THE MITIGATING EFFECT OF EXOGENOUS SELENIUM ON RAPESEED PHYSIOLOGY UNDER CADMIUM STRESS AND ITS INTERACTION WITH CADMIUM AND SELENIUM ACCUMULATION

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Abstract. Cadmium (Cd) contamination in soil poses a significant threat to rapeseed production. A pot experiment was conducted to investigate the growth performance, physiological responses, and accumulation patterns of selenium (Se) and Cd in different tissues of rapeseed (*Brassica napus* L. cv. 'Nanyouza No. 1') under individual and combined stress conditions of Se and Cd. The results showed that 1 mg·kg^{-1} exogenous Se could effectively mitigate the toxicity of ≤ 5 mg·kg^{-1} Cd stress, promote plant growth, increase yield, and help maintain a relatively high chlorophyll content in rapeseed. Under low Cd stress (0 and 5·mg·kg^{-1}), exogenous Se application enhanced the activities of catalase (CAT) and peroxidase (POD). However, the combined Se and Cd treatments did not increase the activity of superoxide dismutase (SOD) but maintained it at a relatively stable level. Cd accumulation in various organs increased with the elevation of Cd concentration. The application of an appropriate dose of Se facilitated the safe accumulation of Se in rapeseed grains. These findings indicate that optimal Se supplementation (1 mg·kg^{-1}) alleviates Cd toxicity (≤5 mg·kg^{-1}) in rapeseed. Conversely, high Se concentrations (5 mg·kg^{-1}) exacerbated Cd-induced damage under high Cd stress (particularly ≥10 mg·kg^{-1}). This research provides a scientific basis for utilizing Se to enhance safe rapeseed production in Cd-contaminated farmland.

Keywords: *Brassica napus L., exogenous selenium, cadmium toxicity mitigation, antioxidant enzyme activity, agronomic traits*

Introduction

Due to the rapid development of industry, agriculture, mining, and urbanization, large amounts of heavy metals and other pollutants have entered the soil, among which cadmium (Cd) is considered one of the most hazardous heavy metals commonly found in the environment (Zhang et al., 2024). Especially in farmland soil, Cd is not only highly toxic but also highly mobile. Its accumulation in crops not only negatively affects crop growth but also allows Cd to enter the human food chain, posing significant health risks and potential threats to life (Chen et al., 2018; Zhang et al., 2023). In 2021, cadmium was identified as the primary soil pollutant and a Class I human carcinogen by the Ministry of Ecology and Environment of the People’s Republic of China (2022). Therefore, reducing the absorption of cadmium by crops through effective measures is of vital importance for controlling human exposure to cadmium.

Selenium (Se) is one of the trace elements closely related to human and animal health, and it is an immune function enhancer (Zhu, 2004). Previous studies have shown that Se has antagonistic effect on heavy metals such as Cd and lead, and proper amount of Se can prevent the absorption and improve the antioxidant enzyme system of heavy metals in

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crops (He et al., 2023). For example, Zhao et al. (2019) found that when plants were grown in Cd contaminated soil, the application of exogenous Se significantly reduced Cd concentration in seed. Se reduced Cd concentration in root, while root morphology showed little variation by comparison with Cd treated alone. Mirza et al. (2012) believe that the exogenous application of Se at low concentrations increases the tolerance of plants to Cd-induced oxidative damage by enhancing their antioxidant defense and MG detoxification systems.

All the above studies showed that the application of selenium on plants was beneficial to reduce the damage caused by cadmium. Meanwhile, some studies have reported the interaction mechanism between selenium and cadmium, which show that spraying Se in rice is beneficial to reduce the content of Cd in brown rice, stems and roots. Among them, brown rice can be reduced by up to 61.6% (Gao et al., 2018). Because the climate, soil pollution, planting methods, Se application amount and application methods are different in different regions of China, the antagonism and cooperation between Se and Cd are different, and the influence mechanism of these interactions still needs further research and analysis.

Rapeseed (*Brassica napus* L.) is a cruciferous plant, which is the main oil crop and commercial crop in China. Its biomass is large and it is a bioenergy material with good development prospects. Relevant research shows that cruciferous crops have strong accumulation ability of Se and Cd. However, the potential reasons for the dose relationship between Cd and Se elements in actual crop production applications, as well as how Se directly affects the toxic effects of Cd on crops, are still unclear. In this study, rapeseed was selected as the test object, and pot culture method was used to explore the effects of Se on agronomic characters, chlorophyll content and enzyme activity of rape under Cd stress, and the effects of Se on regulating the absorption and accumulation of Se and Cd in rape. The purpose is to provide some theoretical basis for formulating cultivation measures to decrease the threats and accumulation of Cd in rape by applying appropriate amount of Se, and also has important research significance for comprehensively utilizing Cd contaminated soil, improving crop safety and maintaining sustainable crop production.

