

<https://doi.org/10.17221/44/2026-PSE>

Selenite alleviates PEG-induced drought stress during rice seed germination through antioxidant regulation and osmotic adjustment

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Citation: Wang Y., Zhu Q., Luo X., Dai G.G., Hou J.W., Yu F.Y., Zhang L.H. (2026): Selenite alleviates PEG-induced drought stress during rice seed germination through antioxidant regulation and osmotic adjustment. *Plant Soil Environ.*, 72: 271–283.

Abstract: Drought stress severely impairs seed germination. Selenium (Se) is a potential mitigator of abiotic stress, but its physiological mechanisms in alleviating osmotic stress during seed germination remain poorly understood. This study investigated how Se alleviates the inhibition of rice seed germination induced by polyethylene glycol (PEG)-simulated drought. The results indicated that co-application of Se and PEG effectively alleviated the PEG-induced suppression of germination. Se significantly increased the activities of superoxide dismutase by 31.0%, peroxidase by 39.0%, catalase by 42.9%, and ascorbate peroxidase by 41.8%, along with elevating the concentrations of glutathione by 19.0% and ascorbate by 38.3%. Consequently, Se attenuated the PEG-induced burst of reactive oxygen species, reducing H₂O₂ by 21.0% and O₂⁻ by 19.1%, and alleviated lipid peroxidation, as reflected by a 20.0% decrease in malondialdehyde concentration. Furthermore, Se partially restored osmotic homeostasis by increasing the accumulation of soluble sugars by 15.9%, soluble proteins by 11.4%, free amino acids by 18.4%, and free proline by 26.3%. It also counteracted PEG-imposed inhibition of hydrolytic enzymes, enhancing α -amylase and protease activities by 26.6% and 11.2%, respectively. Notably, Se accumulation in seeds was reduced under PEG stress, suggesting impaired the penetration of exogenous Se under PEG-simulated drought. Collectively, these results demonstrate that Se alleviates PEG-induced osmotic stress in germinating rice seeds by enhancing antioxidant capacity, maintaining osmotic balance, and sustaining reserve mobilisation.

Keywords: PEG treatment; physiological characteristics; *Oryza sativa* L.; water deficit

Global climate change is increasing the frequency and severity of drought stress, posing a major threat to crop productivity and food security worldwide (Tang et al. 2025). Seed germination is a particularly vulnerable stage, as it determines successful seedling establishment and subsequent yield potential (Lei et al. 2021). Drought stress disrupts germination by disturbing osmotic balance and triggering excessive production of reactive oxygen species (ROS), including superoxide anion (O₂⁻) and hydrogen peroxide

(H₂O₂). This oxidative burst leads to lipid peroxidation, membrane damage, and reduced germination vigor (Zheng et al. 2025), with malondialdehyde (MDA) serving as a key indicator of such oxidative membrane injury (Fei et al. 2020).

Rice (*Oryza sativa* L.) is a staple food for more than half of the global population, yet its high water requirement makes it especially susceptible to drought (Bo et al. 2024). Drought stress reduces rice seed germination rates, thereby compromis-

Supported by the National Natural Science Foundation of China, Project No. U1904114.

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ing subsequent seedling growth (Jarin et al. 2024). This stress primarily affects two interdependent processes essential for germination: the activation of hydrolytic enzymes (e.g., α -amylase and proteases) that mobilise stored reserves to supply energy and biosynthetic precursors to the embryo (Chen et al. 2017), and the maintenance of cellular osmotic and redox homeostasis.

To investigate the physiological mechanisms underlying drought sensitivity during germination, polyethylene glycol (PEG-6000) is widely used as an osmotic agent. As a high-molecular-weight, non-ionic, and non-permeating polymer, PEG-6000 reduces water potential without entering plant tissues, thereby imposing controlled osmotic stress that mimics natural drought conditions (Kilyshbayeva et al. 2025, Yadav et al. 2025). Accordingly, we employed PEG-induced osmotic stress as an experimental model to simulate drought effects on rice seed germination.

Selenium (Se) is a beneficial trace element known to enhance tolerance against various abiotic stresses. At optimal concentrations, exogenous Se improves drought tolerance by modulating multiple defense pathways. It promotes the accumulation of osmolytes such as soluble sugars, proteins, and proline, thereby sustaining cellular water potential (Bocchini et al. 2018, Liu et al. 2023). It also strengthens the antioxidant system by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as elevating the pools of ascorbate (AsA) and glutathione (GSH), which collectively enhance ROS scavenging and protect membrane integrity (Hasanuzzaman et al. 2020). Furthermore, Se can promote hydrolytic enzyme activities involved in reserve mobilisation, supporting early seedling growth (Zeid et al. 2019). Beyond these functions, Se at low concentrations may act as a signaling molecule that primes intrinsic stress resilience mechanisms (Nazir et al. 2023).

Despite these well-documented beneficial roles, several critical knowledge gaps remain. First, the specific physiological mechanisms by which Se alleviates osmotic stress during seed germination, a stage highly sensitive to water deficit, are still poorly understood. Second, it remains unclear whether and how Se affects the activities of reserve-mobilising enzymes (α -amylase and protease) under osmotic stress conditions. Third, the impact of osmotic stress on the penetration of exogenously applied Se through the seed coat has not been investigated, despite its

importance for optimising Se biofortification and stress mitigation strategies under field conditions.

To address these gaps, this study aimed to elucidate the protective mechanisms of Se in alleviating PEG-simulated drought stress during rice seed germination. We hypothesised that an optimal Se concentration mitigates PEG-induced germination inhibition by: (i) activating both enzymatic (SOD, POD, CAT, and APX) and non-enzymatic (GSH and AsA) antioxidant systems; (ii) promoting the accumulation of key osmolytes (soluble sugars, soluble proteins, free proline, and free amino acids); (iii) enhancing the activities of hydrolytic enzymes (α -amylase and protease); and (iv) maintaining tissue Se accumulation under osmotic stress.

MATERIAL AND METHODS

Plant materials and growth conditions. The experiment was performed in a growth chamber under controlled conditions (28 °C, 4 days darkness). Uniform rice seeds (*Oryza sativa* L. cv. Zhonghua 11) were surface-sterilised with 10% (v/v) NaClO for 10 min, thoroughly rinsed with distilled water, and then placed (50 germinating seeds per dish) in Petri dishes lined with double-layer filter paper. Each dish was supplied with 15 mL of the respective treatment solution. To determine the optimal concentrations of polyethylene glycol (PEG) and selenium, two preliminary experiments were conducted. Experiment 1 evaluated the effects of drought stress induced by PEG-6000 at concentrations of 0 (CK) with 5, 10, 15, and 20% (w/v) on seed germination. The corresponding osmotic potentials were -0.06 , -0.17 , -0.32 , and -0.53 MPa, respectively (Michel and Kaufmann 1973); experiment 2 assessed the effects of Se using Na_2SeO_3 at concentrations of 0 (CK), 50, 100, 150, 200, and 250 $\mu\text{mol/L}$ (Na_2SeO_3 was used as the source of Se). Based on the optimal concentrations identified, a third experiment was performed to investigate their interaction under four treatments: CK; 15% PEG-6000 alone; 100 $\mu\text{mol/L}$ Na_2SeO_3 alone, and their combination (15% PEG-6000 + 100 $\mu\text{mol/L}$ Na_2SeO_3).

