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Selenium improves wheat antioxidant capacity, photosynthetic capacity, and growth under cadmium stress

L.M. WU and N.H. LU+

Henan Institute of Science and Technology, 453003 Xinxiang, China

Abstract

Cadmium stress (CS) induced the peroxide damage and inhibited wheat photosynthetic capacity and growth. Compared to CS, selenium (Se) application plus CS bolstered chlorophyll and carotenoid contents, photosynthetic rate, the maximum photochemical efficiency of PSII, the quantum yield of PSII photochemistry, and photochemical quenching, superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, L-galactono-1,4-lactone dehydrogenase, and gamma-glutamylcysteine synthetase activities, ascorbic acid and glutathione contents, AsA/dehydroascorbic acid and GSH/oxidized glutathione, and decreased nonphotochemical quenching (q_N), antioxidant biomarkers malondialdehyde and hydrogen peroxide contents, and electrolyte leakage (EL). At the same time, Se alone declined antioxidant biomarkers contents, q_N and EL, and augmented the rest of the aforementioned indexes. Our research implied that Se upregulated wheat's antioxidant capacity. In this way, Se improved wheat photosynthetic performance and growth, especially for 10 μM sodium selenite (Na_2SeO_3). Consequently, 10 μM Na_2SeO_3 may be considered a useful exogenous substance to reinforce wheat cadmium tolerance.

Keywords: antioxidant enzyme; cadmium treatment; nonenzymatic antioxidant; sodium selenite; *Triticum aestivum*.

Introduction

Pesticide and fertilizer applications and continuous industrial waste discharge lead to soil pollution with cadmium (Cd). The overaccumulation of Cd induces Cd toxicity in plants. Cd toxicity often inhibits photosynthetic activity, disturbs plant metabolism, induces excessive accumulation of active oxygen species (AOS), and reduces plant biomass. Therefore, Cd stress (CS) has serious negative effects on plant growth. Among the aforementioned negative effects of CS, the overproduction of AOS further induces peroxidation in plants (Liu *et al*. 2023, Chi *et al*. 2024). The peroxide damage induces membrane lipid peroxidation, protein oxidation, and DNA damage, thereby seriously affecting plant normal metabolism and growth (Foyer and Noctor 2002). To alleviate peroxidation injury, plants mobilize the antioxidant protection system, including enzymatic and nonenzymatic antioxidants (da Silva *et al*. 2021, Komazec *et al*. 2023). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) are important enzymatic antioxidants. Ascorbic acid

Highlights

- Application of selenium enhances cadmium tolerance of wheat seedlings
- Selenium enhances enzymatic and nonenzymatic antioxidant systems under cadmium stress
- Selenium improves the photosynthetic capacity and growth under cadmium stress

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+Corresponding author
e-mail: luninghai2019@aliyun.com

Abbreviations: AOS – active oxygen species; APX – ascorbate peroxidase; AsA – ascorbic acid; Car – carotenoid; CAT – catalase; Chl – chlorophyll; CS – cadmium stress; DHA – dehydroascorbic acid; DM – dry mass; EL – electrolyte leakage; F_v/F_m – maximum photochemical efficiency of PSII; FM – fresh mass; GalLDH – L-galactono-1,4-lactone dehydrogenase; GR – glutathione reductase; GSH – glutathione; GSSG – oxidized glutathione; MDA – malondialdehyde; Na_2SeO_3 – sodium selenite; P_N – photosynthetic rate; POD – peroxidase; q_N – nonphotochemical quenching; q_P – photochemical quenching; SOD – superoxide dismutase; γ-ECS – gamma-glutamylcysteine synthetase; Φ_{PSII} – the quantum yield of PSII photochemistry.

