0.012 b	4.796 ± 0.065 a	0.248 ± 0.006 a	nd
Root	0	nd	nd	0.127 ± 0.015 d	0.034 ± 0.008 e	nd
	N20	2.245 ± 0.214 d	2.292 ± 0.286 c	3.897 ± 0.240 c	0.888 ± 0.051 d	2.444 ± 0.057 b
	N100	6.318 ± 0.377 b	7.224 ± 0.336 b	5.734 ± 0.250 b	1.759 ± 0.066 b	6.870 ± 0.356 a
	S20	3.613 ± 0.084 c	2.188 ± 0.255 c	3.601 ± 0.178 c	1.046 ± 0.015 c	nd
	S100	11.135 ± 0.716 a	8.698 ± 0.235 a	14.213 ± 0.059 a	1.970 ± 0.021 a	nd

Different letters behind the data indicate significant differences at $p < 0.05$.

concentrations of SeMet and Se^{4+} were lower under SeNPs than those under selenite. In the roots, selenite treatments possessed higher concentrations of $SeCys_2$, MeSeCys, SeMet and Se^{4+} than the SeNPs treatments. Interestingly, SeMet was the dominant Se speciation in the shoots, while the three organic Se forms in the roots, namely, $SeCys_2$, MeSeCys and SeMet, were equivalent in concentration.

MeSeCys: methyl selenocysteine; N100, 100 μM Se nanoparticles; N20, 20 μM Se nanoparticles; nd: not detected; S100, 100 μM selenite;S20, 20 μM selenite; Se^{4+}: selenite; Se^{6+}: selenate; $SeCys_2$: selenocystine; SeMet: selenomethionine.

Contents of chlorophyll and carotenoid

Chlorophyll and carotenoid contents were influenced by SeNPs and selenite treatments. As demonstrated in Figure 3A, SeNPs caused a significant increase in chlorophyll content, whereas selenite treatments (except 80 μM) led to an insignificant increase in chlorophyll content. The changes in carotenoid content were similar to the changes in chlorophyll content. Carotenoid content in soybean sprouts significantly increased following the increase of SeNPs dosages (Figure 3B). Selenite treatments increased carotenoid content as well, although only at concentrations of 10 μM and 80 μM.

Contents of soluble sugar and protein

Treatments with SeNPs and selenite remarkably enhanced the generation of soluble sugar and protein in soybean sprouts (Figure 4). SeNPs and selenite both significantly increased the soluble sugar content of soybean sprouts (Figure 4A). Interestingly, the soluble sugar content of soybean sprouts under 80 μM and 100 μM of selenite treatment was much greater than that of soybean sprouts under 80 μM and 100 μM of SeNPs treatment. Selenite and SeNPs also posed a similar impact on the soluble protein level of soybean sprouts. As shown in Figure 4B, the soluble protein content prominently increased with the increase of SeNPs and selenite concentration. However, 10 μM of SeNPs

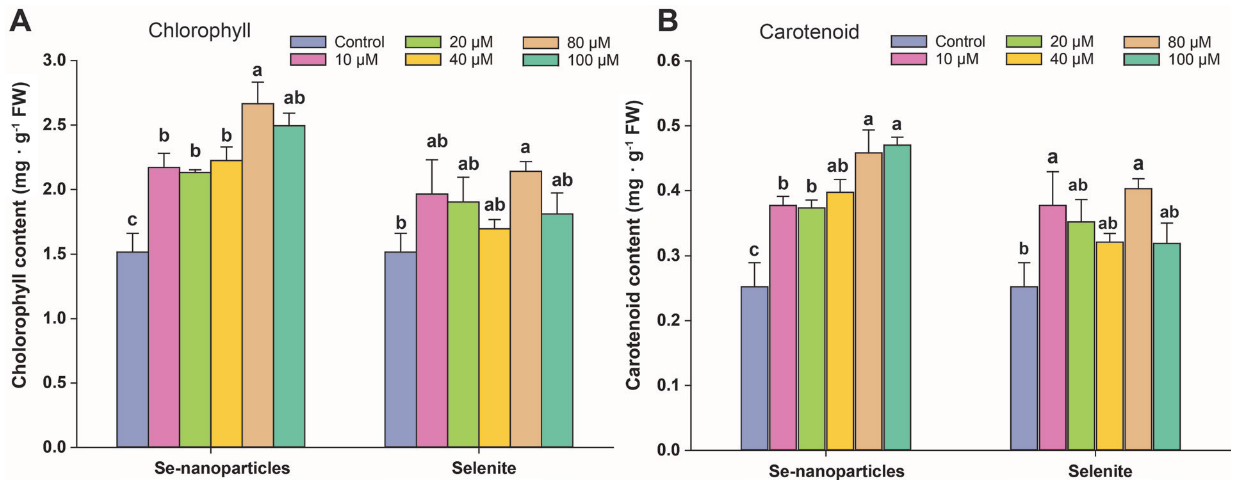

Figure 3. Chlorophyll (A) and carotenoid (B) contents of soybean sprouts treated with SeNPs and selenite. Different letters above the columns indicate significant differences at $p < 0.05$. SeNPs, selenium nanoparticles.

