

Effects of foliar application of different concentrations of lysine-chelated selenite on physiological characteristics of wheat

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Abstract: Understanding how chelated selenium (Se) regulates plant stress tolerance is critical for optimising field applications. We investigated dose-dependent effects of foliar-applied lysine-chelated selenite (0, 15, 30, 45, and 60 g/ha selenite) on antioxidant capacity and Se distribution in wheat (*Triticum aestivum* L.) under field conditions. The results indicated that the optimal application dose (30 g/ha) most effectively suppressed O_2^- and H_2O_2 accumulation by 47.8% and 47.2%, respectively, and reduced MDA concentration by 74.0%. This effect was attributed to enhanced activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), as well as increased concentrations of glutathione and ascorbate. Simultaneously, this treatment inhibited the degradation of photosynthetic pigments and increased the concentrations of soluble sugar, soluble protein, and free amino acids, while reducing proline levels. With increasing application dose, Se concentrations increased and followed the order: roots > leaves > stems > glumes > grains. Our findings demonstrate that lysine-chelated selenite at 30 g/ha was most effective in enhancing antioxidant capacity. This study provides a mechanistic basis for the precise application of chelated Se to simultaneously enhance stress resilience and Se biofortification efficiency.

Keywords: selenium biofortification; AsA–GSH cycle; dose-dependent response; reactive oxygen species; antioxidant defense; Se partitioning

Wheat (*Triticum aestivum* L.) sustains global food security by providing approximately 20% of human dietary calories and protein (Caverzan et al. 2016). However, its productivity is frequently constrained by abiotic stresses – drought, salinity, extreme temperatures, and heavy metal toxicity (Pessarakli 2020) – that disrupt the cellular redox balance. Under stress conditions, the overproduction of reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen

peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$), exceeds the scavenging capacity of plant cells (Gill and Tuteja 2010). These ROS attack unsaturated fatty acids in membranes, triggering lipid peroxidation and generating malondialdehyde (MDA), a reliable proxy for membrane damage. MDA accumulation compromises membrane integrity, increases ion leakage, impairs mitochondrial electron transport and chloroplast photosynthesis, and can ultimately induce programmed cell death (Heath

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and Packer 1968). Therefore, enhancing the antioxidant capacity of crops is a central goal of stress physiology research (Mittler 2017).

The plant antioxidant defence network comprises both enzymatic and non-enzymatic components (Apel and Hirt 2004). The major enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). Among these, APX, with its high affinity for ascorbic acid (AsA), plays an indispensable role in the ascorbate-glutathione (AsA-GSH) cycle, particularly in scavenging H_2O_2 within chloroplasts and the cytosol (Smirnoff 2005). These four enzymes function synergistically to form a complete ROS scavenging network (Hasanuzzaman et al. 2020). In parallel, non-enzymatic antioxidants such as reduced glutathione (GSH) and ascorbate (AsA) directly neutralise ROS and participate in the ASA-GSH cycle, in which AsA is regenerated from its oxidised form (dehydroascorbate) using GSH as the reducing agent (Foyer and Noctor 2011). The concentrations of GSH and AsA, along with their ratio, are key indicators of cellular redox status (Griffith 1980).

Beyond the antioxidant system, plants accumulate osmotic regulatory substances to counteract stress. Proline not only maintains cell turgor and stabilises proteins and membrane structures but also directly scavenges ROS and modulates redox potential (Trovato et al. 2008, Ghosh et al. 2022). Soluble sugars function as both osmolytes and carbon sources, reducing osmotic potential and enhancing water retention. Soluble protein concentration reflects metabolic activity and protein synthesis rates (Bradford 1976). Free amino acids are direct products of nitrogen metabolism and indicate nitrogen assimilation efficiency. Chlorophyll, as the core photosynthetic pigment, directly affects light capture and carbon assimilation. Collectively, these physiological parameters provide a comprehensive assessment of how exogenous substances influence plant physiological activity and antioxidant capacity (Saddhe et al. 2021).

Selenium (Se) is an essential trace element for humans and animals, and while not yet confirmed as essential for plants, its beneficial biological effects are well documented. Appropriate Se levels promote plant growth, increase antioxidant enzyme activities, alleviate membrane lipid peroxidation, and enhance tolerance to various abiotic stresses (Khan et al. 2015). The antioxidant function of Se is primarily mediated by glutathione peroxidase (GSH-Px), which uses selenocysteine as its active centre to reduce

toxic peroxides to non-toxic hydroxyl compounds (Tinggi 2008). However, inorganic Se (e.g., selenite) suffers from low absorption and utilisation efficiency and a narrow safety threshold (Schiavon and Pilon-Smits 2017, Hasanuzzaman et al. 2020); excessive application readily induces toxicity (Reilly 2004). Lysine, an essential amino acid, not only participates in protein synthesis but also acts as a signalling molecule regulating plant growth and metabolism (Gouveia et al. 2026, Shen et al. 2026). By chelating selenite with lysine, the resulting compound exhibits weaker sorptive retention in soil matrices, reduced abiotic interconversion, and improved compatibility with advanced fertigation systems. Previous work has demonstrated that amino acid-chelated selenite can effectively enhance photosynthetic function in crop plants (Wang et al. 2024). Theoretically, lysine-chelated selenite combines the antioxidant function of Se with the nutritional and regulatory roles of lysine, providing synergistic benefits. Nevertheless, systematic studies on the physiological effects of lysine-chelated selenite in crops remain limited, and a comprehensive evaluation of its dose-dependent effects is notably lacking (Liu et al. 2022).

In this study, we investigated the foliar application of lysine-chelated selenite on wheat. We systematically measured ROS concentrations (O_2^- and H_2O_2), MDA levels, activities of antioxidant enzymes superoxide dismutase (SOD, POD, CAT, and APX), non-enzymatic antioxidant concentrations (GSH and AsA), osmotic regulators (soluble sugar, soluble protein, and proline), free amino acids, and chlorophyll concentration in flag leaves at the filling stage. Our objectives were: (i) to elucidate the dose-dependent effects of lysine-chelated selenite on wheat antioxidant capacity and physiological activity; (ii) to determine the optimal application dose; and (iii) to propose a mechanistic basis for its action. This study provides a scientific foundation for developing novel foliar formulations of lysine-chelated selenite to enhance both crop stress resilience and Se biofortification efficiency in wheat production.

MATERIAL AND METHODS

Plant material and field experiment design.

A field experiment was conducted at the experimental farm of Henan University of Science and Technology in Luoyang, China, during the 2025–2026 wheat-growing season. The soil type is calcareous cinnamon soil, and the soil texture is medium loam.

