Effect of Selenium (Se) on Inhibiting Embryo Abortion and Improving Seedling Quality of Red Sandalwood (*Pterocarpus santalinus*)

Chengxiang XU[1,2 *], Shuyi LIU[2], Yupeng LIN[2], Sitong LI[2]

1. Guangdong University of Business and Technology, Zhaoqing 526020, China; 2. College of Life Sciences, Zhaoqing University, Zhaoqing 526061, China

Abstract [**Objectives**] To explore the effect of selenium (Se) on inhibiting embryo abortion and enhancing seedling cultivation quality of Red sandalwood (*Pterocarpus santalinus*). [**Methods**] Based on prior cultivation practices and experimental research, three categories comprising 13 forest soil nutrient management schemes were designed to investigate the synergistic effects of Se, NPK compound fertilizers, and enzyme-microbe fermented organic fertilizers (EFOF) on embryo abortion, winged pod development, and seedling quality of Red sandalwood. [**Results**] Increasing the Se content in the soil, particularly in the form of selenite/Se(IV), within one month following the harvest of Red sandalwood pods and within two months prior to flower withering, significantly reduced embryo abortion percentage (EAP), and consequently improved seed quality and yield per plant. The effect of Se application was markedly greater than that of the single application of nitrogen (N), phosphorus (P), potassium (K), boron (B) fertilizers, or organic fertilizers. Furthermore, when Se was applied in combination with NPK compound fertilizers and EFOF, these beneficial effects were significantly enhanced. When Se(IV) was applied individually, the EAP decreased by 62.4%, reaching 24.8% at 8 weeks after flower withering (compared to 65.9% in the unmanaged control, UMC). Following winged pod maturation, the percentage of empty winged pods (PEWP) declined by 65.2% to 16.8% (UMC: 48.2%), the average individual winged pod weight (IWPW) increased by 69.1% to 0.690 g per fruit (UMC: 0.408 g), and the winged pod yield (WPY) rose by 214.8% to 4.03 kg (UMC: 1.28 kg). Additionally, the blasted seed percentage (BSP) was reduced by 51.2% to 29.9% (UMC: 61.3%), and the 100-seed weight (HSW) increased by 96.0% to 8.37 g (UMC: 4.27 g). Following sowing in the nursery, the seedling emergence rate (SER) increased by 6.57-fold, reaching 59.8% (UMC: 7.9%). Additionally, the whole plant biomass of 6-month-old seedlings increased by 52.9%, attaining 1.56 g (UMC: 1.02 g). The combined application of EFOF + NPK + Se(IV) significantly reduced the EAP, PEWP, and BSP by 56.5%, 46.0%, and 56.3%, respectively, compared to the single application of Se(IV). Furthermore, these percentages decreased by 79.7%, 78.9%, and 71.8%, respectively, relative to the single application of NPK compound fertilizers, and by 79.0%, 74.5%, and 72.1%, respectively, compared to the single application of EFOF. Additionally, the SER increased by 34.6%, 141.0%, and 287.0%, respectively, when compared to the single application of Se(IV), NPK compound fertilizers, and EFOF. [**Conclusions**] Enhancing the nutrient status of forest soils, particularly the concentration of Se(IV), constitutes a critical technical approach to improving the resistance of Red sandalwood to low-temperature stress during its flowering and fruiting stages, thereby preventing embryo abortion.

Key words Red sandalwood (*Pterocarpus santalinus*), Embryo abortion, Sodium selenite, Low-temperature stress, Winged pod development

0 Introduction

Globally, only a limited number of regions possess soils with high selenium (Se) concentrations exceeding 10 mg/kg. Among the highly water-soluble Se-containing salts present in the soil, Se^{4+} is more readily absorbed by plants than Se^{6+} and exhibits a greater capacity to mitigate stress[1]. Se plays a significant role in the growth, development, energy metabolism, and stress resistance of plants, insects, animals, and humans[2]. At low doses, Se benefits plants by promoting growth and development, enhancing the accumulation of active metabolites, activating the antioxidant defense system, and reducing lipid peroxidation[3]. Under conditions of high temperature, drought, and other stressors, Se application primarily protects plant leaves from oxidative damage by enhancing the antioxidant defense system[4]. Additionally, Se application can mitigate the detrimental effects of salt stress on plants by promoting their antioxidant defense mechanisms and improving intracellular ionic homeostasis[5]. The application of Se can mitigate the toxicity of heavy metal ions in plants. This function of Se is considered highly promising and may serve as a potential promoter of plant growth and protector against stress[6-7]. Recent studies have demonstrated that the expression of Se-binding protein (SBP) in plants is correlated with enhanced resistance to both abiotic and biotic stresses. *Theobroma cacao* SBP (TcSBP) is a heat-resistant protein associated with disease resistance against witches' broom. It exhibits affinity for selenite at the active site CSSC and demonstrates thermal stability. TcSBP functions by modulating the redox state of proteins during hydrogen peroxide (H_2O_2) production and programmed cell death in the terminal stage of witches' broom disease[8-9].

Red sandalwood (*Pterocarpus santalinus*) is regarded as the most valuable sandalwood species, serving both as a premium landscaping tree and an important medicinal plant. Nearly 70 years of introduction practices in China demonstrate that Red sandalwood thrives in the tropical and subtropical regions of South Asia, indicating significant potential for further promotion and cultivation[10-11]. The seeds and seedlings of Red sandalwood are notably expensive and scarce. The primary reason is that currently,

Received: August 20, 2025 Accepted: October 9, 2025

Supported by National Natural Science Foundation of China (31270674); Science and Technology Plan Project of Zhaoqing City (2019N012); National Undergraduate Science and Technology Innovation Training Program of China (202210580007).

* Corresponding author. Chengxiang XU, doctoral degree, professor, research fields: plant stress biology.

only a limited number of large, fruit-bearing trees are present in the introduced regions of central and western Hainan. Additionally, the germination rate of Red sandalwood seedlings is considerably low; when assessed using winged pods, the rate is below 20.0%, often around 10.5% or even lower. The highest germination rate observed for aseptically sown selected seeds is 31.7% [12-13]. The likely explanation is that the average percentage of empty winged pods (pods without seeds, PEWP) is 42.6%, and the blasted seed percentage (BSP) exceeds 60%, which differs significantly from that of *P. indicus*, where the average PEWP is only 1.5% and the BSP is less than 5.0%. Additionally, these percentages are substantially higher than the PEWP (8.5%) and BSP (15.8%) of *P. macrocarpus*.

The elevated PEWP and BSP of Red sandalwood are primarily attributable to a high incidence of embryo abortion occurring before the middle stage of fruit setting, specifically by the 10th week following flower withering. Red sandalwood begins flowering within the first ten days of December, attains peak flowering around middle December, and completes the withering process by the last ten days of the month. Embryo abortion percentages (EAP) measured at weekly intervals post flower withering are as follows: 9.2% (week 1), 17.3% (week 2), 26.5% (week 3), 34.6% (week 4), 42.8% (week 5), 58.3% (week 6), 65.2% (week 7), 68.5% (week 8), 71.6% (week 9), and 73.3% (week 10). The highest EAP is observed during the 6th week after flower withering. From the 8th week onward, over 65% of embryos fail to develop, and the rate of increase in EAP subsequently decelerates. Based on cultivation practices and comprehensive analysis, the authors have determined that the flowering and fruiting cycle of Red sandalwood is prolonged. The early stage of fruit development (1-8 weeks after flower withering) coincides with the low-temperature period, during which low-temperature stress is a critical factor contributing to embryo abortion. Therefore, enhancing low-temperature resistance during the reproductive critical period (early to middle stages of fruit development) is essential for improving seed quality and yield, as well as optimizing seedling propagation.