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(AsA) and glutathione (GSH) are important nonenzymatic antioxidant compounds. Meanwhile, AsA content has a close relationship with L-galactono-1,4-lactone dehydrogenase (GalLDH) activity and GSH content has a close relationship with gamma-glutamylcysteine synthetase (γ-ECS) activity (Zhu *et al*. 2021). Previous reports have shown that some exogenous substances can be utilized to mitigate the peroxide damage induced by CS through the above-mentioned antioxidant protection systems, such as melatonin (MT) and brassinosteroids (BRs) (Li *et al*. 2022a, Song *et al*. 2024). For example, BRs bolstered the Cd tolerance of grape plants by activating the levels of both enzymatic and nonenzymatic antioxidants, including SOD, peroxidase (POD), APX, GR, AsA, and GSH (Li *et al*. 2022a). Wheat (*Triticum aestivum* L.) is an important worldwide food crop, which can transport Cd from roots to the aboveground part and accumulate Cd in wheat grain. As Cd cannot be biodegraded in plants, thus, Cd uptake induces CS in wheat crops. In China, Cd pollution is one of the largest and most serious soil pollution problems. The area under wheat cultivation, which is at risk of Cd, exceeds 1.3×10^4 ha in China. Therefore, how to mitigate Cd toxicity in wheat plants has become an important issue for wheat cultivation and production. Aforementioned studies all indicated that corresponding exogenous substances may be utilized to improve wheat Cd tolerance.

Previous research reported that trace element selenium (Se) promoted growth and enhanced the stress tolerance of plants at low concentrations (Basit *et al*. 2023, Hajlaoui *et al*. 2023, Lu *et al*. 2023). For CS, previous research displayed that a suitable concentration of Se alleviated Cd toxicity to sunflower and ramie (Saidi *et al*. 2014, Tang *et al*. 2015). For wheat, previous research mainly focused on the impact of Se on salt, cold, and UV-B tolerance (Chu *et al*. 2010, Yao *et al*. 2010, Elkelish *et al*. 2019). To date, there is very little knowledge about the impact of Se on wheat Cd tolerance. For this reason, it is very important to explore how Se modulates wheat antioxidant ability under Cd treatment, which would provide new evidence for Se utilization in wheat production and cultivation. Besides, CS could impair the photosynthetic performance of alfalfa and wheat crops (Gao *et al*. 2021, Liu *et al*. 2022). Moreover, previous reports have displayed that Se increased plant photosynthetic capacity under CS (Sepehri and Gharehbaghli 2019, Li *et al*. 2020). Therefore, it is meaningful to investigate how Se regulates wheat photosynthetic capacity under CS.

The objective of the present research was to clarify the function of Se in modulating SOD, CAT, APX, GR, GalLDH, and γ-ECS activities, AsA and GSH contents, AsA/dehydroascorbic acid (DHA) and GSH/oxidized glutathione (GSSG), electrolyte leakage (EL), antioxidant biomarkers contents, chlorophyll (Chl) and carotenoid (Car) contents, photosynthetic rate (P_N), chlorophyll fluorescence parameters and growth indexes of wheat seedlings under CS. Through current research, we aimed to add more knowledge for the function of Se in alleviating wheat Cd toxicity and offer the rationale for Se utilization in Cd-resistant cultivation.

Materials and methods

Plant material and treatments: Seeds of winter wheat cultivar Bainong 207 were used as the material. After surface sterilization, seeds were germinated and cultivated in the phytotron under below conditions according to Shan and Ou (2018). When the first leaves were fully unfolded, roots were immersed in the half-strength Hoagland's solution in plastic boxes. Aluminum foil was used to wrap all plastic boxes to keep the roots in a dark environment. The nutrient solution was replaced every second day. After the full expansion of the third leaves, healthy plants with consistent height and leaves were used for subsequent experiments.

To select the suitable Cd concentration, three $CdCl_2$ concentrations (30, 60, and 120 mg L^{-1}) on the wilting phenomenon of seedlings were explored. The control group was only subjected to the nutrient solution. The roots were immersed in corresponding solutions in the beakers for each treatment. Each treatment had three replications. During the whole period of the experiment, all the roots were kept in the dark. After 2 d of Cd exposure, 120 mg($CdCl_2$) L^{-1} led to a significant wilting phenomenon. Seedlings exposed to 30 and 60 mg($CdCl_2$) L^{-1} showed only a nonsignificant wilting phenomenon. Hence, 60 mg($CdCl_2$) L^{-1} was selected as the suitable concentration of Cd treatment. Roots were immersed in 100 mL of 60 mg($CdCl_2$) L^{-1} for 7 d. To explore the impact of Se, three groups of wheat plants were respectively subjected to 5, 10, and 20 μM sodium selenite (Na_2SeO_3) for 12 h and then subjected to $CdCl_2$. The control group was only subjected to the nutrient solution. During the whole period of the experiment, all the roots were kept in the dark. Each treatment had three replications. After 2 d of CS, the top leaves were sampled and frozen by –196°C liquid nitrogen. All samples were stored in a –80°C freezer, and then further used to measure corresponding indexes (Zhao *et al*. 2023). After 7 d of CS, wheat growth indicators were analyzed.