Materials and methods

Test materials

The soil to be tested was taken from paddy soil in agricultural experimental base of Yichun University. Soil samples were air-dried under shade and subsequently sieved to remove foreign matter, including dead branches and gravel. After the soil was broken, it was screened by 2 mm sieve and wrapped with polyethylene film to prevent the pollution and loss of nutrients. Soil texture (sand: 20%, silt: 35%, clay: 45%) was determined using the hydrometer method. The basic physical and chemical properties of the soil are shown in *Table 1*:

Table 1. *The properties of potted soil*

Index	pH value	Organic matter/ $g\cdot kg^{-1}$	Alkaline hydrolysis N/$mg\cdot kg^{-1}$	Available P/$mg\cdot kg^{-1}$	Available K/$mg\cdot kg^{-1}$	Total cadmium/ $mg\cdot kg^{-1}$	Total selenium/ $mg\cdot kg^{-1}$
Measured value	6.78	20.11	137.58	46.15	128.47	0.29	0.25

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Experiment design

The rape variety is Nanyouza 1st. In the experiment, Na_2SeO_3 was used as selenium source, $CdCl_2{\cdot}2.5H_2O$ was used as cadmium source. There were 12 treatments (Se_0Cd_0, Se_0Cd_5, Se_0Cd_{10}, Se_0Cd_{20}, Se_1Cd_0, Se_1Cd_5, Se_1Cd_{10}, Se_1Cd_{20}, Se_5Cd_0, Se_5Cd_5, Se_5Cd_{10}, Se_5Cd_{20}) with three Se levels (0, 1, 5 $mg{\cdot}kg^{-1}$) and four cadmium levels (0, 5, 10, 20 $mg{\cdot}kg^{-1}$), and each treatment was repeated five times, totaling 60 pots.

Test treatment

The pot experiment was conducted in a controlled greenhouse at Yichun University. The environmental parameters maintained as follows: daily temperature ranging from 15-28°C, relative humidity of 60-75%, and a natural photoperiod. In this experiment, 30 cm ×40 cm (diameter× depth) plastic barrels were used, and each pot was filled with 15 kg of air-dried and screened soil. Different Se and Cd treatments were evenly poured into the soil in the form of aqueous solution, and stirred once every other day to prevent uneven distribution of Se and Cd in the soil. The aging time was more than one month. *Brassica napus* was sown on October 20, 2024, and after the seedlings grew stably for about two weeks, the seedlings were thinned (the surplus seedlings were buried in the soil of the respective treatment pots), and 3 seedlings are finally fixed in each pot-only seedlings with consistent height (±5%, measured via a telescopic ruler) and leaf number were selected to minimize initial growth variation. The amount of base fertilizer is N: $0.2g{\cdot}kg^{-1}$, P_2O_5: 0.15 $g{\cdot}kg^{-1}$ and K_2O: $0.2g{\cdot}kg^{-1}$. One week before sowing, the fertilizer required for the test is weighed separately and dissolved in water, and applied to the soil in the form of solution at one time. All treatment groups were watered daily with deionized water (to avoid external heavy metal contamination) at a fixed volume adjusted weekly. Soil moisture was monitored using a soil moisture sensor (Model: WS485, Hengce Cloud Analysis, China) to maintain soil water content at 70% of field capacity. The total weekly water input per pot was recorded to ensure uniformity across all 60 experimental pots. The potted plant is equipped with a rain shelter to prevent rain leaching from damaging and polluting plants and soil. After harvesting the whole plant at maturity (April 25, 2025), the root system was first gently shaken to remove loosely attached soil. For residual soil adhering to root hairs, roots were soaked in deionized water for 5 minutes, then rinsed with a soft-bristled brush (to avoid damaging delicate root tissues). Finally, roots were blotted dry with absorbent filter paper. And the root, stem and grain samples were oven-dryed at 65°C to constant weight. Then all samples were ground and passed through a 1 mm mesh screen, and stored.

Determination items and methods

Measurement of agronomic characters

For agronomic trait measurements (e.g., leaf number, plant height and number of branches), all 3 retained plants in each pot were measured, and the average value of the 3 plants was treated as one biological replicate. With 5 pots per treatment, this design ensures 5 independent replicates while ensuring the appropriate number of replicates. The number of pods per plant, thousand grain weight, and yield of rapeseed were determined at maturity. The specific indicators are measured as follows:

Plant height: Telescopic measuring ruler (accuracy: 0.1 cm, Model: F1B2); Leaf number, branch number, pod number of per plant and number of grains per pod: Manual counting (cross-checked by two researchers to ensure accuracy); Thousand-grain weight:

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Electronic analytical balance (accuracy: 0.001 g, Model: LA203E/A); Yield per 667 m²: Calculated based on total seed weight per pot, scaled to field area using pot soil volume (15 kg/pot) and typical field soil bulk density (1.3 g/cm³).

Determination of chlorophyll content

Cut 0.2 g mature leaves in the middle of rape plant and put them into a mortar, add a certain amount of quartz sand, calcium carbonate powder, and a trace amount of 96% ethanol, grind them into a homogenate in a dark environment, continue to add 10ml of 96% ethanol, and grind until the tissue turns white. Let them stand for 3-5 minutes. Measured the absorbance of leaf tissue at 665 nm and 649 nm, and calculated the content of chlorophyll (Zhao et al., 2024).

Determination of antioxidant enzyme activity

Take 0.5 g fresh rape leaves and cut them into 1-2 cm pieces, then put them in a mortar, add 2-3 mL of phosphate buffer, pre-cooled to 4°C at pH 7.0, along with a small amount of quartz sand, and grind the mixture into a homogenate. Subsequently, transfer the homogenate into a centrifuge tube, and centrifuge them in a low-temperature high-speed centrifuge to obtain the supernatant, i.e. enzyme solution. Catalase (CAT) activity was measured using the ultraviolet absorption method, peroxidase (POD) activity was assessed via the guaiacol method, and superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium photoreduction method (Gao et al., 2011).