Wheat (*Triticum aestivum* L.) cultivar (Keda 1026) was used as the test material. Standard agronomic practices (irrigation, fertilisation, and pest control) were uniformly applied across all plots. The experiment followed a randomised complete block design with five treatments and three replications. The soil properties of the 0–20 cm plough layer were as follows: alkaline hydrolysis nitrogen, 61.34 mg/kg (determined by the alkaline hydrolysis diffusion method) (Lu et al. 2023), available phosphorus 11.37 mg/kg (extracted with 0.5 mol/L NaHCO₃, Olsen method) (Olsen et al. 1954), exchangeable potassium 193.46 mg/kg (extracted with 1 mol/L NH₄OAc) (Hanway and Heidel 1952), organic carbon 11.9 g/kg (determined by the K₂Cr₂O₇ oxidation method) (Walkley and Black 1934), and pH 7.8 (measured in a 1:2.5 soil:water suspension) (Wu et al. 2018). Each plot measured 40 m × 3 m (120 m²), with a 1 m buffer zone between adjacent plots. The wheat was sown at a density of 187.5 kg/ha. Before sowing, base fertilisers were applied: urea (46% N) at 225 kg/ha, diammonium phosphate at 180 kg/ha, and potassium chloride at 112.5 kg/ha. The total base fertiliser application doses were 135.9 kg N/ha, 36.4 kg P/ha, and 56 kg K/ha. At the jointing stage, an additional topdressing of urea at 150 kg/ha (equivalent to 69 kg N/ha) was applied. All fertilisation practices followed local standard agronomic recommendations for wheat production. Treatments consisted of foliar application of lysine-chelated selenite at 0, 15, 30, 45, and 60 g/ha (as selenite), designated as T0, T1, T2, T3, and T4, respectively. Solutions were prepared immediately before use and contained 0.1% (v/v) OP-10 surfactant, including the control, to ensure uniform wetting. Sprays were applied to both leaf surfaces with a handheld sprayer until runoff (675 L/ha) on calm, cloudless afternoons during grain filling. Flag leaves were sampled three days after application. From each plot, leaves from five randomly selected plants were pooled as one biological replicate. Samples were immediately placed on ice and either analysed directly for physiology or stored at –80°C.

Preparation of lysine-chelated selenite. Lysine-chelated selenite was synthesised following the method of Wang et al. (2024) with minor modifications. Briefly, a 4% (w/v) L-lysine solution was prepared by dissolving L-lysine (≥ 98%, China) in distilled water. Separately, a 0.5 mol/L selenite (Na₂SeO₃, ≥ 99%, China) solution was prepared in distilled water. The two solutions were mixed at a volume ratio of 2:1 (L-lysine:Na₂SeO₃). The pH of the mixture was

adjusted to 8.0 using 1 mol/L NaOH, and the reaction was carried out under constant stirring at 60 °C for 60 min. After the reaction, the solution was vacuum-concentrated to one-third of its original volume. Subsequently, 10 volumes of anhydrous ethanol were added to precipitate the chelated product. The mixture was allowed to stand at 4 °C for 12 h, followed by centrifugation at 6 000 × g for 5 min. The resulting precipitate was collected and vacuum freeze-dried to obtain lysine-chelated selenite powder.

Determination of O₂[–] concentration. A 0.2 g sample was homogenised in 5 mL of ice-cold phosphate buffer (50 mmol/L, pH 7.8) and centrifuged at 1 000 × g for 10 min at 4 °C. After a second centrifugation of the supernatant at 16 000 × g for 20 min, 1 mL of the final supernatant was combined with 0.5 mL of phosphate buffer and 0.5 mL of 10 mmol/L hydroxylamine hydrochloride. Following incubation at 25 °C for 30 min, 1 mL each of 17 mmol/L sulfanilamide and 7 mmol/L α-naphthylamine was added. The mixture was left to develop colour at 25 °C for 15 min, and absorbance was measured at 530 nm relative to a blank (water instead of the extract) (Wang et al. 2024).

Determination of H₂O₂ concentration. Approximately 0.2 g of fresh sample was added to 2 mL of cold acetone and ground into a homogenate. The mixture was centrifuged at 1 000 × g for 10 min. One millilitre of the supernatant was mixed with 0.1 mL of 5% titanium sulfate and 0.2 mL of concentrated ammonia water. The precipitate was collected by centrifugation (1 000 × g, 10 min), washed five times with cold acetone, and then dissolved in 5 mL of 2 mol/L sulfuric acid. The absorbance was measured at 415 nm. H₂O₂ concentration was calculated from a standard curve prepared using known H₂O₂ concentrations and expressed as μmol/g FW (Wang et al. 2024).

Determination of MDA concentration. Approximately 0.5 g of the sample was added to 5 mL of 5% (v/v) trichloroacetic acid (TCA) and ground into a slurry. The mixture was centrifuged at 1 000 × g for 20 min. Then, 300 μL of the supernatant and 300 μL of 0.6% thiobarbituric acid (TBA) solution were added to a 2 mL centrifuge tube, mixed thoroughly, heated in a boiling water bath for 15 min, rapidly cooled, and centrifuged. The absorbance was measured at 532, 600, and 450 nm. MDA concentration was calculated using the formula:

$$\text{MDA } (\mu\text{mol/g FW}) = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times V_t / (FW \times V_s)$$

where: V_t – total extraction volume; V_s – aliquot volume, and FW – sample fresh weight (Wang et al. 2024).

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Determination of SOD activity. Approximately 0.2 g of sample was ground into pulp with 1.8 mL of pre-cooled (4 °C) 50 mmol/L phosphate buffer (pH 7.8) and centrifuged at $1\,000 \times g$ for 20 min at 4 °C. The supernatant was used as the crude enzyme extract for SOD, POD, and CAT assays. Then 50 µL of the enzyme solution was collected, and 1.5 mL of 0.05 mol/L phosphate buffer (pH 7.8), 0.3 mL of 130 mmol/L methionine solution, 0.3 mL of 750 µmol/L nitroblue tetrazole (NBT) solution, 0.3 mL of 100 µmol/L EDTA- Na_2 solution, and 0.3 mL of 20 µmol/L riboflavin solution were added sequentially. After mixing, the mixture was exposed to 4 000 lx of light for 20 min. A control reaction without enzyme was conducted under the same conditions. SOD activity 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 activity was expressed as U/g FW (Beauchamp and Fridovich 1971).

Determination of POD activity. The reaction mixture (5 mL total volume) contained 2.9 mL of 50 mmol/L phosphate buffer (pH 5.5), 1.0 mL of 2% H_2O_2 , 1.0 mL of 0.05 mol/L guaiacol, and 0.1 mL of crude enzyme extract. After adding the enzyme solution, the system was immediately incubated in a 37 °C water bath for 15 min, then 2 mL of 20% TCA was added to terminate the reaction. The mixture was centrifuged at $1\,000 \times g$ for 10 min. The absorbance of the supernatant was measured at 470 nm using a spectrophotometer. One unit (U) of POD activity was defined as an increase of 0.01 in absorbance per minute at 470 nm. POD activity was expressed as U/g FW (Wang et al. 2024).