Determination of germination. The rice seeds were incubated in darkness for germination evaluation. Growth conditions were maintained in a controlled chamber at 28 °C with a diurnal cycle of four days of darkness and relative humidity of $60 \pm 5\%$. The germinating seeds were recorded when the radicle length reached 2 mm.

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Determination of SOD activity. Approximately 0.5 g of frozen germinating seeds were homogenised in 1.8 mL of ice-cold 50 mmol/L phosphate buffer (pH 7.8) and centrifuged at $10\,000 \times g$ for 20 min at 4 °C. Then, 0.05 mL of the supernatant was combined with 1.5 mL of phosphate buffer, 0.3 mL of 130 mmol/L methionine, 0.3 mL of 20 µmol/L riboflavin, 0.3 mL of 0.1 mmol/L EDTA- Na_2 , and 0.3 mL of 0.75 mmol/L nitro blue tetrazolium chloride (NBT). The mixture was illuminated at 4 000 lux for 20 min after thorough mixing, and absorbance was determined at 560 nm. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction. SOD specific activity was expressed as U/g protein, based on the protein concentration determined by Wang (2024).

Determination of POD activity. The reaction mixture contained 2.9 mL of 0.05 mol/L phosphate buffer (pH 5.5), 1 mL of 2% (v/v) H_2O_2 , and 1 mL of 50 mmol/L guaiacol. After adding 0.1 mL of enzyme extract, the mixture was immediately incubated at 37 °C for 15 min. The reaction was terminated with 2 mL of 20% (w/v) TCA, followed by centrifugation at $2\,000 \times g$ for 10 min. Absorbance was determined at 470 nm, with one enzyme unit (U) defined as a 0.01 increase in absorbance per minute. POD specific activity was expressed as U/g protein, based on the protein concentration determined by Wang (2024).

Determination of CAT activity. A mixture of 0.05 mL enzyme extract and 1.95 mL of 50 mmol/L phosphate buffer (pH 7.0) was preheated at 25 °C for 3 min. The reaction was initiated by adding 1 mL of 0.3% (v/v) H_2O_2 with immediate mixing. Absorbance was recorded at 240 nm every 30 s for 3 min using an ultraviolet spectrophotometer (Beijing Persee General Instrument Co., Ltd., China). One unit (U) of enzyme activity was defined as a 0.1 increase in absorbance per minute. CAT specific activity was expressed as U/g protein, based on the protein concentration determined by Wang (2024).

Determination of APX activity. The reaction system consisted of 0.1 mL enzyme extract, 2.6 mL of 0.05 mol/L PBS (pH 7.0), and 2.6 mL of 0.1 mmol/L EDTA- Na_2 (pH 7.0). The reaction was initiated by adding 0.15 mL of 5 mmol/L ascorbic acid and 0.15 mL of 20 mmol/L H_2O_2 . The decrease in absorbance at 290 nm was recorded every 30 s for 3 min using an ultraviolet spectrophotometer at 20 °C. One enzyme unit (U) was defined as a 0.1 increase in absorbance per minute under the assay conditions Fei (2020). APX specific activity was expressed as U/g protein,

based on the protein concentration determined by Wang (2024).

Determination of GSH concentration. Approximately 0.2 g of frozen germinating seeds were homogenised in 2.0 mL of 5% (w/v) TCA and centrifuged at $2\,500 \times g$ for 20 min. Then, 0.25 mL of the supernatant was mixed with 2.6 mL of 150 mmol/L phosphate buffer (pH 7.7) and 0.15 mL of 25 mg/mL 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB). After incubation at 30 °C for 5 min, the GSH concentration was determined at 412 nm using a spectrophotometer (Wang et al. 2024).

Determination of AsA concentration. Approximately 0.2 g of frozen germinating seeds were homogenised in 3 mL of 2% (w/v) oxalic acid, and the residue was rinsed with 1% oxalic acid; the combined extracts were transferred to a 100 mL volumetric flask. Then, 1 mL of 30% (w/v) zinc sulfate and 1 mL of 15% (w/v) potassium ferrocyanide were added to precipitate liposoluble pigments. The volume was adjusted to the mark with 1% oxalic acid, mixed, and filtered. 4 mL of the filtrate was mixed with 2 mL of dye solution (2,6-dichlorophenolindophenol in sodium bicarbonate) and 5 mL of xylene in a stoppered tube, shaken vigorously for 0.5 min, and allowed to separate. The absorbance of the upper xylene layer was determined at 500 nm (Lei et al. 2025).

Determination of H_2O_2 concentration. Approximately 0.2 g of frozen germinating seeds were homogenised in 1 mL of pre-cooled acetone at 4 °C and centrifuged at $1\,000 \times g$ for 10 min. A 1 mL of the supernatant was transferred into a tube containing 0.1 mL of 5% (w/v) titanium sulfate and 0.2 mL of 1 mol/L ammonia solution. The mixture was centrifuged again at $1\,000 \times g$ for 10 min, and the supernatant was discarded. The resulting precipitate was washed 3–5 times with 1 mL of cold acetone to remove pigments. After washing, 5 mL of 1 mol/L sulfuric acid was added to dissolve the precipitate. The absorbance was determined at 415 nm using a spectrophotometer once dissolution was complete (Qi et al. 2023).

Determination of O_2^- concentration. Approximately 0.2 g of frozen germinating seeds were homogenised in 5 mL of ice-cold 0.05 mol/L phosphate buffer (pH 7.8). The homogenate was centrifuged at $1\,000 \times g$ for 10 min at 4 °C, and the supernatant was further centrifuged at $16\,000 \times g$ for 20 min at 4 °C to obtain the extract. For the assay, 1 mL of extract was mixed with 0.5 mL of the

same phosphate buffer and 0.5 mL of 10 mmol/L hydroxylamine hydrochloride; a blank replaced the extract with 1 mL of distilled water. After incubation at 25 °C for 30 min, 2 mL of color-developing agent (containing 17 mmol/L sulfanilic acid and 7 mmol/L α -naphthylamine) was added, followed by a 15 min color development at 25 °C. Absorbance was determined at 530 nm against the blank using a spectrophotometer (Luo et al. 2022).

Determination of MDA concentration. Approximately 0.5 g of frozen germinating seeds was homogenised in 5 mL of 5% (w/v) TCA and then centrifuged at 1 000 \times g for 10 min. Then, 2 mL of the supernatant was mixed with 2 mL of 0.67% (w/v) thiobarbituric acid (TBA), boiled for 30 min, and rapidly cooled. After centrifugation, the absorbance of the mixture was determined at 450, 532, and 600 nm using a spectrophotometer (Lei et al. 2025).

Determination of soluble sugar concentration. Approximately 0.5 g of frozen germinating seeds were heated with 15 mL of distilled water in a boiling water bath for 20 min and then filtered. Following extraction, 1 mL of the filtrate was combined with 5 mL of 1 g/L anthrone reagent, heated for 20 min, and cooled. Subsequently, the soluble sugar concentration was determined at 620 nm using a spectrophotometer (Wang et al. 2024).

