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Effects of soaking seeds with selenite on the physiological characteristics and quality of peanut sprouts

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Abstract: The aim of this study was to investigate the physiological characteristics and quality of hypocotyls in the production of selenium-enriched sprouts from peanut seeds soaked in selenium (Se) solution. Peanut seeds were soaked with 0, 2.5, 5.0, 7.5, and 10 μmol/L Na_2SeO_3 for 12 h and then germinated. The results showed that the selenium concentration in peanut shoots increased with increasing levels of selenite soaking, and there existed a crossroads of selenite soaking concentration (5.0 μmol/L) when selenium concentrations in cotyledons and hypocotyls were equal. Below and above this concentration, Se concentrations in shoots were radicle > cotyledon > hypocotyl or cotyledon > radicle > hypocotyl, respectively. In addition, Se significantly promoted the elongation of hypocotyls and radicles, increased shoot biomass, increased the activity of antioxidant enzymes and the concentration of antioxidants in hypocotyls, and decreased malondialdehyde levels. Moreover, Se significantly increased the concentrations of soluble sugars, proteins, free amino acids and resveratrol in hypocotyls. These results indicate that soaking peanut seeds with selenite significantly increased Se concentration, biomass, antioxidant capacity and quality of peanut shoots. This study provides a theoretical basis for the rapid and standardised production of Se-enriched peanut shoots from selenite-soaked seeds.

Keywords: selenium uptake; seed priming; biomass accumulation; oxidative stress reduction; nutritional quality improvement; resveratrol enhancement

Selenium (Se) is an essential trace element for the growth and development of humans and other animals (Prasad and Singh Shivay 2022, Titov et al. 2022). Se has various beneficial effects on the human body, including enhancing immunity, antioxidation, preventing diseases, anticancer, lowering blood sugar, and maintaining male fertility (Rayman 2000). The human body mainly acquires Se from foods, especially plant-based foods. The human body needs 50~60 μg Se per day to meet the nutritional requirements. Approximately one billion people worldwide suffer from Se deficiency (Nothstein et al. 2016). Under normal circumstances, the Se concentration in food is low, and it is difficult to meet human health requirements (Dinh et al. 2018). Therefore, increasing the Se concentrations in the edible parts of plants is of great significance for improving daily Se intake in humans. Peanut is one of the staple oil crops in China. It is mainly processed into peanut oil or fried peanuts. Peanuts contain various beneficial and

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healthy chemical components and are also processed into small snacks due to their excellent taste (Yang and Zheng 2016). However, the consumption pattern of peanuts is relatively simple, which affects their commercial value. Thus, developing new ways of consumption is of great significance for improving the economic value of peanuts. Peanut sprouts are a new type of vegetable that is gradually gaining consumer favour. Peanut sprouts are fresh, tender, and refreshing, and contain resveratrol, vitamins, amino acids, and minerals the human body requires. It has many functions, such as anticancer, anti-aging, and prevention of various diseases (Limmongkon et al. 2017). Compared with peanut seeds, the concentrations of soluble sugars and free amino acids increased because fat, carbohydrates, and proteins in the cotyledons of sprouts were degraded (Li et al. 2014). In addition, new nutrients were also synthesised during the growth process of peanut sprouts (Adhikari et al. 2018). Therefore, peanut sprouts are becoming a favourite vegetable for people with huge market potential. Producing Se-enriched peanut sprouts by increasing Se accumulation is an effective strategy to supplement Se for the human body (Kieliszek and Serrano Sandoval 2023). However, the Se concentration of peanut seeds in the market is not uniform, and Se translocation from cotyledons to hypocotyls and radicles during the growth process of sprouts is different, resulting in a large difference in the Se concentration of peanut sprouts, which is very difficult to standardise the production of Se-enriched peanut sprouts. In addition, producing Se-enriched peanut sprouts using Se-enriched peanuts takes a long production period and is low in efficiency. The Se concentration of peanut seeds in the market is low, and it is challenging to produce peanut sprouts with high Se concentration. The above problems seriously restrict the large-scale production of Se-enriched peanut sprouts. Therefore, developing a new way to produce Se-enriched peanut sprouts is urgent. Se-enriched peanut sprouts can be produced by soaking seeds in Se solutions, where Se enters the seeds through the seed coat, and then Se is transported from the cotyledons to the hypocotyl during the growth of sprouts. However, producing sprouts often face serious problems such as weak growth, inconsistent growth, low yield, and poor quality in the production process. Therefore, it is crucial to increase the stress resistance of peanut sprouts and improve their yield and quality. Se is a beneficial element in plants that enhances the antioxidant capacity of both enzymatic and nonenzymatic systems (Hasanuzzaman et al. 2022). Appropriate concentrations of Se could promote the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbic acid peroxidase (APX), and glutathione reductase (GR) in plants such as quinoa, tomato, and wheat, and reduce the concentrations of malondialdehyde (MDA) and glutathione reductase (Yao et al. 2012, Khalofah et al. 2021, Ishtiaq et al. 2023). In addition, Se could significantly increase the Se concentration in plants, promote the accumulation of carbohydrates, and improve the quality of plants (Turakainen et al. 2006). Se could increase tea yield, total amino acid, and vitamin C concentration, enhance the sweetness and aroma of green tea, and reduce bitterness (Hu et al. 2003). Se could promote various plants' photosynthetic rate, growth, and biomass accumulation (Yin et al. 2019). Se could significantly increase wheat yield and promote dry matter accumulation in shoots and roots (Boldrin et al. 2016).