Determination of CAT activity. The reaction mixture (3 mL total volume) contained 1.95 mL of 50 mmol/L phosphate buffer (pH 7.0), 1.0 mL of 0.3% H_2O_2 , and 0.05 mL of crude enzyme extract. After preheating at 25 °C for 3 min, the reaction was initiated by adding H_2O_2 . The decrease in absorbance at 240 nm was recorded every 30 s for 3 min. One unit (U) of CAT activity was defined as a decrease of 0.01 in absorbance per minute. CAT activity was expressed as U/g FW (Aebi 1984).

Determination of APX activity. The reaction mixture (3 mL total volume) contained 1.8 mL of 50 mmol/L phosphate buffer (pH 7.0), 0.5 mL of 0.5 mmol/L ascorbic acid (AsA), 0.5 mL of 0.1 mmol/L H_2O_2 , and 0.2 mL of crude enzyme extract (extracted in buffer containing 1 mmol/L AsA to protect APX activity). After adding H_2O_2 to initiate the reaction, the absorbance at 290 nm was recorded every 30 s for 2 min. The amount of AsA oxidised was calculated

using an extinction coefficient of 2.8 mL/(µmol·cm). APX activity was expressed as nmol AsA/g FW/min (Wang et al. 2024).

Determination of GSH concentration. Approximately 0.5 g of sample was added to 5 mL of 5% TCA and ground into a homogeneous slurry. The mixture was centrifuged at $1\,000 \times g$ for 20 min. Then 250 µL of the extraction solution was collected, mixed with 2.6 mL of 150 mmol/L Na_2HPO_4 (pH 7.7) and 150 µL of 16.5 mmol/L 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and incubated at 30 °C for 5 min. GSH concentration was determined at 412 nm using a spectrophotometer. GSH concentration was calculated from a standard curve prepared with reduced glutathione and expressed as µmol/g fresh weight (FW) (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 measured at 500 nm (Wang et al. 2024).

Determination of soluble sugar concentration. Approximately 0.5 g of sample was added to 15 mL of distilled water in a test tube and boiled in a water bath for 20 min. After filtration, 1 mL of the extraction solution was mixed with 5 mL of 0.15% anthrone reagent (dissolved in 72% sulfuric acid) and boiled in a boiling water bath for 10 min. After cooling, the absorbance was measured at 620 nm. Soluble sugar concentration was calculated from a standard curve prepared with glucose and expressed as mg/g FW (Wang et al. 2024).

Determination of soluble protein concentration. Approximately 0.5 g of fresh sample was added to 5 mL of 50 mmol/L phosphate buffer (pH 7.8) and ground into a slurry. The homogenate was centrifuged at $1\,000 \times g$ for 20 min at 4 °C. The supernatant was collected, and 0.1 mL of the supernatant was mixed with 5 mL of Coomassie Brilliant Blue G-250 reagent (0.01% G-250, 4.7% ethanol, 8.5% phosphoric acid). After standing for 2 min, the absorbance was measured at 595 nm.

Soluble protein concentration was calculated from a standard curve prepared with bovine serum albumin (BSA) and expressed as mg/g FW (Wang et al. 2024).

Determination of proline concentration. Approximately 0.5 g of fresh sample was added to 5 mL of 3% sulfosalicylic acid solution and boiled in a water bath for 10 min. Then 2 mL of supernatant, 2 mL of glacial acetic acid, and 3 mL of acidic ninhydrin reagent (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6 mol/L phosphoric acid) were mixed thoroughly and boiled in a water bath for 40 min. After cooling, 5 mL of toluene was added to each tube to ensure sufficient oscillation for extraction of the red substance. The absorbance of the toluene phase was measured at 520 nm. Proline concentration was calculated from a standard curve prepared with L-proline and expressed as $\mu\text{g/g}$ FW (Wang et al. 2024).

Determination of free amino acid concentration. Approximately 0.5 g of fresh sample was added to 5 mL of 10% acetic acid, and distilled water was added to 100 mL, mixed thoroughly, and filtered. 1 mL of the filtrate was mixed with 1 mL of distilled water, 3 mL of ninhydrin reagent and 0.1 mL of 0.1% ascorbic acid. The mixture was stirred evenly, heated in a boiling water bath for 15 min, and made up to 20 mL with 60% ethanol. After cooling, the absorbance was measured at 570 nm. Free amino acid concentration was calculated from a standard curve prepared with leucine and expressed as mg/g FW (Wang et al. 2024).

Determination of chlorophyll concentration. Approximately 0.2 g of fresh flag leaf was cut into pieces and extracted with 10 mL of 80% acetone in the dark until the leaves became completely white. The extract was filtered or centrifuged, and the absorbances at 665 and 649 nm were measured. Chlorophyll *a* (Ca), chlorophyll *b* (Cb), and total chlorophyll were calculated using the following formulas (Wellburn 1994):

$$\text{Ca (mg/L)} = 12.19 \times \text{OD}_{665} - 3.45 \times \text{OD}_{649}; \text{Cb}$$

$$(\text{mg/L}) = 21.99 \times \text{OD}_{649} - 5.32 \times \text{OD}_{665}$$

$$\text{Total chlorophyll (mg/L)} = \text{Ca} + \text{Cb. Chlorophyll concentration (mg/g FW)} = (\text{pigment concentration (mg/L)} \times \text{extraction volume (L)}) / \text{sample fresh weight (g)}.$$

Determination of Se concentration. Dried samples were weighed and digested with a 5.0 mL $\text{HNO}_3\text{:HClO}_4$ (4:1, v/v) mixture overnight. Pre-digestion was performed at 100 °C for 60 min, followed by digestion at 150 °C for 240 min. After cooling, 2.5 mL of the acid mixture was added if necessary, and digestion continued at 150 °C for another 120 min. After cooling to below 50 °C, 2.5 mL of 6 mol/L HCl was added, and the mixture

was heated at 100 °C for 180 min for reduction. The digest was cooled and diluted with deionised water to a final volume of 25 mL. The Se concentration was determined by atomic fluorescence spectrometry (PF32, Beijing Purkinje General Instrument Co., Ltd., Beijing, China, 2017) using 5% HCl as carrier solution and a mixture of 1.5% KBH_4 and 0.5% NaOH as reductant. All the reagents used in the analysis were super-pure, guaranteed grade. A standard tea material (GSV-4, 0.072 mg Se/kg, GBW07605) and a blank were simultaneously digested with the test samples, yielding average Se recoveries of 89–93%.

Statistical analysis. All experiments were performed with at least three independent biological replicates. Statistical analyses were conducted using SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). Differences among treatments were assessed using one-way ANOVA, followed by Tukey's multiple comparisons test. Graphs were generated using GraphPad Prism 9.5.0 (GraphPad Software, LLC, San Diego, USA).