Determination of soluble protein concentration. Approximately 0.5 g of frozen germinating seeds were homogenised in 5 mL of 0.05 mol/L phosphate buffer (pH 7.8) and centrifuged at 1 000 \times g for 10 min. The resulting supernatant was then mixed with 5 mL of 0.1 g/L coomassie brilliant blue G-250 solution. After thorough mixing, the absorbance was determined at 595 nm using a spectrophotometer (Wang et al. 2024).

Determination of amino acid concentration. Approximately 0.5 g of frozen germinating seeds were homogenised in 5 mL of 10% acetic acid (v/v), diluted to 100 mL with distilled water, and filtered. Then, 1 mL of filtrate was reacted with 1 mL of ammonia-free distilled water, 3 mL of 0.005 g/mL hydrated ninhydrin, and 0.1 mL of 0.1 mg/mL ascorbic acid in a 20 mL test tube. After boiling for 15 min and rapid cooling, the mixture was diluted to 20 mL with 60% ethanol (v/v). Absorbance was determined at 570 nm using a spectrophotometer (Lei et al. 2025).

Determination of proline concentration. Approximately 0.5 g of frozen germinating seeds were homogenised in 5 mL of 10% (v/v) acetic acid, and the homogenate was diluted to 100 mL with distilled water and filtered. Then, 1 mL of the filtrate was mixed with 1 mL of ammonia-free distilled water, 3 mL of

0.005 g/mL hydrated ninhydrin, and 0.1 mL of 0.1 mg/mL ascorbic acid in a 20 mL test tube. The mixture was boiled in a water bath for 15 min, rapidly cooled, and diluted to 20 mL with 60% (v/v) ethanol. The absorbance was determined at 570 nm using a spectrophotometer (Lei et al. 2025).

Determination of α -amylase activity. Approximately 0.5 g of frozen germinating seeds were homogenised in 5 mL of 0.1 mol/L citrate buffer (pH 5.6) and centrifuged at 1 500 \times g for 20 min. A 0.1 mL of the supernatant was heated at 70 °C for 15 min, cooled rapidly, and mixed with 5 mL of the same buffer. After incubation at 40 °C for 10 min, 2 mL of pre-warmed 2% (w/v) starch solution was added, and the reaction proceeded at 40 °C for 5 min before being stopped with 4 mL of 0.4 mol/L NaOH. Then, 2 mL of DNS reagent (0.01 g/mL) was added, the mixture was boiled for 5 min, cooled, diluted to 25 mL with distilled water, and thoroughly mixed. Absorbance was determined at 540 nm using a spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China). One unit (U) of enzyme activity was defined as the amount that releases 1 mg of maltose per minute under the assay conditions (Zeid et al. 2019).

Determination of protease activity. Approximately 0.2 g of frozen germinating seeds were homogenised in 5 mL of borate buffer (pH 10.5), rinsed with an additional 5 mL of the same buffer, and brought to a final volume of 10 mL. The homogenate was filtered through dry filter paper to obtain the crude enzyme extract. For the assay, 1 mL of extract was added to a series of test tubes; a blank tube was immediately treated with 2 mL of 0.4 mol/L trichloroacetic acid (TCA), while the other tubes received 1 mL of pre-warmed (40 °C) casein solution (10 mg/mL). After incubation for 10 min, the reaction was stopped with 2 mL of 0.4 mol/L TCA (1 mL of casein was added to the blank at this point). Tubes were allowed to stand for 10 min and filtered. Then, 1 mL of each filtrate was mixed with 5 mL of 0.4 mol/L Na_2CO_3 and 1 mL of Folin's reagent, followed by incubation at 40 °C for 20 min for color development. Absorbance was determined at 680 nm against the blank using a spectrophotometer (Zeid et al. 2019).

Se concentration determination. Se concentration in dried germinating seeds was determined by atomic fluorescence spectrometry (Beijing Purkinje General Instrument Co., Ltd., PF32, Beijing, China) following a standardised digestion procedure (Li et al. 2022). Approximately 0.5 g of dried germinating

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seeds were digested with an acid mixture of 4 mL 8 mol/L HNO₃ and 1 mL 12 mol/L HClO₄, predigested overnight at room temperature, and then heated at 150 °C until complete digestion. After cooling, 2.5 mL of 6 mol/L HCl was added, and the samples were heated at 100 °C for 1 h. The digests were diluted to 25 mL with deionised water. Quality control was performed using certified reference material (GSV-4, GBW07605) and blank samples processed concurrently.

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Tukey *HSD* (honestly significant difference) was used to assess significant differences between treatments and the control ($P < 0.05$). Figures were prepared using Prism 10.1.2 (San Diego, USA). All data shown were the means of at least three replications.

RESULTS

Screening of PEG and Se concentrations and the alleviating effect of Se on PEG-induced inhibition of rice seed germination. As shown in Figure 1A,

PEG-induced osmotic stress inhibited rice seed germination in a concentration-dependent manner. Compared to the control (CK, distilled water), germination rates progressively decreased with increasing PEG concentrations (5, 10, 15, and 20%). Treatment with 5% PEG did not significantly reduce germination rate, whereas 10, 15, and 20% PEG treatments caused significant reductions ($P < 0.05$). Among these, 15% PEG exhibited a moderate inhibitory effect, intermediate between the mild inhibition at 10% PEG and the severe inhibition at 20% PEG, and was therefore selected for subsequent experiments. The effect of Se on seed germination is presented in Figure 1B. Treatment with increasing concentrations of Na₂SeO₃ (50, 100, 150, 200, and 250 μmol/L) initially increased germination rates, peaking at 100 μmol/L, and then declined. Accordingly, 100 μmol/L Na₂SeO₃ was selected for subsequent experiments. To investigate the interaction between osmotic stress and Se, a combined treatment was applied (Figure 1C). Seeds were exposed to CK, 15% PEG alone, 100 μmol/L Na₂SeO₃ alone, or a combination of both (PEG + Se). Relative to CK, PEG alone reduced the germination rate by 58.1%, while Se alone increased it by 16.2%