To validate the inference, this study conducted a phased experiment. From 2017 to 2019, forest land management interventions—including water regulation, mowing and mulching, hole expansion and soil loosening, understory vegetation mulching, and increased application of basal fertilizer—were implemented. Although these measures led to an increase in the yield of winged pods, improvements in PEWP and BSP were limited. From 2019 to 2020, the focus shifted to the regulation of multiple elements, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), boron (B), sulfur (S), and Se, with particular emphasis on Se. The results demonstrated that enhancing Se nutrition improved the low-temperature tolerance of Red sandalwood, significantly reduced embryo abortion, and increased both seed fullness and seedling survival rates. This study investigated the regulatory effects of the synergistic interaction between Se, NPK compound fertilizers and enzyme-microbe fermented organic fertilizers (EFOF) on embryo abortion and seedling quality enhancement, thereby providing a foundation for optimizing related agronomic practices.

1 Materials and methods

1.1 Forest site conditions The local average annual temperature is 23.3 ℃, and the annual rainfall measures 1 850 mm. The chemical properties of the 0-30 cm soil layer of laterite, developed from marine sediment parent material, are as follows: pH 4.8; organic matter content 11.5 g/kg; available nitrogen 7.9 g/kg; available phosphorus 1.52 mg/kg; available potassium 60.4 mg/kg; exchangeable calcium 693.5 mg/kg; exchangeable magnesium 80 mg/kg; available zinc 1.8 mg/kg; available boron 0.278 mg/kg; and selenium 0.365 mg/kg. The average diameter at breast height (DBH) of fruiting Red sandalwood in the study was 28.5 cm, with an average tree height of 13.6 m, an average clear bole height of 6.6 m, and an average crown diameter of 6.8 m.

1.2 Experimental design A total of three categories comprising 13 treatments were designed (Table 1). For all fertilizers and chemicals, 50% of the total application amount was applied as a basal fertilizer by middle May, while the remaining 40% was applied as a basal fertilizer by middle October of the same year. The adult Red sandalwood in the local area begin flowering in early December, reaching peak flowering in middle December, with a brief peak flowering period. Fruit ripening occurs at the end of April or the beginning of May of the following year, approximately 20 weeks after peak flowering. Annual forest management practices, aside from the varying fertilization treatments, were consistent across all treatment groups.

1.3 Test content and methods

1.3.1 EAP. The assessment of EAP was conducted at the 2nd, 5th, and 8th weeks following flower withering. Infructescences were randomly collected from the middle outer region of the tree crown using pruning shears. For each treatment and at each time point, 60 fruits were examined. Statistical analyses were performed based on the exact number of seeds observed. Embryo abortion was defined according to the following criteria: complete disappearance of ovules or embryos within the winged pod ventricle; or evidence of severe browning, blackening, shriveling, necrosis; or tissue softening accompanied by pronounced atrophy and wrinkling.

1.3.2 WPY and IWPW. At 18 weeks following flower withering, the winged pods were harvested by individual plants and placed in seed bags made of nylon mesh. These bags were then air-dried in a well-ventilated indoor environment. After 2 weeks, the pods per plant were weighed using an electronic balance with a precision of 0.01 g to determine WPY. For the measurement of IWPW, 20 fruits were randomly selected from each plant and weighed using an electronic balance with a precision of 0.001 g. A total of 180 winged pods were measured for each treatment.

1.3.3 PEWP. A winged pod containing at least one normally developed ovule (seed) is classified as a normally developed embryo fruit. The criteria for this classification include ovules or embryos that are plump, moist, and brittle; milky white in color; transparent internally; free from browning or necrosis; and without evident atrophy or wrinkle-like deformities. Pods not meeting these criteria are considered empty. Observations and statistical analyses should be conducted within one week following fruit ripening. For each plant, 50 winged pods were examined, resulting in a total of 300 fruits assessed per treatment.

Table 1 Experimental design

Category	Treatment	Operation	Specific content
Basic treatment	UMC (Unmanaged control)	No agricultural operations	No other human management practices excluding fertilization, irrigation, and weeding
Basic treatment	UVM (Understory vegetation mulching)	Vegetation mulching beneath tree canopies	Understory vegetation mulching after mowing weeds beneath tree canopies once a month
UVM-derived single fertilization treatment	EFOF (UVM + enzyme-microbe fermented organic fertilizer)	UVM + EFOF	Application of 10 kg of EFOF per plant based on UVM
UVM-derived single fertilization treatment	N	UVM + urea	Application of 400 g of urea (equivalent to 180 g N) per plant based on UVM
UVM-derived single fertilization treatment	P	UVM + superphosphate	Application of 1 000 g of superphosphate (equivalent to 180 g P_2O_5, 150.0 g Ca, and 100.0 g S) per plant based on UVM
UVM-derived single fertilization treatment	K	UVM + potassium chloride	Application of 300 g of potassium chloride (equivalent to 180 g KCl) per plant based on UVM
UVM-derived single fertilization treatment	NPK	UVM + NPK compound fertilizers + Na_2SeO_4	Application of NPK compound fertilizers (equivalent to 180 g N, 180 g P_2O_5, 180 g K_2O) + 8.5 g Na_2SeO_4 (equivalent to 3.6 g Se) per plant based on UVM
UVM-derived single fertilization treatment	B	UVM + boric acid	Application of 10 g of boric acid (equivalent to 1.7 g B) per plant based on UVM
UVM-derived single fertilization treatment	Se(VI)	UVM + Na_2SeO_4	Application of 8.5 g of Na_2SeO_4 (equivalent to 3.6 g Se) per plant based on UVM
UVM-derived single fertilization treatment	Se(IV)	UVM + Na_2SeO_3	Application of 8.5 g of Na_2SeO_3 (equivalent to 3.6 g Se) per plant based on UVM
UVM-derived compound fertilization treatment	EFOF + NPK	UVM + EFOF + NPK compound fertilizers + sodium selenate	Application of 10 kg of EFOF + NPK compound fertilizers (equivalent to 180 g N, 180 g P_2O_5, 180 g K_2O) + 8.5 g of Na_2SeO_4 (equivalent to 3.6 g Se) per plant based on UVM
UVM-derived compound fertilization treatment	NPK + Se(IV)	UVM + NPK compound fertilizers + Na_2SeO_3	Application of NPK compound fertilizers (equivalent to 180 g N, 180 g P_2O_5, 180 g K_2O) + 8.5 g of Na_2SeO_3 (equivalent to 3.6 g Se) per plant based on UVM
UVM-derived compound fertilization treatment	EFOF + NPK + Se(IV)	UVM + EFOF + NPK compound fertilizers + Na_2SeO_3	Application of 10 kg of EFOF + NPK compound fertilizers (equivalent to 180 g N, 180 g P_2O_5, 180 g K_2O) + 8.5 g of Na_2SeO_3 (equivalent to 3.6 g Se) per plant based on UVM

1.3.4 100-seed weight (HSW) and BSP. Seeds obtained from the assessment of PEWP in winged pods were used as the experimental materials. For each trial, the weight of 50 seeds without significant abortion was randomly measured, and the corresponding weight of 100 seeds was subsequently calculated. A total of 150 seeds were evaluated per treatment. BSP was determined using the same seed samples by calculating the percentage of plump seeds per plant. To distinguish blasted seeds, a water selection and grading method was employed: seeds that sank in water were classified as plump, whereas those that floated on the water surface were identified as blasted seeds.

