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Foliar Application of SeNPs for Rice Biofortification: a Comparative Study with Selenite and Speciation Assessment

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ABSTRACT: A significant portion of the global population lacks access to a balanced diet, leading to widespread micronutrient deficiencies. Selenium (Se) deficiency affects approximately 1 billion people worldwide, and agronomic biofortification of food crops using inorganic Se fertilizers or Se nanoparticles (SeNPs) has emerged as a potential solution. However, to ensure food safety, it is critical to assess whether nonbioavailable or toxic Se species are formed when SeNPs are introduced into plants. In this study, pot experiments with rice plants (*Oryza sativa* L.) were conducted to evaluate the effects of foliar applications of selenite (Se(IV)) and SeNPs on Se uptake, translocation, and speciation. Plant growth, chemical, and biochemical parameters were evaluated. Selenium accumulation and speciation were determined using inductively coupled plasma mass spectrometry (ICP-MS) and high-performance liquid chromatography coupled with ICP-MS (HPLC-ICP-MS). The results demonstrated that SeNP treatment did not adversely affect plant growth, grain yield, and oxidative stress or significantly increase the inorganic Se content in rice grains. From a nutritional perspective, grains biofortified with SeNPs had the potential to meet 100% of the recommended daily Se intake. Meanwhile, Se(IV) was more efficient for grain biofortification but increased the concentration of inorganic Se in rice grains by 141% compared to the control group. Regardless of the Se species applied, rice fertilization increased the proportion of selenomethionine while it reduced selenocysteine in grains. The treatment with SeNPs did not compromise the nutritional quality of rice grains but increased As content from 175 to 210 $\mu g\ kg^{-1}$, which remains below the maximum allowable limit of 350 $\mu g\ kg^{-1}$ for husked rice. The foliar application of SeNPs enables the production of Se-enriched rice with Se levels controlled within a safe range for human consumption and without significantly altering inorganic Se concentrations. This approach offers a viable strategy for addressing Se deficiency through biofortified rice.

KEYWORDS: *SeNPs, Oryza sativa L., agronomic biofortification, Se species, HPLC-ICP-MS, sustainable agriculture*

1. INTRODUCTION

Micronutrient deficiency affects approximately 3 billion people worldwide.[1] Selenium (Se) is an essential micronutrient required by most living organisms, including lower plants, animals, and humans, to maintain health.[1−6] It has important functions in the organism, such as regulating the intracellular redox state, supporting human immunity, facilitating thyroid hormone metabolism, and protecting from oxidative damage.[2,5,7−11] Despite its importance, it is estimated that around 1 billion people worldwide—roughly one in every eight— have an insufficient Se intake.[1] The balance between Se deficiency, essentiality, and toxicity is narrow, making its proper management critical.[12] Se toxicity, mobility, and bioavailability depend on its chemical speciation and concentration.[12−15] The dietary reference intake (DRI) of Se in the United States is 55 $\mu g\ day^{-1}$ for adults,[16] while in Europe it ranges from 55 to 70 $\mu g\ day^{-1}$.[6] The tolerable upper intake level (UL) for Se, established by the Institute of Medicine (IOM) in 2000, was 400 $\mu g\ day^{-1}$ for adults.[16] More recently, in 2023, the European Food Safety Authority's (EFSA) Panel on Nutrition, Novel Foods, and Food Allergens established an UL of 255 $\mu g\ day^{-1}$ for Se intake.[17]

Diet is the primary source of Se for humans, including cereals, grains, poultry, bread, fish, eggs, meat, nuts, and broccoli. Se absorption from plant-based foods is generally more efficient than from animal-derived products.[5,18−20] Consequently, human Se intake largely depends on its concentration and bioavailability in edible plants.[3,5,10] Se occurs in more than 15 distinct forms in food, making the determination of total Se content insufficient for evaluating its

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impacts on human health. In general, organic Se compounds exhibit greater bioavailability compared to inorganic forms, with the selenoamino acid selenomethionine (SeMet) being particularly effective in increasing tissue Se levels.[10,19,21] Regarding toxicity, both organic and inorganic Se compounds can be harmful at high doses.[21] However, inorganic precursors are more toxic, requiring smaller doses to induce poisoning compared to organic Se.[16,19]