During the germination process, seeds, starch, protein, and fat are hydrolysed into small molecules such as sugars and amino acids. From the late stage of germination to the growth stage, seeds differentiate into various tissues and begin vegetative growth (Rosental et al. 2014). Therefore, it is postulated that Se can improve the stress resistance, quality, and yield of peanut sprouts. However, how Se affects peanut sprouts' physiological characteristics and quality needs to be clarified. This study investigated the effects of selenite-soaked levels on the physiological characteristics and quality of peanut sprouts. It will provide a theoretical basis for rapid and standardised production of Se-enriched peanut sprouts.

MATERIAL AND METHODS

Plant materials and treatment. Peanut seeds (cv. Yuhua 22), full and uniform in size, were selected and washed three times with ozone water and soaked with 0, 2.5, 5, 7.5, and 10.0 μmol/L Na_2SeO_3. After 12 h, the seeds were rinsed with deionised water filled with ozone. When the radicles of the peanut sprouts grew to 1.0–2.0 cm, peanut sprouts with consistent growth were transferred to Hoagland's solution and cultured in a 25 °C incubator in the dark and rinsed with ozone water three times daily. On the 7th day, samples were collected to determine the Se concentration in cotyledons, radicles, and hypocotyls, biomass, the activities of SOD, POD, and

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CAT in the hypocotyl, as well as the concentrations of reduced glutathione (GSH), vitamin C, proline, MDA, soluble sugars, soluble proteins, free amino acids, and resveratrol of peanut sprouts.

Measurement of the lengths and thicknesses of peanut sprouts. The lengths of radicles and hypocotyls and thicknesses of peanut sprouts were determined using a ruler and vernier calliper, and the fresh weight was measured using a one-thousandth scale.

Determination of Se concentration. About 0.5 g of dried samples were weighed and placed into 100 mL digestion tubes, and a 5 mL acid mixture (HNO_3:$HClO_4$; 4:1, *v*/*v*) was added. The samples were predigested overnight and thoroughly digested at 150–165 °C in a digestion oven. After cooling, a 2.5 mL 6 mol/L HCl was added to reduce SeO_4^{2-} to SeO_3^{2-} at 100 °C. The digests were diluted with millipore water to a final volume of 25 mL. Se concentrations were determined by atomic fluorescence spectrometry (Beijing Purkinje General Instrument Co., Ltd., PF32, Beijing, China). Standard tea material (GSV-4, 0.072 mg Se/kg, GBW07605) and a blank were simultaneously digested with the test samples for quality control.

Determination of GSH concentration. About 0.5 g of the sample was added to 5 mL of 5% TCA (trichloroacetic acid) and ground into a homogenate. The mixture was centrifuged at 10 000 *r*/min for 20 min. 250 µL of the extraction solution was collected, 2.6 mL of 150 mmol Na_2HPO_4 (pH 7.7), and 150 µL of 16.5 mmol DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) were added and mixed thoroughly after being kept at 30 °C for 5 min. GSH concentration was determined at a wavelength of 412 nm with a spectrophotometer.

Determination of vitamin C concentration. About 10.0 g of fresh sample was added into 5 mL 2% hydrochloric acid, ground into a homogenate, made up to 50 mL, and filtered. 5 mL of the filtrate was collected and added into 0.5 mL of 1% KI, 2.0 mL of 0.5% starch solution, and distilled water up to 10 mL. The mixture solution was titrated with 0.001 mol/L KIO_3 solution until a slight blue colour did not fade. The dosage of KIO_3 was recorded, and vitamin C concentration was calculated.

Determination of malondialdehyde concentration. About 0.5 g of the sample was added into 5 mL of 5% trichloroacetic acid and ground into a homogenate. The mixture was centrifuged at 10 000 *r*/min for 20 min, and 300 µL of the supernatant and 300 µL of 0.6% TBA solution were added into a 2 mL centrifuge tube, mixed thoroughly, reacted on a boiling water bath for 15 min, cooled rapidly, and then centrifuged. MDA concentration was determined at 532, 600, and 450 nm wavelengths, respectively.

Determination of proline concentration. About 0.5 g of the fresh cut sample was added into 5 mL of 3% sulfosalicylic acid solution and was boiled in a water bath for 10 min. 2 mL of supernatant, 2 mL of glacial acetic acid, and 3 mL of colourimetric solution were mixed thoroughly and boiled in a water bath for 40 min. After cooling, 5 mL of toluene was added to each tube for sufficient oscillation to extract the red substance. Proline concentration was determined at a wavelength of 520 nm.

Determination of superoxide dismutase activity. About 0.2 g of the sample was ground into pulp with 1.8 mL phosphate buffer pre-cooled at 4 °C and centrifuged at 10 000 *r*/min for 20 min at 4 °C. 50 µL of the above enzyme solution was collected and sequentially added 1.5 mL of 0.05 mol/L phosphate buffer, 130 mmol/L methionine solution, 750 µmol/L nitroblue tetrazole (NBT) solution, 100 µmol/L EDTA-Na_2 solution, and 20 µmol/L riboflavin solution, with 0.3 mL each. After mixing, reacted under 4000 Lx-ray for 20 min. Superoxide dismutase activity was determined at a wavelength of 560 nm.

Determination of peroxidase activity. In a 5 mL reaction system, 2.9 mL of PBS with pH 5.5, 1.0 mL of 2% H_2O_2, and 1 mL of 0.05 mol/L guaiacol were added. After adding enzyme solution (extracted as above) to the reaction system, it was immediately kept in a 37 °C water bath for 15 min, then 2 mL of 20% trichloroacetic acid was added to terminate the reaction. The mixture solution was centrifuged at 5 000 *r*/min for 10 min. Peroxidase activity was determined at a wavelength of 560 nm with a spectrophotometer.

Determination of catalase activity. 0.05 mL of the extracted enzyme solution (extracted as above) and 1.95 mL of phosphate buffer were collected and placed in a centrifuge tube. Preheating was performed in a 25 °C water bath for 3 min, followed by adding 1 mL of 0.3% H_2O_2 and rapid mixing. CAT activity was determined at a wavelength of 240 nm every 30 s for 3 min.