Determination of soil physical and chemical indexes

Measured by conventional methods, including soil pH value, organic matter, alkali-hydrolyzed nitrogen, available phosphorus and available potassium (Bao, 2000).

Determination of selenium and cadmium content

The Se content of soil samples and rape samples was determined using microwave digestion atomic fluorescence spectrometry, and the content of Cd was determined by microwave digestion-graphite furnace atomic absorption spectrophotometry (Wu et al., 2015). Certified reference materials (CRM: NIST 1573a for plant tissues) were used to validate the accuracy of Cd and Se measurements. Recovery rates ranged between 92–105% for Cd and 89–98% for Se.

The instruments used in the experiment are as follows: Chlorophyll content / Antioxidant enzyme (CAT, POD, SOD) activity: UV-Vis spectrophotometer (Model: UV-721, Thermo Fisher Scientific, China); Soil pH: pH meter (Model:Push-button desktop pH meter P901, Shanghai Youke Instrument & Meter Co., LTD, China). Cd/Se content: Microwave digestion-graphite furnace atomic absorption spectrophotometer (Microwave digester: Model MWD-500, Shanghai Yuanxi Instrument Co., LTD, China; Atomic absorption spectrophotometer: Model AA-SFG3, Shenzhen Sanli Technology Co., LTD, China).

Data analysis

Data processing and statistical analysis were performed using Microsoft Excel 2021 and IBM SPSS Statistics 27.0, respectively. Two-way analysis of variance (ANOVA) was employed to evaluate the main effects of Se (0, 1, 5 $mg \cdot kg^{-1}$), Cd (0, 5, 10, 20 $mg \cdot kg^{-1}$), and their interaction (Se×Cd) on rapeseed agronomic traits, chlorophyll content,

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antioxidant enzyme activity, Se and Cd accumulation. Post hoc comparisons were conducted using Duncan's multiple range test with a significance level of $P<0.05$.

Results

Effects of Se on agronomic traits and yield of rape in Cd contaminated soil

As shown in *Table 2*, under the condition of no exogenous selenium addition, with the increase of cadmium stress, the plant height of rapeseed showed a trend of first increasing and then decreasing; under Cd_0 and Cd_{10} treatment conditions, 1 $mg \cdot kg^{-1}$ Se addition was beneficial to the increase of rapeseed plant height, while under 20 $mg \cdot kg^{-1}$ cadmium stress, the effect of exogenous selenium addition on plant height was not significant. Regarding the effect on the number of rapeseed leaves, under the same selenium level, no significant difference was observed in the leaf count of rapeseed between adjacent cadmium stress treatments. Furthermore, treatments with different Se and Cd concentrations did not affect the number of branches in rapeseed, which may be primarily attributed to the dominant role of genetic factors in regulating branch number.

Table 2. *The change of agronomic shapes of rape at maturity under Se-Cd treatments*

Treatments	Height/cm	Leaves	Branches	Pods	Seeds	1000-grain weight/g
Se_0Cd_0	139.00±1.87e	17.33±1.25abc	7.33±1.25a	148.00±1.63e	17.63±0.51ab	3.45±0.34a
Se_0Cd_5	156.33±1.70c	19.67±0.47a	7.33±0.47a	124.50±2.04f	15.21±1.27cd	3.32±1.52ab
Se_0Cd_{10}	125.50±1.08fg	18.00±0.82ab	8.00±0.82a	108.00±1.63g	17.49±1.57ab	3.08±2.41ab
Se_0Cd_{20}	124.00±1.63g	15.67±0.47bc	7.33±0.47a	93.50±1.18h	16.05±1.15bc	3.11±0.63ab
Se_1Cd_0	161.67±1.70b	18.33±0.58ab	8.33±0.47a	179.50±2.86b	18.32±2.59a	3.53±0.56a
Se_1Cd_5	149.00±3.56d	19.67±2.08a	7.00±0.82a	233.50±3.87a	18.95±2.68a	3.48±1.51a
Se_1Cd_{10}	163.67±2.62b	17.00±1.00abc	8.00±0.00a	160.00±3.27c	13.65±2.53d	3.41±4.01a
Se_1Cd_{20}	127.17±2.25fg	18.00±1.00ab	7.00±0.82a	106.50±2.04g	15.03±0.54cd	3.14±2.64ab
Se_5Cd_0	147.67±7.23d	17.67±1.53abc	8.25±3.21a	164.00±1.41c	13.99±0.97d	3.36±3.85ab
Se_5Cd_5	159.67±0.94bc	18.33±2.52ab	7.41±1.15a	152.50±2.86d	15.51±0.55cd	3.45±2.74a
Se_5Cd_{10}	169.33±7.51a	14.67±3.51c	7.67±1.15a	137.50±0.41f	11.73±0.91e	3.13±0.93ab
Se_5Cd_{20}	129.00±5.29f	15.33±1.15bc	8.00±1.00a	109.50±2.27g	10.19±3.31e	2.94±0.89b

Note: Different lowercase letters in each column of the data represent significant differences in the comparison between different treatments. Two-factor analysis of variance and Duncan's multiple comparisons were used for all data

Under no exogenous Se conditions, the number of pods per plant decreased with increasing Cd stress. In no exogenous Cd addition, exogenous Se application significantly increased the number of pods per plant, with Se_1Cd_0 treatment exhibiting a significantly higher pod number than Se_5Cd_0. At the same Cd concentration, the seeds per pod in the no exogenous Se treatment were significantly higher than that in the high-Se treatment. Notably, both the number of pods per plant and seeds per plant were significantly higher in Se_1Cd_0 and Se_1Cd_5 treatments compared with all other treatments. The lowest seeds per pod were observed under the Se_5Cd_{20} treatment. Under the treatment conditions with the same selenium concentration and same cadmium concentration, no significant difference was observed in the thousand-seed weight of rapeseed.