1.3.5 Seedling emergence rate (SER). Sowing and seedling cultivation were conducted during the 2nd week following fruit harvest. Prior to sowing, the thinner marginal portions (wings) of the winged pods were removed, and the pods were soaked in tap water for 24 h. Seedlings were grown in non-woven fabric seedling bags measuring 25 cm in height and 6.5 cm in diameter. The seedling substrate consisted of 60% yellow heart soil, 35% coconut coir, and 5% perlite by volume. One pod was sown per seedling bag. Sixty days after sowing, the final SER was determined, expressed as the percentage of emerged fruits relative to the total number of sown fruits.

1.3.6 Growth of seedlings. When the seedlings reached 180 d of age (calculated from 30 d post sowing), 30 plants were selected from each treatment in accordance with the experimental design. Seedling height and basal diameter were measured using a steel ruler and a vernier caliper, respectively. Based on the measurements and subsequent analysis of seedling height and basal diameter, five seedlings were randomly chosen from each treatment to assess the length of the primary roots. Following drying to a constant weight in a forced-air drying oven, the dry weights of the roots, stems and leaves were recorded separately for each treatment, and the total dry weight of each whole plant was subsequently calculated.

1.4 Data statistics and analysis The mean, standard deviation, and graphical representations were computed using Microsoft Office Excel 2013 software. The significance of differences was assessed employing Duncan's new multiple range test at a significance level of $P < 0.05$.

2 Results and analysis

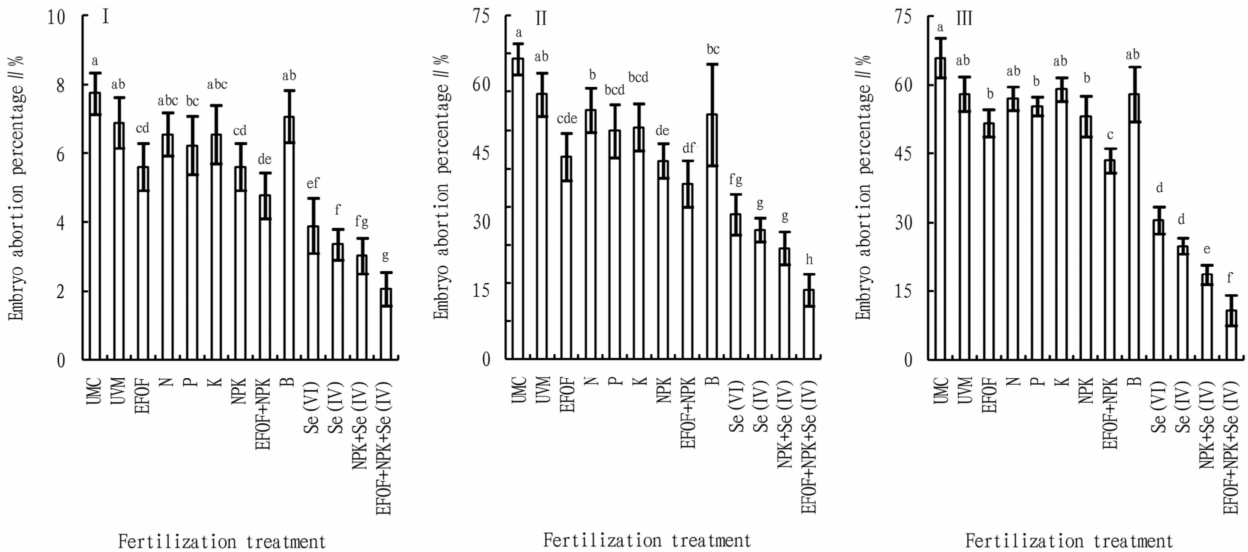
2.1 Effect of Se on the EAP of Red sandalwood Observations and statistical analyses conducted during the 2nd, 5th, and 8th weeks following flower withering revealed that various fertilization treatments did not alter the temporal differentiation patterns of embryo abortion of Red sandalwood. Nevertheless, compared to UMC during the same periods, all 12 forest soil management practices resulted in a reduction of EAP. The single application of B, N, and K fertilizers did not produce a significant decrease in the EAP of Red sandalwood. In contrast, the single application of EFOF, P, and NPK compound fertilizers significantly reduced EAP; however, these reductions were markedly less pronounced than those of single application of Se(IV) or Se (VI). Furthermore, the efficacy of Se(IV) surpassed that of Se(VI). Notably, the combined application of EFOF and NPK compound fertilizers alongside Se (IV) further enhanced the fertilization efficiency of Se(IV).

As illustrated in Fig. 1-I, 2 weeks following flower withering, the EAP of UMC was 19.3%. In comparison, the EAP of UVM and the single application of N, K, and B fertilizers based on UVM were 17.2%, 16.4%, 16.4%, and 17.6%, respectively; these differences were not statistically significant relative to UMC. Conversely, the EAP of the single application of P fertilizer was significantly lower than that of UMC, exhibiting a reduction of 19.5%. Furthermore, based on UVM, the EAP of the single application of EFOF and NPK compound fertilizers were both 14.0%, representing a 27.6% decrease compared to UMC; however, these percentages did not differ significantly from the treatment involving only P fertilizer application. The EAP of the EFOF + NPK treatment was 11.9%, which was significantly lower than that of the EFOF + P treatment. When Se(IV) was applied individually, the EAP was slightly higher than of the single application of Se(VI); however, both decreased by 49.7% and 56.5%, respectively, compared to UMC, and were significantly lower than the EAP of the EFOF + NPK treatment. The EAP of the EFOF + NPK + Se(IV) treatment was lower than that of the EFOF + Se (IV) treatment. Both treatments exhibited the EAP significantly lower than those of the single application of P fertilizer or the EFOF + NPK treatment, as well as lower than those of the single application of Se(VI) or Se(IV), all being below 10.0%. The EAP of the EFOF + NPK + Se(IV) treatment was the lowest, showing a reduction of 73.3% compared to UMC.

Five weeks after flower withering, the differences in EAP among the various treatments further increased (Fig. 1-II). Eight weeks after flower withering, when EAP tended to stabilize and no longer decreased significantly, these differences reached their maximum. As illustrated in Fig. 1-III, at 8 weeks following flower withering, the EAP of UVM and the single application of N fertilizer, K fertilizer, and Se(VI) based on UVM were comparable, showing no significant difference from that of UMC (65.9%). In contrast, the EAP of the single application of P fertilizer, EFOF, and NPK compound fertilizers were similar to each other but significantly lower than that of UMC. The EFOF + NPK treatment demonstrated a significantly greater efficacy in inhibiting embryo abortion compared to the other treatments evaluated. The EAP of this treatment was 27.2% lower than that of UMC, yet remained substantially higher than that of the four Se treatments. Specifically, the two treatments with the single application of Se, Se(VI) and Se(IV), reduced the EAP by 46.9% and 54.8%, respectively, relative to UMC. These findings further suggest that Se application exerts the most pronounced and effective role in preventing embryo abortion of Red sandalwood, surpassing the effects of B, N, K, P fertilizers, as well as EFOF and NPK compound fertilizers applied individually.