Determination of soluble sugar concentration. About 0.5 g of sample was added to 15 mL of distilled water in a test tube and boiled in a water bath for 20 min. After filtration, 1 mL of extraction solution was mixed with 5 mL of anthrone reagent and boiled in a boiling water bath for 10 min. The concentration

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of soluble sugar was determined at a wavelength of 620 nm.

Determination of soluble protein concentration. About 0.5 g of fresh sample was added to the phosphate buffer to grind into a slurry, and 5 mL of Coomassie Brilliant Blue G-250 reaction solution and an appropriate amount of phosphate buffer with pH 7.8 was added to the supernatant. The concentration of soluble protein was determined at a wavelength of 595 nm.

Determination of amino acid concentration. About 0.5 g of fresh samples were added to 5 mL of 10% acetic acid, distilled water to 100 mL, and mixed thoroughly and filtered. 1 mL of the filtrate, 1 mL of distilled water, 3 mL of hydrazine trione, and 0.1 mL of ascorbic acid were added. The mixture solution was stirred evenly and heated in a boiling water bath for 15 min, and made up to 20 mL with 60% ethanol. The concentration of amino acid was determined at a wavelength of 570 nm with a spectrophotometer.

Determination of resveratrol concentration. About 1.0 g of fresh sample was weighed, and after grinding with liquid nitrogen, 10 mL 90% ethanol was added and treated with ultrasonic wave for 30 min. After filtration, 10 mL 90% ethanol was added to the residue and treated with ultrasonic wave repeatedly. The two filtrates were combined and mixed thoroughly. Resveratrol concentration was determined at a wavelength of 306 nm with spectrophotometry.

Statistical analysis. Data analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA) to determine the significant differences ($P < 0.05$) between control and treatments. Statistical differences were assessed using the Student's *t*-test. Graphs were generated using Origin 2021 (OriginLab Corporation, Northampton, USA).

RESULTS

Effects of selenite-soaked levels on Se concentration of peanut sprouts. The Se concentrations in peanut sprouts were strongly affected by the selenite-soaked levels (Figure 1). The Se concentration in cotyledons, hypocotyls, and radicles gradually increased with the increase in selenite-soaked levels. When selenite-soaked levels were low, the Se concentrations in the radicles were higher than those in the hypocotyls and cotyledons. However, the Se concentrations in cotyledons increased faster with the increases of selenite-soaked levels compared with the Se concentration in the radicles, resulting in the Se concentration in cotyledons and radicles being equal at a certain selenite-soaked level. When the selenite-soaked level was below this concentration, the Se concentration in sprouts from high to low was radicle > cotyledon > hypocotyl; when the selenite-soaked level was above this concentration, the Se concentration in sprouts was cotyledon > radicle > hypocotyl. In addition, the distribution of Se in peanut sprouts was also affected by the selenite-soaked levels (Figure 2). As the selenite-soaked levels increased, the distribution ratio of Se in cotyledons gradually increased. In contrast, the distribution ratio in hypocotyls and radicles gradually decreased.

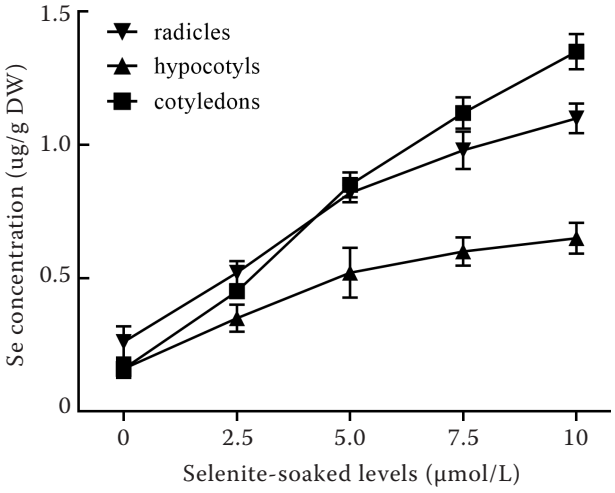

Figure 1. Effects of selenite-soaked levels on the selenium (Se) concentration in radicles, cotyledons, and hypocotyls of peanut sprouts. DW – dry weight

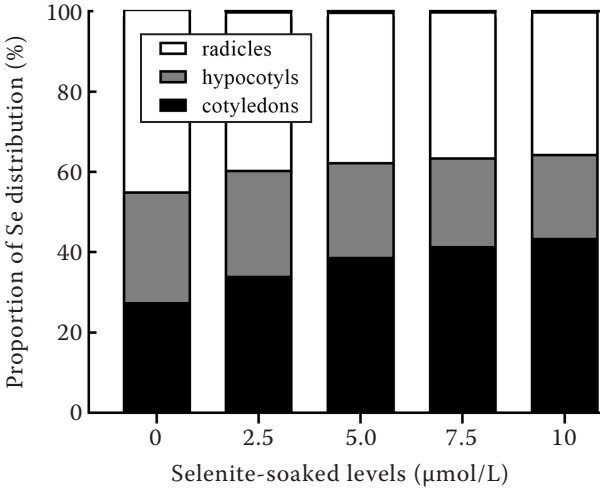

Figure 2. Effects of selenite-soaked levels on the distribution ratio of selenium (Se) in radicles, cotyledons, and hypocotyls of peanut sprouts

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Table 1. Effects of selenite-soaked levels on the length of radicle and hypocotyl, thickness, and biomass of peanut sprouts

