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Exogenous 24-Epibrassinolide Alleviated Selenium Stress in Peach Seedling

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Abstract

Selenium stress can adversely affect plants by inhibiting growth, impairing oxidative stress resistance, and inducing toxicity. In this experiment, we investigated the effect of exogenous 24-epibrassinolide (24-EBL; 2.0 mg/L), a brassinosteroid (BR), on alleviating selenium stress in peach trees by analyzing its impact on biomass, selenium accumulation, and the expression of selenium metabolism-related genes in peach seedlings. The results demonstrated that 24-EBL could effectively mitigate biomass loss in peach seedlings exposed to selenium stress. Compared to the Se treatment alone, the 24-EBL+Se treatment resulted in a significant 16.55% increase in root selenium content and a more pronounced 30.39% increase in selenium content in the aboveground parts. Regarding the subcellular distribution, the cell wall was the primary site of Se deposition, accounting for 42.3% and 49.8% in the root and aboveground parts, respectively, in the Se treatment. 24-EBL further enhanced Se distribution at this site, reaching 42.9% and 63.2% in root and aboveground parts, respectively, in the 24-EBL+Se treatment. The 24-EBL+Se treatment significantly increased the contents of different chemical forms of Se, including ethanol-soluble, water-soluble, and salt-soluble Se. The quantitative real-time PCR (qRT-PCR) results indicated that the Se treatment promoted the expression of organic Se assimilation genes (*SATs*, *OAS-TL B*, and *OAS-TL C*), and 24-EBL application further increased their expression. Meanwhile, the Se-only treatment up-regulated the organic Se metabolism gene *CGS1*. Consequently, we propose that 24-EBL alleviates Se stress in peach seedlings by enhancing Se uptake and assimilation, and by adjusting subcellular distribution and chemical forms.

Keywords: brassinosteroids; selenium determination; gene expression

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1. Introduction

Selenium is an essential trace element for human health, but the human body cannot synthesize it and must obtain it from external sources [1,2]. However, naturally occurring selenium cannot be directly absorbed or utilized by humans. Plants serve as a critical intermediary in selenium metabolism by absorbing inorganic selenium from the soil and converting it into bioavailable organic forms [3]. As a result, crops, fruits, and vegetables are ideal dietary sources of selenium, constituting the primary route of human selenium intake [4]. This has led to the widespread use of selenium-enriched fertilizers in agriculture to enhance the selenium content of food products [5].

However, excessive application of selenium fertilizers in agriculture can lead to environmental selenium pollution, posing risks to human health, disrupting ecosystems, and harming agricultural productivity [5]. For example, an intake of >400 μg/day is generally considered the threshold for toxic levels for humans [6,7]. The widespread use of selenium-enriched fertilizers may result in the gradual accumulation of selenium in soil and groundwater [8]. Elevated selenium levels can adversely affect plants by inhibiting growth, impairing oxidative stress resistance, and inducing toxicity [9]. Specifically, it disrupts root physiology, damages antioxidants, suppresses antioxidant enzyme activity, and destabilizes the plant's antioxidant defense system [9,10].

Selenium induces stress in plants through multiple mechanisms. The bioavailable forms of selenium for plant uptake in soil are primarily inorganic selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) [11]. These inorganic selenium species induce oxidative stress by generating excessive reactive oxygen species (ROS), disrupting intracellular antioxidant defenses, and causing phytotoxicity [12]. Furthermore, selenite is readily assimilated into organic forms. Excessive accumulation of organic selenium compounds, particularly selenocysteine (SeCys) and selenomethionine (SeMet), can lead to nonspecific incorporation into proteins, where they displace their sulfur (S) analogues. This substitution alters protein structure, impairs functionality, and disrupts enzymatic processes critical for plant metabolism [13].

Brassinosteroids (BRs) are a class of ubiquitous plant sterol hormones. They can enhance plant stress resistance by boosting antioxidant enzyme activity, optimizing antioxidant systems, and maintaining intracellular homeostasis [14,15]. These properties of BRs enable plants to tolerate various abiotic stresses such as extreme temperatures, salinity, drought, heavy metals, and pesticides [16]. Specifically, BRs have been shown to strengthen antioxidant defense systems in rice (*Oryza sativa*) under heavy metal (cadmium; Cd) stress [17] and reduce Cd and also phenanthrene (PAH) accumulation in tomato (*Solanum lycopersicum*) leaves and roots [18]. Recent studies demonstrated that synthesized BR (24-epibrassinolide; 24-EBL) alleviated zinc (Zn) stress in mung bean (*Vigna radiata*) seedlings, significantly improving germination rate, germination velocity, and seedling vigor index [19]. Our previous work also demonstrated the alleviating effect of 24-epibrassinolide on Se toxicity [20,21]. However, the metabolic mechanisms by which 24-EBL alleviates Se-induced stress in plants remain unclear.