Staple crops such as rice, wheat, beans, maize, cassava, barley, and corn are the most common plant-based foods in the human diet.[5,22] Although Se is not essential for higher plants, it is considered a beneficial element. Optimal Se concentration in plant tissues, which stimulate growth and enhance stress resistance, range of 1 to 10 mg kg^{-1} DW, while toxic effects are typically observed at concentrations exceeding 100 mg kg^{-1} DW.[23] Increasing the bioavailable Se concentration in edible crop products through agronomic biofortification has become a promising strategy in modern agriculture.[5,22] Agronomic biofortification with Se can be achieved using seed coating and priming, soil fertilization, or foliar application. Common Se fertilizers are based on inorganic Se sources, primarly selenate (Se (VI)) and selenite (Se (IV)).[3,5,24−26] These compounds are highly soluble, ensuring rapid uptake by plants. While the cost of Se-enriched fertilizers is relatively low, their continuous application poses risks of soil and water contamination through leaching.[27] Additionally, the high mobility of inorganic Se contributes to environmental toxicity.[28] Organic Se fertilizers, such as Se-containing amino acids, have shown promise in producing Se-enriched rice with increased biomass and grain quality;[29] as well as in enhancing cabbage nutrition and defense against crop diseases.[30] Unlike inorganic Se, organic compounds are not actively leached but degrade rapidly after application.[27] Biofortification with Se nanoparticles (SeNPs) is a relatively novel approach that is gaining attention.[4,5] SeNPs exhibit lower solubility and toxicity, allowing for controlled Se release, reduced leaching, and prolonged Se availability in soil and plants.[6,27] Although the initial production cost of SeNPs is higher compared to inorganic fertilizers, their lower reapplication frequency may offset these costs over time.

Previous studies have demonstrated that SeNPs can be absorbed by rice roots and leaves.[31−33] Once internalized, Se is transported both upward and downward within the plant.[32,33] Inside a biological system, NPs may undergo transformations that alter their physicochemical properties. For instance, SeNPs can be converted into more bioavailable Se species within plant tissues, thereby influencing Se speciation.[31] The mechanisms of NPs uptake, translocation, bioaccumulation, and biotransformation in plants remain not fully elucidated.[34,35] Understanding the biotransformation of SeNPs in plants is essential to assess their metabolic pathway and their potential impact on human health. Further research is needed to address these critical knowledge gaps.[36,37]

Considering the essential role of selenium (Se) in human nutrition and the widespread consumption of rice, it is hypothesized that SeNPs could be a safe and effective nanofertilizer for rice biofortification. The main objectives of thist study were as follows: (1) to utilize nanotechnology to produce Se-enriched rice grains; (2) to evaluate the uptake, accumulation, translocation, and biotransformation of SeNPs in plants, compared to selenite (Se(IV)); (3) to assess the effects of Se enrichment on the agronomic, chemical, and biochemical parameters of rice plants.

2. MATERIALS AND METHODS

2.1. SeNPs Synthesis and Characterization. In this study, sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are referred to as Se (IV) and Se (VI), respectively. SeNPs were synthesized using a chemical reduction method as previously described.[38,39] Briefly, SeNPs were prepared by reduction of Se (IV) (Sigma-Aldrich, St-Louis, USA) by ascorbic acid (Synth, Diadema, Brazil) using poly(vinyl alcohol) (30,000 to 70,000 MW, Sigma-Aldrich, St-Louis, USA) as a stabilizer. The synthesized SeNPs were characterized by ultraviolet−visible spectrophotometry (Agilent 8454, Palo Alto, USA), transmission electron microscopy (JEM-2100 Plus, 200 kV, JEOL, Peabody, USA), dynamic light scattering (Nano ZS Zetasizer, Malvern Instruments Co, Worcestershire, UK), Fourier transform infrared spectroscopy (PerkinElmer, Waltham, MA, USA), ICP-MS (Agilent 7900, Hachioji, Japan), and single particle ICP-MS (data not shown). The sample preparation and instrumental parameters for each analytical technique have been detailed in previous studies.[39,40]