Selenite-soaked level	Radicle length	Hypocotyl length	Hypocotyl thickness	Fresh weight
	(cm)			(g)
CK	10.43 ± 0.15^{c}	7.48 ± 0.33^{d}	0.86 ± 0.04^{a}	4.69 ± 0.20^{b}
2.5	10.75 ± 0.22^{c}	8.48 ± 0.15^{c}	0.88 ± 0.01^{a}	5.46 ± 0.24^{a}
5.0	11.70 ± 0.11^{b}	8.82 ± 0.17^{bc}	0.88 ± 0.02^{a}	5.45 ± 0.24^{a}
7.5	12.27 ± 0.09^{a}	9.21 ± 0.14^{a}	0.89 ± 0.01^{a}	5.60 ± 0.19^{a}
10.0	10.60 ± 0.17^{c}	7.77 ± 0.30^{d}	0.87 ± 0.02^{a}	4.74 ± 0.17^{b}

Different lowercase letters in the same column indicated significant differences in the length of radicle and hypocotyl, thickness, and biomass of peanut sprouts ($P < 0.05$)

Effects of selenite-soaked levels on the biomass of peanut sprouts. The effects of selenite-soaked levels on the length of peanut radicles and hypocotyls and the biomass of sprouts were indicated in Table 1. Compared with the control, 5.0 and 7.5 μmol/L Na_2SeO_3 significantly promoted the elongation of peanut radicles by 12.17% and 17.60%, respectively ($P < 0.05$), while no significant difference existed between 2.5 and 10.0 μmol/L Na_2SeO_3 treatments. When peanut seeds were soaked with 2.5, 5.0, and 7.5 μmol/L Na_2SeO_3, the lengths of the hypocotyl of peanut sprouts significantly increased by 13.36, 17.86, and 23.07% compared with the control, respectively ($P < 0.05$). At the same time, there was no significant difference between 2.5 and 5.0 μmol/L Na_2SeO_3 treatments. When peanut seeds were soaked with 2.5, 5.0, and 7.5 μmol/L Na_2SeO_3, the fresh weight of peanut sprouts significantly increased by 16.57, 16.36, and 19.56%, respectively ($P < 0.05$). In contrast, different selenite-soaked levels had no significant effects on sprout thickness.

Effects of selenite-soaked levels on GSH concentration. GSH is an important antioxidant in the non-enzymatic defense system, which can eliminate excess reactive oxygen species (ROS) produced in cell metabolism and reduce the damage caused by membrane lipid peroxidation to cells. The results indicated that GSH concentration significantly increased with the increase of selenite-soaked levels, exhibiting a characteristic of first increasing and then decreasing (Figure 3). Compared with the control, 2.5, 5.0, 7.5, and 10.0 μmol/L Na_2SeO_3 significantly increased the GSH concentration of the hypocotyl by 29.04, 47.1, 55.19, and 31.0%, respectively ($P < 0.05$). There was no significant difference in GSH concentration in the hypocotyls soaked with 2.5 and 10.0 μmol/L Na_2SeO_3 or 5.0 and 7.5 μmol/L Na_2SeO_3. However, they were significantly lower than those in the hy-

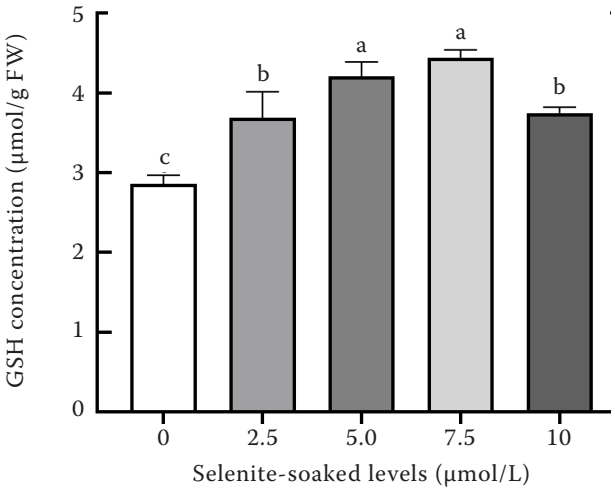

Figure 3. Effect of selenite-soaked levels on glutathione (GSH) concentration in hypocotyls. Different lowercase letters indicated significant differences in GSH concentration in hypocotyls ($P < 0.05$). FW – fresh weight

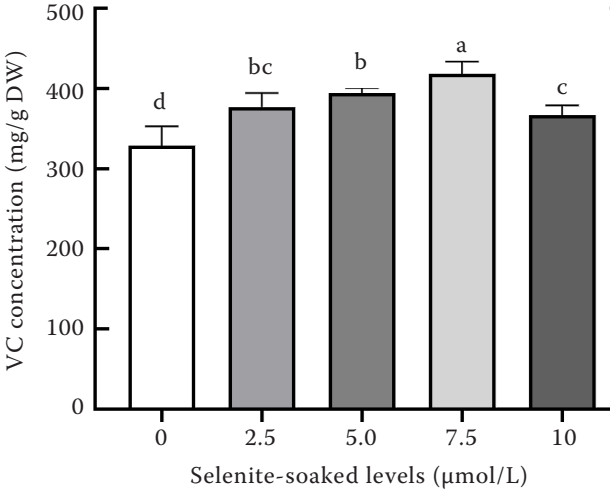

Figure 4. Effects of selenite-soaked levels on vitamin C (VC) concentrations in hypocotyls. Different lowercase letters indicated significant differences in vitamin C concentration in hypocotyls ($P < 0.05$). DW – dry weight

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pocotyls soaked with 5.0 and 7.5 μmol/L Na_2SeO_3. The differences in GSH concentration between 2.5 and 10.0 μmol/L Na_2SeO_3 or between 5.0 and 7.5 μmol/L Na_2SeO_3 were not significant.