Peach fruit has gained global popularity due to its delightful taste and nutritional benefits. As the foundation for nutrient absorption in peach trees, rootstock selection is crucial for cultivation. Fluffy peach ("MaoTao"), a native Chinese germplasm, serves as one of the most widely used rootstocks in peach production regions of China. This study investigated the potential of BRs to mitigate selenium stress in peach plants using fluffy peach seedlings as experimental material. We examined the effects of exogenous 24-EBL application on plant biomass and total selenium accumulation, subcellular selenium distribution patterns, chemical forms of selenium, and expression profiles of selenium metabolism-related genes. Our findings provide valuable insights into using 24-EBL as a potential treatment for enhancing plant tolerance to selenium stress, offering practical references for peach cultivation under selenium-rich conditions.

2. Materials and Methods

2.1. Plant Material and Growth Condition

Fluffy peach (*Prunus persica* (L.) Batsch) seeds were obtained from Chengdu, Sichuan Province, China. The experiment was conducted from March to May 2024 in the greenhouse of Sichuan Agricultural University, Chengdu Campus (30°43′ N, 103°52′ E), with day/night temperatures maintained at 25/20 °C, relative humidity at 70%/90%, and a 14/10 h photoperiod at 10,000 lx intensity. Seeds were germinated in perlite-filled trays under

controlled conditions. After emergence, seedlings were irrigated with Hoagland nutrient solution (Betensh, Hubei, China) every 3 days. When seedlings reached 10 cm height, uniform plants were transplanted into 32-cell plug trays (4 × 8 arrangement), containing perlite substrate, continuing with triweekly Hoagland solution irrigation. The Hoagland nutrient solution was pH 6.0. The main nutrient elements in the Hoagland solution were as follows: Ca^{2+} 23.625 mEq/L, K^{+} 15.425 mEq/L, $NH_4{}^{+}$ 2.375 mEq/L, Mg^{2+} 20.54 mEq/L, NO^{3-} 34.8 mEq/L, and $PO_4{}^{3-}$ 1.15 mEq/L. Each treatment was replicated in three trays.

2.2. Experimental Treatments

Ten days post-transplantation, three treatments were initiated: (1) Control (CK) receiving standard Hoagland solution; (2) Selenium treatment (Se) with 0.1 mg/L sodium selenite in Hoagland solution applied every 3 days [22]; and (3) Combined treatment (24-EBL+Se) receiving both the selenium solution and foliar sprays of 2.0 mg/L 24-EBL weekly for 4 weeks [21]. Each treatment included three biological replicates (trays). After one month of treatment, when seedlings reached 32–38 cm height with 21–25 leaves, complete plants (aboveground part and root) were harvested from all treatments for subsequent analysis of growth parameters, selenium content, subcellular distribution, chemical form, and gene expression related to selenium metabolism.

2.3. Plant Biomass Determination

The aboveground parts and roots were washed with tap water, followed by repeated rinsing with deionized water. Plant materials were then deactivated at 105 °C in a drying oven for 15 min and dried to constant weight at 70 °C. The biomass of roots and aboveground parts was measured separately using an electronic balance.

2.4. Total Selenium Content Determination

Dried plant materials were ground and sieved through a 100-mesh sieve. Exactly 0.500 g samples were weighed into Erlenmeyer flasks containing 9 mL concentrated nitric acid (analytical grade) and 1 mL perchloric acid (analytical grade). After 12 h of predigestion, samples were digested on an electric hot plate until the solution became transparent. After complete cooling, the solution was diluted to 25 mL with potassium ferricyanide solution (100 g/L). Selenium concentration was determined using an atomic fluorescence spectrometer (AFS-9700, Beijing Haiguang Instrument Co., Ltd., Beijing, China).

2.5. Inorganic Se and Organic Se Contents Determination

Dry plant materials (0.20–0.50 g) were digested with 20 mL 6 mol/L HCl at 70 °C for 2 h in a water bath shaker. After cooling and volume adjustment, the mixture was filtered through degreased cotton. The filtrate was heated in a boiling water bath for 20 min, cooled, and 1 mL was transferred to a 25 mL stoppered colorimetric tube. Following dilution to 10 mL with deionized water, 2 mL concentrated HCl and 1 mL potassium ferricyanide solution (100 g/L) were added to obtain the inorganic Se (Inorg-Se) content. Organic Se (Org-Se) content was calculated as the difference between total Se and inorganic Se. Selenium concentration was determined using an atomic fluorescence spectrometer (AFS-9700, Beijing Haiguang Instrument Co., Ltd., Beijing, China).