2.2. Plant Material and Growth Conditions. Rice seeds (*Oryza sativa* L. ssp. *indica* cv. BRS PAMPA) used in this study were provided by EMBRAPA (Brazilian Agricultural Research Corporation, Temperate Climate Center, Pelotas, RS, Brazil). The BRS PAMPA cultivar was developed by EMBRAPA in partnership with the Rice Institute of Rio Grande do Sul (Brazil) and is characterized by high genotypic grain yield, adaptability, favorable agronomic traits, and an early growth cycle.[41]

The pot experiment was conducted in a greenhouse at the Federal University of ABC (UFABC, São Bernardo do Campo, Brazil) from November 2021 to April 2022, under controlled humidity (60 to 75%) and temperature (30 ± 5 $C°$). Approximately 150 seeds of *O. sativa* were immersed in water in the dark for 48 h. The seeds were then germinated on moistened paper sheets at 28 °C (SL-100, Solab, Brazil). After 72h, germinated seeds (radicle length >2 mm) were selected and planted in germination trays containing organic soil. After 10 days, uniform seedlings were transplanted into individual 8 L plastic pots (one seed per pot) containing a mixture of 70% organic soil and 30% topsoil.

The soil used in the experiment was characterized for its general physicochemical properties according to the methodologies described by Raij et al.[42] and da Silva.[43] The soil was classified as clay loam with a pH of 7.5 ± 0.2. The composition analysis showed 370 ± 17 g kg^{-1} of clay, 343 ± 26 g kg^{-1} of sand (180 ± 20 g kg^{-1} of coarse sand and 163 ± 8 20 g kg^{-1} of fine sand), and 287 ± 43 g kg^{-1} of silt. The organic matter content was 36.0 ± 2.6 g dm^{-3}, and the cation exchange capacity was 182 ± 7 mmolc dm^{-3}. Additional soil physicochemical properties included: base saturation, 96.3 ± 0.6%; sum of bases, 175 ± 7 mmolc dm^{-3}; exchangeable K, 15.4 ± 2.1 mmolc dm^{-3}; exchangeable Ca, 131 ± 7 mmolc dm^{-3}; exchangeable Mg, 28.7 ± 5.7 mmolc dm^{-3}; H+Al, 9.00 ± 0.00 mmolc dm^{-3}; electrical conductivity, 5.33 ± 2.26 ds m^{-1}; total C, 21.0 ± 1.7 g dm^{-3}; and P (resin), 28.0 ± 3.5 mg dm^{-3}.

The plants were grown under flooded conditions, maintaining a water level of 3−4 cm, until the grains reached full maturation. Agricultural practices and fertilizer applications (urea-N 45% and NPK 4−14−8) were standardized across all pots throughout the experiment.

2.3. Plant Treatments and Growth Measurements. The experimental design consisted of a randomized setup with three treatments, each containing 10 biological replicates randomly distributed within the greenhouse. A 0.1% w v^{-1} solution of Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) was used as a surfactant in all dilutions to enhance the efficiency of the foliar treatments. The treatment groups were as follows: (i) Control group: Triton X-100 at 0.1%; (ii) SeNP group: SeNPs at 0.5 mg L^{-1} of Se diluted in 0.1% Triton X-100; (iii) Se (IV) group: Se (IV) at 0.5 mg L^{-1} of Se diluted in 0.1% Triton X-100. The Se concentration was selected based on previous studies conducted by our group.[40] The SeNP suspension was freshly prepared by diluting the original suspension after 1 min of sonication (KQ3200, 120 W, 40 kHz, Kunshan, China).

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The treatments were applied via foliar spraying in the morning (7:00 to 9:00), beginning at the flowering stage of each plant. To prevent SeNPs from leaching into the soil, the soil surface was covered with aluminum foil during the applications.[44] Plants were sprayed until the solution was evenly distributed across the leaf surface. Each plant received a total of four applications, spaced 5 days apart.