Effects of selenite-soaked levels on vitamin C concentration. The result indicated that the vitamin C concentration increased first and then decreased as the Se concentration increased (Figure 4). Compared with the control, the vitamin C concentration in peanut sprouts soaked with 7.5 μmol/L Na_2SeO_3 increased significantly, by 27.38% ($P < 0.05$). 2.5, 5.0, and 10.0 μmol/L Na_2SeO_3 also increased vitamin C concentration significantly compared with the control, with improvements of 14.79, 20.10, and 11.70%, respectively ($P < 0.05$). Differences in vitamin C concentrations were significant between 5.0 and 7.5 μmol/L Na_2SeO_3 treatments or between 5.0 and 10.0 μmol/L Na_2SeO_3 treatments. However, the difference in vitamin C concentration between 2.5 μmol/L Na_2SeO_3 treatment and 10 μmol/L Na_2SeO_3 treatment was not significant.

Effects of selenite-soaked levels on MDA concentrations. MDA concentration is an essential indicator of membrane lipid peroxidation and the final product of membrane lipid peroxidation. The change in MDA concentration is related to the degree of cell peroxidation. The more it is, the greater the degree of damage to the cells. The result indicated the MDA concentration significantly decreased with the increase of selenite-soaked levels (Figure 5). Compared with the control, 2.5, 5.0, 7.5, and 10.0 μmol/L Na_2SeO_3 significantly reduced the MDA concentration of the hypocotyl, with reductions of 9.20, 13.25, 24.34, and 12.85%, respectively ($P < 0.05$). There was no significant difference in MDA concentration in the hypocotyls soaked with 2.5, 5.0, and 7.5 μmol/L Na_2SeO_3, but they were all significantly lower than those soaked with 7.5 μmol/L Na_2SeO_3.

Effects of selenite-soaked levels on proline concentration. Proline is one of the components of plant proteins, which is abundant in the plant in a free state. The accumulation of proline plays a role in regulating osmotic potential in the cytoplasm, stabilising the structure of biological macromolecules, and regulating cellular redox status. The results indicated that proline concentration exhibited a characteristic of first increasing and then decreasing as the selenite-soaked levels increased (Figure 6). Compared with the control, 2.5, 5.0, and 7.5 μmol/L Na_2SeO_3 exhibited significant differences, with proline concentration increased by 25.21, 42.50, and 55.34%, respectively ($P < 0.05$). There was no significant difference in proline concentration between 10.0 μmol/L and the control or 2.5 μmol/L Na_2SeO_3.

Effects of selenite-soaked levels on POD activity. POD is a highly active plant enzyme closely related to respiration, photosynthesis, and auxin oxidation. It can reflect changes in plant metabolism during a certain period and is also one of plants' main defence enzyme systems. As selenite-soaked levels increased,

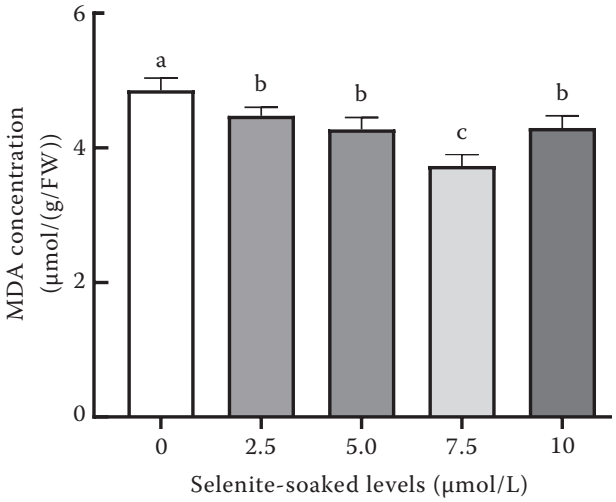

Figure 5. Effects of selenite-soaked levels on malondialdehyde (MDA) concentration in hypocotyls. Different lowercase letters indicated significant differences in MDA concentration in hypocotyls ($P < 0.05$). FW – fresh weight

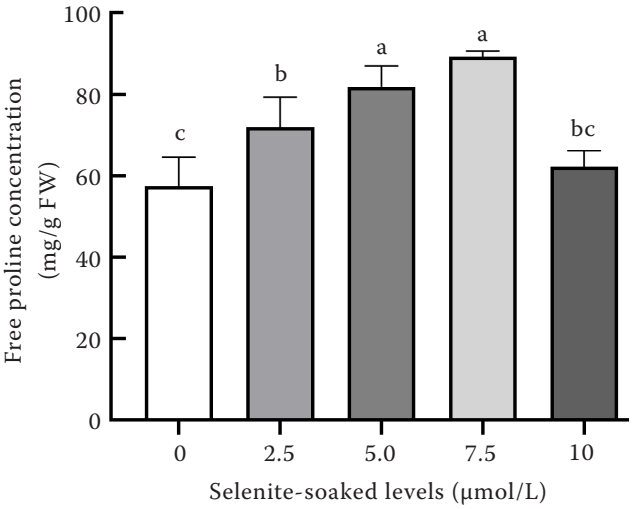

Figure 6. Effects of selenite-soaked levels on proline concentration in hypocotyls. Different lowercase letters indicated significant differences in proline concentration in hypocotyls ($P < 0.05$). FW – fresh weight

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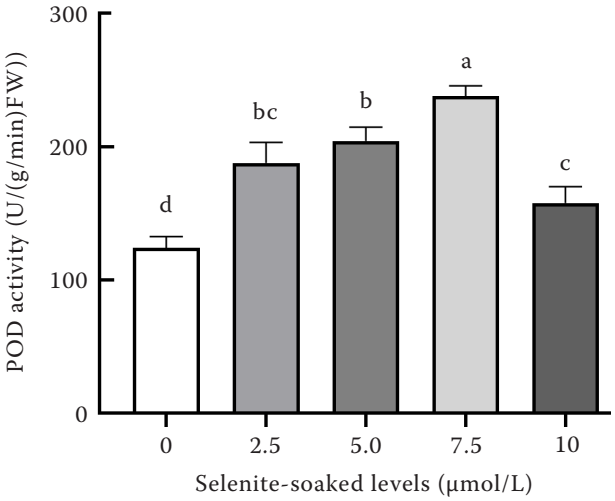