2.6. Selenium Content Determination in Plant Subcellular Distribution

Subcellular fractions from peach aboveground parts were extracted by differential centrifugation. Exactly 1.0 g of fresh root tissue was weighed and homogenized in a precooled mortar with extraction buffer (250 mmol/L sucrose, 50 mmol/L Tris-HCl [pH 7.5], and 1.0 mmol/L dithioerythritol) at a 1:10 (*w*/*v*) ratio. The homogenate was filtered through cheesecloth, with the residue washed into a beaker to obtain the cell wall fraction (CW-Se).

The filtrate was centrifuged at 20,000× *g* for 45 min at 4 °C to separate organelle fraction (O-Se, excluding vacuoles) in the pellet from the free fraction (F-Se) in the supernatant. All fractions were analyzed for selenium content by atomic fluorescence spectrometer (AFS-9700, Beijing Haiguang Instrument Co., Ltd., Beijing, China).

2.7. Determination of Selenium Chemical Forms

The analysis of selenium chemical forms was performed using a sequential extraction method. Dried aboveground part and root samples were defatted by stirring with acetone (1:10 *w*/*v*) for 4 h, repeated three times. After each defatting step, centrifugation was performed at 5000 rpm for 40 min. The combined supernatants yielded the water-soluble Se form (Fw). The residue was then extracted with 0.5 mol/L NaCl solution (2 h stirring) followed by centrifugation (5000 rpm, 4 °C, 20 min), repeated three times to obtain the salt-soluble Se form (Fs). Subsequent extractions with 0.6 mol/L acetic acid (HAC) solution and 80% ethanol under identical conditions yielded the weak acid-soluble (F_A) and ethanol-soluble (F_E) forms, respectively. The final residue represented the residual Se form (F_R). All speciations were analyzed for selenium content by atomic fluorescence spectrometer (AFS-9700, Beijing Haiguang Instrument Co., Ltd., Beijing, China).

2.8. Gene Expression Analysis Using Quantitative Real-Time PCR (qRT-PCR)

The expression levels of 14 selenium metabolism-related genes (*SULTR2;1*, *SULTR3;1*, *SULTR3;4*, *SULTR4;1*, *APS1*, *APR1*, *SAT3;1*, *SAT2;2*, *SAT1;1*, *OAS-TL B*, *OAS-TL C*, *NFS1*, *CGS1*, and *MS1*) in peach seedlings were analyzed using the primers listed in Table S1. Total RNA was extracted from leaves using the RNAprep Pure Plant Total RNA Extraction Kit (Tiangen Biotech, Beijing), followed by first-strand cDNA synthesis with the EasyScript One-Step gDNA Removal and cDNA Synthesis Kit (TransGen Biotech, Beijing, China). qRT-PCR was performed using TransStart Top Green qPCR SuperMix (TransGen Biotech, Beijing, China). The reaction system consisted of 1 μL cDNA, 0.4 μL forward primer, 0.4 μL reverse primer, 5 μL Green qPCR SuperMix, and 3.2 μL water. The thermal cycling program was as follows: 95.0 °C for 1 min, followed by 95.0 °C for 5 s and 55.0 °C for 30 s, repeated for 39 cycles, and finally, 95.0 °C for 10 s and 65.0 °C for 5 s. The *actin* gene was used as the internal reference gene. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with three technical replicates.

2.9. Statistical Analysis

Statistical analyses were performed using SPSS software SPSS 19.0 (IBM Corporation, Armonk, NY, USA). Significant differences among treatments were assessed using one-way analysis of variance (ANOVA) followed by *F*-tests, with a significance threshold of $p < 0.05$. Post hoc multiple comparisons were conducted using the Least Significant Difference (LSD) method.

3. Result

3.1. Biomass of Peach Seedlings

Under selenium stress conditions, both root and aboveground biomass of peach seedlings showed significant reductions ($p < 0.05$) compared to the control, decreasing by 15.24% and 6.24%, respectively (Figure 1). However, when selenium-stressed plants were treated with 24-EBL (24-EBL+Se treatment), both root and aboveground part biomass increased significantly by 7.84% and 3.69% relative to the selenium-only treatment, respectively ($p < 0.05$). These results demonstrated that foliar application of 24-EBL can effectively mitigate biomass loss in peach seedlings exposed to selenium stress.