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Synthesizing the responses across all agronomic traits, it can be preliminarily concluded that lower levels of both Se and Cd facilitate enhanced growth performance in rapeseed.

Figure 1 clearly shows the variations in rapeseed yield across different treatments. As shown in the figure, rapeseed yield ranged from 101.9 to 199.5 kg·667 m^{-2}, with an average yield of 148.46 kg·667 m^{-2}. The Se_1Cd_5 treatment achieved the highest yield (199.5 kg·667 m^{-2}), and Duncan's multiple range test ($P < 0.05$) confirmed that the yield of this treatment was significantly higher than that of all other treatments. Under single Cd treatment, the yield of rapeseed decreased progressively with increasing Cd concentration, and the yield of Cd_{20} treatment decreased by 24.09% compared with that of Cd_0 treatment. For single selenium treatment, compared with the Se_0Cd_0, the rapeseed yield in the 1 mg·kg^{-1} Se treatment was significantly increased by 7.20%, while the yield in the 5 mg·kg^{-1} Se treatment was significantly decreased by 12.58%. These results indicate that the main effect of Se on rapeseed yield is concentration-dependent.

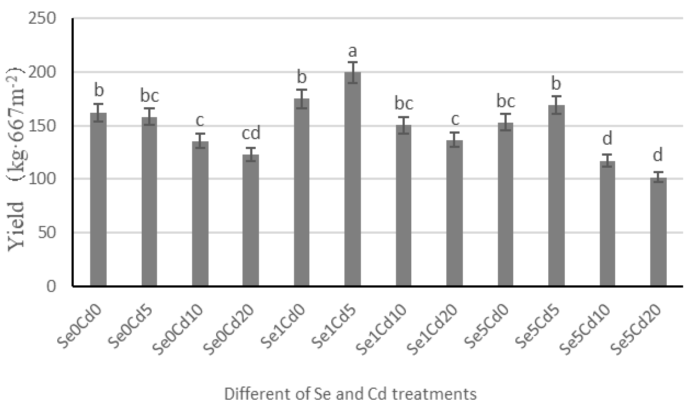

Figure 1. *The effect of Se on rape yield in Cd contaminated soil. Note: Error bars denote SD (n=5). Different lowercase letters above bars indicate significant differences between treatments (P<0.05) according to Duncan's multiple range test*

In the combined treatments of Se and Cd, with the increase in Cd addition level, the rapeseed yield in all Se concentration groups showed a significant downward trend, confirming that Cd stress has a significant inhibitory main effect on yield. Further multiple comparison results showed that, at the same Cd concentration, the yields of the 1 mg·kg^{-1} Se combined with different Cd concentrations were significantly higher than those of the 5 mg·kg^{-1} Se combined with corresponding Cd concentrations. This suggests that low concentrations of Se can alleviate the inhibitory effect of Cd stress on yield, while high concentrations of Se exacerbate this inhibitory effect, indicating a significant interactive effect between Se and Cd on yield.

Effect of Se on chlorophyll content of rape in Cd contaminated soil

Table 3 presents the alteration of chlorophyll content in rapeseed grown in soils treated with different levels of selenium and cadmium. In the no exogenous Se addition, the chlorophyll a (Chl a) content showed a trend of first increasing and then decreasing with the increase of Cd concentration. Under the condition of 1 mg·kg^{-1} exogenous Se, the Chl a contents in the Se_1Cd_5 and Se_1Cd_{10} treatments were higher than those in the other treatments, however, no significant differences were detected among the treatments. At

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the 5 mg·kg^{-1} exogenous Se, the Se_5Cd_{10} treatment showed the lowest Chl a content, indicating that the synergistic toxic effects of Se and Cd under this treatment condition impaired the synthesis of Chl a.

Table 3. *Effects of different Se-Cd treatments on photosynthetic pigment content in rape*

Treatments	Chlorophyll a (mg·L^{-1})	Chlorophyll b (mg·L^{-1})	Total Chl. (mg·L^{-1})
Se_0Cd_0	3.17±0.15abc	0.79±0.20ab	3.97±0.32ab
Se_0Cd_5	3.43±0.06ab	0.78±0.10ab	4.21±0.06ab
Se_0Cd_{10}	3.04±0.39abc	1.08±0.44ab	4.13±0.82ab
Se_0Cd_{20}	2.53±0.22bc	0.66±0.04b	3.19±0.26b
Se_1Cd_0	3.28±0.95abc	1.33±1.41ab	4.61±2.12ab
Se_1Cd_5	3.92±0.47a	1.09±0.28ab	4.45±1.04ab
Se_1Cd_{10}	3.71±0.67a	1.01±0.32ab	4.72±0.91ab
Se_1Cd_{20}	3.36±0.94abc	1.27±0.55ab	5.19±0.97a
Se_5Cd_0	3.56±0.38ab	1.68±0.47a	5.24±0.81a
Se_5Cd_5	3.08±0.45abc	0.98±0.18ab	4.00±0.60ab
Se_5Cd_{10}	2.26±0.43c	0.67±0.07b	2.94±0.42b
Se_5Cd_{20}	2.81±0.96abc	0.75±0.11ab	3.56±1.06ab

Note: Different lowercase letters in each column of the data represent significant differences in the comparison between different treatments. Two-factor analysis of variance and Duncan's multiple comparisons were used for all data

The dynamic response of Chl b content differed from that of Chl a. Among all treatments, the Se_5Cd_0 treatment exhibited the highest Chl b content, which was higher than that in the other treatments . This indicates that under no exogenous Cd conditions, the high-dose Se alone can strongly promote the accumulation of Chl b. Under different Cd stress, the 1 mg·kg^{-1} exogenous Se helped maintain Chl b levels, with no significant differences observed among these treatments.