Figure 7. Effects of selenite-soaked levels on peroxidase (POD) activity in hypocotyls. Different lowercase letters indicated significant differences in POD activity in hypocotyls ($P < 0.05$). FW – fresh weight

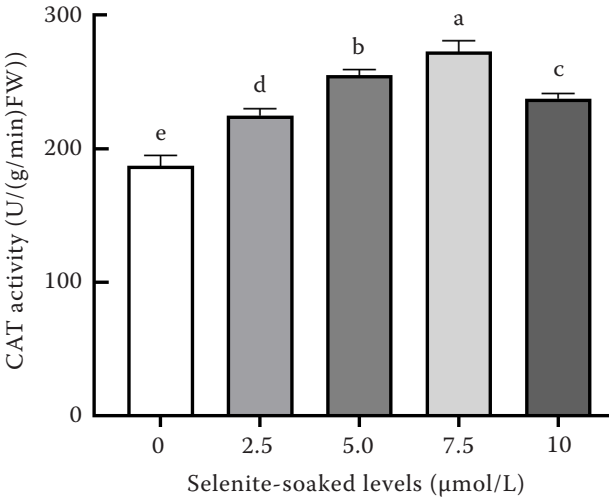

Figure 8. Effects of selenite-soaked levels on catalase (CAT) activity in hypocotyls. Different lowercase letters indicated significant differences in CAT activity in hypocotyls ($P < 0.05$). FW – fresh weigh

the POD activity exhibited a characteristic of first increasing and then decreasing (Figure 7). When the selenite-soaked level was 7.5 μmol/L, the POD activity increased the most significantly by 92.40% ($P < 0.05$) compared to the control and 42.11% ($P < 0.05$) compared to 5.0 μmol/L Na_2SeO_3 and was significantly higher than other treatments. The difference between 2.5 and 5.0 μmol/L treatments or between 2.5 and 10.0 μmol/L treatments was not significant.

Effects of selenite-soaked levels on CAT activity. CAT is widely present in all tissues of plants, and it is related to the intensity of plant metabolism and antioxidant capacity. It can remove ROS in the plant and prevent biomembrane damage. The results indicated that the CAT activity increased first and then decreased as the selenite-soaked level increased (Figure 8). Compared with the control, 2.5, 5.0, 7.5, and 10.0 μmol/L Na_2SeO_3 significantly increased the CAT activity by 20.20, 36.24, 45.85, and 26.88%, respectively ($P < 0.05$). Among them, 7.5 μmol/L Na_2SeO_3 treatment increased the CAT activity to the largest extent.

Effects of selenite-soaked levels on SOD activity. SOD is one of the antioxidant protective enzymes in plants that scavenge free radicals. It works in synergy with POD and CAT to defend against damage to cell membranes caused by ROS or other peroxide free radicals. The SOD activity exhibited a characteristic of first increasing and then decreasing as the Se concentration increased (Figure 9). Compared with the control, 2.5, 5.0, 7.5, and 10.0 μmol/L Na_2SeO_3 significantly increased SOD activity by 23.03, 43.44, 57.42, and 18.22%, respectively ($P < 0.05$). Among them, 7.5 μmol/L Na_2SeO_3 was significantly higher than other treatments, and there was no significant difference between 2.5 and 10.0 μmol/L Na_2SeO_3 treatments.

Effects of selenite-soaked levels on the quality of peanut sprouts. The results indicated that the soluble sugar concentration exhibited a characteristic of first increasing and then decreasing as selenite-soaked levels increased (Table 2). Compared with the control, 2.5, 5.0, 7.5, and 10.0 μmol/L Na_2SeO_3 signifi-

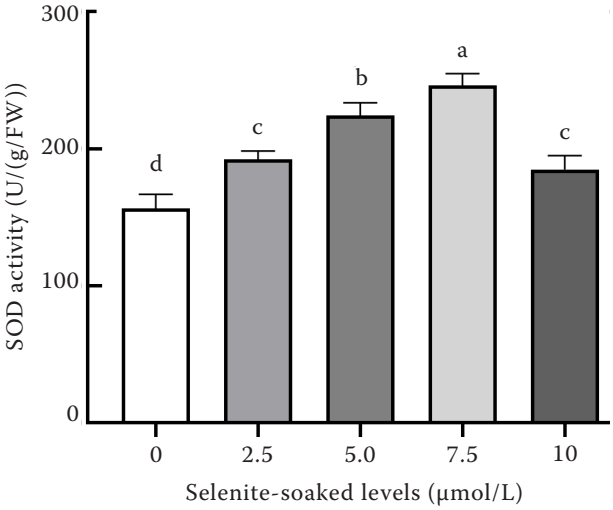

Figure 9. Effects of selenite-soaked levels on superoxide dismutase (SOD) activity in hypocotyls. Different lowercase letters indicated significant differences in SOD activity in hypocotyls ($P < 0.05$). FW – fresh weight

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Table 2. Effects of selenite-soaked levels on the concentrations of soluble sugars, soluble proteins, free amino acids, and resveratrol in hypocotyls