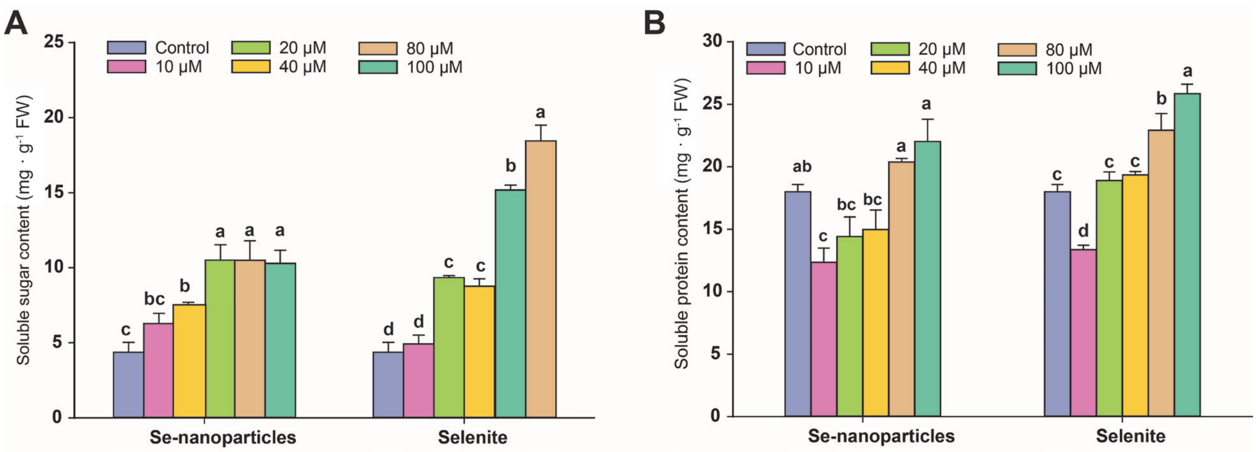

Figure 4. Soluble sugar (A) and protein (B) contents of soybean sprouts treated with SeNPs and selenite. Different letters above the columns indicate significant differences at $p < 0.05$. SeNPs, selenium nanoparticles.

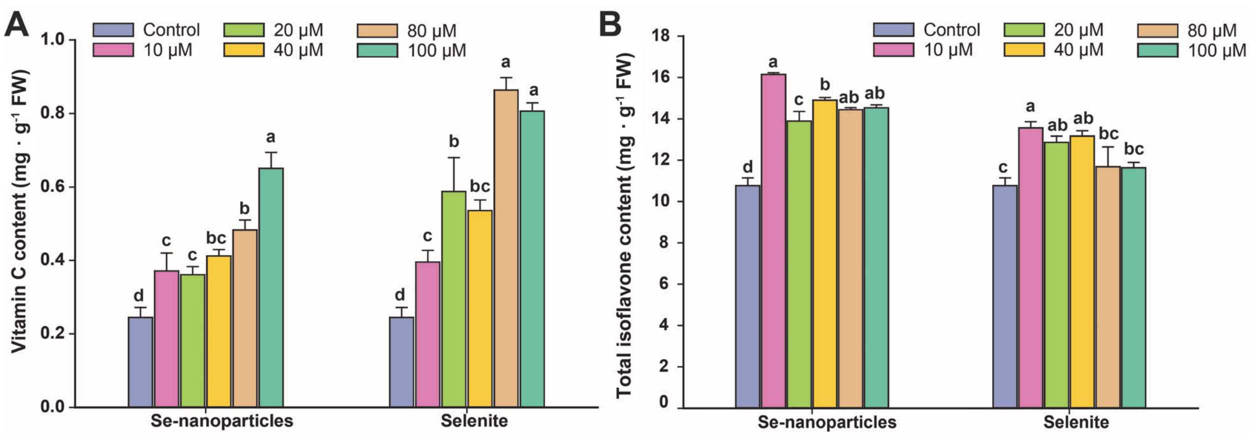

Figure 5. Vitamin C (A) and total isoflavone (B) contents of soybean sprouts treated with SeNPs and selenite. Different letters above the columns indicate significant differences at $p < 0.05$. SeNPs, selenium nanoparticles.

or selenite conspicuously reduced the soluble protein content when compared with the control.

Contents of vitamin C and total isoflavone

Vitamin C and isoflavone are the two kinds of important nutrient compounds in soybean sprouts. As shown in Figure 5A, SeNPs and selenite remarkably boosted the vitamin C content of soybean sprouts. For instance, the vitamin C content in soybean sprouts was 0.25 mg · g^{-1} FW in the control group, but it was 0.65 mg · g^{-1} FW and 0.81 mg · g^{-1} FW under 100 µM of SeNPs and selenite, respectively. They were 2.6-fold and 3.4-fold, respectively, of that under the control treatment. The total isoflavone content of soybean sprouts was also altered by SeNPs and selenite (Figure 5B). The total isoflavone content of soybean sprouts treated with SeNPs was significantly higher than that of the control. Notably, 10–40 µM of selenite remarkably enhanced the total isoflavone content of soybean sprouts, but 80 µM and 100 µM of selenite showed no significant influences compared with the control.

MDA and antioxidant indexes

The changes in MDA and five antioxidant indexes, namely, GSH, CAT, POD, SOD and APX, were determined to evaluate the effects of SeNPs and selenite treatments on the membrane oxidation status and antioxidant properties of soybean sprouts. Results showed that SeNPs reduced MDA content in soybean sprouts (Figure 6A). Compared with the control, 40 µM and 80 µM of SeNPs led to a significant decrease in MDA content. The MDA concentration of soybean sprouts was dramatically decreased by 20 µM selenite treatment, but the MDA content was significantly elevated by 80 µM and 100 µM selenite treatment. SeNPs and selenite increased the GSH content in soybean sprouts (Figure 6B). GSH levels were 37.8% and 38.2% higher under 100 µM of SeNPs and 80 µM of selenite, respectively, than those under control.