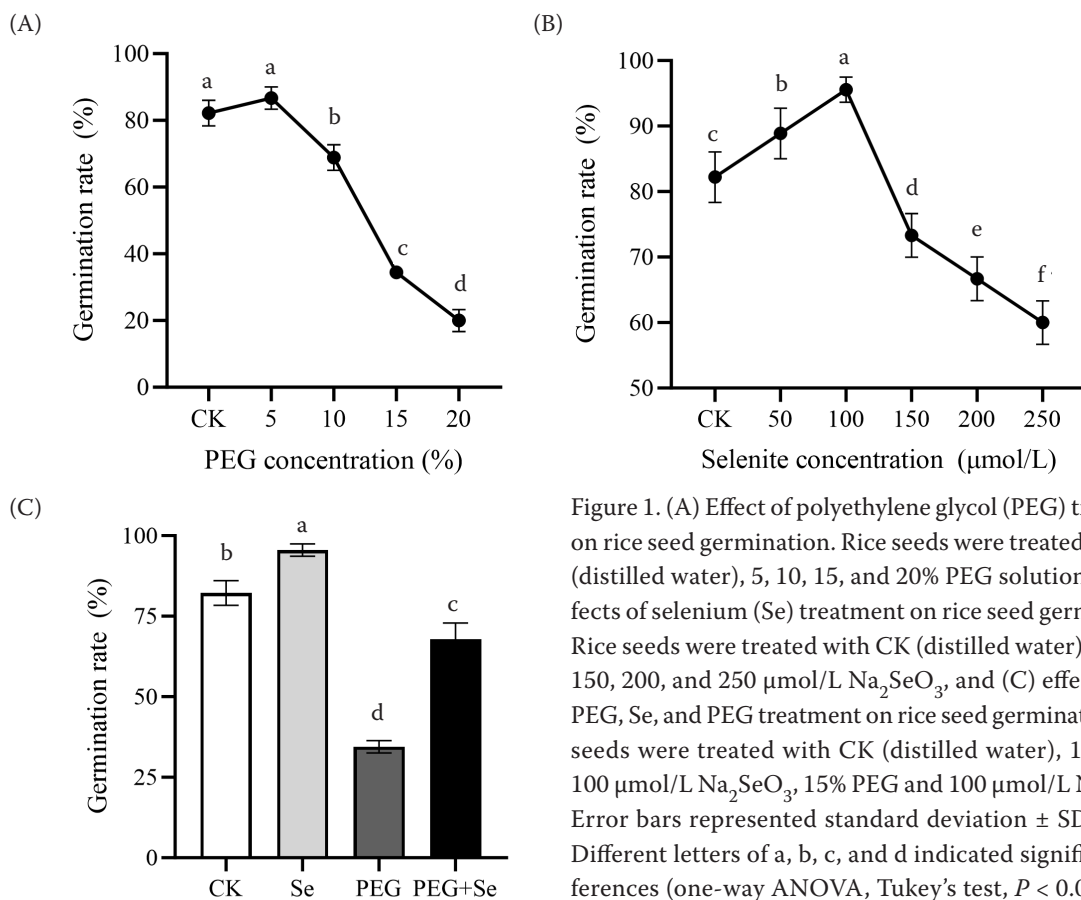


Figure 1. (A) Effect of polyethylene glycol (PEG) treatment on rice seed germination. Rice seeds were treated with CK (distilled water), 5, 10, 15, and 20% PEG solutions; (B) effects of selenium (Se) treatment on rice seed germination. Rice seeds were treated with CK (distilled water), 50, 100, 150, 200, and 250 μmol/L Na₂SeO₃, and (C) effects of Se, PEG, Se, and PEG treatment on rice seed germination. Rice seeds were treated with CK (distilled water), 15% PEG, 100 μmol/L Na₂SeO₃, 15% PEG and 100 μmol/L Na₂SeO₃. Error bars represented standard deviation ± SD ($n = 3$). Different letters of a, b, c, and d indicated significant differences (one-way ANOVA, Tukey's test, $P < 0.05$)

($P < 0.05$). When applied together, Se significantly alleviated the inhibitory effect of PEG, indicating that Se mitigates PEG-induced germination stress under the selected concentrations.

Se partially reverses PEG-induced suppression of antioxidant enzyme activities. As shown in Figure 2, the activities of key antioxidant enzymes varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased the activities of SOD, POD, CAT, and APX by 18.4, 17.4, 22.6, and 19.3%, respectively ($P < 0.05$), whereas treatment with 15% PEG alone significantly decreased them by 38.5, 42.9, 45.7, and 46.3%, respectively ($P < 0.05$). Under the combined PEG and Se treatment (PEG + Se), the activities of these enzymes were significantly higher than those in the PEG-alone group, with increases of 31.0, 39.0, 42.9, and 41.8%, respectively, though they remained lower than in the CK and Se-alone groups ($P < 0.05$). These results indicate that Se partially mitigates the PEG-induced suppression of the antioxidant enzyme system.

Se partially restores the PEG-diminished GSH pool. As shown in Figure 3A, GSH concentrations

varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased GSH concentration by 10.1% ($P < 0.05$), whereas treatment with 15% PEG alone significantly decreased it by 22.9% ($P < 0.05$). Under the combined PEG and Se treatment (PEG + Se), the GSH level was 19.0% higher than that in the PEG-alone group, though it remained lower than in the CK and Se-alone groups ($P < 0.05$). These results indicate that Se partially restores the PEG-diminished GSH pool.

Se alleviates the PEG-induced decline in AsA concentration. As shown in Figure 3B, AsA concentrations varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased AsA concentration by 17.6% ($P < 0.05$), whereas treatment with 15% PEG alone significantly decreased it by 36.6% ($P < 0.05$). Under the combined PEG and Se treatment (PEG + Se), the AsA level was 38.3% higher than that in the PEG-alone group, though it remained lower than in the CK and Se-alone groups ($P < 0.05$). These results indicate that Se partially alleviates the PEG-induced decline in AsA.

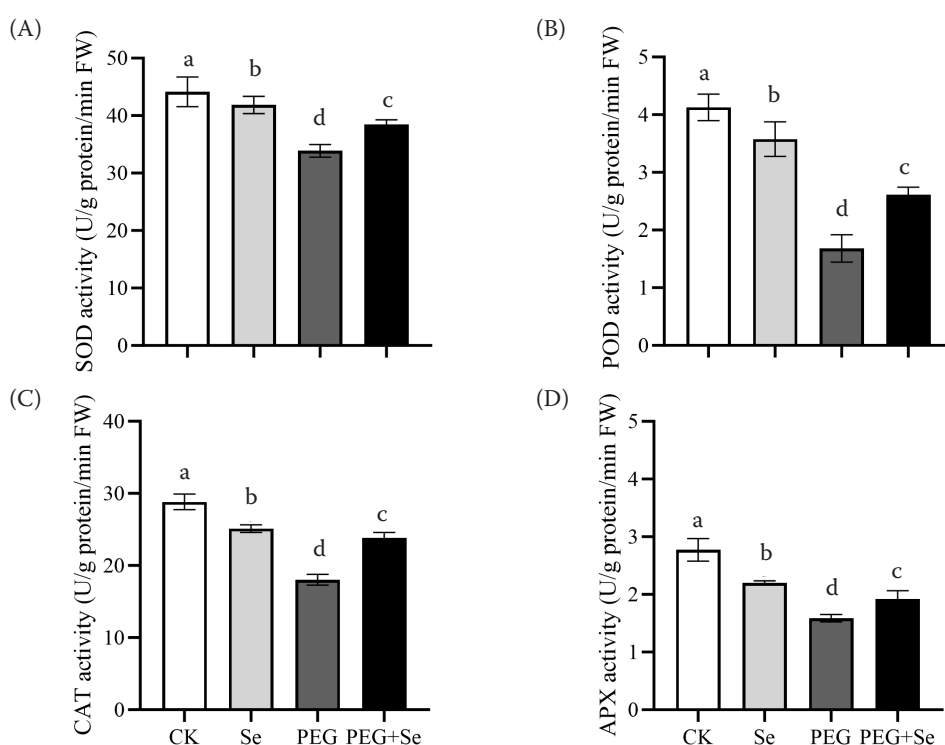


Figure 2. Effect of selenium (Se), polyethylene glycol (PEG), Se, and PEG treatment on the activities of (A) superoxide dismutase (SOD); (B) peroxidase (POD); (C) catalase (CAT), and (D) ascorbate peroxidase (APX) in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 $\mu\text{mol/L}$ Na_2SeO_3 , 15% PEG and 100 $\mu\text{mol/L}$ Na_2SeO_3 . Error bars represented standard deviation \pm SD ($n = 3$). Different letters of a, b, c, and d indicated the differences among different treatments (one-way ANOVA, Tukey's test, $P < 0.05$). FW – fresh weight