Antioxidant enzymes were all analyzed through the spectrophotometric method. The nitroblue tetrazolium method was applied to determine SOD (EC 1.15.1.1) activity and the absorbance at 560 nm was recorded (Giannopolitis and Ries 1977). CAT (EC 1.11.1.6) activity was analyzed according to Aebi (1984). APX (EC 1.11.1.11) activity was analyzed according to Nakano and Asada (1981). GR (EC 1.6.4.2) activity was analyzed as reported by Grace and Logan (1996). The amount of enzyme required to cause a 50% inhibition of nitroblue tetrazolium reduction was defined as one unit of SOD. The changes in the absorbance value by 0.1 per min were defined as one unit of other enzymes. The specific activity was represented as U g^{-1} fresh mass (FM).

Key biosynthetic enzymes of AsA and GSH: GalLDH (EC 1.3.2.3) and γ-ECS (EC 6.3.2.2) were extracted and measured as reported by Shan and Liang (2010). One unit of GalLDH and γ-ECS was defined according to Shan and Liang (2010). The specific activity was represented as U g^{-1}(FM).

AsA and GSH contents and their redox state: AsA and dehydroascorbic acid (DHA) were analyzed as reported by Hodges *et al*. (1996). Its redox state was represented as the ratio of AsA/DHA. Oxidized glutathione (GSSG) and GSH were analyzed as reported by Griffith (1980). Its redox state was represented as the ratio of GSH/GSSG.

Electrolyte leakage (EL), malondialdehyde (MDA) and H_2O_2 content: MDA content was analyzed as reported by Heath and Packer (1968). EL and H_2O_2 content was analyzed as reported by Brennan and Frenkel (1977).

Photosynthetic performance: Top fully expanded leaves were used to measure Chl and Car contents as reported by Song *et al*. (2016). P_N was measured and recorded using the photosynthesis system (*Licor-6400*, USA). In the leaf chamber, the conditions of measurement were set as light intensity of 1,000 μmol(photon) m^{-2} s^{-1} and CO_2 concentration of 400 ppm. Maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_P), nonphotochemical quenching (q_N), and quantum efficiency of PSII photochemistry (Φ_{PSII}) were measured and recorded using the fluorometer (*Yaxin-1161G*, *Yaxin*, China). P_N and chlorophyll fluorescence parameters were measured from 10:00 to 11:30 h.

Growth indicators: Plant height was measured using the ruler. The fresh mass of each seedling was weighed and recorded and then they were placed in the oven to be dried for 72 h at 80°C until the constant mass. After 72 h, the dry mass of each seedling was weighed and recorded as the biomass.

Statistical analysis: Data were the mean of three replications. The *Excel* software was used for data organization and table drawing. All the data were analyzed by applying a one-way analysis of variance (*ANOVA*) and *Duncan*'s test (P<0.05) using *SPSS* software (*version 25.0*, Chicago, USA).

Results

Antioxidant enzymes: CS markedly reinforced SOD, CAT, APX, and GR activities compared to control (Table 1). In comparison with CS, 5 and 10 μM Na_2SeO_3 significantly increased the activities of the antioxidant enzymes of wheat seedlings under CS. Whereas, 20 μM Na_2SeO_3 plus CS showed a nonsignificant impact on the activity of the antioxidant enzymes. Among three concentrations, 10 μM Na_2SeO_3 showed a more positive impact on above antioxidant enzymes. Compared to CS alone, 10 μM Na_2SeO_3 plus Cd augmented SOD, CAT, APX, and GR activities by 27.6, 92.9, 54.6, and 65.8%, respectively. Compared with the control, Na_2SeO_3 alone also augmented the activities of the antioxidant enzymes, especially for 10 μM Na_2SeO_3. Compared to the control, 10 μM Na_2SeO_3 alone augmented SOD, CAT, APX, and GR activities by 62.7, 91.7, 59.2, and 60.3%, respectively. The results indicated that Na_2SeO_3 positively impacted the activities of the antioxidant enzymes at low concentrations under CS.