In soybean sprouts, the activity of three antioxidant enzymes (CAT, POD and SOD) was measured to access how they responded to SeNPs and selenite treatment. The results indicated that 10–40 µM of SeNPs and 10–20 µM of selenite remarkably enhanced CAT activity in soybean sprouts, but 100 µM of SeNPs and 80–100 µM of selenite dramatically decreased CAT activity when compared with the control (Figure 6C). Unlike CAT, POD activity increased as exogenous Se concentrations increased, as illustrated in Figure 6D. POD activity of soybean sprouts treated with 100 µM of SeNPs and selenite increased 71.7% and 175.5%, respectively, when compared with 0 µM of Se treatment. In contrast, the SOD activity of soybean sprouts was not significantly affected by SeNPs, but 40–100 µM of selenite remarkably decreased the SOD activity compared with the control (Figure 6E). SeNPs and selenite significantly increased the activity of APX. As shown in Figure 6F, AXP activity under 100 µM of SeNPs and selenite treatment was 2.86 and 2.56-fold of that under the control, respectively.

DISCUSSION

This study compared the effects of two Se forms, namely, SeNPs and selenite, on the growth and quality of soybean sprouts. Gui et al. (2021) also reported that 0.1–1.6 mM of Se yeast and selenite significantly increased and decreased the growth of broccoli florets, respectively. Here, similar results were obtained that selenite inhibited the growth of soybean sprouts. SeNPs are inorganic Se form, but Se yeast contains kinds of Se forms (mainly as SeMet) and other chemical compounds (Gui et al., 2021), implying that they would pose different influences on crops. Noticeably, as a novel type of Se source, SeNPs showed no significant influence on the growth of soybean sprouts (Figure 1).

A high concentration of Se accumulating in the plant body may hinder plant development and even be toxic (Kolbert et al., 2019). For example, 800 mg · L^{-1} of selenite significantly reduced the biomass of *Cardamine violifolia* (Wu et al., 2020). Here, soybean sprouts treated with 100 µM of SeNPs or selenite exhibited the