The total chlorophyll content in the Se_1Cd_{20} and Se_5Cd_0 treatments was higher than that in the other treatments , indicating that both the combined treatment of 1 mg·kg^{-1} Se with 20 mg·kg^{-1} Cd stress and the treatment of 5 mg·kg^{-1} Se without exogenous Cd stress are conducive to increasing the total chlorophyll content. Furthermore, the lowest total chlorophyll content was observed in the Se_0Cd_{20} and Se_5Cd_{10} treatments, suggesting that among all tested treatments, the antagonistic effect of these two treatments on total chlorophyll content was relatively pronounced.

Se on the Cd contaminated soil rape antioxidant enzyme activity changes

Table 4 shows the effects of exogenous Se on the activities of three antioxidant enzymes in rapeseed grains cultivated in Cd-contaminated soils.

The effect on CAT activity was that exogenous Se application enhanced CAT activity in rapeseed at equivalent Cd levels. In the no exogenous Se, increasing Cd concentration induced a biphasic response in CAT activity, characterized by an initial increase followed by a subsequent decline. Under the combined treatment of low-dose Se (1 mg·kg^{-1}) and Cd, CAT activity generally exhibited a trend of initial decrease followed by recovery as Cd stress intensified. In contrast, under high-dose Se (5 mg·kg^{-1}) in combination with Cd, CAT activity showed an initial increase followed by a decrease, and then increasing again with escalating Cd stress. Notably, the Se_5Cd_{20} treatment resulted in the highest CAT

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activity among all 12 treatments, with statistically significant differences compared to all other treatments ($P < 0.05$).

Table 4. *The change of antioxidant enzyme activity of rape under Se-Cd treatments*

Treatments	CAT activity ($U\ g^{-1}$)	POD activity ($U\ g^{-1}$)	SOD activity ($U\ g^{-1}$)
Se_0Cd_0	132.25±3.58i	211.15±1.82f	300.00±14.29c
Se_0Cd_5	197.25±4.83ef	251.00±5.72d	633.33±34.08a
Se_0Cd_{10}	254.25±7.04c	260.85±6.16cd	514.29±21.99b
Se_0Cd_{20}	139.75±2.67i	294.00±2.45a	300.00±29.48c
Se_1Cd_0	275.00±4.32b	231.00±12.83e	247.62±23.64c
Se_1Cd_5	270.75±1.27b	272.85±7.07b	347.62± 8.24c
Se_1Cd_{10}	149.00±2.16g	158.30±4.50g	323.81± 8.24c
Se_1Cd_{20}	191.75±3.97f	226.85±4.14e	338.10±29.74c
Se_5Cd_0	201.00±1.63de	259.15±2.30cd	304.76±20.17c
Se_5Cd_5	207.75±3.91d	279.35±1.22b	319.05± 8.24c
Se_5Cd_{10}	175.0.±4.32g	151.70±2.59g	342.92±24.69c
Se_5Cd_{20}	405.00±5.10a	269.00±0.82bc	495.24±17.24b

Note: Different lowercase letters in each column of the data represent significant differences in the comparison between different treatments. Two-factor analysis of variance and Duncan's multiple comparisons were used for all data

The effects of exogenous Se on POD activity were as follows: In the absence of exogenous Cd and under low Cd level (5 $mg{\cdot}kg^{-1}$), exogenous Se addition enhanced POD activity in rapeseed leaves. Under single Cd stress, POD activity in rapeseed leaves increased with the increase of soil Cd application rate, which was significantly 20.00%–39.33% higher than that in the control group. When only exogenous Se was applied, POD activity in rapeseed increased significantly with the elevation of Se concentration, and the POD activity in the Se (5 $mg{\cdot}kg^{-1}$) treatment was 1.23 times higher than that in the treatment without exogenous Se. Under the combined Se and Cd treatments, POD activity in rapeseed showed a trend of first decreasing and then increasing with the intensification of Cd stress. For the combined treatments of Se (1 $mg{\cdot}kg^{-1}$) and different Cd concentrations, significant differences were observed between adjacent treatments ($P < 0.05$).

The effects of exogenous Se on SOD activity were as follows: Under single Cd stress, SOD activity showed a trend of first increasing and then decreasing with the elevation of Cd concentration, and significant differences were observed among the treatments ($P < 0.05$). Additionally, no significant difference in SOD activity was detected under single Se treatments. Under the condition of combined treatment with selenium and cadmium, exogenous Se addition exerted no significant effect on SOD activity (except for the Se_5Cd_{20} treatment, this treatment exhibited the highest activity and was significantly different from all other treatments).