Activities of key enzymes of AsA and GSH biosynthesis: CS significantly reduced GalLDH and γ-ECS activities compared with the control (Table 2). In comparison with CS alone, 5 and 10 μM Na_2SeO_3 significantly augmented the activities of these two enzymes in seedlings under CS. Nevertheless, 20 μM Na_2SeO_3 plus CS showed a nonsignificant impact on the above enzymes. Among the three concentrations used, 10 μM Na_2SeO_3 showed a more positive effect on these enzymes. Compared to CS alone, 10 μM Na_2SeO_3 plus CS augmented GalLDH and γ-ECS activities by 86.7 and 70.0%, respectively. Compared with the control, Na_2SeO_3 treatment alone also augmented the activities of the above enzymes, especially for 10 μM Na_2SeO_3. In comparison with the control, 10 μM Na_2SeO_3 alone augmented GalLDH and γ-ECS activities by 34.4 and 34.8%, respectively. This implied that Na_2SeO_3 positively impacted the key biosynthetic enzymes of AsA and GSH at low concentrations under CS.

AsA and GSH contents and their redox state: CS significantly declined AsA and GSH contents and AsA/DHA and GSH/GSSG ratios in seedlings compared to the control (Table 3). Compared to CS alone, 5 and 10 μM Na_2SeO_3 markedly augmented above indexes

Table 1. Effects of sodium selenite (Na_2SeO_3) on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activities under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d. Values are means ± SD, n = 3. *Different lowercase letters* indicate significant differences between treatments (P<0.05).

Treatment	SOD [U g^{-1}(FM)]	CAT [U g^{-1}(FM)]	APX [U g^{-1}(FM)]	GR [U g^{-1}(FM)]
Control	17.7 ± 1.03^{d}	1.33 ± 0.15^{e}	1.25 ± 0.12^{d}	1.06 ± 0.09^{e}
CS	22.8 ± 1.18^{c}	1.83 ± 0.11^{d}	1.52 ± 0.14^{c}	1.49 ± 0.11^{cd}
5 μM Se + CS	23.9 ± 1.29^{bc}	2.60 ± 0.20^{b}	1.91 ± 0.12^{b}	2.05 ± 0.15^{b}
10 μM Se + CS	29.1 ± 1.67^{a}	3.53 ± 0.18^{a}	2.35 ± 0.16^{a}	2.47 ± 0.17^{a}
20 μM Se + CS	19.0 ± 1.02^{d}	1.44 ± 0.10^{e}	1.60 ± 0.13^{c}	1.64 ± 0.13^{c}
5 μM Se	26.0 ± 0.97^{b}	2.28 ± 0.10^{c}	1.75 ± 0.10^{c}	1.45 ± 0.10^{d}
10 μM Se	28.8 ± 1.10^{a}	2.55 ± 0.15^{b}	1.99 ± 0.12^{b}	1.70 ± 0.12^{c}
20 μM Se	25.0 ± 1.11^{b}	2.03 ± 0.12^{d}	1.54 ± 0.09^{c}	1.22 ± 0.08^{e}

Table 2. Effects of sodium selenite (Na_2SeO_3) on gamma-glutamylcysteine synthetase (γ-ECS) and L-galactono-1,4-lactone dehydrogenase (GalLDH) activities under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d. Values are means ± SD, $n = 3$. *Different lowercase letters* indicate significant differences between treatments ($P<0.05$).

Treatment	γ-ECS [U g^{-1}(FM)]	GalLDH [U g^{-1}(FM)]
Control	1.38 ± 0.10^{c}	1.80 ± 0.13^{c}
CS	1.10 ± 0.10^{d}	1.50 ± 0.12^{d}
5 μM Se + CS	1.60 ± 0.11^{b}	2.15 ± 0.16^{bc}
10 μM Se + CS	1.87 ± 0.15^{a}	2.80 ± 0.20^{a}
20 μM Se + CS	1.21 ± 0.10^{cd}	1.40 ± 0.14^{d}
5 μM Se	1.66 ± 0.10^{b}	2.19 ± 0.11^{bc}
10 μM Se	1.86 ± 0.14^{a}	2.42 ± 0.15^{b}
20 μM Se	1.54 ± 0.12^{bc}	2.04 ± 0.13^{c}

under CS. Whereas, 20 μM Na_2SeO_3 plus CS showed a nonsignificant impact on these indexes. Among three concentrations, 10 μM Na_2SeO_3 showed a more positive impact on indexes mentioned above of plants under CS and in comparison with CS alone, 10 μM Na_2SeO_3 plus CS augmented AsA content, GSH content, AsA/DHA, and GSH/GSSG by 75.7, 89.1, 25.3, and 17.9%, respectively. Compared to control, Na_2SeO_3 alone also augmented these indexes, especially for 10 μM Na_2SeO_3. Compared with the control, 10 μM Na_2SeO_3 alone increased AsA content, GSH content, AsA/DHA, and GSH/GSSG by 25.3, 33.3, 12.5, and 8.6%, respectively. The results demonstrated that Na_2SeO_3 could improve AsA and GSH contents and keep their redox state at low concentrations under CS.