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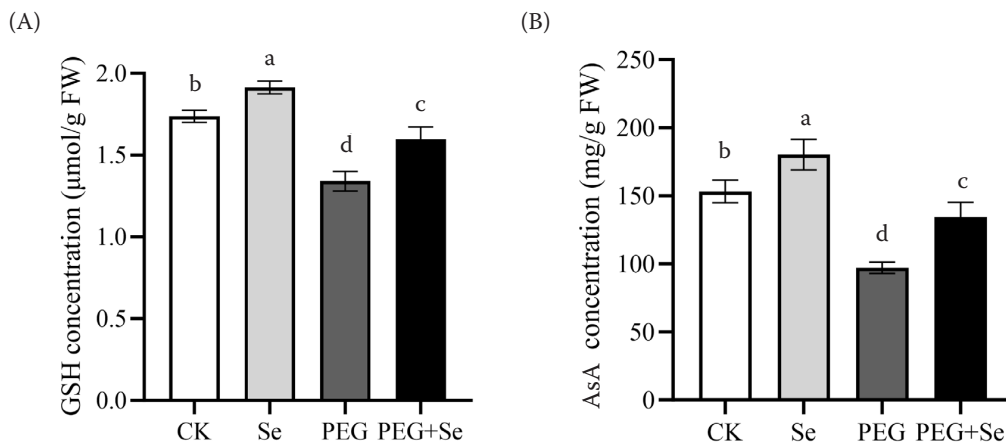


Figure 3. Effects of selenium (Se), polyethylene glycol (PEG), and Se, and PEG treatment on (A) glutathione (GSH) and (B) ascorbate (AsA) concentration in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 μmol/L Na₂SeO₃, 15% PEG and 100 μmol/L Na₂SeO₃. Error bars represented standard deviation ± SD (*n* = 3). Different letters of a, b, c, and d indicated the differences among different treatments (one-way ANOVA, Tukey’s test, *P* < 0.05). FW – fresh weight

Se reduces PEG-induced overproduction of H₂O₂. As shown in Figure 4A, H₂O₂ concentrations in germinating rice seeds varied significantly among treatments. Relative to CK, treatment with 100 μmol/L Na₂SeO₃ alone significantly decreased H₂O₂ concentration by 36.7% (*P* < 0.05), whereas treatment with 15% PEG alone significantly increased

it by 71.6% (*P* < 0.05). Under the combined PEG and Se treatment (PEG + Se), the H₂O₂ concentration was 21.0% lower than that in the PEG-alone group, though it remained higher than in the CK and Se-alone groups (*P* < 0.05). These results indicate that Se supplementation partially alleviates PEG-induced oxidative burst.

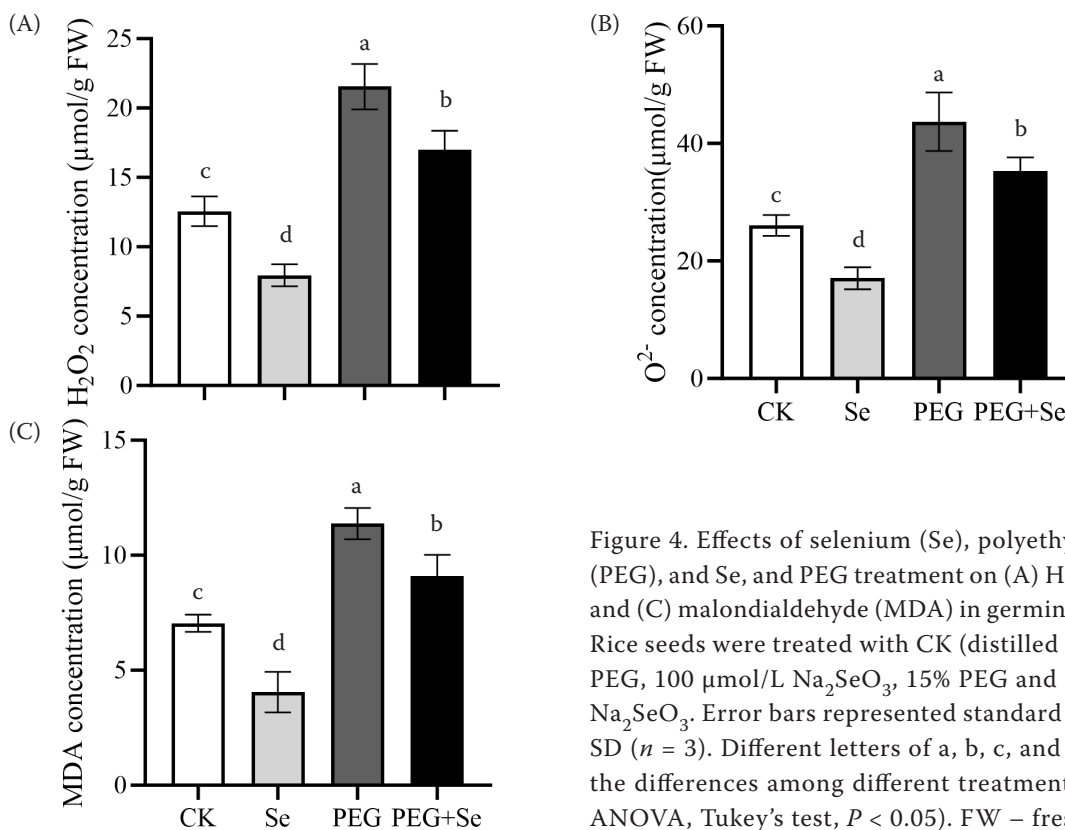


Figure 4. Effects of selenium (Se), polyethylene glycol (PEG), and Se, and PEG treatment on (A) H₂O₂; (B) O₂⁻, and (C) malondialdehyde (MDA) in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 μmol/L Na₂SeO₃, 15% PEG and 100 μmol/L Na₂SeO₃. Error bars represented standard deviation ± SD (*n* = 3). Different letters of a, b, c, and d indicated the differences among different treatments (one-way ANOVA, Tukey’s test, *P* < 0.05). FW – fresh weight

Se partially restores metabolite levels reduced by PEG-induced osmotic stress. The concentrations of key metabolites in germinating rice seeds varied significantly among treatments (Figure 5). Relative to the CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased soluble sugar, soluble protein, free amino acids, and free proline by 13.3, 10.3, 15.3, and 17.0%, respectively ($P < 0.05$). In contrast, 15% PEG alone significantly reduced these metabolites by 22.3, 19.1, 30.1, and 36.6%, respectively ($P < 0.05$). Under combined PEG and Se treatment (PEG + Se), the levels of these four metabolites were significantly increased by 15.9, 11.4, 18.4, and 26.3%, respectively, compared to PEG alone, reaching concentrations intermediate between the PEG-alone and CK groups ($P < 0.05$). These results indicate that Se partially alleviates PEG-induced metabolic suppression.