RESULTS

Dose-dependent effects of lysine-chelated selenite on ROS accumulation and membrane lipid peroxidation in wheat leaves. To evaluate the effects of lysine-chelated selenite on oxidative stress markers, we measured the concentrations of O_2^- and H_2O_2 as indicators of ROS accumulation, and MDA as a marker of membrane lipid peroxidation in wheat flag leaves following foliar application at different application doses (Figure 1). Compared with the control, all lysine-chelated selenite treatments reduced O_2^- accumulation in wheat leaves, albeit to different extents. T2 (30 g/ha) resulted in the most pronounced decrease (47.8%), followed by T1 (34.2%) and T3 (31.4%). T4 (60 g/ha) showed only a modest 9.8% reduction compared with the control. Low- to medium-concentration lysine-chelated selenite reduced O_2^- accumulation more effectively than the highest concentration tested (60 g/ha). A similar dose-dependent pattern was observed for H_2O_2 . T1, T2, and T3 reduced H_2O_2 concentration by 32.3, 47.2, and 22.5%, respectively, while T4 reduced it by only 7.7%. T2 (30 g/ha) resulted in the lowest H_2O_2 concentration among all treatments. Further increasing the application dose beyond 30 g/ha led to a progressive increase in H_2O_2 concentration. All treatments significantly reduced MDA concentration compared with the control, indicating alleviation of membrane lipid peroxidation. T2 achieved the greatest reduction (74.0%), followed by T3 (52.2%), T4

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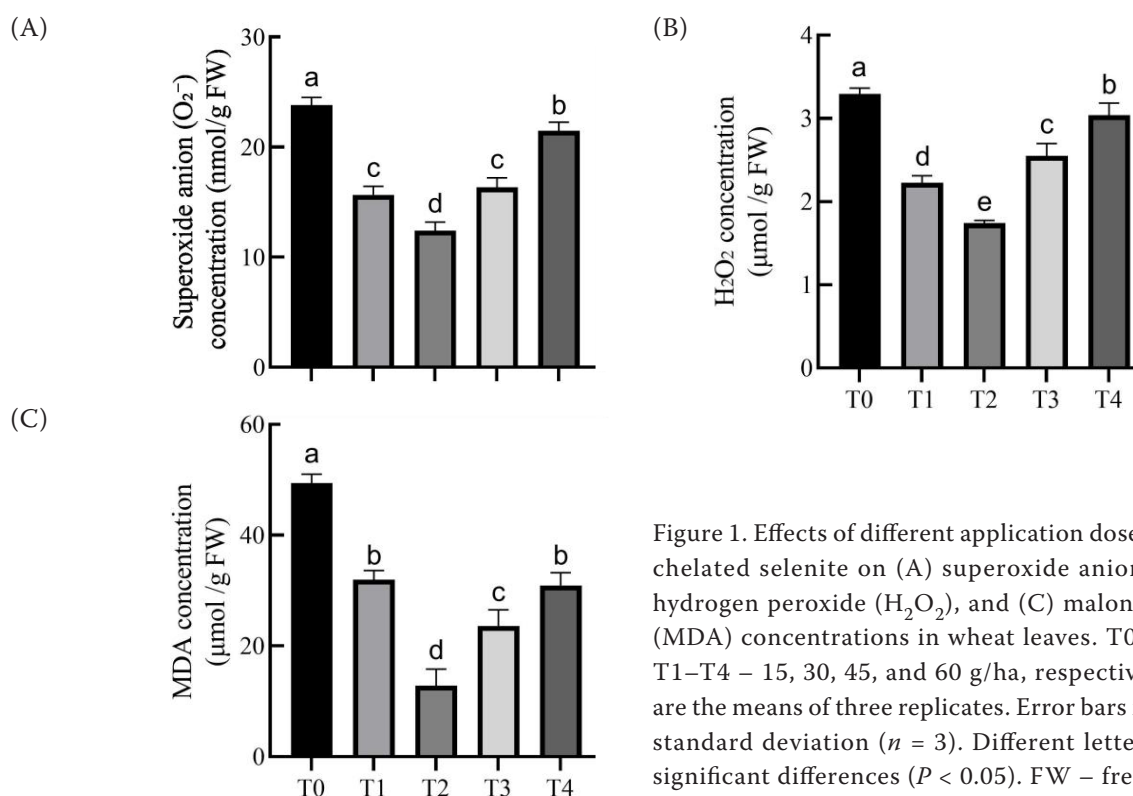


Figure 1. Effects of different application doses of lysine-chelated selenite on (A) superoxide anion (O_2^-); (B) hydrogen peroxide (H_2O_2), and (C) malondialdehyde (MDA) concentrations in wheat leaves. T0 – control; T1–T4 – 15, 30, 45, and 60 g/ha, respectively. Values are the means of three replicates. Error bars represent $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). FW – fresh weight

(37.4%), and T1 (35.3%). T2 resulted in the greatest reduction in MDA concentration (74.0%), followed by T3 (52.2%), T4 (37.4%), and T1 (35.3%). T2 (30 g/ha) consistently showed the largest reductions in O_2^- , H_2O_2 , and MDA among all treatments, while T4 (60 g/ha) showed the smallest reductions.

Dose-dependent activation of antioxidant enzyme activities by lysine-chelated selenite. To determine whether lysine-chelated selenite enhances the enzymatic antioxidant defence system, we measured the activities of SOD, POD, CAT, and APX in wheat flag leaves following foliar application at different doses (Figure 2). Foliar application of lysine-chelated selenite modulated SOD activity in a dose-dependent manner. Compared with the control, T1, T2, and T3 increased SOD activity by 5.42, 15.91, and 6.01%, respectively. In contrast, T4 (60 g/ha) resulted in a slight decrease of 1.11% relative to the control. T2 (30 g/ha) exhibited the highest SOD activity among all treatments, whereas T4 (60 g/ha) showed a slight decrease relative to the control. A similar dose-response pattern was observed for POD activity. T1, T2, and T3 increased POD activity by 15.74, 23.66, and 13.56%, respectively, while T4 decreased it by 1.29%. Again, T2 yielded the largest increase (23.66%), consistent with the optimal effect observed at the medium concentration and a decline at higher concentrations. All tested concen-

trations significantly enhanced CAT activity compared with the control. T1, T2, T3, and T4 increased CAT activity by 19.1, 42.4, 22.7, and 7.0%, respectively. T2 showed the highest CAT activity, suggesting that the medium concentration most effectively induces CAT to accelerate H_2O_2 decomposition. APX activity was markedly enhanced by lysine-chelated selenite across all treatments. T1, T2, T3, and T4 increased APX activity by 220.2, 393.4, 217.8, and 105.4%, respectively. T2 (30 g/ha) resulted in the most pronounced increase, nearly four-fold higher than the control. APX activity showed the largest increase at T2 (30 g/ha), reaching 393.4% above the control. Collectively, the activities of the four antioxidant enzymes followed a consistent pattern: T2 (30 g/ha) exhibited the strongest inductive effect, followed by T1, with T3 showing a decline and T4 the weakest, or even inhibitory, effect. The activities of SOD, POD, CAT, and APX were highest at T2 (30 g/ha), showing a concentration-dependent response.