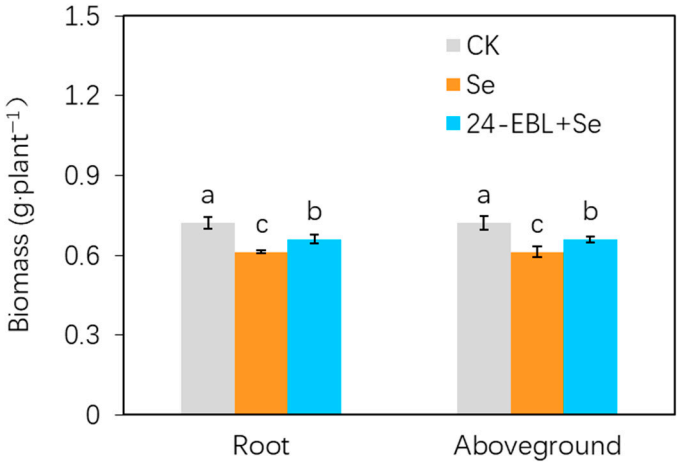


Figure 1. The biomass of root and aboveground parts. The lowercase letters a, b, and c denote statistically significant differences ($p < 0.05$).

3.2. Selenium Content in Peach Seedling

In both Se and 24-EBL+Se treatments, the majority of absorbed selenium accumulated in the root system (2.682 mg/kg and 3.126 mg/kg, respectively), with significantly lower concentrations detected in aboveground parts (0.362 mg/kg and 0.472 mg/kg, respectively) (Figure 2). Compared to the Se treatment alone, the 24-EBL+Se treatment resulted in a significant 16.55% increase in root selenium content (from 2.682 to 3.126 mg/kg) and a more pronounced 30.39% increase in selenium content in the aboveground part (from 0.362 to 0.472 mg/kg). In both the root and aboveground parts, the organic selenium content was significantly higher than the inorganic selenium content (Figure 2).

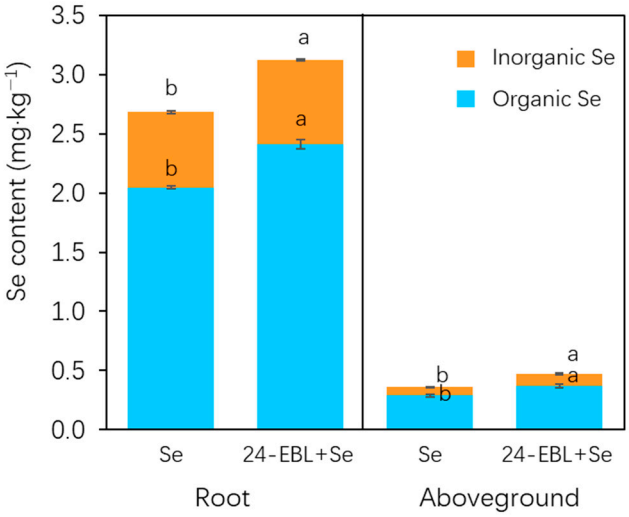


Figure 2. The selenium contents in root and aboveground parts. The lowercase letters a and b denote statistically significant differences ($p < 0.05$).

3.3. Subcellular Distribution of Selenium in Peach Seedlings

Both CW-Se and F-Se selenium fractions in 24-EBL+Se treatment showed significantly higher concentrations compared to the Se-only treatment, while the O-Se remained unchanged (Figure 3). In the aboveground part, 24-EBL+Se treatment also significantly increased CW-Se fractions (Figure 3). Consequently, in roots, the proportional Se distribution under Se treatment was CW-Se (42.3%), O-Se (23.1%), and F-Se (34.6%), which was similar to that in the 24-EBL+Se treatment: CW-Se (42.9%), O-Se (21.6%), and F-Se (35.5%) (Figure 4). By comparison, the distribution pattern shifted from CW-Se (49.8%),

O-Se (20.9%), and F-Se (29.3%) in the Se treatment to CW-Se (63.2%), O-Se (16.4%), and F-Se (20.4%) in the 24-EBL+Se treatment, with the cell wall-bound Se fraction increasing sharply while the organelle-bound and free Se fractions decreased proportionally (Figure 4). This suggested that the cell wall is the primary site of Se deposition, and 24-EBL further enhanced Se accumulation in this site.