Effect of exogenous selenium on the cadmium content in various parts of rapeseed under different cadmium contaminated soil

Figure 2 shows the variations in Cd accumulation in roots, stems, and seeds of mature rapeseed under different Se and Cd treatments. Cd accumulation in rapeseed roots increased with increasing Cd concentration in the soil. In the absence of exogenous Cd,

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no significant difference in root Cd accumulation was observed among rapeseed plants. Under Cd stress at the same concentration, the application of 1 mg·kg^{-1} Se significantly reduced Cd accumulation in the roots, and the mitigating effect on root Cd accumulation was more pronounced than that of 5 mg·kg^{-1} Se.

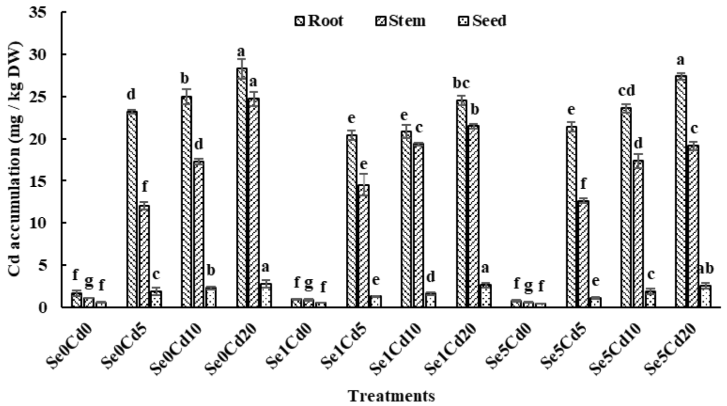

Figure 2. *The change of Cd concentrations in different parts of rape under Se-Cd treatments. Note: Error bars denote SD (n=5). Different lowercase letters above bars indicate significant differences between treatments (P<0.05) according to Duncan's multiple range test*

The pattern of Cd accumulation in rapeseed stems was not entirely consistent with that in the roots. Under the same Cd concentration treatment, Cd accumulation in stems increased with increasing Cd concentration in the soil. In the presence of exogenous Cd, Cd accumulation in stems treated with 5 mg·kg^{-1} Se was lower than that treated with 1 mg·kg^{-1} Se ($P < 0.05$). At the Cd_{20} level, compared with the no exogenous Se treatment, the application of 1 or 5 mg·kg^{-1} Se significantly reduced Cd accumulation in stems ($P < 0.05$).

The effect on seed Cd accumulation was as follows: under Cd_5 and Cd_{10} conditions, the addition of exogenous Se significantly reduced grain Cd accumulation ($P < 0.05$). However, at the Cd_{20} level, no significant difference in grain Cd accumulation was observed between the treatments with 1 or 5 mg·kg^{-1} Se addition and the treatment without exogenous Se. These results indicate that under high Cd stress, Se addition failed to reduce Cd accumulation in rapeseed grains.

Effect of exogenous selenium on the selenium content in various parts of rapeseed under different cadmium contaminated soil

As shown in *Figure 3*, among the 12 experimental treatments evaluated, selenium (Se) concentration was highest in the roots. In the absence of exogenous Se application, although variations in Se content were observed across different parts of rapeseed, these differences were not statistically significant. Under various cadmium (Cd) stress conditions with 1 mg·kg^{-1} Se supplementation, the Se concentration in all plant tissues was significantly higher compared to treatments without Se addition, yet significantly lower than those receiving 5 mg·kg^{-1} Se supplementation. In treatments supplemented with 5 mg·kg^{-1} exogenous Se, the Se distribution followed a descending order: root > grain > stem. Furthermore, as Cd stress intensity increased, root Se content initially

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increased and subsequently decreased, whereas Se levels in stems and grains exhibited a consistent decline with increasing Cd concentration.

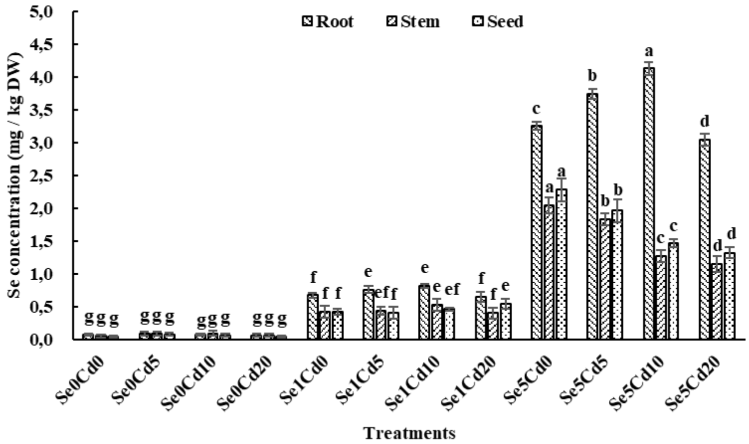

Figure 3. *The change of Se concentrations in different parts of rape under Se-Cd treatments. Note: Error bars denote SD (n=5). Different lowercase letters above bars indicate significant differences between treatments (P<0.05) according to Duncan's multiple range test*

Discussion

Cd pollution in soil is an important factor restricting agricultural safety production. Previous research has confirmed that heavy metal cadmium stress can inhibit the increase in plant height and interfere with plant reproductive growth (Rizwan et al., 2019). The results showed that under the stress of no Cd (0 mg·kg^{-1}), low Se (1 mg·kg^{-1}) promoted the increase of plant height. High Se and Cd inhibit the plant height growth of rape. There was no significant difference in 1000-grain weight between Se and Cd single treatment and compound treatment. Under single Cd treatment, the number of pods per plant and yield of rape decreased with the increase of Cd content. However, under the compound treatment of Se (1 mg·kg^{-1}) and Cd, with the increase of Cd content, the number of pods per plant and the yield of rape first increased and then decreased. Se1Cd5 treatment can make rape get a higher number of pods per plant and yield (199.5 kg·667 m^{-2}). These results are consistent with the conclusion of Lin et al. (2012), that Se can improve the plant growth under the condition that appropriate doses of Cd and Se coexist. However, when the Cd content is constant and the Se content continues to increase, the overall change of rape yield tends to increase first and then decrease, indicating that the Se content has a dual effect on the growth and yield of rape in Cd-contaminated soil.