Effects of lysine-chelated selenite on non-enzymatic antioxidants, osmotic regulators, nitrogen metabolites, and photosynthetic pigments. To comprehensively evaluate the physiological responses of wheat leaves to lysine-chelated selenite, we measured the concentrations of non-enzymatic antioxidants (GSH and AsA), osmotic regulators (soluble sugar,

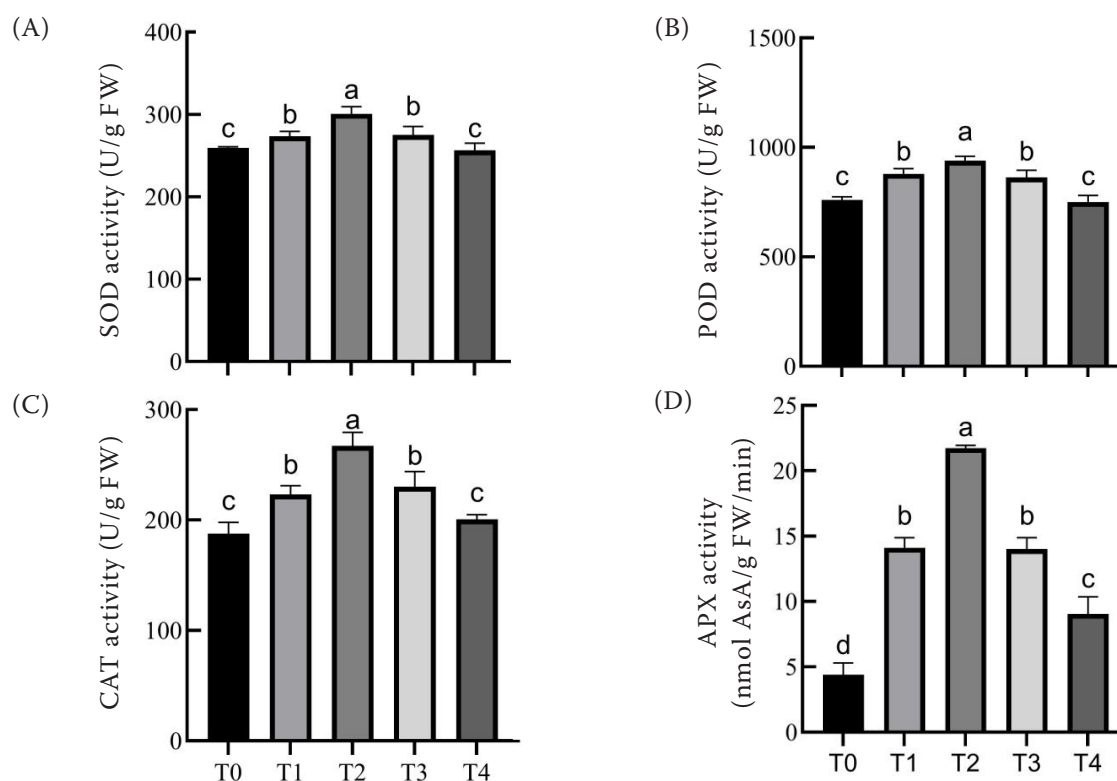


Figure 2. Effects of different application doses of lysine-chelated selenite on (A) superoxide dismutase (SOD); (B) peroxidase (POD); (C) catalase (CAT), and (D) ascorbate peroxidase (APX) activities in wheat leaves. T0 – control; T1–T4 – 15, 30, 45, and 60 g/ha, respectively. Values are the means of three replicates. Error bars represent $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). FW – fresh weight

soluble protein, and proline), free amino acids, and total chlorophyll following foliar application of different application doses (Figures 3–5). All treatments increased glutathione (GSH) concentration compared with the control, albeit to varying extents (Figure 3). T1, T2, T3, and T4 elevated GSH concentration by

24.9, 74.3, 15.6, and 2.2%, respectively. T2 (30 g/ha) resulted in the most substantial increase (74.3%).

AsA concentration followed a similar pattern, with increases of 15.2, 69.8, 44.9, and 15.8% in T1, T2, T3, and T4, respectively. Both non-enzymatic antioxidants reached their highest levels under T2

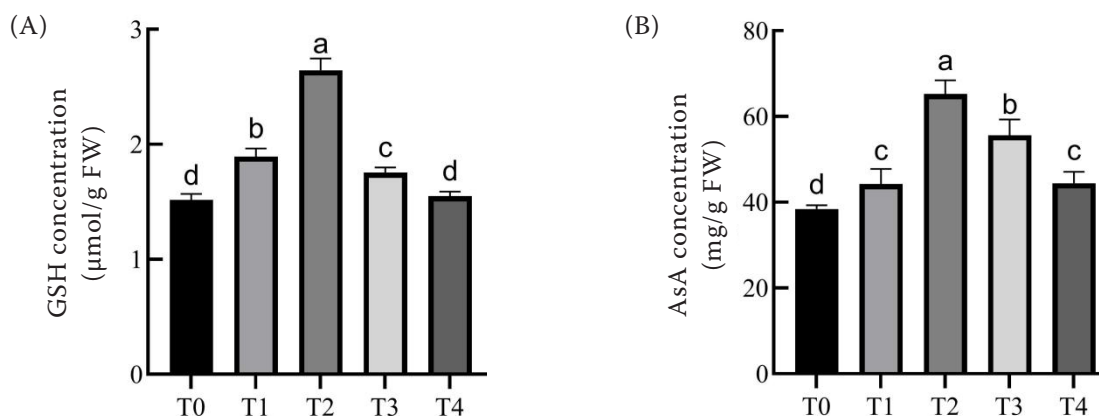


Figure 3. Effects of different application doses of lysine-chelated selenite on (A) glutathione (GSH) and (B) ascorbic acid (AsA) concentrations in wheat leaves. T0 – control; T1–T4 – 15, 30, 45, and 60 g/ha, respectively. Values are the means of three replicates. Error bars represent $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). FW – fresh weight

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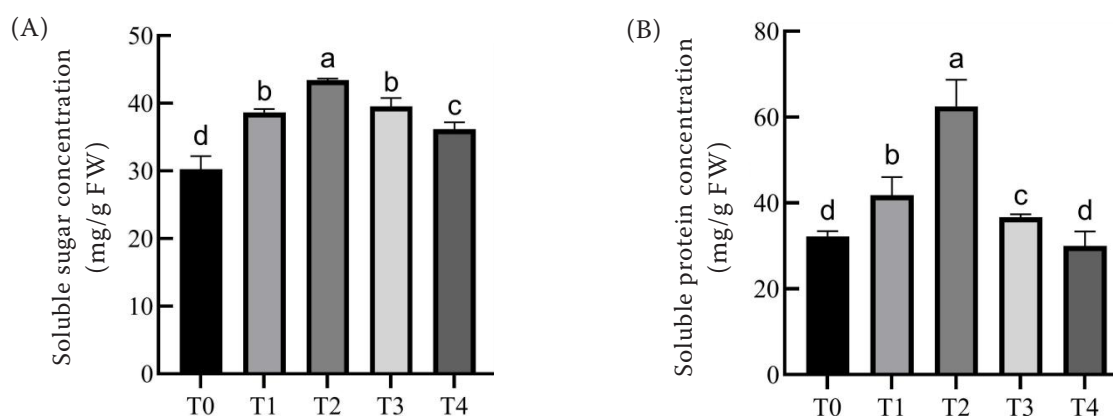