Following the four applications, flag leaves were collected from three replicates per treatment and stored at −80 °C for biochemical analysis (refer to Section 2.4). After grain maturity (approximately five months), the number of productive panicles was recorded, and grains were harvested. Shoot length was measured with a ruler, and shoots were cut 2 cm above the soil surface. Plant tissues were harvested and separated into roots and aerial parts (shoots + leaves). These tissues were individually washed with tap water and rinsed five times with ultrapure water (resistivity 18.2 MΩ cm, MS2000, Gehaka, São Paulo, Brazil). A portion of the fresh roots was stored at −80 °C for subsequent biochemical analysis (refer to Section 2.4). The roots, grains, and aerial parts were oven-dried at 45 °C (SL-100, Solab, Piracicaba, Brasil) until constant weight. The dried tissues were then weighed to determine their dry weight (DW). Roots and aerial parts were roughly cut into small pieces using scissors. Grains were manually husked, and both grains and husks were ground separately in an analytic mill (IKA A1, Staufen, Germany) before sieving (particle size <250 μm). Samples were stored until further analysis for total Se content or Se speciation (refer to Section 2.5 and 2.6, respectively).

2.4. Biochemical Analysis. The following subsections detail the biochemical parameters measured in this study. All experiments were performed in triplicate. Absorbance measurements were performed using a UV−visible spectrophotometer (Agilent 8454, Palo Alto, CA, USA).

2.4.1. Antioxidant Enzyme Activity. Enzymatic extracts were prepared following the methodology described by Pelegrino et al.[45] to quantify the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD). Briefly, 100 mg fresh roots or flag leaves, previously stored at −80 °C, were macerated in liquid nitrogen with 1 mL of extraction buffer. The extraction buffer consisted of 1 mmol L^{-1} EDTA (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 mol L^{-1} potassium phosphate buffer (Labsynth, Diadema, São Paulo, Brazil) at pH 6.8. The extract was sonicated for 10 min (KQ3200, 120 W, 40 kHz, China) and centrifuged for 10 min (12,100 rpm, Mini Spin, Eppendorf, Hamburg, Germany). The supernatant was collected for enzyme activity determination.

SOD activity was measured according to its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT),[46,47] following the procedure described by Freire et al.[48] Absorbance was measured at 560 nm after 10 min of reaction under 300 μmol m^{-2} s^{-1} of photosynthetic active radiation. One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50%.[47,49]

APX activity was determined by measuring H_2O_2-dependent ascorbate oxidation,[47] following the methods of Pelegrino et al.[45] and Freire et al.[48] Absorbance was recorded at 290 nm 2 min after preparing the reaction mixture. Enzyme activity was calculated using the Lambert−Beer law (ε = 2.8 L $mmol^{-1}$ cm^{-1}).[50]

POD activity was determined according to Pelegrino et al.[45] and Freire et al.[48] Absorbance was measured at 420 nm 1 min after preparing the reaction mixture. Enzyme activity was calculated using the Lambert−Beer law (ε = 2.47 L $mmol^{-1}$ cm^{-1}).[46]

2.4.2. H_2O_2 Generation. For hydrogen peroxide (H_2O_2) quantification, 100 mg of fresh leaves or roots previously frozen at −80 °C were macerated with 5 mmol L^{-1} of *N*-ethylmaleimide in 1 mL of potassium phosphate buffer. The extract was sonicated (40 kHz) for 10 min and centrifugated at 12,100 rpm for 10 min. The measurement was performed using a free radical analyzer (WPI TBR4100/1025 Ammeter, World Precision Instruments Inc., Sarasota FL, USA) equipped with an ISO−HPO-2 sensor. Quantification was achieved using a 100 mmol L^{-1} PBS solution and an H_2O_2 calibration curve.[45,51]