Selenite-soaked level	Soluble sugar (mg/g FW)	Soluble protein (mg/g FW)	Free amino acid (μg/g FW)	Resveratrol (μg/mL FW)
CK	4.15 ± 0.05^{c}	22.41 ± 0.47^{b}	329.72 ± 16.13^{c}	2.45 ± 0.10^{c}
2.5	4.81 ± 0.10^{c}	23.48 ± 0.48^{b}	352.26 ± 11.47^{bc}	2.50 ± 0.13^{c}
5.0	5.04 ± 0.06^{b}	24.59 ± 0.66^{a}	369.01 ± 13.82^{ab}	2.52 ± 0.08^{c}
7.5	5.22 ± 0.15^{a}	25.50 ± 0.82^{a}	389.94 ± 10.13^{a}	2.75 ± 0.12^{b}
10.0	4.74 ± 0.06^{c}	23.43 ± 0.47^{b}	344.99 ± 14.40^{bc}	3.04 ± 0.10^{a}

Different lowercase letters in the same column indicated significant differences in selenite-soaked levels in the concentrations of soluble sugars, soluble proteins, free amino acids, and resveratrol in hypocotyls ($P < 0.05$). FW – fresh weight

cantly increased the soluble sugar concentration by 15.73, 21.36, 25.63, and 14.19% ($P < 0.05$), with 5.0 and 7.5 μmol/L Na_2SeO_3 treatments being more significant compared to the control, while 2.5 and 10.0 μmol/L Na_2SeO_3 treatments were not significant. The results indicated that the soluble protein concentration significantly increased with the increase of selenite-soaked levels, exhibiting a characteristic of first increasing and then decreasing (Table 2). Compared to the control, 5.0 and 7.5 μmol/L Na_2SeO_3 significantly increased it by 9.73% and 13.76%, respectively ($P < 0.05$); 2.5 and 10.0 μmol/L Na_2SeO_3 increased it by 4.77% and 4.53% ($P < 0.05$), with no significant difference compared to the control. The difference between 5.0 and 7.5 μmol/L Na_2SeO_3 treatments was not significant, and between 2.5 and 10.0 μmol/L Na_2SeO_3 treatments and the control was not significant. The results indicated that free amino acid concentration significantly increased with the increase of selenite-soaked levels, exhibiting a characteristic of first increasing and then decreasing (Table 2). Compared with the control, 5.0 and 7.5 μmol/L Na_2SeO_3 significantly increased by 11.92% and 18.26%, respectively ($P < 0.05$). 2.5 and 10.0 μmol/L Na_2SeO_3 increased it by 6.84% and 4.63%, respectively ($P < 0.05$), which had no significant difference compared with the control. The result indicated that the resveratrol concentration significantly increased with selenite-soaked levels (Table 2). Compared with the control, 7.5 and 10.0 μmol/L Na_2SeO_3 significantly increased in the hypocotyls by 12.45% and 24.0%, respectively ($P < 0.05$), with 10.0 μmol/L Na_2SeO_3 treatment significantly higher than 7.5 μmol/L Na_2SeO_3 treatment ($P < 0.05$).

DISCUSSION

Se concentration in peanut sprouts was strongly affected by selenite-soaked levels. This study found that the Se concentrations in the cotyledons, hypocotyls, and radicles of sprouts increased differently with the selenite-soaked levels. Compared with the radicles, the Se concentration in the cotyledons increased rapidly with the selenite-soaked levels. When the selenite-soaked levels reached a concentration point, the Se concentration in the cotyledons and radicles was equal. When the selenite-soaked level was below this concentration, the Se concentrations in sprouts were radicles > cotyledons > hypocotyls from high to low. When the selenite-soaked level was above this concentration, the Se concentration in sprouts was cotyledons > radicles > hypocotyls. The above results indicated that the Se concentration in peanut sprouts was strongly affected by selenite-soaked levels.

Previous studies indicated that selenite was readily converted into organic Se after entering plants (Zhang et al. 2019). Literature reported that Se in the cotyledons, hypocotyls, and radicles of peanuts mainly existed in the forms of selenocystine, selenite, and selenomethionine after soaking peanut seeds with selenite, indicating that most selenite was converted into selenocystine and selenomethionine in the cotyledons during peanut seed germination (Han et al. 2024). Therefore, Se in cotyledons is mainly transported to the hypocotyl and radicle in the forms of selenocystine, selenite, and selenomethionine. When peanut seeds were soaked in lower concentrations of selenite solutions, the amount of Se entering the cotyledons through the seed coat was less. The Se concentration in the cotyledons was lower, resulting in the Se concentration potential and motive potential between the cotyledon and hypocotyl or radicle being small. The amount of Se transported from the cotyledons to the hypocotyls or radicles was less. After Se was transported from

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cotyledons to hypocotyls and radicles for 7 days, the Se concentration in cotyledons was lower than in radicles but higher than in hypocotyls. Thus, the Se concentration in peanut sprouts from high to low was in the order of radicles > cotyledons > hypocotyls. The relationship between cotyledon and radicle was that between source and sink. Under normal circumstances, the Se concentration in the source is higher than in the sink, but the Se concentration in the radicle was higher than in the cotyledons. It was postulated that Se transported from the cotyledons to the radicle was rapidly fixed in the form of selenomethionine in the replace of methionine in protein synthesis rather than in the form of free Se that could be transported (Erika et al. 2014). Otherwise, Se could be transported back from the radicles to the cotyledons in the form of selenocystine, selenite, and selenomethionine. When peanut seeds were soaked in higher concentrations of selenite, a higher amount of Se entered the cotyledons through the seed coat, resulting in a higher accumulation of Se in the seeds. Therefore, there was a significant difference in Se concentration between the cotyledon and hypocotyl, as well as the hypocotyl and radicle, resulting in a higher motive potential. As a result, more Se was transported from the cotyledons to the hypocotyls and radicles. However, the transport of Se from cotyledons to hypocotyls and radicles was limited within 7 days, and most Se was still retained in cotyledons. Therefore, the concentration of cotyledons was always higher than that of radicles, indicating a characteristic of cotyledons > radicles > hypocotyls.