EL, MDA, and H_2O_2: CS significantly augmented MDA and H_2O_2 accumulation and EL levels, compared to the control (Table 4). Compared with CS alone, 5 and 10 μM Na_2SeO_3 significantly reduced these indexes in Cd-stressed seedlings. Whereas, 20 μM Na_2SeO_3 plus CS showed a nonsignificant impact on the above indexes. Among three concentrations, 10 μM Na_2SeO_3 had more positive roles in reducing these indicators of seedlings exposed to CS. Compared to CS, 10 μM Na_2SeO_3 plus CS declined MDA content, H_2O_2 content, and EL level by 48.9, 39.8, and 31.3%, respectively. Compared to the control, Na_2SeO_3 treatment alone also decreased the above indicators, especially for 10 μM Na_2SeO_3. Compared with the control, 10 μM Na_2SeO_3 alone reduced the contents of MDA, H_2O_2, and EL by 21.9, 33.3, and 21.6%, respectively. These findings displayed that Na_2SeO_3 showed a positive impact on wheat Cd tolerance at low concentrations.

Photosynthetic performance and growth parameters: CS significantly decreased Chl and Car contents, F_v/F_m, q_P, and Φ_{PSII}, and reduced P_N compared to the control (Tables 5, 6). Meanwhile, CS significantly increased q_N and reduced plant height and biomass. When compared to CS alone, 5 and 10 μM Na_2SeO_3 markedly reduced q_N and improved other indicators of Cd-stressed wheat seedlings. Moreover, 20 μM Na_2SeO_3 plus CS showed a nonsignificant impact on these indicators. Among the three concentrations, 10 μM Na_2SeO_3 influenced positively wheat photosynthetic performance and growth under CS. In comparison with CS alone, 10 μM Na_2SeO_3 plus CS reduced q_N by 28.6%, and increased Chl content, Car content, F_v/F_m, q_P, Φ_{PSII}, P_N, plant height, and biomass by 22.7, 60.0, 36.5, 37.5, 30.0, 23.1, 16.3, and 17.0%, respectively. Compared to the control, Na_2SeO_3 treatment alone also decreased q_N and increased other indicators, especially for 10 μM Na_2SeO_3. Compared to the control, 10 μM Na_2SeO_3 alone decreased q_N by 28.6% and increased Chl content, Car content, F_v/F_m, q_P, Φ_{PSII}, P_N, plant height, and biomass by 25.0, 10.8, 6.0, 14.5, 17.0, 14.5, 16.3, and 14.8%, respectively. Our findings implied that Na_2SeO_3 enhanced wheat photosynthetic performance and promoted growth under CS and normal conditions at low concentrations.

Discussion

Peroxide damage is an important injury induced by Cd toxicity in plants (Nasirzadeh *et al*. 2022, Zhou *et al.*

Table 3. Effects of sodium selenite (Na_2SeO_3) on ascorbic acid (AsA) and glutathione (GSH) contents and their redox state under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d. Values are means ± SD, $n = 3$. *Different lowercase letters* indicate significant differences between treatments ($P<0.05$).

Treatment	AsA [μmol g^{-1}(FM)]	GSH [μmol g^{-1}(FM)]	AsA/DHA	GSH/GSSG
Control	2.85 ± 0.18^{d}	1.41 ± 0.06^{d}	25.6 ± 1.20^{b}	25.5 ± 1.41^{b}
CS	2.30 ± 0.15^{e}	1.10 ± 0.04^{e}	18.2 ± 1.10^{d}	18.4 ± 1.11^{d}
5 μM Se + CS	3.10 ± 0.10^{cd}	1.66 ± 0.07^{c}	22.0 ± 1.30^{c}	21.0 ± 1.00^{c}
10 μM Se + CS	4.04 ± 0.25^{a}	2.08 ± 0.05^{a}	22.8 ± 1.25^{c}	21.7 ± 1.48^{c}
20 μM Se + CS	2.42 ± 0.13^{e}	1.20 ± 0.06^{e}	19.2 ± 1.35^{d}	18.8 ± 1.09^{d}
5 μM Se	3.18 ± 0.12^{c}	1.70 ± 0.06^{c}	27.0 ± 1.30^{ab}	26.6 ± 1.16^{ab}
10 μM Se	3.57 ± 0.20^{b}	1.88 ± 0.08^{b}	28.8 ± 1.07^{a}	27.7 ± 1.01^{a}
20 μM Se	3.05 ± 0.16^{cd}	1.60 ± 0.05^{c}	26.2 ± 1.22^{b}	26.0 ± 1.24^{ab}