For most plants, the inhibition of photosynthesis in higher plants is another major aspect of Cd toxicity (Ying et al., 2010; Wang et al., 2016). The content of photosynthetic pigments directly influences the photosynthetic rate of plants (Xiong et al., 2021), and subsequently affects the physiological growth of plants. Hence, the initial symptoms of Cd stress can be distinctly reflected by studying the photosynthetic pigment content and photosynthesis changes of plants.

However, after a lot of research, it was found that proper amount of Se enters plants in the form of selenate or selenite through roots in soil, which enhances the increase of photosynthetic pigment content in plants and restores the photosynthetic performance of plants to varying degrees, thus helping plants resist Cd stress (Shekari et al., 2019; Wan

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et al., 2021; Si et al., 2021). The results of this study indicate that the addition of low-dose Se (1 $mg \cdot kg^{-1}$) to different Cd-contaminated soil was beneficial for maintaining chlorophyll content at a relatively high level. In contrast, either high Cd (Se_0Cd_{20}) stress or the combined treatment of high Se and high Cd (Se_5Cd_{20}) was unfavorable for chlorophyll synthesis and compromised rapeseed photosynthesis. These findings indicate that Se addition can indeed enhance the photosynthetic capacity of rapeseed leaves in Cd-contaminated soil, but there is an optimal concentration range of Se for this beneficial effect.

In addition, Cd can also interfere with the scavenging system of active oxygen in plants, which can directly or indirectly lead to excessive accumulation of active oxygen in plants (Gill et al., 2011) and cause damage to plants. Se has the ability to enhance the antioxidant defense system of plants and improve the antioxidant capacity of crops (Silva et al., 2018; Wang et al., 2020). The results of this study showed that under no exogenous Se treatments, the activities of three antioxidant enzymes would increase to some extent with the increase of Cd stress, but the activities of CAT and SOD would decrease with the continuous increase of Cd dose. Adding a certain amount of Se (1 $mg \cdot kg^{-1}$) can help to resist the harm of active oxygen to rape. Furthermore, in the Cd_0 and Cd_5 conditions, the activities of CAT and POD in rapeseed leaves were higher in the treatments with exogenous Se addition than those in the treatment without Se. However, the change in SOD activity was the opposite. These variations in enzyme activities might be attributed to the complex intracellular physiological changes induced by different Se and Cd treatments, which could be either antagonistic or synergistic. The specific mechanisms underlying these observations need further investigation at the molecular level.

Different Se and Cd treatments have different effects on Se and Cd content in different parts of rape. Appropriate addition of Se can not only promote the absorption of Se by plants, but also alleviate the toxicity of Cd to plants (Zhou et al., 2017; Ling et al., 2023). In the study on winter wheat, it was discovered that the increase in Se content could notably reduce the Cd content in winter wheat, particularly under moderate Cd (5 $\mu mol \cdot L^{-1}$) stress (Qin et al., 2018). This study showed that under all kinds of treatments, the Cd content in rape grains was the lowest, followed by roots and stems. Adding low amount of Se (1 $mg \cdot kg^{-1}$) is helpful to reduce the Cd toxicity in this variety of rape and increase the Se content in all parts, which may provide a theoretical basis for exploring how to plant rape in Cd-contaminated soil to reduce Cd toxicity and increase the Se content in grains. However, with the continuous increase of Se content (5 $mg \cdot kg^{-1}$), the Cd content in parts of rape increases trend, which is not conducive to the growth of rape, indicating that there is a certain synergistic effect between Se and Cd (Yu et al., 2017). However, some studies have shown that the accumulation and distribution of Cd in Chinese cabbage by Se decrease with the growth time of Chinese cabbage (Yu et al., 2019). It shows that the regulation of Se on Cd in soil environment may change with the change of environmental conditions (Chen et al., 2019).

In this experiment, sodium selenite was selected as the source of exogenous selenium for the following reasons: First, selenite is more prone to complexation reactions with cadmium compared to other selenium species, forming stable complexes that reduce plant absorption of cadmium. Second, upon uptake, selenite predominantly accumulates in plant roots with limited translocation to aerial parts, thereby minimizing the risk of selenium toxicity in plants. Nevertheless, different selenium forms exhibit varying effects on plants (e.g., rapeseed) under cadmium stress. In future studies, we aim to systematically investigate and compare the impacts of selenite, selenate, and organic

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selenium on cadmium uptake and subcellular distribution in rapeseed, enabling a more comprehensive analysis of the underlying mechanisms by which selenium influences rapeseed under cadmium stress.

In this study, it was found that with the change of Se content, the accumulation of Cd and Se in rape was regulated to some extent, and the low Se content (1 $mg \cdot kg^{-1}$) was beneficial to the reduction of Cd accumulation and the increase of Se content. However, this experiment is only a one-year pot culture experiment, and the variety of rape is relatively single. In practical application, soil environment factors, crop variety characteristics, Se species and application amount should be considered. Therefore, to obtain the ideal regulatory effect of Se on Cd more effectively, the experiment requires further in-depth exploration.