This study also found that when peanut seeds were soaked in different levels of selenite solutions, the Se concentration in the hypocotyl of sprouts always remained the lowest. As the Se concentration increased, the distribution ratio of Se in cotyledons increased, while the distribution ratio in hypocotyls and radicles decreased. The higher the selenite-soaked level, the less Se was accumulated in the hypocotyl. The hypocotyl is the main edible part of sprouts, and increasing Se accumulation in hypocotyl is helpful in enhancing Se intake in the human body. However, the hypocotyl is a pathway for the transport of Se from cotyledon to the radicle. The tissue structure of the hypocotyl differs from the cotyledon or radicle and is unsuitable for storing Se. Otherwise, Se or other nutrients in the cotyledon will be retained in the hypocotyl and be difficult to transport to the radicles. Therefore, improving Se accumulation in hypocotyl by optimising culture technology and breeding new cultivars is still a problem that needs to be solved for producing Se-enriched peanut sprouts.

The biomass of peanut sprouts was enhanced by soaking seeds with selenite. Biomass is an important indicator for determining plant growth and development. Compared with the control, the fresh weight of peanut sprouts increased with the increase of selenite-soaked levels from 2.5 to 7.5 μmol/L. When the soaking level was 7.5 μmol/L, the biomass reached its maximum. Previous studies indicated that low concentrations of Se could promote growth, while high concentrations of Se inhibit growth (Khaliq et al. 2015). This study found that 10 μmol/L Se did not inhibit the growth of peanut sprouts, indicating that selenite-soaked concentration did not reach the level of toxicity. It was reported that appropriate concentrations of Se could increase the concentrations of soluble sugars, soluble proteins, and free amino acids in plants (Sun et al. 2020). Soluble sugars and free amino acids provide more carbohydrates and nitrogen compounds for the growth of peanut sprouts, which promotes the elongation of radicles and hypocotyls and improves the biomass of peanut sprouts. The increase in peanut sprout biomass by Se may also be related to promoting radicle growth and taking up more nutrients from nutrient solutions (Kathpalia and Bhatla 2018). However, there were no carbon compounds in the nutrient solution. In addition, peanut sprouts grew in a dark environment and did not perform photosynthesis. Therefore, the soluble sugars in the hypocotyl mainly came from the hydrolysis and transport of carbohydrates in the cotyledons of peanuts. The nitrogen in amino acids and soluble proteins came from both amino acids formed by protein degradation in seeds and ammonium and nitrate in nutrient solutions. Thus, Se could promote the degradation of proteins and carbohydrates in cotyledons, thereby promoting the increase of peanut sprout biomass.

The antioxidant capacity of peanut sprouts was enhanced by soaking seeds with selenite. The antioxidant capacity of plants is mainly dependent on the enzymatic and non-enzymatic systems, which are commonly present in all tissues of plants, playing important roles in eliminating free radicals and preventing oxidation in the cells. Sprouts grew vigorously and metabolised violently, producing a large amount of ROS. High levels of ROS can cause damage to the biomembrane. In this study, Se could significantly increase the activities of CAT, POD,

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and SOD in the hypocotyl, as well as increase the concentrations of vitamin C and GSH. It indicated that Se could enhance the antioxidant capacity of sprouts by increasing antioxidant enzyme activity, vitamin C, and GSH concentration. The MDA concentration can reflect the degree of damage to the biomembrane. Further study suggested that selenite significantly reduced the MDA concentration of hypocotyls, indicating that Se removed excessive ROS and reduced damage to the biomembrane in hypocotyl cells by enhancing the antioxidant capacities of both enzymatic and non-enzymatic systems (Atencio et al. 2009). In addition, Se is an important component of glutathione peroxidase in humans and animals. It forms the enzyme's active site and plays an important role in enhancing its activity. Therefore, Se is crucial in regulating ROS and redox states in cells (Hoffmann and Berry 2008). However, Se is not a component of glutathione peroxidase in plants. It can participate in the enzyme composition by replacing sulfur, thereby removing ROS. This study also found that Se could increase proline concentration, indicating that Se could maintain osmotic potential balance by regulating proline level, which was of great significance for improving plant stress resistance and cultivating robust sprouts.

Effects of selenite-soaked levels on the quality of peanut sprouts. In this study, it was found that Se significantly increased the concentrations of soluble sugars, soluble proteins, free amino acids, vitamin C, and resveratrol in hypocotyls; the maximum improvement was achieved when selenite-soaked concentration was at 7.5 μmol/L. Se could increase the concentrations of free amino acids and soluble proteins in the hypocotyl, which was related to promoting protein degradation in peanut cotyledons, thereby promoting more amino acids transport to the hypocotyl. It might also be due to promoting the uptake of more nitrogen from the nutrient solution by the radicles, thereby increasing the concentration of amino acids and soluble proteins in the hypocotyl. Literature reported that Se could promote carbohydrate accumulation and increase yield (Turakainen et al. 2004). In this study, the nutrient solution did not contain carbon compounds. Hence, the carbohydrates in the hypocotyl of peanut sprouts came from the degradation of carbon compounds such as fat and starch in cotyledons. It suggested that Se stimulated the degradation of fat and starch in cotyledons. Resveratrol is a natural polyphenolic compound with health benefits such as anti-aging, anti-inflammatory, and anti-cancer, which has attracted much attention (Ghanim et al. 2011). It was found that the concentration of resveratrol in peanut sprouts increased with the increase of selenite-soaked concentration, indicating that Se could promote the synthesis of resveratrol. Thus, Se could significantly increase the nutrient concentration in the hypocotyl, thereby improving the quality of peanut sprouts.

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