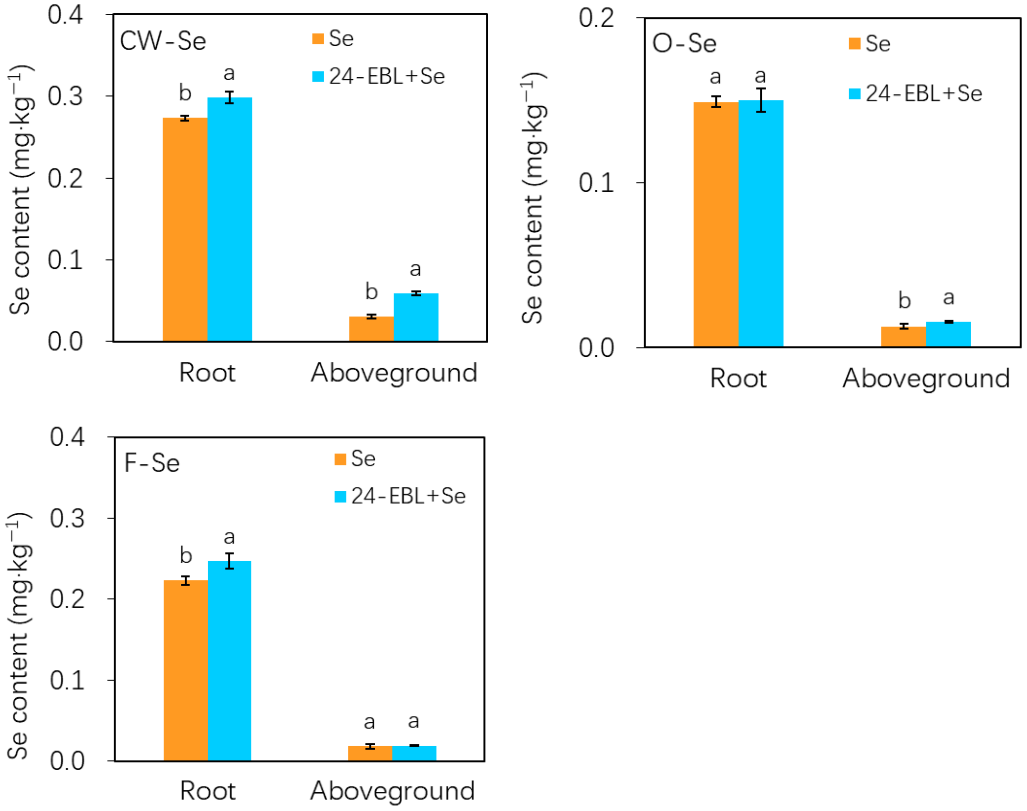


Figure 3. The selenium contents in subcellular fractions in root and aboveground parts. CW-Se, cell wall fraction Se; O-Se, organelle fraction Se; F-Se, free fraction Se. The lowercase letters a and b denote statistically significant differences ($p < 0.05$).

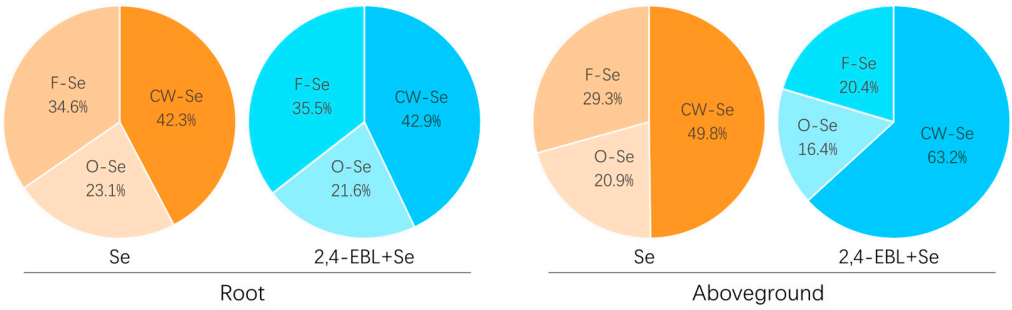


Figure 4. The proportional Se subcellular distribution in root and aboveground parts. CW-Se, cell wall fraction Se; O-Se, organelle fraction Se; F-Se, free fraction Se.

3.4. Chemical Forms of Selenium in Peach Seedlings

The 24-EBL+Se treatment significantly increased the contents of F_E (0.714 vs. 0.567 mg/kg), F_W (0.575 vs. 0.448 mg/kg), and F_S (0.440 vs. 0.380 mg/kg) in roots compared to the Se treatment alone (Figure 5). Similarly, in the aboveground part, the contents of F_E (0.151 vs. 0.104 mg/kg), F_W (0.065 vs. 0.051 mg/kg), and F_S (0.056 vs. 0.048 mg/kg) were significantly higher in the 24-EBL+Se treatment than in the Se-only treatment (Figure 5). However, the F_A content in both roots and aboveground parts significantly decreased

under 24-EBL+Se treatment, while no significant differences in F_R content were observed between the two treatments.