2.5. Total Element Content in Rice Tissues. Acid digestion was performed to determine the concentrations of Se, Na, Mg, P, K, Ca, Mn, Co, Ni, Cu, Zn, As, and Cd in rice tissues, following the method described by Freire et al.[40] Dried samples (six biological replicates per treatment) were weighed (150 mg for grains and husks, 100 mg for aerial parts, and 50 mg for roots) into 50 mL conical tubes. Subdistilled (DST-100-Savillex, USA) HNO_3 (65% w w^{-1}, Synth, São Paulo, Brazil) was then added (2 mL for grains and husks, 1.5 mL for aerial parts, and 1 mL for roots), and the tubes were left for predigestion at room temperature (~25 °C) for 48 h. After predigestion, samples were heated in a digestion block (Easy Digest, Analab, Hoenheim, France) at 95 °C for 4 h. Once cooled, the volumes were made up to 30 mL (grains and husks) or 20 mL (roots and aerial parts) with deionized water (resistivity 18.2 MΩ cm, Master System All, Gehaka, Brazil).

Elemental determination was conducted using an ICP-MS (Agilent 7900, Hachioji, Japan) according to Freire et al.[40] The sample introduction system consisted of a Mira Mist nebulizer and a Scott (double pass) spray chamber. Helium was used in the collision reaction cell (CRC) to reduce spectral interferences, with flow rates of 5 mL min^{-1} for Mg, Co, Ni, Cu, Zn, As, Se, and Cd, and 10 mL min^{-1} for Na, P, K, Ca, and Mn. Other operating conditions included RF power at 1550 W, plasma gas flow at 15.0 L min^{-1}, and nebulizer gas flow at 1.01 L min^{-1}. The monitored isotopes were ^{23}Na, ^{24} Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{55} Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{68}Zn, ^{75}As, ^{78}Se, and ^{112}Cd.

The instrumental limits of detection (LOD) were calculated as the mean of the analytical blank concentration values plus three times the standard deviation of these measurements (n = 10), in accordance with guidelines from the National Institute of Metrology, Quality, and Technology.[52] The calculated LODs (in μg L^{-1}) were as follows: 1.9 (Na), 0.38 (Mg), 88 (P), 4.8 (K), 8.3 (Ca), 0.032 (Mn), 0.002 (Co), 0.038 (Ni), 1.7 (Cu), 1.1 (Zn), 0.060 (As), 0.50 (Se), and 0.004 (Cd).

To ensure quality assurance and control (QA/QC) of the analytical protocol, four standard reference materials (SRM) were analyzed: tomato leaves (NIST 1573a, National Institute of Standard and Technology Gaithersburg, MD, USA), aquatic plants (BCR-670, Institute for Reference Materials and Measurements, Geel, Belgium), rice flour (1568b, NIST, Gaithersburg, MD, USA), and brown rice (C1001a, CRM-Agro- Certified Reference Materials for Agriculture, Livestock and Toxicology, CENA-USP, Piracicaba, SP, Brazil). These SRMs were subjected to the same digestion procedure as the samples, and recovery rates ranged from 80 and 120%, meeting U.S. Food & Drug Administration guidelines for elemental determination in food samples using spectrometric techniques.[53] For Se, the measured content in SRM NIST 1568b was 0.410 ± 0.015 mg kg^{-1} compared to the certified value of 0.365 ± 0.029 mg kg^{-1}, resulting in a recovery rate of 112%.

2.6. Se Speciation in Rice Grains. Selenium speciation in rice grains was determined using high-performance liquid chromatography (HPLC, Agilent 1200 Infinity II, Waldbronn, Germany) coupled with ICP-MS (HPLC-ICP-MS). The extraction of Se species was performed as previously described by Fang et al.,[54] with slight modifications. Briefly, 250 mg of milled rice (<250 μm) was weighed into a 50 mL centrifugal tube (four replicates per treatment), and 25 mg of α-amylase (Sigma-Aldrich, St. Louis, MO, USA) along with 4 mL of deionized water were added. The mixture was incubated in an ultrasonic bath at 37 °C for 30 min, followed by continuous shaking (100 rpm, SL 223, Solab, Brazil) for 2 h. Subsequently, 20 mg of Protease XIV (Sigma-Aldrich, St. Louis, MO, USA) was added, and the mixture was incubated in an ultrasonic bath at 45 °C for another 2 h. The extract was then centrifuged (SL 699, Solab, Brazil) at 3500 rpm for 20 min, the supernatant was collected, filtered through a 0.22 μm cellulose filter (Sartorius, Burlington, MA, USA), and stored at −20 °C for subsequent Se speciation analysis according to Paniz et al.[55]