2.2 Effect of Se on the WPY, IWPW and PEWP of Red sandalwood Fig. 2-I illustrated that, in comparison to UMC, the application of UVM and the single application of K, B, P, and N fertilizers resulted in the smallest increases in the WPY of Red sandalwood, with respective increases of 3.1%, 5.5%, 5.5%, 11.7%, and 27.3%. No significant differences were observed among these treatments. Furthermore, the single application of EFOF based on UVM led to a significant WPY increase of 53.9% compared to UMC. The single application of NPK compound fertilizers produced a significantly greater yield increase than the six aforementioned treatments. Although the WPY was significantly enhanced relative to UVM, the increase rates were 30.3% and 58.0% lower, respectively, than those of the single application of Se(VI) and Se(IV) based on UVM. Overall, Se application demonstrated a markedly superior effect on yield enhancement. The NPK + Se(IV) treatment and the EFOF + NPK + Se(IV) treatment resulted in a greater increase in WPY. Specifically, the WPY increased by 98.0% and 71.2%, respectively, compared to that of the single application of NPK compound fertilizers or the combined application of EFOF and NPK compound fertilizers. Compared to UMC, the WPY increased by 294.5% and 335.9%, with the yields reaching 5.05 and 5.58 kg/plant, respectively, demonstrating a highly significant effect.

The effects of various fertilization treatments on the IWPW of Red sandalwood exhibited a response pattern similar to that observed for the WPY of individual plants. Although the differences were diminished, they remained statistically significant. As illustrated in Fig. 2-II, both UVM and the single application of B and K fertilizers resulted in an increase in IWPW relative to UMC; however, this increase was modest, averaging 12.5%, and did not reach statistical significance. On the basis of UVM, the single application of N, P, NPK compound fertilizers, and EFOF did not produce significant differences in IWPW, yet each treatment demonstrated a significant increase compared to the previously mentioned four treatments, with an average enhancement of 37.6% relative to UMC. Regarding the two treatments involving the single application of Se(VI) and Se(IV), the IWPW were comparable and both exhibited significant increases compared to the nine aforementioned treatments. The respective increases relative to UMC were 61.0% and 69.1%. The application of elevated levels of NPK compound fertilizers combined with Se(IV) and the subsequent combined application of EFOF further increased the IWPW to 0.765 and 0.805 g/fruit, respectively. Compared to the single application of NPK compound fertilizers and the combined application of EFOF + NPK compound fertilizers, the IWPW increased by 37.1% and 36.4%, respectively. Relative to UMC, these treatments resulted in increases of 87.5% and 97.3%, respectively.



NOTE I: Two weeks after flower withering; II: Five weeks after flower withering; III: Eight weeks after flower withering. UMC: Unmanaged control; UVM: Understory vegetation mulching; EFOF: Enzyme-microbe fermented organic fertilizer; N: Single application of urea based on UVM; P: Single application of calcium superphosphate based on UVM; K: Single application of potassium chloride based on UVM; NPK: Single application of NPK compound fertilizers (CF) based on UVM; EFOF + NPK: Application of EFOF and NPK-CF based on UVM; B: Single application of boric acid based on UVM; Se(VI): Single application of Na_2SeO_4 based on UVM; Se(IV): Single application of Na_2SeO_3 based on UVM; NPK + Se(IV): Application of NPK-CF and Na_2SeO_3 based on UVM; EFOF + NPK + Se(IV): Application of EFOF, NPK-CF and Na_2SeO_3 based on UVM. The same below.

Fig. 1 Effect of fertilizer chemical composition on the EAP of Red sandalwood

The effects of various fertilization treatments on the PEWP of Red sandalwood exhibited trends opposite to those of WPY and IWPW, with these differences reaching statistical significance. As illustrated in Fig. 2-III, the average PEWP subjected to UVM and the single application of N, K, NPK, and B fertilizers was 43.9%, showing no significant difference compared to UMC. Similarly, the single application of EFOF and P fertilizers did not lead to significant changes in PEWP, and no notable differences were observed when compared to the single application of NPK compound fertilizers and B fertilizer. However, relative to UMC, the PEWP were significantly reduced by 19.7% and 26.1%, respectively. The combined application of EFOF + NPK compound fertilizers further decreased PEWP, resulting in a value 41.3% lower than that of UMC. No significant difference in PEWP was detected when compared to the single application of EFOF; however, the PEWP remained significantly higher than those of the four Se application treatments. Among these four selenium treatments—namely, the single application of Se(VI) and Se(IV), as well as the combined application of NPK + Se(IV) and EFOF + NPK + Se(IV)—no significant differences in PEWP were found. Nonetheless, these treatments demonstrated significantly lower PEWP than the other nine treatments, with reductions of 64.5%, 65.2%, 73.0%, and 81.2% relative to UMC, respectively.

2.3 Effect of Se on the HSW, BSP and SER of Red sandalwood Compared to UMC, UVM and the other 11 treatments based on UVM all resulted in an increase in the HSW of Red sandalwood. However, no significant difference in HSW (ranging from 4.27 to 4.96 g) was observed between UVM, the single application of K fertilizer, and UMC. In contrast, the HSW for the remaining 10 treatments differed significantly from that of UMC. The single application of B, N, and K fertilizers led to increases in HSW, although these differences were not statistically significant. Notably, the single application of P fertilizer increased the HSW by 14.2% compared to the single application of N fertilizer; however, this increase was less pronounced than that of the single application of EFOF and NPK compound fertilizers. The combined application of EFOF + NPK compound fertilizers resulted in increases in HSW of 13.6% and 6.9%, respectively, compared to their single application, and an increase of 76.3% relative to UMC. However, these increases were significantly lower than those observed in the four Se application treatments. As illustrated in Fig. 3-I, when Se(VI) and Se(IV) were applied individually, the HSW of the single application of Se(VI) was 3.7% higher than that of the single application of Se(IV), although this difference was not statistically significant. Both treatments increased HSW by 88.9% and 96.0%, respectively, compared to UMC. Furthermore, the combined application of NPK compound fertilizers + Se(IV), and subsequent addition of EFOF, led to further significant increases in HSW, exceeding those of the single application of Se(IV). Specifically, the HSW increased by 110.8% and 115.5%, respectively, compared to UMC.

The effects of various fertilization treatments on the BSP of

Red sandalwood exhibited an inverse relationship with the trend observed for seed HSW, with a more pronounced difference; specifically, higher HSW corresponded to lower BSP. As illustrated in Fig. 3-I, no significant differences in BSP were observed among treatments associated with UVM, the single application of K fertilizer, and UMC, which recorded BSP of 61.2%, 60.8%, and 56.4%, respectively. In contrast, the single application of B, N, P, NPK compound fertilizers, and EFOF resulted in significantly lower BSP compared to UMC, with reductions of 10.0%, 10.4%, 13.4%, 14.2%, and 14.5%, respectively; however, no significant differences were detected among these treatments. The combined application of EFOF + NPK compound fertilizers significantly reduced BSP compared to the eight previously mentioned treatments, exhibiting a 22.1% reduction relative to the control. However, this reduction was significantly less pronounced than those of the four treatments involving Se application. As illustrated in Fig. 3-II, the single application of Se(IV) resulted in a slightly higher BSP than the single application of Se(VI), yet both were significantly lower than UMC, with an average reduction of 49.2%. Furthermore, the the combined application of NPK compound fertilizers + Se (IV), supplemented with EFOF, produced a more substantial decrease in BSP, with reductions of 66.2% and 77.8% relative to UMC, respectively. These percentages were also significantly lower than those of the single application of Se(VI).