Se decreases MDA concentration under PEG-induced oxidative stress. As shown in Figure 4C, MDA concentrations varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly decreased MDA concentration by 42.5% ($P < 0.05$), whereas treatment

with 15% PEG alone significantly increased it by 61.5% ($P < 0.05$). Under the combined PEG and Se treatment (PEG + Se), the MDA concentration was 20.0% lower than that in the PEG-alone group, though it remained higher than in the CK and Se-alone groups ($P < 0.05$). These results indicate that Se application partially alleviates PEG-induced lipid peroxidation.

Se partially restores metabolite levels reduced by PEG-induced osmotic stress. The concentrations of key metabolites in germinating rice seeds varied significantly among treatments (Figure 5). Relative to the CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased soluble sugar, soluble protein, free amino acids, and free proline by 13.3, 10.3, 15.3, and 17.0%, respectively ($P < 0.05$). In contrast, 15% PEG alone significantly reduced these metabolites by 22.3, 19.1, 30.1, and 36.6%, respectively ($P < 0.05$). Under combined PEG and Se treatment (PEG + Se), the levels of these four metabolites were significantly increased by 15.9, 11.4, 18.4, and 26.3%, respectively, compared to PEG alone, reaching concentrations intermediate between the PEG-alone and CK groups ($P < 0.05$).

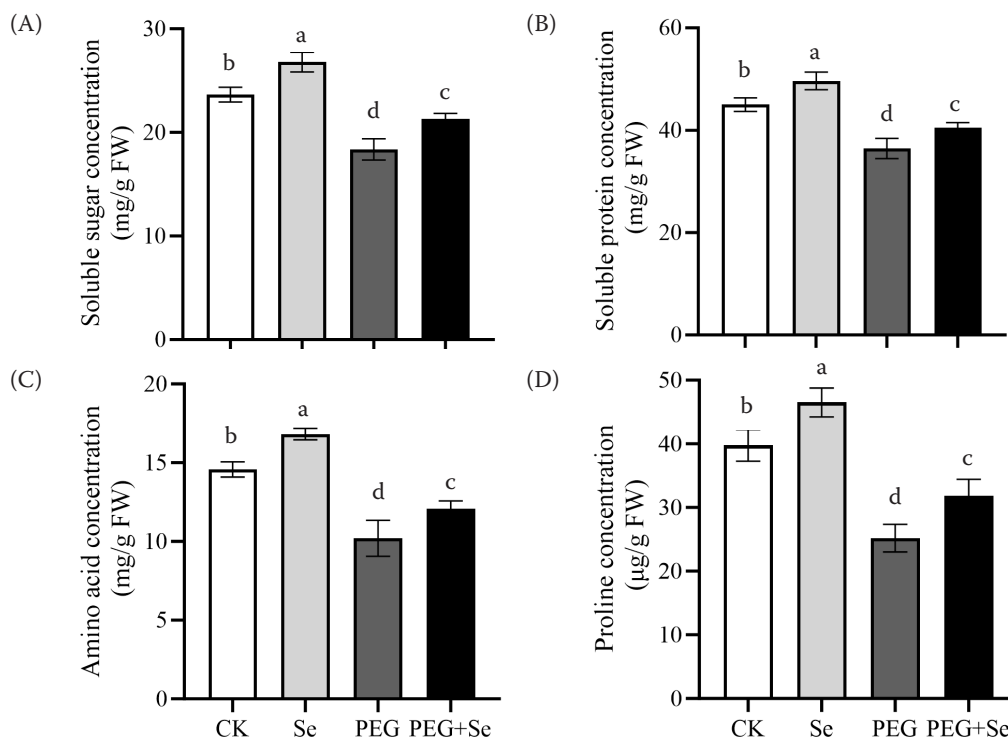


Figure 5. Effects of selenium (Se), polyethylene glycol (PEG), and Se, and PEG treatment on the concentration of (A) soluble sugar; (B) soluble protein; (C) free amino acid, and (D) free proline in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 $\mu\text{mol/L}$ Na_2SeO_3 , 15% PEG and 100 $\mu\text{mol/L}$ Na_2SeO_3 . Error bars represented standard deviation \pm SD ($n = 3$). Different letters of a, b, c, and d indicated the differences among different treatments (one-way ANOVA, Tukey's test, $P < 0.05$). FW – fresh weight

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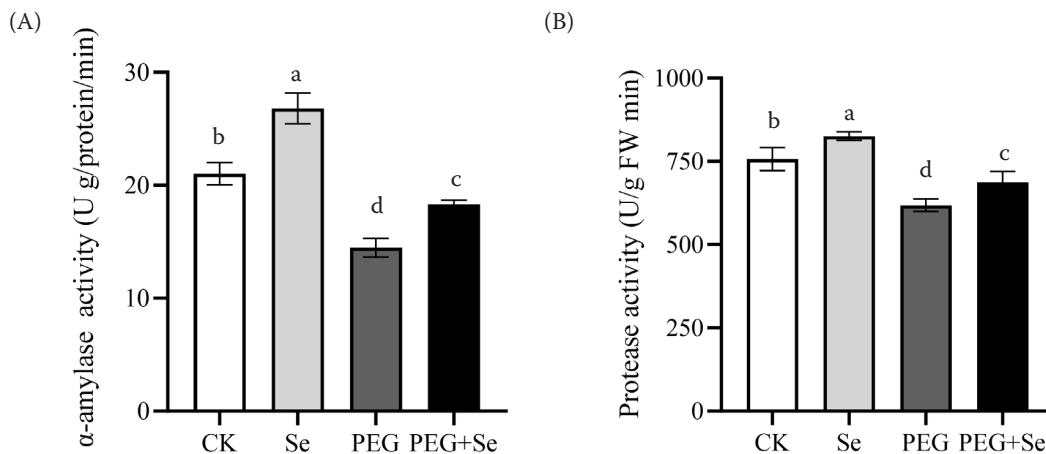


Figure 6. Effect of selenium (Se), polyethylene glycol (PEG), Se, and PEG treatment on (A) α -amylase and (B) protease activity in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 $\mu\text{mol/L}$ Na_2SeO_3 , 15% PEG and 100 $\mu\text{mol/L}$ Na_2SeO_3 . The activities of α -amylase and protease are expressed on a fresh weight (FW) basis. Error bars represented standard deviation \pm SD ($n = 3$). Different letters of a, b, c, and d indicated significant differences (one-way ANOVA, Tukey's test, $P < 0.05$)

($P < 0.05$). These results indicate that Se partially alleviates PEG-induced metabolic suppression.

Se alleviates PEG-induced inhibition of α -amylase activity. As shown in Figure 6A, α -amylase activity in germinating rice seeds varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased α -amylase activity by 27.6% ($P < 0.05$), whereas treatment with 15% PEG alone significantly decreased it by 31.1% ($P < 0.05$). Under the combined PEG and Se treatment (PEG + Se), α -amylase activity was 26.6% higher than that in the PEG-alone group, though it remained lower than in the CK and Se-alone groups ($P < 0.05$). These results indicate that Se alleviates PEG-induced inhibition of α -amylase activity during rice seed germination.

Se alleviates PEG-induced inhibition of protease activity. As shown in Figure 6B, protease activity in germinating rice seeds varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$ Na_2SeO_3 alone significantly increased protease activity by 9.6% ($P < 0.05$), whereas treatment with 15% PEG alone significantly decreased it by 18.3% ($P < 0.05$). Under the combined PEG and Se treatment, protease activity was 11.2% higher than that in the PEG-alone group ($P < 0.05$). These results indicate that Se alleviates the inhibitory effect of PEG-induced stress on protease activity during rice seed germination.