Table 4. Effects of sodium selenite (Na_2SeO_3) on electrolyte leakage (EL) and malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d. *Different lowercase letters* indicate significant differences between treatments (P<0.05).

Treatment	EL [%]	MDA [nmol g^{-1}(FM)]	H_2O_2 [μmol g^{-1}(FM)]
Control	10.2 ± 1.00^{d}	3.2 ± 0.23^{d}	0.60 ± 0.04^{d}
CS	20.8 ± 1.37^{a}	9.4 ± 0.55^{a}	1.33 ± 0.10^{a}
5 μM Se + CS	16.5 ± 1.11^{b}	5.7 ± 0.42^{b}	1.06 ± 0.07^{b}
10 μM Se + CS	14.3 ± 1.20^{c}	4.8 ± 0.30^{c}	0.80 ± 0.05^{c}
20 μM Se + CS	19.0 ± 1.13^{a}	8.8 ± 0.61^{a}	1.23 ± 0.08^{a}
5 μM Se	8.7 ± 0.82^{de}	2.8 ± 0.17^{de}	0.47 ± 0.03^{e}
10 μM Se	8.0 ± 0.77^{e}	2.5 ± 0.14^{e}	0.40 ± 0.02^{f}
20 μM Se	9.1 ± 0.58^{de}	2.9 ± 0.17^{de}	0.52 ± 0.03^{e}

Table 5. Effects of sodium selenite (Na_2SeO_3) on chlorophyll fluorescence parameters under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d to measure these indexes. F_v/F_m – maximum photochemical efficiency of PSII; q_N – nonphotochemical quenching; q_P – photochemical quenching; Φ_{PSII} – the quantum yield of PSII photochemistry. *Different lowercase letters* indicate significant differences between treatments (P<0.05).

Treatment	F_v/F_m	q_P	q_N	Φ_{PSII}
Control	0.83 ± 0.06^{a}	0.55 ± 0.03^{b}	0.20 ± 0.01^{d}	0.47 ± 0.02^{b}
CS	0.52 ± 0.04^{c}	0.32 ± 0.02^{d}	0.35 ± 0.02^{a}	0.30 ± 0.02^{d}
5 μM Se + CS	0.63 ± 0.05^{bc}	0.39 ± 0.03^{cd}	0.29 ± 0.02^{b}	0.35 ± 0.02^{cd}
10 μM Se + CS	0.71 ± 0.05^{b}	0.44 ± 0.04^{c}	0.25 ± 0.01^{c}	0.39 ± 0.03^{c}
20 μM Se + CS	0.59 ± 0.04^{c}	0.35 ± 0.02^{d}	0.31 ± 0.03^{ab}	0.33 ± 0.02^{d}
5 μM Se	0.86 ± 0.06^{a}	0.60 ± 0.03^{ab}	0.18 ± 0.01^{d}	0.52 ± 0.04^{ab}
10 μM Se	0.88 ± 0.07^{a}	0.63 ± 0.04^{a}	0.15 ± 0.01^{e}	0.55 ± 0.04^{a}
20 μM Se	0.84 ± 0.05^{a}	0.58 ± 0.03^{ab}	0.19 ± 0.02^{d}	0.50 ± 0.03^{ab}

Table 6. Effects of sodium selenite (Na_2SeO_3) on chlorophyll (Chl) and carotenoid (Car) contents, photosynthetic rate (P_N), plant height, and biomass under cadmium stress (CS). Wheat plants were treated as below: Control, half-strength Hoagland's solution; CS, 60 mg L^{-1} $CdCl_2$; 5 μM Se + CS, 5 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 10 μM Se + CS, 10 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$; 20 μM Se + CS, 20 μM Na_2SeO_3 + 60 mg L^{-1} $CdCl_2$. Wheat plants were first treated by Na_2SeO_3 for 12 h and then exposed to CS for 2 d to measure Chl and Car contents and P_N, and 7 d to measure growth indexes. *Different lowercase letters* indicate significant differences between treatments (P<0.05).