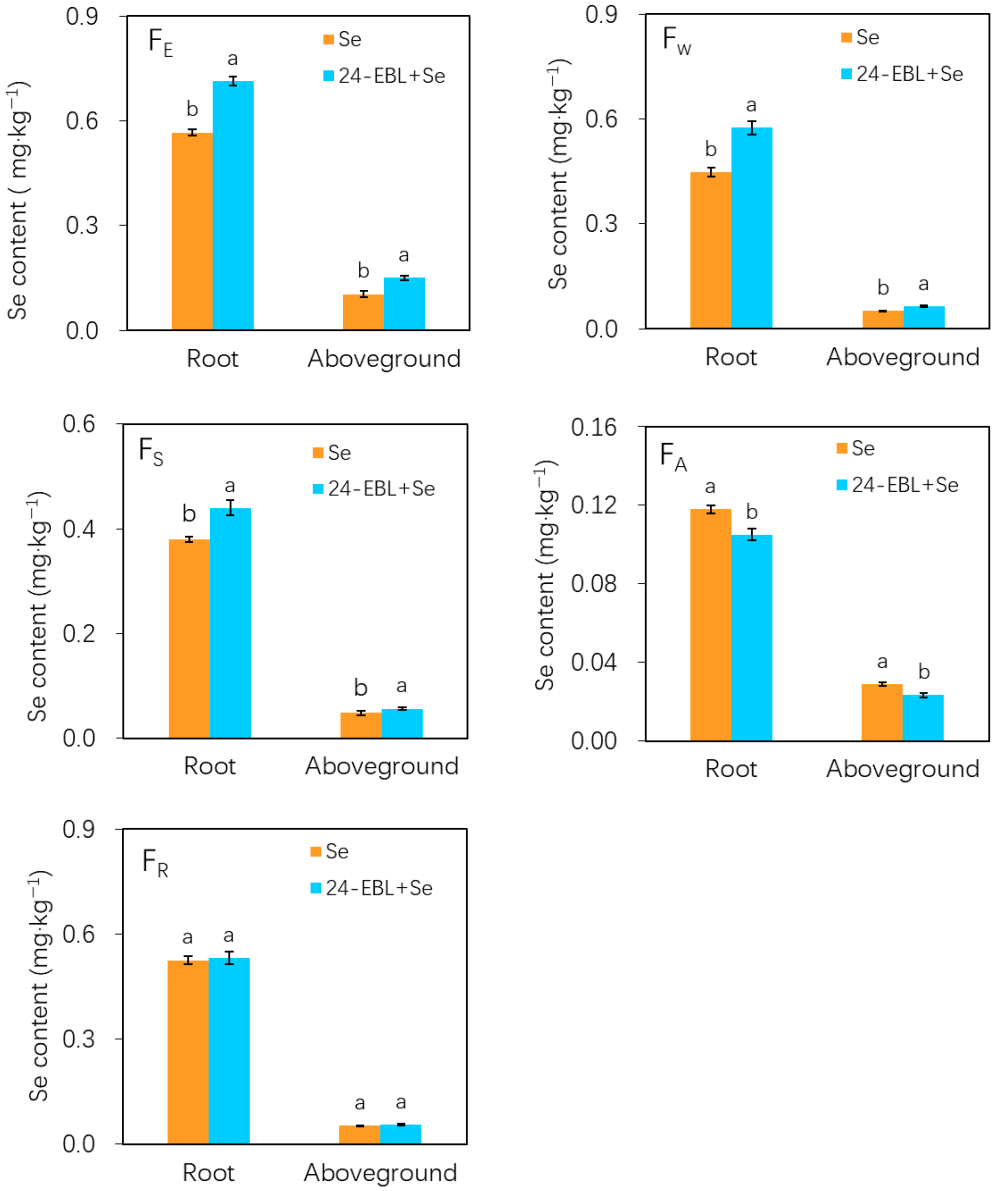


Figure 5. The selenium contents in different chemical forms in the root and aboveground parts. F_E, ethanol-soluble Se; F_W, water-soluble Se; F_S, salt-soluble Se; F_A, weak acid-soluble Se; F_R, residual Se. The lowercase letters a and b denote statistically significant differences ($p < 0.05$).

3.5. Expression Analysis of Selenium Metabolism-Related Genes in Peach Seedlings

Among the 14 genes tested, Se stress induced down-regulation of *SULTR2;1*, *SULTR3;4*, *APS1*, and *APR1* genes, while up-regulating *SAT3;1*, *SAT2;2*, *SAT1;1*, *OAS-TL C*, and *CGS1* (Figures 6 and 7) compared to the control. In the 24-EBL+Se treatment, similar expression patterns for the aforementioned genes were observed. Additionally, it significantly up-regulated *SULTR3;1* and *OAS-TL B* but did not alter the expression levels of *APS1* and *CGS1* significantly. Compared to the Se treatment, 24-EBL+Se significantly up-regulated the expression of *SULTR2;1*, *APS1*, *SAT3;1*, *OAS-TL B*, and *OAS-TL C*, while significantly down-regulating *SULTR3;1*, *APR1*, *NFS1*, and *CGS1* (Figures 6 and 7).