The effects of various fertilization treatments on the SER of Red sandalwood were inverse to those observed for BSP, aligning with the response pattern of HSW; however, the differences among treatments were more pronounced. As illustrated in Fig. 3-III, the SER of UVM and the single application of B, K, and N fertilizers ranged from 9.4% to 12.2%. Although these figures represented an increase compared to UMC, the differences were not statistically significant. In contrast, the single application of P fertilizer and EFOF resulted in an average SER of 19.2%, which did not differ significantly between these two treatments but was significantly higher than those of UMC and the other four treatments mentioned above. The SER of the single application of NPK compound fertilizers and the combined application of EFOF were 33.4% and 39.6%, respectively. Although no significant difference was observed between these two treatments, both figures were significantly higher than those of UMC and the other 6 treatments mentioned. When Se(VI) and Se(IV) were applied individually, the SER were 54.6% and 59.8%, respectively. While the difference between these two treatments was not statistically significant, both exhibited significantly higher SER compared to UMC and the previously mentioned treatments. Specifically, these SER figures represented increases of 591.1% and 656.9% compared to UMC, respectively. Furthermore, the combined application of NPK + Se (IV), as well as the addition of EFOF to this combination, resulted in a further significant increase in SER, surpassing that of the single application of Se(IV).

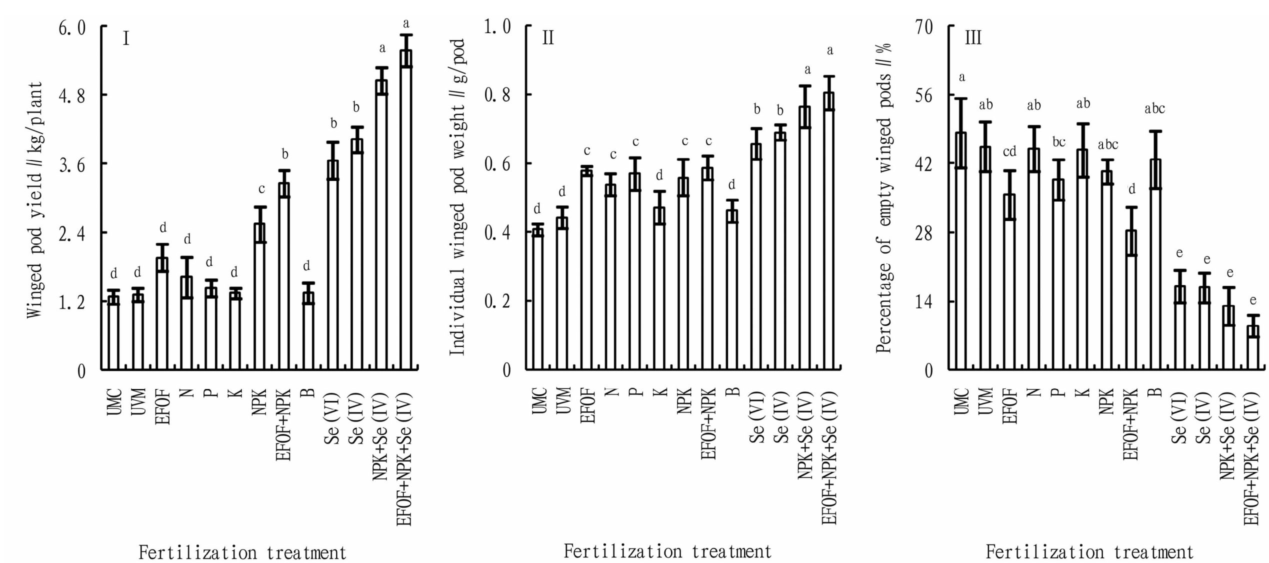


Fig. 2 Effect of fertilizer chemical composition on the WPY (I), IWPW (II) and PEWP (III) of Red sandalwood

2.4 Effect of Se on the seedling growth of Red sandalwood

Seeds from Red sandalwood subjected to various fertilization treatments were sown. Six months following mass germination (middle December of the same year), assessments revealed significant differences in seedling height, basal diameter, and taproot length among seedlings derived from seeds of trees exposed to different fertilization treatments. The most pronounced effect was observed on plant height, followed by taproot length, whereas the impact on basal diameter was comparatively minor. Furthermore, the response patterns of these three growth parameters generally corresponded with those of fruit production and seed development across various fertilization treatments. Although Se-treated trees produced seeds that significantly enhanced seedling growth post germination, this effect differed somewhat from the more substantial impacts previously noted on fruit yield and seed quality. The magnitude of these effects was closely associated with specific growth indicators.

As illustrated in Fig. 4-I, the seedling heights of Red sandalwood grown from seeds treated exclusively with UVM or concurrently with the single application of B, K, or P fertilizers were similar and did not differ significantly from the control group.

Seedlings derived from seeds treated with the single application of N fertilizer, EFOF, or NPK compound fertilizers also exhibited similar heights; however, these heights were significantly greater than those observed in the previously mentioned five treatments. Seedlings grown from Red sandalwood seeds treated individually with Se(VI) or Se(IV) exhibited greater height compared to those treated exclusively with NPK compound fertilizers. Their height was comparable to seedlings treated with NPK + Se(IV), yet remained shorter than those derived from seeds treated with NPK + Se(IV) + EFOF.

As illustrated in Fig. 4-II, the basal diameter of seedlings grown from Red sandalwood seeds subjected to UVM and the single application of B, K, P, and N fertilizers was slightly greater than that of UMC. Seedlings under the remaining seven treatments exhibited basal diameters larger than that of UMC; however, no significant differences were detected among these six treatments. Regarding taproot length, seedlings treated individually with B, K, and N fertilizers did not differ significantly from that in UMC. In contrast, the other nine treatments demonstrated significant increases in taproot length compared to UMC, although no significant differences were found among these eight treatments (Fig. 4-III).