Treatment	Chl [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]	P_N [μmol m^{-2} s^{-1}]	Plant height [cm]	Plant biomass [mg per plant]
Control	2.23 ± 0.16^{ab}	0.69 ± 0.05^{b}	24.8 ± 1.20^{b}	16.0 ± 0.94^{b}	120.0 ± 6.74^{b}
CS	1.63 ± 0.11^{c}	0.35 ± 0.03^{e}	18.2 ± 1.05^{d}	12.3 ± 0.70^{d}	90.6 ± 4.20^{d}
5 μM Se + CS	1.84 ± 0.10^{bc}	0.48 ± 0.03^{d}	21.0 ± 1.12^{c}	13.9 ± 0.80^{c}	100.0 ± 4.38^{c}
10 μM Se + CS	2.00 ± 0.10^{b}	0.56 ± 0.04^{c}	22.4 ± 1.15^{c}	14.3 ± 0.75^{c}	106.0 ± 5.60^{c}
20 μM Se + CS	1.72 ± 0.11^{c}	0.40 ± 0.04^{e}	18.8 ± 1.03^{d}	12.5 ± 0.50^{d}	93.0 ± 3.95^{d}
5 μM Se	2.37 ± 0.11^{a}	0.76 ± 0.04^{b}	27.0 ± 1.07^{ab}	17.4 ± 0.80^{ab}	132.0 ± 5.00^{ab}
10 μM Se	2.47 ± 0.13^{a}	0.87 ± 0.06^{a}	28.4 ± 1.13^{a}	18.6 ± 0.98^{a}	137.8 ± 5.07^{a}
20 μM Se	2.30 ± 0.10^{a}	0.70 ± 0.05^{b}	26.0 ± 1.10^{b}	16.8 ± 0.72^{b}	127.4 ± 4.44^{b}

2022). More and more reports proved this fact. The level of peroxide damage is usually evaluated by EL, MDA, and AOS contents (Bano *et al*. 2023, He *et al*. 2023). Currently, research findings displayed that CS noticeably raised the contents of MDA, H_2O_2, and EL, which suggested that CS led to peroxide damage in wheat, which agreed with previous research (Tang *et al*. 2015). Previous studies displayed that CS increased SOD, CAT, APX, and GR activities in wheat (Li *et al*. 2022b). We also found that CS improved the activity level of antioxidant enzymes in wheat, including SOD, CAT, APX, and GR, which agreed with previous studies.

In plants, Se can alleviate peroxide damage caused by salt stress, CS, and drought stress (Sajedi *et al*. 2011, Diao *et al*. 2014, Tang *et al*. 2015). For wheat plants, previous research also displayed that Se alleviated CS-caused peroxide damage (Zembala *et al*. 2010, Zhou *et al*. 2021). Zhou *et al*. (2021) demonstrated that Se alleviated CS-caused peroxide damage in wheat crops by enhancing SOD and POD activities. However, more details about the influences of Se on the responses of wheat antioxidant metabolism to CS are still not yet fully uncovered. We currently found that Na_2SeO_3 improved wheat antioxidant metabolism by enhancing SOD activity under CS, which agreed with Zhou *et al*. (2021). Besides, we uncovered that Na_2SeO_3 improved the antioxidant metabolism of Cd-stressed wheat seedlings by strengthening CAT, APX, and GR activities. Wu *et al*. (2017) documented that Se augmented AsA and GSH contents in flowering Chinese cabbage exposed to CS. We found that Se improved AsA and GSH contents in wheat plants exposed to CS, which agreed with Wu *et al*. (2017) for flowering Chinese cabbage. Therefore, current results indicated that Se could alleviate CS-induced peroxide damage to wheat plants by bolstering both enzymatic and nonenzymatic antioxidant protection systems, which provided more details for the impact of Se on the responses of wheat antioxidant metabolism to CS.