The HPLC instrument conditions were as follows: an anion exchange column (150 mm × 4.6 mm, 5 μm particle size, PRP-X100, Hamilton, Switzerland) operated at room temperature; the mobile phase consisted of 1% v v^{-1} Methanol in 99% v v^{-1} 10 mM

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

ammonium citrate (pH 5.0) at a flow rate of 0.4 mL min^{-1}; and the injection volume was 20 μL. The ICP-MS operating conditions were: RF power of 1550 W, sampling depth of 8.0 mm, nebulizer gas flow rate of 1.06 L min^{-1}, and H_2 as the reaction gas in the CRC at a flow rate of 3.5 mL min^{-1}. Selenium was monitored at the mass-to-charge ratio (m/z) ^{78}Se, with ^{103}Rh used as internal standard. The integration time was set to 0.4 s.

Four standard Se compounds (SeMet: seleno-DL-methionine, $SeCys_2$: seleno-L-cystine, Se^{4+}: selenite, and Se^{6+}: selenate, all from Sigma-Aldrich, USA) were used for calibration. Stock solutions of each compound containing 1000 mg L^{-1} of Se were prepared by dissolving the standards in 0.2% v v^{-1} HCl. These solutions were subsequently acid-digested, and the Se concentrations were verified by ICP-MS. Recovery rates for the four Se species ranged from 104% to 106%, indicating no chemical interferences during Se determination. The individual Se species solutions were mixed to prepare calibration standards with concentrations ranging from 0 to 100 μg of Se L^{-1} for HPLC-ICP-MS analysis. Selenium species were identified by their retention times and quantified based on the peak areas in the calibration curve. For quality control, the SRM NIST 1568b rice flour (National Institute of Standards and Technology, Gaithersburg, MD, USA) was analyzed.

2.7. Data Analysis. The translocation factor (TF) of Se from part "a" to part "b" of the rice plant was calculated using eq 1:[56]

$$TF_{a-b} = \frac{C_b}{C_a} \quad (1)$$

where C_a and C_c represent Se concentration in part "a" and part "b" of the rice plant (μg kg^{-1}), respectively. Parts "a" and "b" can refer to roots, aerial parts, or grains.

The estimated daily intake (EDI) was calculated to evaluate whether rice biofortification influenced Se intake through rice consumption (husked grain). The EDI (μg day^{-1}) was determined using eq 2:

$$EDI = C_{Se} \times M_{rdc} \quad (2)$$

where C_{Se} is the mean Se concentration in rice grains (μg kg^{-1}), and M_{rdc} is the average daily rice consumption in Brazil, assumed to be 0.1314 kg day^{-1}.[57] The calculated EDI was compared to the DRI of 55 μg day^{-1}.[16]

The proportions of Se species in rice grains ($\%Se_{sp}$) were calculated using eq 3:

$$\%Se_{sp} = \frac{(A_{sp} \times 100)}{\Sigma_A} \quad (3)$$

where A_{sp} is the integrated area of each species (Se^{4+}, Se^{6+}, SeMet, and $SeCys_2$), and Σ_A is the sum of the integrated areas of all detected Se species.

2.8. Statistical Analysis. The results were analyzed using one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons. Statistical analyses were performed using GraphPad Prism 10.2.2 (GraphPad Software, San Diego, CA, USA), with a significance level of $p < 0.05$. Graphical representations were generated using Origin Pro 8.5 (OriginLab Corp, Northampton, MA, USA).