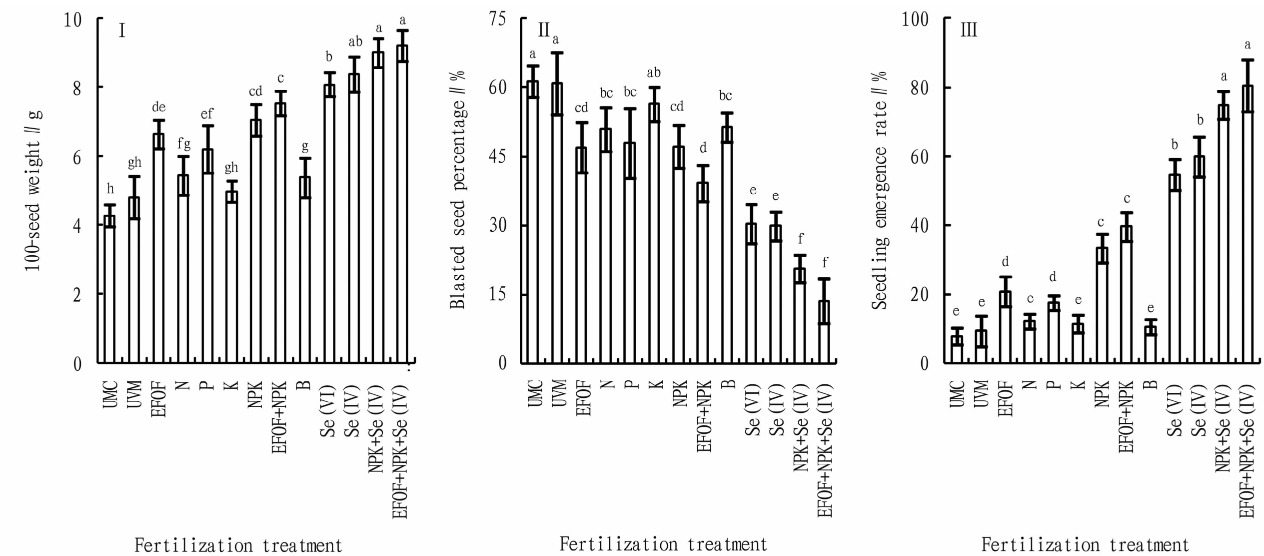
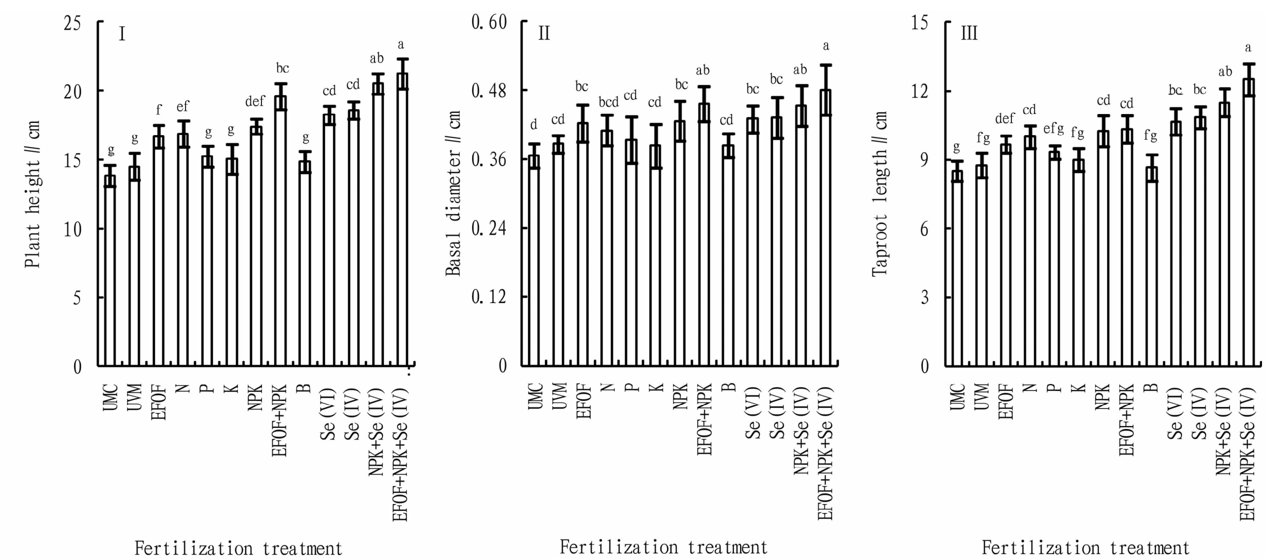


Fig. 3 Effect of fertilizer chemical composition on the HSW (I), BSP (II) and SER (III) of Red sandalwood



NOTE Seedling age: 6 months; I: Plant height; II: Basal diameter; III: Taproot length.

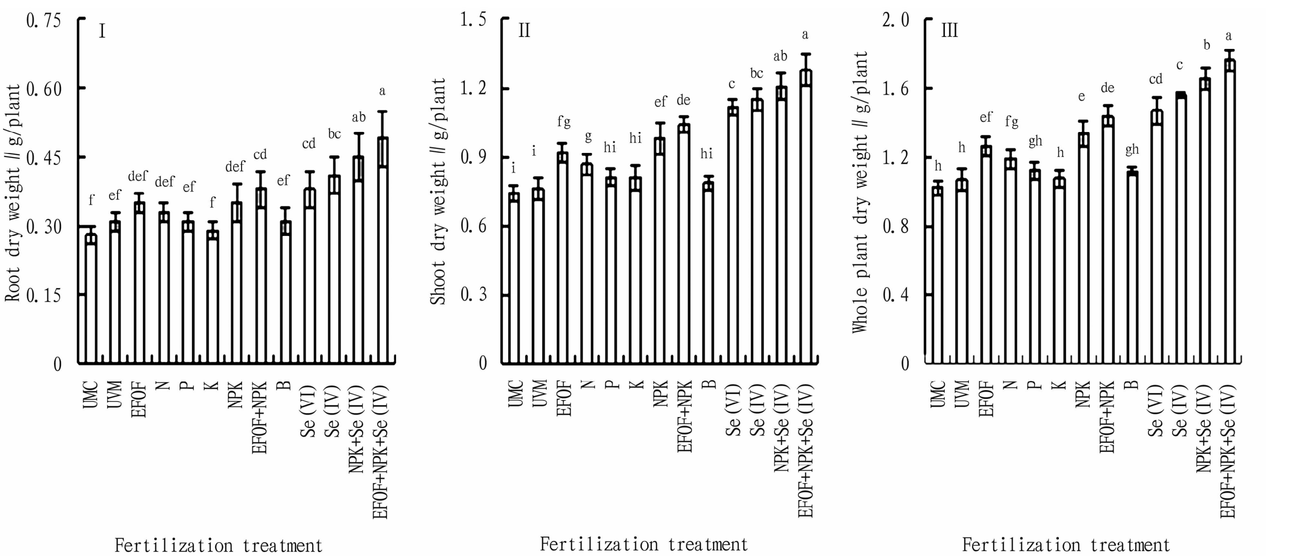
Fig. 4 Effect of fertilizer chemical composition on the seedling growth of Red sandalwood

2.5 Effect of Se on the seedling biomass of Red sandalwood

Seedlings grown from the seeds of Red sandalwood subjected to seven treatments, including UVM and the single application of K, B, P, N, EFOF, and NPK compound fertilizers based on UVM, exhibited comparable root dry weights, all of which increased relative to UMC. Notably, the single application of EFOF and NPK compound fertilizers resulted in increases of 25.0% and 28.6%, respectively; however, these differences were not statistically significant compared to UMC. In contrast, the root dry weight of seedlings cultivated from seeds treated with the other five treatments was significantly greater than that of UMC. The root dry weights of seedlings grown from seeds of trees treated individually with Se(VI) and Se(IV) were 39.3% and 46.4% greater, respectively, than that of UMC. Additionally, these weights were 2.6% and 7.9% higher, respectively, than those of seedlings from trees treated with the combined application of EFOF + NPK compound fertilizers. Notably, the root dry weights of seedlings derived from seeds of trees treated with the combined application of NPK + Se(IV) and EFOF + NPK + Se(IV) exhibited further increases, showing enhancements of 60.7% and 75.0%, respectively, compared to UMC, and increases of 9.8% and 19.5%, respectively, relative to the single application of Se(IV) (Fig. 5-I).

The stem and leaf dry weights of seedlings grown from the seeds of Red sandalwood treated with UVM and the single application of B, K, or P fertilizers were comparable to each other and did not differ significantly from the control. However, these weights were significantly lower than those of the single application of N fertilizer, EFOF, NPK compound fertilizers, and the combined application of EFOF + NPK compound fertilizers. Seedlings derived from seeds treated individually with Se(VI) and Se(IV) exhibited a significant increase in stem and leaf dry weight, exceeding the figures observed in the aforementioned nine treatments, with increases of 49.3% and 55.4% compared to UMC. Furthermore, the root dry weights of seedlings cultivated from seeds subjected to the combined application of NPK + Se(IV) and EFOF + NPK + Se(IV) increased by 60.7% and 75.0%, respectively, compared to the control (Fig. 5-II).