Conclusion

This study investigated the effects of Se, Cd, and their combined treatments on the biological traits of *Brassica napus* L. The results showed that the Se_1Cd_5 treatment achieved the optimal growth and the highest yield. Regarding photosynthetic capacity, low-dose Se alleviated Cd stress by increasing chlorophyll content. Under Cd_0 and Cd_5 stress conditions, the addition of 1 $mg \cdot kg^{-1}$ exogenous Se resulted in higher CAT and POD activities compared with other treatments. At the high Cd level (20 $mg \cdot kg^{-1}$), exogenous Se application enhanced SOD activity, thereby mitigating the oxidative damage caused by Cd stress in rapeseed. Analysis of heavy metal contents in different parts of rapeseed revealed that Cd accumulation was the lowest in seeds, moderate in stems, and the highest in roots. Furthermore, compared with the treatment without exogenous Se, the addition of 1 $mg \cdot kg^{-1}$ Se under different Cd concentrations significantly reduced Cd accumulation in all tissues of rapeseed while improving Se uptake by the plant.

In conclusion, when the Cd concentration in soil reaches a certain threshold, it exerts significant toxic effects on the growth and physiology of rapeseed. Therefore, the appropriate application of a specific dose of Se is conducive to promoting rapeseed growth, effectively alleviating Cd toxicity, and simultaneously increasing Se content in rapeseed.

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APPENDIX

Figure S1. *Potted plant experiment. Note: a: Cadmium-contaminated soil balance; b: Rapeseed sowing; c: The bud-bolting period of rape; d: The flowering period of rape*

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Table S1. *Variance analysis of the effect of selenium-cadmium interaction on yield of rape*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	24376.767a	11	2216.070	41.994	0.000
Intercept	793435.562	1	793435.562	15035.494	0.000
Se Concentration	5644.205	2	2822.103	53.478	0.000
Cd Concentration	17419.507	3	5806.502	110.032	0.000
Se Concentration * Cd Concentration	1313.055	6	218.843	4.147	0.005
Error	1266.500	24	52.771		
Total	819078.830	36			
Corrected Total	25643.267	35			

Table S2. *Variance analysis of the effect of selenium-cadmium interaction on cadmium content in rape root*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	3674.152	11	334.014	924.819	0.000
Intercept	11879.910	1	11879.910	32893.152	0.000
Se Concentration	48.756	2	24.378	67.498	0.000
Cd Concentration	3611.284	3	1203.761	3332.980	0.000
Se Concentration * Cd Concentration	14.111	6	2.352	6.512	0.000
Error	8.668	24	0.361		
Total	15562.730	36			
Corrected Total	3682.820	35			

Table S3. *Variance analysis of the effect of selenium-cadmium interaction on cadmium content in rape stem*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	2304.868	11	209.533	1842.458	0.000
Intercept	6492.331	1	6492.331	57087.981	0.000
Se Concentration	18.450	2	9.225	81.118	0.000
Cd Concentration	2239.296	3	746.432	6563.483	0.000
Se Concentration * Cd Concentration	47.122	6	7.854	69.058	0.000
Error	2.729	24	0.114		
Total	8799.928	36			
Corrected Total	2307.598	35			

Table S4. *Variance analysis of the effect of selenium-cadmium interaction on cadmium content in rape seed*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	23.643	11	2.149	105.274	0.000
Intercept	96.138	1	96.138	4708.801	0.000
Se Concentration	1.187	2	0.593	29.058	0.000
Cd Concentration	21.751	3	7.250	355.126	0.000
Se Concentration * Cd Concentration	0.705	6	0.117	5.752	0.001
Error	0.490	24	0.020		
Total	120.271	36			
Corrected Total	24.133	35			

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Table S5. *Variance analysis of the effect of selenium-cadmium interaction on selenium content in rape root*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	83.854	11	7.623	2574.396	0.000
Intercept	75.053	1	75.053	25346.345	0.000
Se Concentration	81.634	2	40.817	13784.402	0.000
Cd Concentration	0.979	3	0.326	110.225	0.000
Se Concentration * Cd Concentration	1.240	6	0.207	69.812	0.000
Error	0.071	24	0.003		
Total	158.978	36			
Corrected Total	83.925	35			

Table S6. *Variance analysis of the effect of selenium-cadmium interaction on selenium content in rape stem*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	20.582	11	1.871	636.061	0.000
Intercept	20.657	1	20.657	7022.218	0.000
Se Concentration	18.765	2	9.383	3189.544	0.000
Cd Concentration	.545	3	0.182	61.810	0.000
Se Concentration * Cd Concentration	1.271	6	0.212	72.025	0.000
Error	0.071	24	0.003		
Total	41.310	36			
Corrected Total	20.652	35			

Table S7. *Variance analysis of the effect of selenium-cadmium interaction on selenium content in rape seed*

Source	Sum of Squares	df	Mean Square	F-value	Sig.
Corrected Model	20.782	11	1.889	482.365	0.000
Intercept	20.566	1	20.566	5250.951	0.000
Se Concentration	18.966	2	9.483	2421.236	0.000
Cd Concentration	0.465	3	0.155	39.581	0.000
Se Concentration * Cd Concentration	1.350	6	0.225	57.466	0.000
Error	0.094	24	0.004		
Total	41.442	36			
Corrected Total	20.876	35			

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