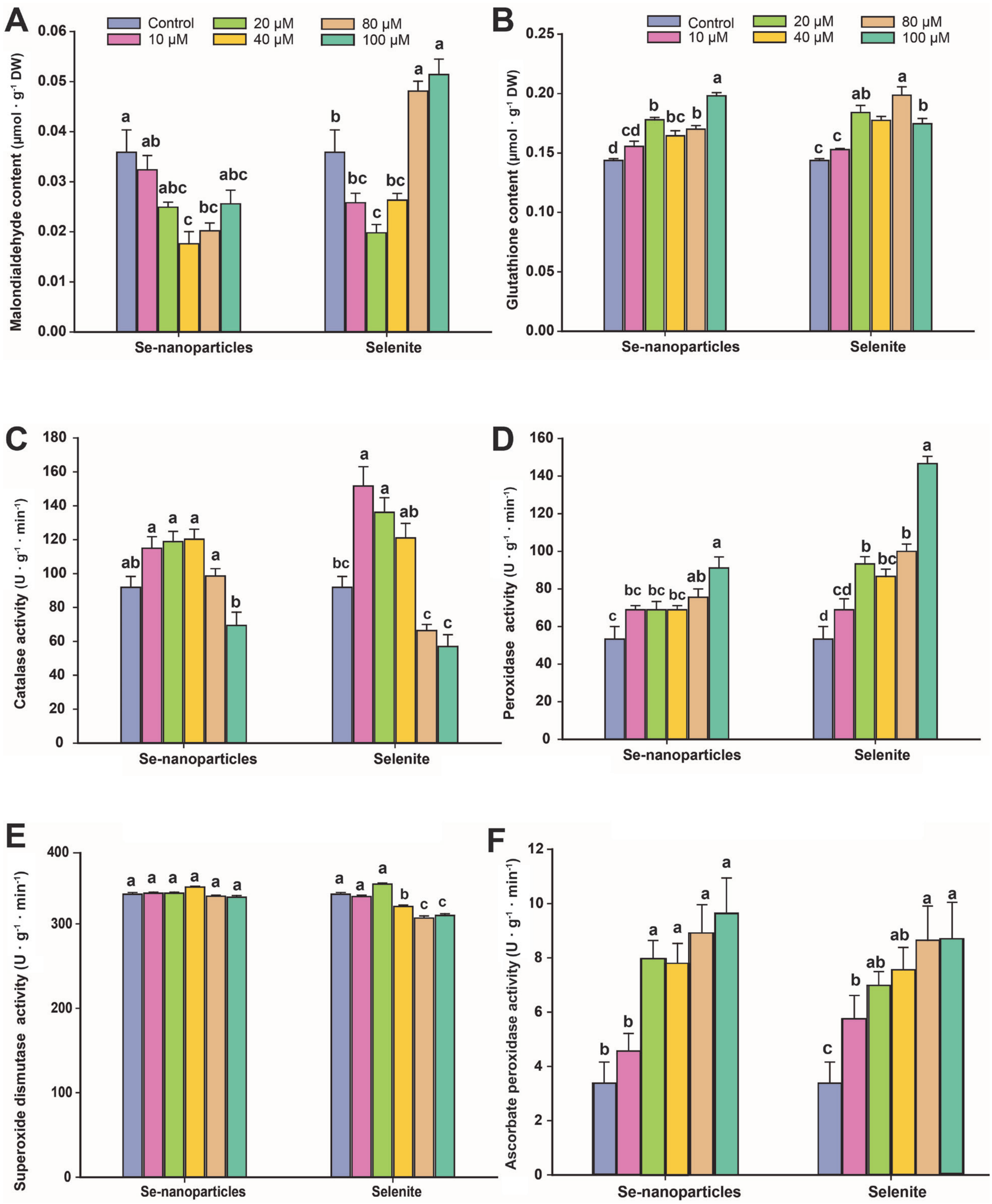

Figure 6. Changes in the MDA and antioxidant system of soybean sprouts treated with SeNPs and selenite. (A) MDA content; (B) GSH content; (C) CAT activity; (D) POD activity; (E) SOD activity and (F) APX activity. Different letters above the columns indicate significant differences at $p < 0.05$. APX, ascorbate peroxidase; CAT, catalase; GSH, glutathione; MDA, malonaldehyde; POD, peroxidase; SeNPs, selenium nanoparticles; SOD, superoxidase dismutase.

lowest fresh weight. In contrast, 10 μM of SeNPs or selenite significantly promoted the growth of soybean sprouts, demonstrating that a modest concentration of exogenous Se may boost the growth of soybean sprouts. Moreover, SeNPs treatments resulted in lower total Se levels in both shoots and roots than selenite treatments (Figure 2), indicating that soybean sprouts take up selenite more effectively than SeNPs. Although there is no conclusive evidence of how SeNPs enter the plant body, there are indications that aquaporin may play a role in SeNPs absorption in rice (Wang et al., 2020). Studies reveal that plants may take up selenite via silicon influx transporters (Zhao et al., 2010) or phosphate transporters (Zhang et al., 2014) in rice. On the one hand, $SeCys_2$, SeMet and MeSeCys were found in both SeNPs and selenite-treated soybean sprouts in this study. Given the efficiency of these three Se forms in safeguarding human health (Schoenmakers and Chatterjee, 2020; Nasim et al., 2021), this study establishes SeNPs as a viable Se source for producing Se-enriched soybean sprouts. On the other hand, Se^{6+} was only found in soybean sprouts treated with SeNPs (Table 1). This finding implies that SeNPs can be oxidised to Se^{6+} in soybean sprouts, but the underlying mechanism of this phenomenon still needs elucidation.

Application with Se can differently affect the biosynthesis of chlorophyll in various plant species. For instance, leaf yellowing was observed in cucumber treated with 80 μM of selenite or 20 μM of selenate (Hawrylak-Nowak et al., 2015). Spraying or irrigating selenate showed no significant influences on chlorophyll content in lamb's lettuce (Hawrylak-Nowak et al., 2018). This study revealed that 0–100 μM of SeNPs or selenite has no reverse effects on photosynthesis of soybean. Carotenoids are important pigments that give leaves their red, orange or yellow colour and are important nutritional components for humans (Loladze et al., 2019). Here, SeNPs substantially increased the content of carotenoid in soybean shoots, thus promoting the nutrient quality of soybean sprouts. Soluble sugar is an essential component of vegetables and fruits (Wang et al., 2018; Hu et al., 2019). Several studies demonstrate that exogenous Se application causes an increase in soluble sugar content in some crops, such as selenite on broccoli (Gui et al., 2021), Se yeast on cabbage (Liao et al., 2021) and amino acid-chelated Se-enriched foliar fertiliser on grapefruits (Zhu et al., 2017). Similar to the results reported by these previous studies, the present study found that SeNPs and selenite stimulated the accumulation of soluble sugar (Figure 4A). Different plant species have various soluble protein accumulation patterns when they were treated with Se. For example, high dosages of Se applied on rice plants showed decreased soluble proteins (Mostofa et al., 2017), but treatment with selenate posed no significant influences on soluble protein level in *C. violifolia* (Rao et al., 2021b). Here, the low concentration (e.g., 10 μM) of SeNPs or selenite decreased the soluble protein content, but high concentrations increased soluble protein content. This not only reflects the variations in the responses of different species to Se but also may relate to the breakdown of dysfunctional proteins in soybean plants exposed to high dosages of Se. However, the potential mechanism requires further investigation. Vitamin C is a vital antioxidant that not only helps plants scavenge reactive oxygen species (ROS) but also plays a crucial function in the human body (van Gorkom et al., 2019). SeNPs and selenite promoted the accumulation of vitamin C in soybean sprouts (Figure 5A), signifying that not only the antioxidant level of soybean sprouts but also their edibleness for humans was improved. Isoflavones are the characteristic secondary metabolites in soybeans and show bioactivity in the prevention and treatment of cancer (Hatono et al., 2021) and cardiovascular disease (Ferreira et al., 2019). Here, the total isoflavone content in soybean sprouts was found to be promoted by SeNPs and selenite treatments. However, SeNPs showed more positive effects on the total content of isoflavones than selenite (Figure 5B). Isoflavones are biosynthesised by the phenylpropanoid pathway in plants (Sugiyama et al., 2017; Cho et al., 2020); SeNPs may enhance the activities of several catalytic enzymes in the phenylpropanoid pathway in soybean sprouts.