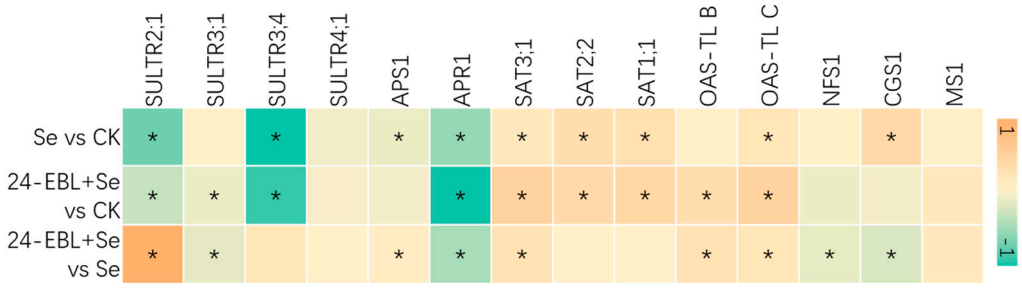


Figure 6. The qRT-PCR analysis of selenium metabolism-related genes. The colors show the $\log_{10}$ of different treatment ratios. "*" denotes the differentially expressed genes with a significance threshold of $p < 0.05$.

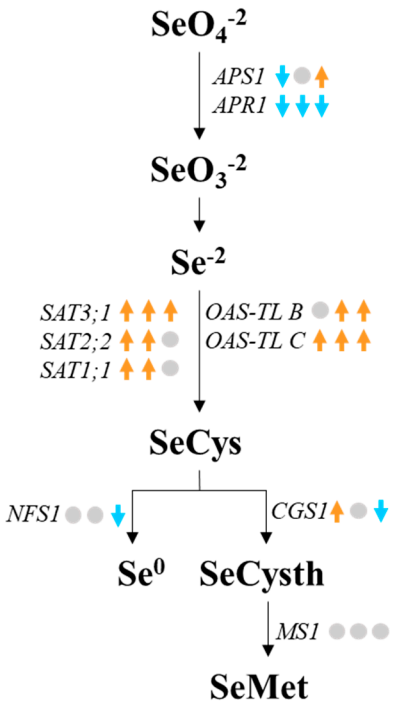


Figure 7. Schematic model of Se metabolism in peach seedling. The three arrows/circles next to gene names are Se vs. CK, 24-EBL+Se vs. CK, and 24-EBL+Se vs. Se. The orange up arrows indicate significantly up-expression, blue down arrows indicate significantly down-expression, and grey circles indicate no significant change.

4. Discussion

In this study, we used exogenous BR (24-EBL) to alleviate the effects of Se stress on the growth of peach seedlings, as indicated by the increased biomass in the 24-EBL+Se treatment compared to the Se-only treatment (Figure 1). By measuring the Se content in the plants, we found that the 24-EBL+Se treatment promoted the uptake of Se by the plants rather than inhibiting it (Figure 2). Therefore, 24-EBL might have activated certain metabolic mechanisms in the peach seedlings to prevent excessive damage caused by the absorbed Se. Similar functions of BRs have also been reported in alleviating the stress caused by cadmium, phenanthrene, and zinc [17–19]. Moreover, it indicated that the 24-EBL+Se treatment induced a more pronounced increase in organic Se content compared to inorganic Se (Figure 2). Since the organic Se in plants is derived from the assimilation of inorganic Se [23,24], these findings in the present study demonstrated that 24-EBL enhanced the assimilation process of inorganic Se in peach seedlings.

Plants employ subcellular distribution as a strategy to remove heavy metal toxicity [25,26]. Se can be bound to the cell wall, thereby limiting its effect on other intracellular

physiological and metabolic processes [27]. Our findings demonstrated that 24-EBL treatment significantly enhanced selenium sequestration in cell walls (CW-Se), particularly in the aboveground parts (Figures 3 and 4). This indicated that 24-EBL might mitigate selenium toxicity through preferential distribution to the cell wall, thereby protecting vital organelles from selenium-induced damage.