Seedlings grown from seeds produced by Red sandalwood subjected to various fertilization treatments demonstrated greater variability in whole plant dry biomass compared to root dry weight or stem-leaf dry weight. This variability was predominantly driven by stem-leaf dry weight, which showed response patterns closely mirroring those observed in whole plant dry biomass. As illustrated in Fig. 5-III, the total biomass of seedlings derived from the seeds of Red sandalwood treated with UVM and the single application of K, B, and P fertilizers was comparable, with no significant difference observed relative to UMC. Notably, the biomass of seedlings originating from seeds of the four tree types treated with Se was significantly greater than that observed in the nine aforementioned treatments. The biomass of seedlings grown from seeds of trees treated individually with Se(VI) and Se(IV) increased by 44.1% and 52.9%, respectively, compared to UMC. Furthermore, the combined application of NPK + Se(IV) and EFOF + NPK + Se(IV) enhanced the whole plant biomass of the cultivated seedlings by 62.7% and 72.5%, respectively, relative to UMC.



NOTE Seedling age: 6 months; I: Root dry weight; II: Shoot dry weight; III: Whole plant dry weight.

Fig. 5 Effect of fertilizer chemical composition on the seedling biomass of Red sandalwood

3 Discussion

The zygote, formed by the fusion of sperm and egg in plants, is a totipotent cell that initiates the primary cell lineage and tissue formation through cell division and cell-specific regulatory mechanisms during early embryonic development. During the subsequent morphogenetic stage, full-size embryos are produced, which can subsequently develop into complete seeds. Although plant embryos exhibit considerable conservation during the early stages of development, they display substantial diversity at maturity, particularly among angiosperms. Domestication plays a significant role in influencing seed quality traits established during the embryonic phase. Additionally, it facilitates the capacity of seeds to germinate under a variety of environmental conditions. The formation, development, and growth of this process represent an exceedingly complex and delicate biological phenomenon, influenced by a multitude of internal and external factors[14]. Among these factors, mineral nutrition holds significant importance and is closely associated with embryo abortion, exerting profound and extensive effects[15-17].

This study demonstrated that Se supplementation, particularly in the form of Se^{4+}, in the forest soil of Red sandalwood significantly reduced the EAP, PEWP, and BSP, markedly enhances the WPY, IWPW, and HSW, and significantly promoted the seed germination, emergence rate, and seedling growth of Red sandalwood. Notably, these beneficial effects were not observed in the single application of NPK compound fertilizers, increased soil organic matter, increased application of EFOF, or the single application of N, P, K, Ca, B, and S fertilizers. Although Se cannot yet be classified as an essential element for the growth and development of Red sandalwood, the findings clearly indicate that Se is a critical mineral nutrient that prevents embryo abortion, supports proper seed development, and ensures seed yield and quality. Among all treatments, the effect of EFOF ranks second only to that of Se addition, while the impact of increasing NPK compound fertilizers does not achieve a comparable effect. However, when exogenous Se is applied in conjunction with EFOF, the synergistic effect of Se is markedly enhanced. This finding suggests that EFOF exhibits a strong synergistic interaction with Se, an effect that is not observed when EFOF is applied alone or combined with other mineral elements. This discovery represents a significant advancement in the scientific study of Red sandalwood cultivation, bearing considerable theoretical and practical implications. Currently, the limited number of Red sandalwood introduced and cultivated over the past two decades in Hainan, Guangdong, Guangxi, and Yunnan are approaching their fruiting stage, highlighting the importance of further exploration and application of this technology.

Embryo abortion during plant development involves complex embryological mechanisms[18], physiological and biochemical processes[19], and is significantly influenced by gene regulation[20]. Based on previous observational studies and the experiments conducted in this research, it is evident that male sterility, female sterility, and inadequate pollination and fertilization have minimal or no significant impact on the embryological mechanism underlying embryo abortion of Red sandalwood. The primary issue is the continuous embryo abortion occurring before the 8^{th} week after flower withering, with an abortion rate approaching 60%. The main manifestations include rapid atrophy, depolymerization, and degradation of embryonic tissue. It is hypothesized that intense cellular oxidation and autophagy may be involved, potentially resulting from low Se content in the soil or insufficient uptake by plants. These factors likely trigger a series of physiological and biochemical responses that promote embryo abortion immediately following ovule fertilization, culminating in a peak abortion rate at the 8^{th} week after flower withering.

Among the physiological and biochemical mechanisms underlying embryo abortion, endogenous hormone levels, the activity of antioxidant enzymes, polyamines, and phenolic compounds all influence the normal development of embryos. *Ziziphus jujuba* is a widely recognized woody fruit tree characterized by a notably high EAP. Research has indicated that embryo abortion is influenced by disparities in hormone concentrations between the embryo and the surrounding pulp. Specifically, embryo abortion occurs when the levels of zeatin (ZT), gibberellic acid (GA_3), and indole-3-acetic acid (IAA) in the seed are lower than those in the pulp[21]. Studies on the embryonic development of *Litchi chinensis* have demonstrated that the concentrations of IAA, GA_3, and cytokinins (CTK) are relatively elevated in aborted embryos, whereas these levels are lower in normal embryos. Furthermore, elevated abscisic acid (ABA) content appears to impede the normal development of young embryos. Additionally, increased levels of phenolic compounds may disrupt the balance between growth-promoting and growth-inhibiting substances and could play a role in the regulation of DNA synthesis[22]. The synergistic effects of multiple hormones in regulating embryo abortion in plant species have been well proved[23]. Research findings indicate that the Se nutrient status in forest soil significantly influences the content and ratio of endogenous hormones during seed development of Red sandalwood.

Test analysis demonstrated that, in 13 types of adult Red sandalwood treated with nutrients in forest land, Se treatment significantly increased the contents of IAA, GA_3, and ABA, and the combined levels of IAA and GA_3, as well as the ratio of $(IAA + GA_3)/ABA$ at 2, 5, 10, and 15 weeks following flower withering. Prior to the middle stage of seed growth and development, Se nutritional status exerts a pronounced influence on the ratio of $(IAA + GA_3)/ABA$ in seeds. Additionally, the Se nutrient status in forest soil significantly affects the activities of antioxidant enzymes of Red sandalwood seeds. Specifically, at 2, 5, 10, and 15 weeks following flower withering, the activities of catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) of the seeds from Se-treated plants were significantly elevated compared to those of the control group. Notably, even 15 weeks after flower withering, the Se-dependent enzyme GR maintained relatively high activity levels. Se is involved in the composition of GR and serves as the primary flavin enzyme responsible for maintaining glutathione (GSH) levels within cells[24]. Path analysis results demonstrated that the EAP of Red sandalwood seeds at various developmental stages was highly significantly correlated with the concentrations of the three aforementioned endogenous hormones and the activities of four antioxidant enzymes. Although the relevant study has not yet been published, it offers valuable insights into the mechanism by which Se mitigates the high EAP in Red sandalwood.

The combination of EFOF and Se enhances the inhibitory effect of Se on embryo abortion of Red sandalwood, representing a noteworthy phenomenon whose underlying mechanism warrants further investigation. The authors hypothesize that EFOF facilitates the transformation of Se in the soil, thereby promoting its absorption and utilization by the root system of Red sandalwood. Additionally, Se may positively influence the physiological activity of EM enzyme-microbial communities. EFOF comprises a compound preparation of live bacteria and a beneficial microbial community that includes bacteria, actinomycetes, yeasts, and filamentous bacteria, all capable of producing various catalytic decomposition enzymes. This preparation exhibits a robust aerobic fermentation decomposition capacity and generates numerous highly active enzymes, which effectively decompose and degrade a wide range of soil components[25]. Microorganisms play a crucial role in the morphological transformation of Se, thereby affecting its bioavailability and geochemical cycling. They are also intimately involved in the absorption, transformation, and accumulation of Se by plants. In this context, we intend to pursue further research.