Plants are continuously defending themselves from oxidation. MDA is a useful metric for measuring the level of lipid peroxidation in plant membranes (Morales and Munné-Bosch, 2019). SeNPs treatment results in lower MDA levels than selenite treatment, implying that soybean sprouts have superior membrane integrity under SeNPs treatment than selenite treatment. GSH is a potent antioxidant molecule that plays a role in a wide range of environmental stressors in plants (Park et al., 2021). Both SeNPs and selenite increased the GSH levels in soybean sprouts, indicating that the two Se forms are beneficial to enhance the antioxidant activities. CAT plays an essential role in the defence reactions of plants because it can catalyse the extinction of hydrogen peroxide and protect cells (Anjum et al., 2016). Here, results of CAT activity indicate that a low dosage of SeNPs or selenite enhances the elimination of hydrogen peroxide, but a high dosage may aggravate the accumulation of peroxides in soybean sprouts. POD is another key antioxidase that participates in the scavenging of ROS, including hydrogen peroxide (Lukacova et al., 2021). Given that POD activity showed the highest in 100 μM SeNPs or selenite treatments (Figure 6D), POD may be the dominant peroxide remover in soybean sprouts treated with a high dosage of Se. SOD is a superoxide dismutase enzyme that includes multiple isozymes, including copper/zinc-SOD and iron-SOD (Saed-Moucheshi et al., 2021). Here, SeNPs showed no significant influence on SOD activity, but selenite posed a slight decrease in the SOD activity of soybean sprouts, indicating that SOD activity may be stable when soybean sprouts are exposed to Se. APX plays an important role in scavenging ROS, especially hydrogen peroxide in chloroplasts and relates to the redox reaction of vitamin C in plants (Li et al., 2022). Changes in APX activity were consistent with vitamin C content in the present study, indicating that Se treatments enhanced the antioxidant property of soybean sprouts. Taking the analysis together, we suggest that soybean sprouts can regulate ROS scavenging by adjusting the activity of some key antioxidases when they are treated with SeNPs and selenite.

CONCLUSIONS

This study revealed that both SeNPs and selenite significantly influenced the growth of soybean sprouts. The low dosage promoted the growth but the high dosage prohibited it. Both SeNPs and selenite remarkably increased the total Se concentration in soybean sprouts, although the former was less effective than the latter in terms of uptake efficiency. Five Se speciations, namely, $SeCys_2$, SeMet, MeSeCys, Se^{4+} and Se^{6+}, were detected in the shoots or roots of soybean sprouts treated with SeNPs. Four Se speciations were found in soybean sprouts treated with selenite, excluding Se^{6+}. SeNPs and selenite increased the contents of chlorophyll, carotenoid, soluble sugar, soluble protein, vitamin C and isoflavones in soybean sprouts. SeNPs and selenite treatments differently affected MDA content and activities of several antioxidant enzymes. SeNPs treatments resulted in lower MDA content when compared with selenite. Both of SeNPs and selenite increased the GSH content. The modest dose of exogenous Se stimulated CAT activity, while the large dose inhibited it. SeNPs and selenite both increased the activity of POD and APX. SeNPs did not affect SOD activity; on the other hand, selenite reduced SOD activity. In conclusion, SeNPs may be a more suitable Se source than selenite for application in Se-enriched soybean sprouts.

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AUTHOR CONTRIBUTIONS

S.R. plotted the figures and wrote the manuscript. X.X. performed the experiments and analysed the data. Y.W. and Y.X. contributed to the experiments and data acquisition. H.C. reviewed the manuscript and polished the language. L.L. and S.C. designed the experiments. S.R and X.X contributed equally to this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K., Mohamed, A. A., Thangavel, P., Devi, G. D., Vasudhevan, P., Sofo, A., Khan, N. A., Misra, A. N., Lukatkin, A. S., Singh, H. P., Pereira, E., and Tuteja, N. (2016). Catalase and ascorbate peroxidase – Representative H_2O_2-detoxifying heme enzymes in plants. *Environmental Science and Pollution Research*, *23*, 19002–19029, doi: 10.1007/s11356-016-7309-6.

Bi, W., Zhao, G., Zhou, Y., Xia, X., Wang, J., Wang, G., Lu, S., He, W., Bi, T., and Li, J. (2022). Metabonomics analysis of flavonoids in seeds and sprouts of two Chinese soybean cultivars. *Scientific Reports*, *12*(1), 5541, doi: 10.1038/s41598-022-09408-1.

Chan, Q., Afton, S. E., and Caruso, J. A. (2010). Selenium speciation profiles in selenite-enriched soybean (*Glycine max*) by HPLC-ICPMS and ESI-ITMS. *Metallomics*, *2*, 147–153, doi: 10.1039/B916194E.

Chen, M., Zeng, L., Luo, X., Mehboob, M. Z., Ao, T., and Lang, M. (2019). Identification and functional characterization of a novel selenocysteine methyltransferase BjSMT from *Brassica juncea* L. *Journal of Experimental Botany*, *7*, 6401–6416, doi: 10.1093/jxb/erz390.

Cho, C. H., Jung, Y. S., Nam, T. G., Rha, C. S., Ko, M. J., Jang, D., Kim, H. S., and Kim, D. O. (2020). pH-adjusted solvent extraction and reversed-phase HPLC quantification of isoflavones from soybean (*Glycine max* (L.) *Merr.*). *Journal of Food Science*, *85*, 673–681, doi: 10.1111/1750-3841.15051.

Deng, X., Liao, J., Zhao, Z., Qin, Y., and Liu, X. (2022). Distribution and speciation of selenium in soybean proteins and its effect on protein structure and functionality. *Food Chemistry*, *370*, 130982, doi: 10.1016/j.foodchem.2021.130982.