Figure 4. Effects of different application doses of lysine-chelated selenite on (A) soluble sugar and (B) soluble protein concentrations in wheat leaves. T0 – control; T1–T4 – 15, 30, 45, and 60 g/ha, respectively. Values are the means of three replicates. Error bars represent $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). FW – fresh weight

(30 g/ha). GSH concentration increased by only 2.2% under T4 compared with the control.

Soluble sugar concentration increased significantly across all treatments (Figure 4). T1, T2, T3, and T4 increased soluble sugar concentration by 27.8, 43.7, 30.8, and 19.7%, respectively, with T2 exhibiting the largest increase (43.7%). Soluble protein concentration increased by 30.0, 94.0, and 13.9% in T1, T2, and T3, respectively, whereas T4 decreased by 7.0% relative to the control.

In contrast to the other osmotic regulators, proline concentration was lower than the control in all treatments (Figure 5). T1, 2, 3, and 4 decreased proline concentration by 41.8, 56.4, 28.8, and 14.3%, respectively, with T2 showing the largest reduction. Free amino acid concentration was elevated in all treatments compared with the control (Figure 5). T1, 2, 3, and 4 increased free amino acid concentration by 16.9, 30.5, 14.2, and 1.94%, respectively. T2 (30 g/ha) resulted in the largest increase (30.5%).

Total chlorophyll concentration was higher than the control in all treatments (Figure 5). T1, 2, 3, and 4 increased total chlorophyll concentration by 20.8, 32.5, 16.4, and 6.0%, respectively. T2 exhibited the most pronounced promotional effect (a 32.5% increase). Although the increase was the smallest under T4 (6.0%), it remained above the control level. In summary, T2 (30 g/ha) showed the greatest improvements across all measured parameters, whereas T4 (60 g/ha) showed the smallest.

Se distribution across different wheat tissues within each treatment. As shown in Figure 6, foliar application of lysine-chelated selenite significantly

increased Se concentrations in all wheat tissues in a dose-dependent manner, with distinct tissue-specific accumulation patterns. The Se concentrations varied substantially among organs, with the highest accumulation consistently observed in roots, followed by leaves, stems, glumes, and grains, respectively. Across all application doses, Se concentrations in roots were significantly higher than those in other organs (T4). The tissue-specific distribution of Se within each treatment exhibited consistent patterns across application doses (Figure 6). The grain Se concentration increased progressively with application dose, from 0.0564 $\mu\text{g/g DW}$ in control to 0.0736, 0.1047, 0.1280, and 0.1582 $\mu\text{g/g DW}$ at T1, T2, T3, and T4, respectively. In the control (Figure 6A), Se concentration followed the order: roots > leaves > glumes > stems $\approx$ grains. Under lysine-chelated selenite application (Figure 6B–E), the hierarchy shifted to roots > leaves > stems > glumes > grains in all treated groups. Notably, roots consistently maintained the highest Se concentration across all treatments. At the same time, grains consistently exhibited the lowest, confirming that although foliar-applied Se is absorbed through leaf surfaces, only a limited fraction is ultimately partitioned to the grains.

DISCUSSION

In this study, we demonstrated that foliar application of lysine-chelated selenite at an optimal concentration (30 g/ha) coordinately enhances the antioxidant defence system and physiological performance of wheat leaves under field conditions. Lysine-chelated selenite alleviates oxidative stress

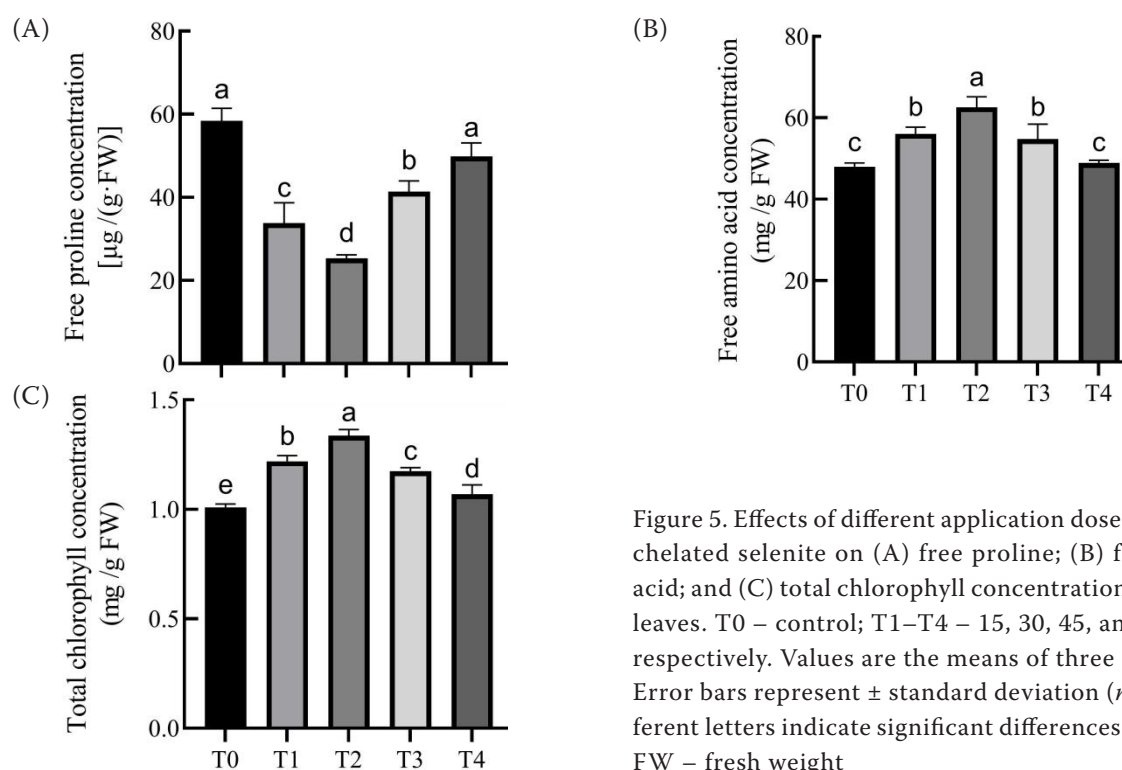


Figure 5. Effects of different application doses of lysine-chelated selenite on (A) free proline; (B) free amino acid; and (C) total chlorophyll concentrations in wheat leaves. T0 – control; T1–T4 – 15, 30, 45, and 60 g/ha, respectively. Values are the means of three replicates. Error bars represent $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($P < 0.05$). FW – fresh weight

through coordinated activation of enzymatic and non-enzymatic antioxidants. Excessive accumulation of ROS is a primary cause of oxidative damage in plants (Mittler 2017). In our study, foliar application of lysine-chelated selenite significantly reduced O_2^- and H_2O_2 concentrations and concurrently decreased MDA levels, indicating effective alleviation of membrane lipid peroxidation. This finding is consistent with previous reports showing that exogenous Se can lower ROS levels (Chu et al. 2010, Liu et al. 2022).