The plant redox state is related to AsA/DHA and GSH/GSSG ratios and is modulated by key enzymes in charge of AsA and GSH recycling and biosynthetic pathways. The recycling pathway is named the ascorbate–glutathione (AsA–GSH) cycle, in which APX and GR are key enzymes. In plants, GalLDH and γ-ECS are key enzymes in charge of AsA and GSH biosynthesis, respectively. We currently uncovered that CS reinforced APX and GR activities, and decreased GalLDH and γ-ECS activities in seedlings. Moreover, we found that CS significantly decreased AsA and GSH contents and AsA/DHA and GSH/GSSG ratios. As AsA content has a negative correlation with APX activity and a positive correlation with GalLDH activity, current results implied that CS reduced AsA content and AsA/DHA ratio by regulating APX and GalLDH activities and inducing AOS accumulation. As GSH content showed a positive correlation with GR and γ-ECS activities, current findings implied that CS reduced GSH content and GSH/GSSG ratio by inhibiting γ-ECS activity and inducing AOS accumulation in wheat seedlings. It is still unclear whether Se regulated AsA/DHA and GSH/GSSG ratios and the activities of the enzymes in charge of AsA and GSH recycling and biosynthesis in Cd-stressed wheat seedlings. Currently, we found that pretreatment with low concentrations of Na_2SeO_3 increased AsA/DHA and GSH/GSSG ratios and APX, GalLDH, γ-ECS, and GR activities of wheat plants exposed to CS. As a result, our current study indicated that Na_2SeO_3 could increase AsA/DHA and GSH/GSSG ratios by modulating the AsA–GSH cycle and AsA and GSH biosynthetic pathway, which in turn augmented the wheat redox state under CS.

Many reports proved that peroxide damage destroyed photosynthetic performance by degrading photosynthetic pigments, which decreased P_N and plant growth (Rady *et al*. 2023, Zhang *et al*. 2023). Therefore, photosynthetic performance has a close relationship with plant antioxidant capacity under stresses. According to above results for the effects of Na_2SeO_3 on the antioxidant capacity of Cd-stressed wheat seedlings, our current study indicated that Se could improve wheat photosynthetic performance by enhancing the antioxidant capacity. Besides, Car is also an important type of antioxidant in removing AOS. Thus, higher Car content in plants also means higher antioxidant capacity. In this study, we showed that Se could increase the contents of Car and Chl to improve wheat photosynthetic performance under CS. Chlorophyll fluorescence parameters are important indicators used to evaluate the efficiency and physiological status of plants. F_v/F_m reflects the overall health status of photosynthetic apparatus. q_P is the photochemical quenching of PSII under light conditions. Φ_{PSII} is the effective quantum yield of photochemical energy conversion in PSII under light conditions. q_N represents the degree of nonphotochemical quenching and reflects the degree of damage in the photosynthetic system. We displayed that Se increased F_v/F_m, q_P, and Φ_{PSII} and decreased q_N of wheat seedlings exposed to CS, which indicated that Se improved wheat photosynthetic performance under CS. It has been reported that there was a close relationship between q_N and thermal energy dissipation through the xanthophyll cycle (Shin *et al*. 2021). Meanwhile, the xanthophyll cycle also plays an important role in clearing ROS. We currently uncovered that CS simultaneously augmented q_N and H_2O_2 content, indicating that wheat plants could enhance the xanthophyll cycle to improve both heat dissipation capacity and AOS-scavenging ability. After Se application, q_N and H_2O_2 content simultaneously decreased under CS, which further suggested a close relationship between oxygen radical production and the q_N pathway. Thus, our study proved that CS impaired the photosynthesis activity and Se improved the photosynthesis activity by modulating the antioxidant capacity, heat dissipation capacity, and the absorption and conversion ability of light energy through PSII, which further affected wheat growth. Besides, the impact of the CS on chlorophyll fluorescence parameters was greater than antioxidant enzyme activities. Meanwhile, there was no proper correlation between photosynthetic activity and antioxidant enzyme activity under Se application. This phenomenon may be also due to the above reasons. Thus, it is meaningful to further explore the impact of both Cd treatment and Se application plus Cd treatment on the chloroplast structure of this tested wheat variety.

In a word, current findings implied that Na_2SeO_3 enhanced wheat antioxidant ability at low concentrations, which further reduced the stress level and promoted wheat photosynthetic performance and growth. Current results showed new insights into the modulatory role of Se in enhancing wheat Cd tolerance and offered a theoretical basis for its application in wheat production. Meanwhile, the results of our research indicated that 10 μM Na_2SeO_3

could be used in wheat production and cultivation, especially for those grown in Cd-polluted soil.

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