Fernandez-Llamosas, H., Castro, L., Blazquez, M. L., Diaz, E., and Carmona, M. (2016). Biosynthesis of selenium nanoparticles by *Azoarcus* sp. CIB. *Microbial Cell Factories*, *15*, 109, doi: 10.1186/s12934-016-0510-y.

Ferreira, L. L., Silva, T. R., Maturana, M. A., and Spritzer, P. M. (2019). Dietary intake of isoflavones is associated with a lower prevalence of subclinical cardiovascular disease in postmenopausal women: Cross-sectional study. *Journal of Human Nutrition and Dietetics*, *32*, 810–818, doi: 10.1111/jhn.12683.

Gui, J. Y., Rao, S., Gou, Y., Xu, F., and Cheng, S. (2021). Comparative study of the effects of selenium yeast and sodium selenite on selenium content and nutrient quality in broccoli florets (*Brassica oleracea* L. var. *italica*). *Journal of the Science of Food and Agriculture, 102,* 1707–1718, doi: 10.1002/jsfa.11511.

Hariharan, S., and Dharmaraj, S. (2020). Selenium and selenoproteins: It's role in regulation of inflammation. *Inflammopharmacology*, *28*, 667–695, doi: 10.1007/s10787-020-00690-x.

Hatfield, D. L., Tsuji, P. A., Carlson, B. A., and Gladyshev, V. N. (2014). Selenium and selenocysteine: Roles in cancer, health, and development. *Trends in Biochemical Science*, *39*, 112–120, doi: 10.1016/j.tibs.2013.12.007.

Hatono, M., Ikeda, H., Suzuki, Y., Kajiwara, Y., Kawada, K., Tsukioki, T., Kochi, M., Suzawa, K., Iwamoto, T., Yamamoto, H., Shien, T., Yamane, M., Taira, N., Doihara, H., and Toyooka, S. (2021). Effect of isoflavones on breast cancer cell development and their impact on breast cancer treatments. *Breast Cancer Research and Treatment*, *185*, 307–316, doi: 10.1007/s10549-020-05957-z.

Hawrylak-Nowak, B., Dresler, S., Rubinowska, K., Matraszek-Gawron, R., Woch, W., and Hasanuzzaman, M. (2018). Selenium biofortification enhances the growth and alters the physiological response of lamb's lettuce grown under high temperature stress. *Plant Physiology and Biochemistry*, *127*, 446–456, doi: 10.1016/j.plaphy.2018.04.018.

Hawrylak-Nowak, B., Matraszek, R., and Pogorzelec, M. (2015). The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. *Acta Physiologiae Plantarum*, *37*, 41, doi: 10.1007/s11738-015-1788-9.

He, E., Shen, R., Wang, W., Chang, Q., and Guan, Q. (2013). Determination of isoflavones in extraction of soybean dregs using ultraviolet spectrophotometry. *Soybean Science*, *32*, 818–824, doi: 10.1016/0264-410X(93)90184-Y.

Hu, L., Yang, C., Zhang, L., Feng, J., and Xi, W. (2019). Effect of light-emitting diodes and ultraviolet irradiation on the soluble sugar, organic acid, and carotenoid content of postharvest sweet oranges (*Citrus sinensis* (L.) Osbeck). *Molecules*, *24*, 3440, doi: 10.3390/molecules24193440.

Kim, M. A., and Kim, M. J. (2020). Isoflavone profiles and antioxidant properties in different parts of soybean sprout. *Journal of Food Science*, *85*, 689–695, doi: 10.1111/1750-3841.15058.

Kolbert, Z., Molnár, Á., Feigl, G., and Van Hoewyk, D. (2019). Plant selenium toxicity: Proteome in the crosshairs. *Journal of Plant Physiology*, *232*, 291–300, doi: 10.1016/j.jplph.2018.11.003.

Li, C., Li, J., Du, X., Zhang, J., Zou, Y., Liu, Y., Li, Y., Lin, H., Li, H., Liu, D., and Lu, H. (2022). Chloroplast thylakoidal ascorbate peroxidase, PtotAPX, has enhanced resistance to oxidative stress in *Populus tomentosa*. *International Journal of Molecular Science*, *23*(6), 3340, doi: 10.3390/ijms23063340.

Liao, X., Rao, S., Yu, T., Zhu, Z., Yang, X., Xue, H., Gou, Y., Cheng, S., and Xu, F. (2021). Selenium yeast promoted the Se accumulation, nutrient quality and antioxidant system of cabbage (*Brassica oleracea* var. *capitata* L.). *Plant Signaling and Behavior*, *16*, 1907042, doi: 10.1080/15592324.2021.1907042.

Lima, L. W., Pilon-Smits, E. A. H., and Schiavon, M. (2018). Mechanisms of selenium hyperaccumulation in plants: A survey of molecular, biochemical and ecological cues. *Biochimica et Biophysica Acta-General Subjects*, *1862*, 2343–2353, doi: 10.1016/j.bbagen.2018.03.028.

Loladze, I., Nolan, J. M., Ziska, L. H., and Knobbe, A. R. (2019). Rising atmospheric CO_2 lowers concentrations of plant carotenoids essential to human health: A meta-analysis. *Molecular Nutrition AND Food Research, 63*, 1801047, doi: 10.1002/mnfr.201801047.