PEG stress reduces the accumulation of exogenous Se. As shown in Figure 7, Se concentrations in germinating rice seeds varied significantly among treatments. Relative to CK, treatment with 100 $\mu\text{mol/L}$

Na_2SeO_3 alone significantly increased Se accumulation by 2.8-fold ($P < 0.05$), whereas treatment with 15% PEG alone did not cause a significant change. Under the combined PEG and Se treatment (PEG + Se), the Se concentration was significantly lower than that in the Se-alone group, though it remained slightly higher than the CK level ($P < 0.05$), indicating that PEG-induced stress reduces the penetration of exogenous Se.

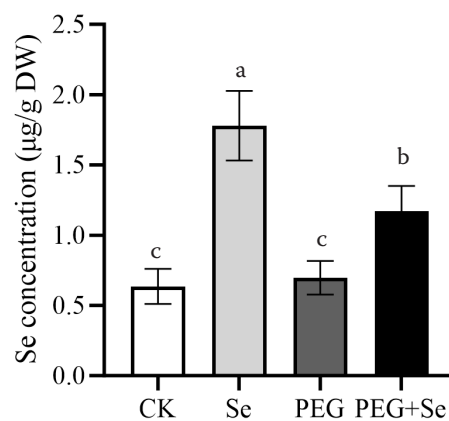


Figure 7. Effects of selenium (Se), polyethylene glycol (PEG), and Se and PEG treatment on Se concentration in germinating seeds. Rice seeds were treated with CK (distilled water), 15% PEG, 100 $\mu\text{mol/L}$ Na_2SeO_3 , 15% PEG and 100 $\mu\text{mol/L}$ Na_2SeO_3 . Se concentration is expressed on a dry weight (DW) basis. Error bars represented standard deviation \pm SD ($n = 3$). Different letters of a, b, c, and d indicated the differences among different treatments (one-way ANOVA, Tukey's test, $P < 0.05$)

DISCUSSION

Our findings demonstrate that exogenous Se effectively mitigates PEG-induced osmotic stress (Figure 5), thereby improving rice seed germination. This protective effect is mediated through a coordinated enhancement of the antioxidant system, maintenance of osmotic homeostasis, and preservation of reserve mobilisation capacity, even under conditions where Se uptake itself is compromised. Mechanistically, Se acts not merely as an antioxidant but as a signaling molecule that orchestrates a multi-layered defense response.

Se reinforces the antioxidant defense system through transcriptional regulation of key enzymes. The primary inhibitory effect of PEG-induced stress was a severe suppression of germination, concomitant with a marked oxidative burst, evidenced by elevated levels of O_2^- , H_2O_2 , and the lipid peroxidation product MDA (Figure 4). This aligns with the established model in which osmotic imbalance disrupts cellular redox homeostasis, leading to oxidative damage that impairs critical germination processes (Zhang et al. 2025). Se application notably counteracted this trend. Mechanistically, Se acts as a signaling molecule that upregulates the expression of genes encoding antioxidant enzymes. Studies have shown that Se activates the expression of SOD, CAT, and POD through the modulation of mitogen-activated protein kinase (MAPK) cascades and the induction of transcription factors such as WRKY and NAC (Deng et al. 2025). This transcriptional regulation provides a sustained enhancement of enzymatic defenses, enabling seeds to rapidly neutralise ROS upon stress perception. Our data reveal that Se upregulates both enzymatic (SOD, POD, CAT, and APX) (Figure 2) and non-enzymatic (GSH and AsA) (Figure 3) components of the antioxidant machinery. The activities of key enzymes – SOD (which dismutates O_2^- to H_2O_2), POD and CAT (which detoxify H_2O_2), and APX (which utilises AsA to reduce H_2O_2) – were enhanced by Se treatment under both normal and stress conditions (Nawaz et al. 2021, Gomathi et al. 2025). Crucially, Se also positively regulated the pools of the non-enzymatic antioxidants GSH and AsA. The decline in GSH and AsA following PEG treatment alone reflects the typical depletion of antioxidant reserves under drought stress (Chen et al. 2026). Se restored and even enhanced their levels, which is functionally significant within the framework of the ascorbate-glutathione (AsA-GSH) cycle, a central pathway for H_2O_2 scavenging (Saleem et al. 2025).

This restoration can be attributed to Se-induced enhancement of the AsA-GSH cycle, in which Se up-regulates the expression of key cycle enzymes, such as dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Saleem et al. 2025), thereby reinforcing the cycle's capacity to sustain redox balance under prolonged stress. Moreover, GSH is known to affect seed germination beyond its antioxidant function, potentially modulating hormone signalling and protein redox status through S-glutathionylation, a post-translational modification that regulates the activity of key signalling proteins (Koramutla et al. 2021). Thus, Se appears to orchestrate a multi-layered defense, mitigating oxidative damage, preserving membrane integrity, and supporting redox-dependent developmental signaling.

Se contributes to osmotic adjustment and metabolic homeostasis *via* regulation of proline metabolism. Beyond oxidative stress management, Se supported osmotic adjustment, a vital strategy for maintaining cell turgor. PEG stress depleted key osmolytes, including soluble sugars, soluble proteins, free amino acids, and proline (Figure 5). This depletion likely reflects a diversion of resources or a disruption in synthesis pathways under stress. Se treatment counteracted this effect, leading to higher concentrations of these compatible solutes in the PEG + Se group than in the PEG-alone group. Soluble sugars and proline are well-established osmolytes that stabilise cellular structures and proteins, thereby helping to maintain water potential (Fedotova 2019). From a mechanistic perspective, Se-mediated proline accumulation is likely driven by the transcriptional upregulation of Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) and ornithine- δ -aminotransferase (OAT), key enzymes in proline biosynthesis, while simultaneously downregulating proline dehydrogenase (ProDH), which catalyses proline degradation (Mushtaq et al. 2023). This regulatory shift enables seeds to maintain favourable osmotic potential and protect macromolecular structures under low water availability. The increased accumulation of proline, a potent osmoprotectant and radical scavenger, further underscores this adaptive response (Luo et al. 2022). By bolstering the osmolyte pool through coordinated transcriptional regulation of biosynthetic and catabolic genes, Se helps preserve cellular hydration, which is fundamental for sustaining metabolic activity essential for germination.

Preservation of reserve mobilisation by key hydrolytic enzymes is linked to gibberellin signaling. The hydrolysis of stored reserves is imperative for provid-

<https://doi.org/10.17221/44/2026-PSE>

ing energy and building blocks to the growing embryo (Alves-de-Oliveira et al. 2023). PEG stress severely inhibited the activities of α -amylase and protease, enzymes critical for starch and protein mobilisation, respectively (Figure 6). Se application significantly alleviated this inhibition. Mechanistically, the maintenance of α -amylase activity under combined stress may be associated with Se-mediated preservation of the gibberellin (GA) signalling pathway. In cereal seeds, α -amylase expression is tightly controlled by GA_3 , which is perceived by the *GID1* receptor, leading to the degradation of *DELLA* repressors and subsequent activation of transcription factors such as *GAMYB*, which drive α -amylase gene expression (Hedden 2025). GA signaling is sensitive to cellular redox status, and oxidative stress can impair this pathway (Chen et al. 2022). It has been reported that Se can activate transcription factors in the GA signalling pathway, potentially by maintaining redox homeostasis, thereby enabling proper GA perception and signal transduction (Zeeshan et al. 2025). Similarly, protease activity, which is also influenced by the balance of GA and abscisic acid (ABA) during germination, may be sustained through Se-mediated modulation of hormone signalling. Thus, by alleviating oxidative stress and modulating GA-ABA crosstalk, Se indirectly sustains the hydrolytic capacity required for endosperm mobilisation.