Mechanistically, the scavenging of O_2^- and H_2O_2 was closely linked to the enhanced activities of antioxidant enzymes. SOD, as the first line of defence, converts O_2^- to H_2O_2 and O_2 . At the optimal concentration (30 g/ha), SOD activity increased by 15.91%, effectively reducing the precursor supply for H_2O_2 . Subsequently, POD, CAT, and APX decompose H_2O_2 into water, thereby forming an efficient ROS-scavenging network (Chu et al. 2010). APX is the first and rate-limiting enzyme of the AsA-GSH cycle. Notably, APX activity increased nearly fourfold (393.4%) at 30 g/ha, far exceeding the increases observed for other enzymes. This strong induction suggests that lysine-chelated selenite may preferentially activate the ascorbate-glutathione (AsA-GSH) cycle, which is particularly important for H_2O_2 scavenging in chloroplasts and the cytosol (Smirnoff 2005, Lanza and Reis 2021).

Se is an essential cofactor for glutathione peroxidase (GSH-Px), and exogenous Se can participate in peroxide scavenging through this enzyme pathway (Tinggi 2008). Selenite is indeed the most commonly used inorganic form of Se in crop biofortification.

However, ionic selenite has limited stability in aqueous spray solutions and is susceptible to rapid interconversion with other ions, potentially reducing its efficacy in foliar application (Pei et al. 2025). Compared with ionic selenite, lysine-chelated selenite exhibits greater stability in spray solution, slower interconversion, and improved compatibility with modern foliar application systems. These inherent properties make it a promising candidate for more efficient Se biofortification *via* foliar application (Dinh et al. 2018). In addition, lysine, as an essential amino acid, may improve plant nitrogen metabolism or act as a signalling molecule to induce the expression of antioxidant genes, thereby enhancing enzyme activities (Galili 2002). The superior effect of lysine-chelated selenite compared with previously reported applications of selenite may be attributed to the chelation with lysine, which improves the bioavailability of Se. At the same time, the amino acid itself may also participate in osmotic regulation and metabolic regulation.

GSH and AsA are important non-enzymatic antioxidants that function synergistically in the AsA-GSH

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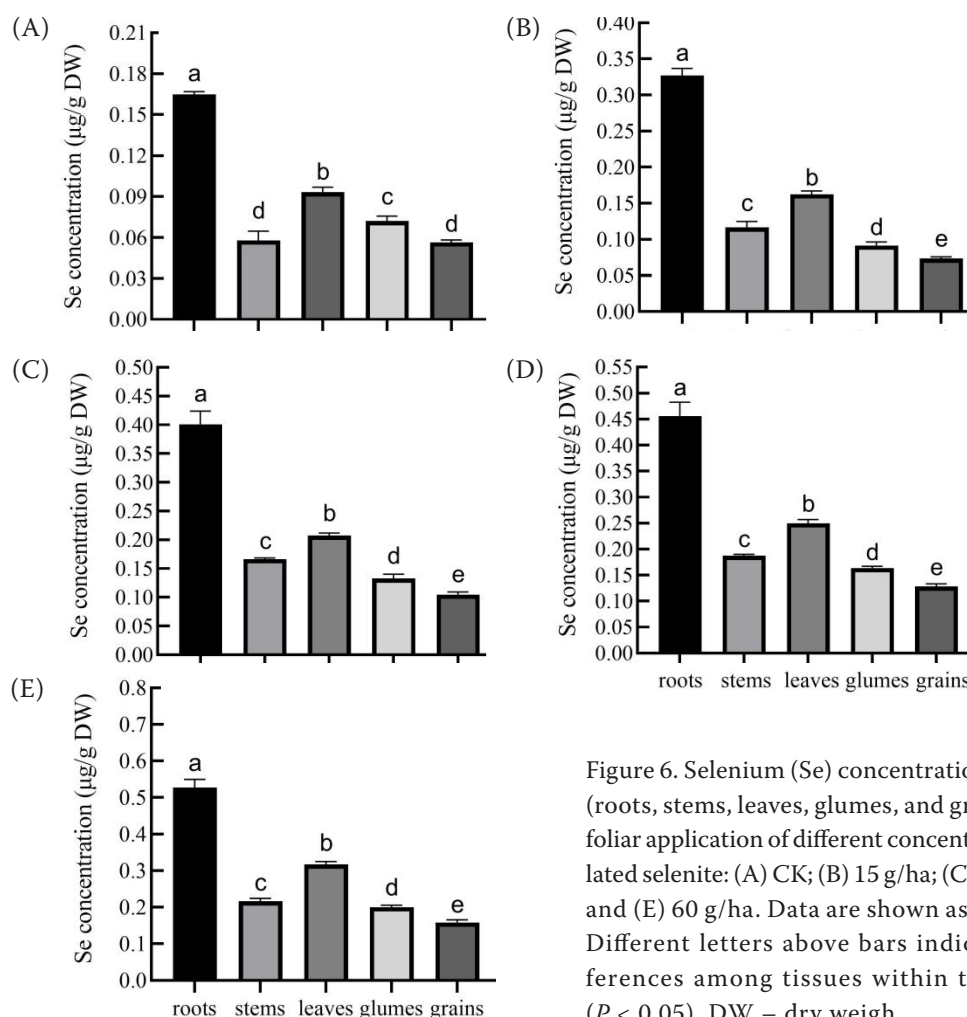


Figure 6. Selenium (Se) concentration in different tissues (roots, stems, leaves, glumes, and grains) of wheat under foliar application of different concentrations of lysine-chelated selenite: (A) CK; (B) 15 g/ha; (C) 30 g/ha; (D) 45 g/ha; and (E) 60 g/ha. Data are shown as means $\pm$ SD ($n = 3$). Different letters above bars indicate significant differences among tissues within the same treatment ($P < 0.05$). DW – dry weight

cycle to scavenge H_2O_2 (Foyer and Noctor 2011). In our study, GSH and AsA concentrations at 30 g/ha increased by 74.3% and 69.8%, respectively – substantial increments that likely result from Se-induced enhancement of glutathione synthase activity and serve as substrate support for the efficient operation of the AsA-GSH cycle (Ikram et al. 2024). However, at the high concentration (60 g/ha), the increase in GSH dropped sharply to 2.2% and that of AsA to 15.8%. This reduction may be explained by excessive Se causing GSH overconsumption by GSH-Px or by inducing mild oxidative stress that inhibits synthetic pathways (Yao et al. 2009).