Lu, X., He, Z., Lin, Z., Zhu, Y., Yuan, L., Liu, Y., and Yin, X. (2018). Effects of Chinese cooking methods on the content and speciation of selenium in selenium bio-fortified cereals and soybeans. *Nutrients*, *10*, 317, doi: 10.3390/nu10030317.

Lukacova, Z., Bokor, B., Vavrova, S., Soltys, K., and Vaculik, M. (2021). Divergence of reactions to arsenic (As) toxicity in tobacco (*Nicotiana benthamiana*) plants: A lesson from peroxidase involvement. *Journal of Hazardous Materials*, *417*, 126049, doi: 10.1016/j.jhazmat.2021.126049.

Morales, M., and Munné-Bosch, S. (2019). Malondialdehyde: Facts and artifacts. *Plant Physiology*, *180*, 1246–1250, doi: 10.1104/pp.19.00405.

Mostofa, M. G., Hossain, M. A., Siddiqui, M. N., Fujita, M., and Tran, L. P. (2017). Phenotypical, physiological and biochemical analyses provide insight into selenium-induced phytotoxicity in rice plants. *Chemosphere*, *178*, 212–223, doi: 10.1016/j.chemosphere.2017.03.046.

Nasim, M. J., Zuraik, M. M., Abdin, A. Y., Ney, Y., and Jacob, C. (2021). Selenomethionine: A pink trojan redox horse with implications in aging and various age-related diseases. *Antioxidants*, *10*, 882, doi: 10.3390/antiox10060882.

Park, S., Kim, J., Kim, H., Kim, Y., and Yoon, H. (2021). Enhanced glutathione content improves lateral root development and grain yield in rice plants. *Plant Molecular Biology*, *105*, 365–383, doi: 10.1007/s11103-020-01093-w.

Radomska, D., Czarnomysy, R., Radomski, D., Bielawska, A., and Bielawski, K. (2021). Selenium as a bioactive micronutrient in the human diet and its cancer chemopreventive activity. *Nutrients*, *13*, 1649, doi: 10.3390/nu13051649.

Rahman, M., Rahman, K., Sathi, K. S., Alam, M. M., Nahar, K., Fujita, M., and Hasanuzzaman, M. (2021). Supplemental selenium and boron mitigate salt-induced oxidative damages in *Glycine max* L. *Plants*, *10*, 2224, doi: 10.3390/plants10102224.

Rao, S., Gou, Y., Yu, T., Cong, X., Gui, J., Zhu, Z., Zhang, W., Liao, Y., Ye, J., Cheng, S., and Xu, F. (2021a). Effects of selenate on Se, flavonoid, and glucosinolate in broccoli florets by combined transcriptome and metabolome analyses. *Food Research International*, *146*, 110463, doi: 10.1016/j.foodres.2021.110463.

Rao, S., Yu, T., Cong, X., Zhang, W., Zhu, Z., Liao, Y., Ye, J., Cheng, S., and Xu, F. (2021b). Effects of selenate applied at two growth stages on the nutrient quality of *Cardamine violifolia*. *Scientia Horticultrae*, *288*, 110352, doi: 10.1016/j.scienta.2021.110352.

Rao, S., Yu, T., Cong, X., Xu, F., Lai, X., Zhang, W., Liao, Y., and Cheng, S. (2020). Integration analysis of PacBio SMRT- and Illumina RNA-seq reveals candidate genes and pathway involved in selenium metabolism in hyperaccumulator *Cardamine violifolia*. *BMC Plant Biology*, *20*, 492, doi: 10.1186/s12870-020-02694-9.

Saed-Moucheshi, A., Sohrabi, F., Fasihfar, E., Baniasadi, F., Riasat, M., and Mozafari, A. A. (2021). Superoxide dismutase (SOD) as a selection criterion for triticale grain yield under drought stress: A comprehensive study on genomics and expression profiling, bioinformatics, heritability, and phenotypic variability. *BMC Plant Biology*, *21*, 148, doi: 10.1186/s12870-021-02919-5.

Santesmasses, D., Mariotti, M., and Gladyshev, V. N. (2020). Bioinformatics of selenoproteins. *Antioxidant & Redox Signaling*, *33*, 525–536, doi: 10.1089/ars.2020.8044.

Schiavon, M., Nardi, S., Dalla Vecchia, F., and Ertani, A. (2020). Selenium biofortification in the 21st century: Status and challenges for healthy human nutrition. *Plant and Soil*, *453*, 245–270, doi: 10.1007/s11104-020-04635-9.

Schoenmakers, E., and Chatterjee, K. (2020). Human disorders affecting the selenocysteine incorporation pathway cause systemic selenoprotein deficiency. *Antioxidant and Redox Signaling*, *33*, 481–497, doi: 10.1089/ars.2020.8097.

Siddiqui, M. H., Al-Whaibi, M. H., Sakran, A. M., Basalah, M. O., and Ali, H. M. (2012). Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. *International Journal of Molecular Science*, *13*, 6604–6619, doi: 10.3390/ijms13066604.

Sugiyama, A., Yamazaki, Y., Hamamoto, S., Takase, H., and Yazaki, K. (2017). Synthesis and secretion of isoflavones by field-grown soybean. *Plant & Cell Physiology*, *58*, 1594–1600, doi: 10.1093/pcp/pcx084.

Tian, M., Xu, X., Liu, Y., Xie, L., and Pan, S. (2016). Effect of Se treatment on glucosinolate metabolism and health-promoting compounds in the broccoli sprouts of three cultivars. *Food Chemistry*, *190*, 374–380, doi: 10.1016/j.foodchem.2015.05.098.