The results of selenium chemical speciation in the present study revealed that the 24-EBL+Se treatment significantly enhanced the levels of water-soluble, ethanol-soluble, and salt-soluble Se forms (Figure 5). The water-soluble form contains predominantly inorganic Se species along with small selenoamino acids, including selenolanthionine, glutamyl-methylselenocysteine (γGMSC), se-methylselenocysteine (MeSeCys), SeCys, and SeMet [28,29]. The ethanol-soluble form comprises monosaccharides, disaccharides, and low-molecular-weight organic Se compounds, while the salt-soluble form includes protein-bound Se and exchangeable Se species [29]. The findings in the present study demonstrated that the organic Se that 24-EBL+Se treatment promoted in peach seedlings was particularly stored as small-molecule organic Se, which contained several low-toxicity compounds, such as MeSeCys [30], selenocystathionine (SeCysth) [31], selenolanthionine [32], and γGMSC [33]. This shift in Se speciation likely contributed to the improvement in Se tolerance in peach seedlings under 24-EBL treatment. Concurrently, the increased incorporation of Se into proteins, as evidenced by elevated ethanol- and salt-soluble Se forms, might explain the relatively minor impact on biomass accumulation in the 24-EBL+Se treatment compared to the control (Figure 1).

The sulfate transporter gene *SULTR1;2*, known to mediate selenium/sulfate uptake from soil and their subsequent distribution to leaf tissues [34], exhibited low transcriptional expression in peach seedlings under both control (CK) and selenium stress conditions in the present research. Notably, 24-EBL application significantly upregulated *SULTR1;2* expression relative to both CK and selenium-treated plants (Figure 6), demonstrating that BRs enhance selenium acquisition through sulfate transport systems while facilitating root-to-shoot selenium translocation.

The SAT-OASTL enzyme complex (serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OASTL)) is responsible for the assimilation of inorganic Se into organic Se [35,36]. The present study revealed that under Se stress, the expression of *SATs*, *OAS-TL C*, and *OAS-TL B* was upregulated (Figures 6 and 7). Following 24-EBL application, the expression of these genes was further enhanced. This upregulation might ultimately lead to a significant increase in organic Se content in the 24-EBL+Se treatment (Figure 2).

One of the mechanisms of selenium toxicity in plants is the misincorporation of organic selenium species (e.g., SeCys and SeMet) into cysteine and methionine residues, then disrupting normal metabolic processes [37–39]. In contrast, the accumulation of water-soluble, non-protein-bound selenium forms is less toxic [6]. Other detoxification strategies in plants involve reducing selenate to elemental Se (Se^0) and selenide (Se^{2-}), which can then be volatilized as dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) [40]. We investigated three key genes in organic selenium metabolism, cysteine desulfurase (*NFS1*), cystathionine γ-synthase (*CGS1*), and methionine synthase (*MS1*) (Figures 6 and 7). NFS1 catalyzes the reduction of SeCys to Se^0, while CGS1 and MS1 mediate the conversion of SeCys to SeMet [27]. Notably, Se treatment alone significantly upregulated *CGS1* expression, suggesting enhanced SeMet biosynthesis. However, in the 24-EBL+Se treatment, *CGS1* expression levels showed no significant difference from the control and were even significantly lower than in Se-treated plants, indicating that attenuated SeMet production likely contributed to the reduction in selenium toxicity. Furthermore, our selenium chemical speciation analysis (Figure 5) suggested that 24-EBL promotes the accumulation of less

toxic water-soluble small selenoamino acids, providing the enhanced selenium tolerance observed in 24-EBL-treated peach seedlings.

5. Conclusions

This study demonstrated that 24-EBL application effectively mitigated selenium stress while promoting selenium accumulation in peach seedlings. Foliar application of 24-EBL enhanced plant biomass and total selenium content under selenium stress, facilitated organic selenium translocation from roots to aboveground, reduced selenium toxicity through cell wall sequestration of inorganic selenium, and upregulated key selenium metabolism genes (*Sultr1;2*, *SATs*, *OAS-TLs*, and *CGS1*). These molecular and physiological modifications collectively regulated organic selenium assimilation and conversion, minimized damage from excessive selenocysteine accumulation, and enhanced plant selenium enrichment capacity. Our findings provide a scientific foundation for employing 24-EBL, offering practical applications for selenium-enriched agricultural production systems. Future studies should employ more precise metabolite profiling (e.g., high-performance liquid chromatography (HPLC)) and comprehensive transcriptome analysis to better understand the mechanisms of 24-EBL in alleviating Se stress.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae11080909/s1, Table S1: Primers for qRT-PCR.

Author Contributions: Z.H.: Formal analysis, Investigation, Resources, Writing—original draft. Q.C.: Investigation, Resources, Writing—original draft. Y.D.: Investigation, Resources, Writing—original draft. J.Z.: Resources, Supervision, Writing—review and editing. L.L.: Resources, Supervision. M.Z.: Conceptualization, Writing—review and editing. X.W.: Formal analysis, Conceptualization, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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