Implications of restricted Se accumulation under stress: a signalling perspective. A pivotal finding was that PEG-induced stress markedly inhibited the accumulation of externally supplied Se in germinating seeds (Figure 7). This suggests that osmotic stress impedes the penetration of exogenous Se due to reduced hydraulic conductivity. This observation suggests that the significant protective effects of Se were achieved at a substantially lower tissue Se concentration under combined stress than when Se was applied alone. This strongly indicates that the mechanism of protection depends not simply on Se accumulation, but rather on its efficacy as a signalling molecule or priming agent that triggers and potentiates endogenous plant defence pathways. At low concentrations, Se can act as a signalling molecule by modulating the redox state of the cell, influencing the activity of redox-sensitive transcription factors, and inducing systemic acquired resistance-like responses (Nazir et al. 2023). This priming effect allows seeds to mount a faster and stronger defense response upon subsequent stress exposure. However, our data further reveal a critical difference: while

exogenous Se effectively mitigated PEG-induced stress, the protective effect in the PEG + Se treatment was consistently inferior to that observed with Se treatment alone (Figures 1C–6D). This pattern aligns with the observation that Se accumulation in germinating seeds under combined stress (PEG + Se) was approximately 1.5-fold lower than under Se treatment alone, despite both receiving the same exogenous Se concentration (Figure 7). This suggests that PEG-induced osmotic stress impairs physical penetration through the seed coat, which occurs *via* osmotic diffusion. Consequently, the lower tissue Se concentration in the PEG + Se group compared to the Se-alone group resulted in a diminished capacity to activate downstream protective mechanisms. For instance, the activities of key antioxidant enzymes (SOD, POD, CAT, and APX) and the accumulation of non-enzymatic antioxidants (GSH and AsA) were significantly higher in the Se-alone group than in the PEG + Se group, correlating with the differential Se levels (Figures 2A–3B). Similarly, the accumulation of osmolytes (soluble sugars and proline) and the activities of hydrolytic enzymes (α -amylase and protease) followed the same trend, with the Se-alone treatment showing the most pronounced improvement (Figures 5A–6B). This gradient of physiological responses – from optimal in Se-alone to moderate in PEG + Se – underscores that the degree of Se-mediated protection is directly proportional to the actual Se load achieved within the seed, which in turn determines the magnitude of signal activation. Importantly, despite this impaired accumulation under stress, Se still provided significant protection (compared to PEG alone), highlighting its potent efficacy as a priming agent, capable of activating defence pathways even at reduced tissue concentrations. This finding has practical implications for Se biofortification and stress mitigation strategies, as it suggests that the efficacy of Se application under drought conditions may be constrained by reduced Se uptake, necessitating optimised application methods (e.g., seed priming prior to stress exposure) to maximise its protective potential.

In conclusion, Se alleviates PEG-mediated inhibition of rice seed germination through multifaceted mechanisms that improve antioxidant capacity, promote the accumulation of osmotic adjustment substances, and enhance reserve mobilisation (Figure 8). These insights deepen our understanding of Se-mediated abiotic stress tolerance and support its potential application as an eco-friendly seed priming agent to enhance

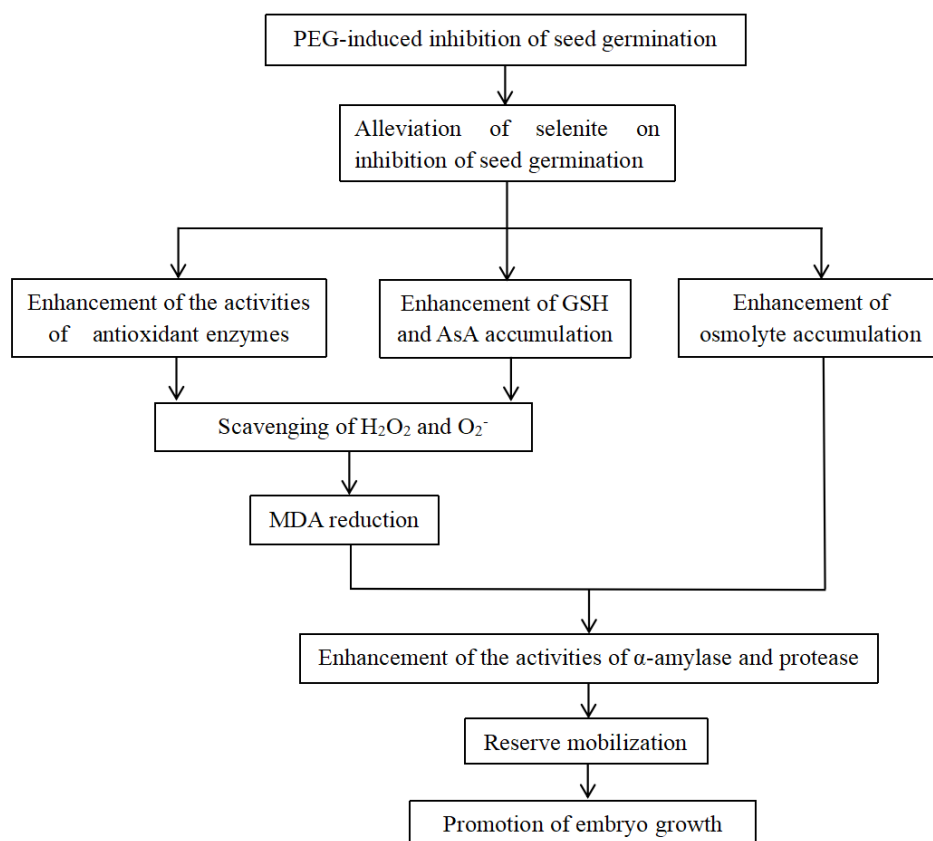


Figure 8. Physiological mechanisms of selenium (Se) alleviating polyethylene glycol (PEG)-mediated inhibition of rice seed germination. GSH – glutathione; AsA – ascorbate; H_2O_2 – hydrogen peroxide; O_2^- – superoxide anion; MDA – malondialdehyde

crop seedling establishment under water-deficient conditions. However, the experiments were conducted in a controlled growth chamber (e.g., constant temperature and humidity), which may not fully reflect the complex and variable environmental factors present in natural field conditions. Future research should elucidate the early molecular signaling events triggered by Se and validate the field-level efficacy of Se seed priming.

Acknowledgement. We thank the Experimental Teaching Center of Agricultural University for its strong support for the experiment. This study was supported by the National Natural Science Foundation of China-Henan Joint Fund, Project No. U1904114.

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Received: January 26, 2026

Accepted: April 16, 2026

Published online: May 25, 2026