4 Conclusions

Two months following the harvest of winged pods of Red sandalwood and prior to flowering, the application of additional selenium fertilizer—particularly in the form of Se(IV)—to the forest soil can significantly reduce EAP during the reproductive phase. This intervention substantially decreases the PEWP and BSP of mature winged pods in the subsequent year. Concurrently, it markedly enhances the IWPW, WPY, and HSW of Red sandalwood . Furthermore, Se application promotes seed germination and seedling emergence post sowing, as well as seedling growth. These effects are significantly more pronounced than those of the single application of N, P, K, Ca, B, S and other individual elements, or NPK compound fertilizers. The application of Se fertilizer in conjunction with NPK compound fertilizers, particularly with EFOF, exhibits a pronounced synergistic effect in preventing embryo abortion and enhancing the quality of seeds and seedlings. Enhancing Se nutrition in forest soils represents a critical technical strategy to prevent embryo abortion of Red sandalwood and to ensure the production of high-yield, high-quality seeds, thereby holding substantial practical significance.

References

[1] TRAN TAT, DINH QT , CUI Z, *et al*. Comparing the influence of selenite (Se^{4+}) and selenate (Se^{6+}) on the inhibition of the mercury (Hg) phytotoxicity to Pakchoi [J]. Ecotoxicology and Environmental Safety, 2018, 147: 897 – 904.

[2] FAROOQ MU, ISHAAQ I, BARUTCULAR C, *et al*. Mitigation effects of selenium on accumulation of cadmium and morpho-physiological properties in rice varieties[J]. Plant Physiology and Biochemistry, 2022, 170: 1 – 13.

[3] ELKELISH AA, SOLIMAN MH, ALHAITHLOUL HA, *et al*. Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism[J]. Plant Physiology and Biochemistry, 2019, 137: 144 – 153.

[4] ANDRADEA FR, DA SILVAB GN, GUIMARÃESC KC, *et al*. Selenium protects rice plants from water deficit stress[J]. Ecotoxicology and Environmental Safety, 2018, 164: 562 – 570.

[5] ALVES AMM, MENEZESA SP, LIMA EL, *et al*. The selenium-binding protein of *Theobroma cacao*: A thermostable protein involved in the witches' broom disease resistance[J]. Plant Physiology and Biochemistry, 2019, 142: 472 – 481.

[6] ULHASSANA Z, GILLB RA, HUANGC H, *et al*. Selenium mitigates the chromium toxicity in *Brassicca napus* L. by ameliorating nutrients uptake, amino acids metabolism and antioxidant defense system[J]. Plant Physiology and Biochemistry, 2019, 145: 142 – 152.

[7] AZIMI F, ORAEI M, GOHARI G, *et al*. Chitosan-selenium nanoparticles (Cs-Se NPs) modulate the photosynthesis parameters, antioxidant enzymes activities and essential oils in *Dracocephalum moldavica* L. under cadmium toxicity stress [J]. Plant Physiology and Biochemistry, 2021, 167: 257 – 268.

[8] VALASSAKIS C, LIVANOS P, MINOPETROU M, *et al*. Promoter analysis and functional implications of the selenium binding protein (SBP) gene family in *Arabidopsis thaliana* [J]. Journal of Plant Physiology, 2018, 224 – 225: 19 – 29.

[9] ALVES AMM, MENEZESA SP, LIMA EL, *et al*. The selenium-binding protein of *Theobroma cacao*: A thermostable protein involved in the witches' broom disease resistance[J]. Plant Physiology and Biochemistry, 2019, 142: 472 – 481.

[10] XU CX, ZENG J, CUI TC, *et al*. Introduction, growth performance and ecological adaptability of Hongmu tree species (*Pterocarpus* spp.) in China[J]. Journal of Tropical Forest Science, 2016, 28(3): 260 – 267.

[11] XU CX, MA YP, DAN SY, *et al*. Effects of silicon on mineral element absorption, micro-distribution and ion pump activity of red sandalwood seedlings[J]. Scientia Silvae Sinicae, 2019, 55(10): 1 – 9.

[12] XU CX, MA YP, SHEN JN. Experimental study on formation and development of red sandalwood heartwood by grafting[J]. Agricultural Biotechnology, 2018, 7(2): 1 – 4.

[13] XU CX, MA YP, PENG BL, *et al*. Tissue culture and rapid propagation of red sandalwood plantlets[J]. Journal of Southern Agriculture, 2019, 50(12): 2741 – 2748.

[14] FILEK M, SIEPRAWSK A, KOSCIELNIAK J, *et al*. The role of chloroplasts in the oxidative stress that is induced by zearalenone in wheat plants: The functions of 24-epibrassinolide and selenium in the protective mechanisms[J]. Plant Physiology and Biochemistry, 2020, 137: 84 – 92.

[15] ARMENTA-MEDINA A, GILLMORPENG CS, GAO P, *et al*. Developmental and genomic architecture of plant embryogenesis: From model plant to crops[J]. Plant Communications, 2021, 2: 100136, 1 – 12.

[16] EBADI A, SARIKHANI H, ZAMANI Z. Effect of male parent and application of boric acid on embryo rescue in some seedless grapevine (*Vitis vinifera*) cultivars[J]. Acta Horticulturae, 2004, 640: 255 – 260.

[17] LIU JF, CHENG YQ, YAN K, *et al*. The relationship between reproductive growth and blank fruit formation in *Corylus heterophylla* Fisch [J]. Scientia Horticulturae, 2012, 136: 128 – 134.

[18] KHOSHANDAM L, BANEH HD, MARANDI RJ. Effect of BA and ovule developmental stages on embryo rescue in perlette grape (*Vitis Vinifera* L.) cultivar[J]. European Online Journal of Natural and Social Sciences, 2017, 6(1): 1 – 9.

[19] YANG XY, MA HY, LIU GC, *et al*. Anatomical study on the multi-ovule development and abortion of Hanfu apple[J]. Journal of Integrative Agriculture, 2014, 13(4): 770 – 777.

[20] JIAO Y, ZHANG K, CAI G, *et al*. Fine mapping and candidate gene analysis of a major locus controlling ovule abortion and seed number per silique in *Brassica napus* L. [J]. Theoretical and Applied Genetics, 2021, 134(8): 2517 – 2530.

[21] WANG JR, LIANG HY, LIU MJ. The relationship between endogenous hormone changes and embryo abortion during fruit development of Chinese jujube male sterile germplasm [J]. Journal of Plant Genetic Resources, 2008, 9(3): 367 – 371.

[22] GLAIVE AS, MODJINOU T, VERSACE DL, *et al*. Design of antibacterial and sustainable antioxidant networks based on plant phenolic derivatives used as delivery system of carvacrol ortannic acid[J]. ACS Sustainable Chemistry & Engineering, 2017, 5(3): 2320 – 2329.

[23] LI SP, CHEN L, ZHANG LW, *et al*. BnaC9. SMG7b functions as a positive regulator of the number of seeds per silique in *Brassica napus* by regulating the formation of functional female gametophytes [J]. Plant Physiology, 2015, 169(4): 2744 – 2760.

[24] FENG R, WEI C, TU S. The roles of selenium in protecting plants against abiotic stresses [J]. Environmental and Experimental Botany, 2013, 87: 58 – 68.

[25] ZHAO HX, LI SM, JIANG Y X, *et al*. Independent and combined effects of antibiotic stress and EM microbial agent on the nitrogen and humus transformation and bacterial community successions during the chicken manure composting [J]. Bioresource Technology, 2022, 354: 127237.