Ullah, A., Yin, X., Wang, F., Xu, B., Mirani, Z. A., Xu, B., Chan, M. W. H., Ali, A., Usman, M., Ali, N., Muhammad, U., Nawazish, A., and Muhammad, N. (2021). Biosynthesis of selenium nanoparticles (via *Bacillus subtilis* BSN313), and their isolation, characterization, and bioactivities. *Molecules*, *26*, 5559, doi: 10.3390/molecules26185559.

Van Gorkom, G. N. Y., Lookermans, E. L., Van Elssen, C. H. M. J., and Bos, G. M. J. (2019). The effect of vitamin C (ascorbic acid) in the treatment of patients with cancer: A systematic review. *Nutrients*, *11*, 977, doi: 10.3390/nu11050977.

Wang, H., Gong, M., Xin, H., Tang, L., Dai, D., Gao, Y., and Liu, C. (2018). Effects of chilling stress on the accumulation of soluble sugars and their key enzymes in *Jatropha curcas* seedlings. *Physiology and Molecular Biology of Plants*, *24*, 857–865, doi: 10.1007/s12298-018-0568-6.

Wang, K., Wang, Y., Li, K., Wan, Y., Wang, Q., Zhuang, Z., Guo, Y., and Li, H. (2020). Uptake, translocation and biotransformation of selenium nanoparticles in rice seedlings (*Oryza sativa* L.). *Journal of Nanobiotechnology*, *18*, 103, doi: 10.1186/s12951-020-00659-6.

Wang, K., Wang, Y., Wan, Y., Mi, Z., Wang, Q., Wang, Q., and Li, H. (2021). The fate of arsenic in rice plants (*Oryza sativa* L.): Influence of different forms of selenium. *Chemosphere*, *264*, 128417, doi: 10.1016/j.chemosphere.2020.128417.

Winkel, L. H. E., Johnson, C. A., Lenz, M., Grundl, T., Leupin, O. X., Amini, M., and Charlet, L. (2012). Environmental selenium research: From microscopic processes to global understanding. *Environmental Science & Technology*, *46*, 571–579, doi: 10.1021/es203434d.

Wu, Z., Bañuelos, G. S., Lin, Z., Liu, Y., Yuan, L., Yin, X., and Li, M. (2015). Biofortification and phytoremediation of selenium in China. *Frontiers in Plant Science*, *6*, 136, doi: 10.3389/fpls.2015.00136.

Wu, M., Cong, X., Li, M., Rao, S., Liu, Y., Guo, J., Zhu, S., Chen, S., Xu, F., Cheng, S., Liu, L., and Yu, T. (2020). Effects of different exogenous selenium on Se accumulation, nutrition quality, elements uptake, and antioxidant response in the hyperaccumulation plant *Cardamine violifolia*. *Ecotoxicology and Environmental Safety*, *204*, 111045, doi: 10.1016/j.ecoenv.2020.111045.

Zhang, L., Hu, B., Li, W., Che, R., Deng, K., Li, H., Yu, F., Ling, H., Li, Y., and Chu, C. (2014). OsPT2, a phosphate transporter, is involved in the active uptake of selenite in rice. *New Phytologist, 201*, 1183–1191, doi: 10.1111/nph.12596.

Zhang, Z., and Li, X. (2006). *Experiments in plant physiology* (5th Ed.). Beijing, China: Higher Education Press.

Zhao, X. Q., Mitani, N., Yamaji, N., Shen, R. F., and Ma, J. F. (2010). Involvement of silicon influx transporter OsNIP2,1 in selenite uptake in rice. *Plant Physiology*, *153*, 1871–1877, doi: 10.1104/pp.110.157867.

Zhu, S., Liang, Y., An, X., Kong, F., Gao, D., and Yin, H. (2017). Changes in sugar content and related enzyme activities in table grape (*Vitis vinifera* L.) in response to foliar selenium fertilizer. *Journal of the Science of Food and Agriculture*, *97*, 4094–4102, doi: 10.1002/jsfa.8276.

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SUPPLEMENTARY MATERIALS

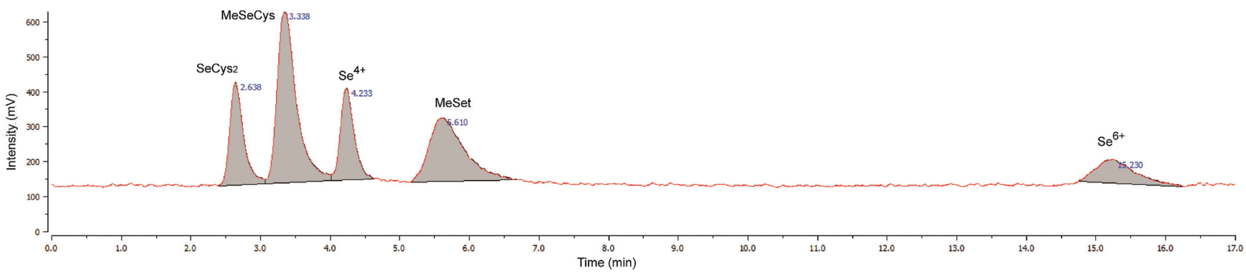

Figure S1. The spectrogram of the Se speciations by LC-AFS. LC-AFS, liquid chromatography-atomic fluorescence spectrometry; Se^{4+}, selenite; Se^{6+}, selenate; $SeCys_{2,}$ selenocystine; SeMeCys, methyl selenocysteine; SeMet, selenomethionine.