The optimal selenite concentration enhanced osmotic adjustment and photosynthetic capacity. Soluble sugar and soluble protein concentrations at 30 g/ha increased by 43.7% and 94.0%, respectively, indicating that lysine-chelated selenite promotes carbohydrate accumulation and protein synthesis, thereby helping to maintain cellular osmotic balance

and metabolic activity. The increase in soluble sugar is likely related to enhanced photosynthetic capacity, as chlorophyll concentration increased by 32.5% under the same treatment. The increase in soluble protein is consistent with enhanced nitrogen metabolism, supported by a 30.5% increase in free amino acids.

Notably, proline concentration was lower than the control in all treatments, with the largest decrease at 30 g/ha. This finding contrasts with the classic phenomenon of proline accumulation under abiotic stress, where drought and salinity typically induce massive proline synthesis to maintain osmotic balance (Ghosh et al. 2022). However, our experiment was conducted under normal growth conditions without imposing stress. Under such conditions, the decrease in proline actually reflects good growth status and very low internal stress levels. Previous studies have shown that adequate nitrogen supply can reduce proline accumulation (Saddhe et al. 2021). In our study, the fertiliser contained calcium nitrate as a nitrogen

source, which may further improve nitrogen nutrition and thus reduce the demand for proline as an osmotic regulator. The increase in total free amino acids (30.5% at optimal concentration) confirms enhanced nitrogen metabolism. Therefore, the decrease in proline concentration is not contradictory to improved antioxidant capacity; rather, it serves as an indirect indicator of improved overall plant health.

The increases in roots and glumes indicate that Se continued to accumulate in all non-sink tissues without preferential redistribution to grains. These tissues function as accumulation sites because Se was continuously distributed to all plant parts, resulting in continuous accumulation in all non-sink tissues. The progressive increase in stem Se concentration indicates that the stem functioned as an efficient transport path throughout the tested concentration range. This may be attributed to the chelation of Se with lysine, which enhances Se mobility and reduces premature immobilisation in vascular tissues. The sustained increase in stem Se concentration, coupled with the continuous accumulation in grains, supports the conclusion that lysine-chelated selenite was efficiently translocated toward developing seeds.

The dose-dependent increases in leaves and grains demonstrate that both tissues serve as Se sinks, albeit through different mechanisms. Leaves, as the direct targets of foliar sprays, accumulate Se passively through cuticular and stomatal absorption, with no apparent saturation even at the highest concentration. Grains, as the ultimate sink organs, exhibit the highest accumulation priority; Se continues to be loaded into grains even when the plant experiences oxidative stress at application dose 4. The continuous increase in grain Se concentration – despite the physiological decline at T4 – has important implications for Se biofortification: even at concentrations that slightly compromise plant health, grain Se enrichment continues, suggesting that the optimal concentration for biofortification may differ from that for plant growth. The disproportionate increase in stem Se concentration relative to grains highlights a potential transport bottleneck in the source-sink pathway. While the stem serves as an efficient conduit, the disproportionate accumulation of Se in the stem relative to grains suggests that the efficiency of Se transport from the stem to the grain may decline at higher application doses, although the absolute amount of Se loaded into grains continued to increase. This observation aligns with findings from Di et al. (2023), who demonstrated that grain Se ac-

cumulation largely depends on the transfer efficiency from the node to the internode. Future strategies to enhance biofortification should therefore focus not only on increasing leaf Se concentration but also on improving phloem loading and unloading efficiency to overcome this translocation bottleneck.

The grain Se concentration obtained in the present study is comparable to values reported for wheat following foliar selenite application. A field study reported that foliar selenite application at 100 g Se/ha resulted in substantial Se accumulation in mature wheat grains (Galinha et al. 2015). In addition, foliar application of 5 mg/L selenite was reported to be the most efficient strategy for producing Se-biofortified wheat flour (Wang et al. 2022). In the present study, the grain Se concentration at a Se application rate of 30 g/ha was 0.1047 µg/g DW, which falls within the safe range for grain Se concentrations following foliar selenite application in these studies. The dose-dependent increase in grain Se concentration indicates that lysine-chelated selenite is effective for the Se biofortification of wheat grain.

When integrated with the antioxidant and metabolic data (Figures 1–5), these tissue-specific Se distribution patterns reveal a close association between plant physiological status and Se partitioning. At T2 (30 g/ha) – the concentration that maximised antioxidant enzyme activities, minimised oxidative damage, and optimised osmotic and photosynthetic performance – Se accumulation in grains reached 85.8% over control, indicating efficient translocation to the ultimate sink. At T4 (60 g/ha), despite continued Se accumulation in grains, the plant exhibited clear signs of oxidative stress (reduced SOD and POD activities, diminished soluble protein), indicating that the physiological cost of high Se accumulation outweighed the antioxidant benefits. This suggests that while Se transport to grains remained efficient even at the highest dose, the associated physiological burden limits the practical utility of doses above 30 g/ha. This biphasic response reinforces the conclusion that 30 g/ha represents the optimal balance between plant health and effective Se translocation, and that stem Se concentration can serve as a practical indicator for assessing Se transport efficiency in wheat breeding and agronomic programs.

As a trace element, Se has a narrow beneficial concentration range. When the application dose exceeds a certain threshold, excessive Se may replace sulfur in proteins, interfere with the electron transport chain, or induce excessive ROS production, leading

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to pro-oxidant effects (Hartikainen et al. 2000, Reilly 2004). In our study, APX activity at 60 g/ha remained 105.4% higher than the control, whereas SOD and POD activities had shifted to inhibition (Mechora et al. 2017). This differential response indicates that different antioxidant enzymes have varying sensitivities to Se concentration, with APX being more robustly induced even at higher doses. This finding has practical implications: in agricultural production, the foliar application dose should be strictly controlled, and our recommended rate is 30 g/ha of lysine-chelated selenite under the conditions tested.

In conclusion, this study demonstrates that foliar application of lysine-chelated selenite at an optimal concentration (30 g/ha) effectively enhances the overall physiological activity of wheat leaves by coordinately upregulating antioxidant enzymes, alleviating membrane lipid peroxidation, enhancing osmotic adjustment, and promoting photosynthetic pigment synthesis. Moreover, the tissue-specific Se distribution analysis reveals that this concentration results in substantial Se translocation to grains (an 85.8% increase) while maintaining physiological balance, providing a basis for Se biofortification strategies. These findings provide a scientific basis for the development of lysine-chelated selenite as a novel foliar conditioner in wheat production. However, further mechanistic and agronomic validation is warranted before large-scale application.

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