3. RESULTS AND DISCUSSION

3.1. SeNPs Characterization. The characterization of SeNPs has been previously reported.[39,40] Briefly, the results confirmed the successful synthesis of spherical and monodisperse SeNPs with an average diameter of 50.1 ± 5.6 nm, as determined by TEM. A representative TEM image of synthesized SeNPs is shown in Figure 1. The formation efficiency of SeNPs was 75%. SeNPs were also characterized in the medium used for foliar applications, and it was observed that the addition of Triton X-100 as a surfactant enhanced SeNP stability by reducing the zeta potential from −3.63 ± 2.58 to −9.85 ± 0.39 mV.[40]

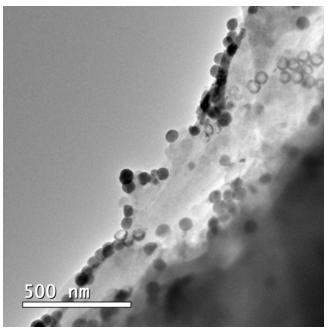

Figure 1. Transmission electron microscopy image of SeNPs showing spherical nanoparticles. The white bar indicates 500 nm.

3.2. Effects of Different Se Forms on Plant Agronomic Parameters. A previous study by our group suggested that the foliar application of SeNPs at 0.5 mg L^{-1} represents an optimal dose for rice biofortification, as it increased Se content in rice tissues without negatively affecting plant growth.[40] Therefore, this concentration was used in pot experiments with plants cultivated through their entire life cycle to evaluate whether, under this optimal concentration, different Se forms influence plant agronomic, chemical, and biochemical parameters.

Various agronomic indicators of rice growth and productivity were measured at the final harvest, and the results are shown in Table 1. The number of panicles remained unchanged across treatments. Shoot length was not significantly different between Se-biofortified plants and the control group; however, Se(IV)-treated plants exhibited a 12% increase in shoot length compared to SeNP-treated plants.

In contrast, root, shoot, and grain DW were differently affected by the form of Se applied. Se (IV)-treated plants showed a 35% increase in shoot and grain DW compared to the control group, while root DW increased by 98%. In plants treated with SeNPs, these parameters showed slight increases, but the differences were not statistically significant ($p > 0.05$).

It is worth mentioning that the stimulatory or inhibitory effects of Se in rice plants are highly dose-dependent, showing a hormetic effect, that is, beneficial at a low doses but toxic or inhibitory at higher doses.[40,58,59] Other factors, such as the form of Se applied (e.g., ionic or nanoparticulate), experimental conditions, treatment duration, and the developmental stage at which Se is applied, also influence the response. Consistent with our findings, previous studies have demonstrated the stimulatory effects of ionic Se on plant growth and productivity. D'Amato et al.[58] reported that cultivating rice sprouts in a Se (IV) solution at 15 mg L^{-1} increased fresh biomass and root length, while higher concentrations inhibited growth, suggesting a hormetic response. Similarly, the foliar application of Se (IV) and Se (VI) at 50 μmol L^{-1} in rice plants was shown to enhance grain yield.[60] In contrast, the application of SeNPs as a nanofertilizer may not significantly affect plant growth under stress-free conditions. Freire et al.[40] observed that foliar application of SeNPs at 0.5 mg L^{-1} in rice seedlings had no significant impact on growth parameters, apart from a slight reduction in root DW. However, under abiotic stress conditions (e.g., drought or saline stress), SeNPs have been shown to promote plant growth and productivity, mitigating the effects of stress.[44,61] In the present study, plants were cultivated under stress-free

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Table 1. Effect of Foliar Application of SeNPs and Se (IV) on Agronomic Parameters of Rice Plants Harvested after Complete Maturation of Grains[a]

Treatment	Shoot length (cm)	Number of panicles	Root weight (g DW)	Shoot weight (g DW)	Grain weight (g DW)
Control	87.6 ± 6.6^{ab}	15.6 ± 3.2^{a}	24.4 ± 10.2^{b}	16.9 ± 4.8^{b}	24.5 ± 5.4^{b}
SeNPs	84.6 ± 6.8^{b}	17.5 ± 3.2^{a}	30.3 ± 11.6^{ab}	18.7 ± 4.3^{ab}	28.6 ± 5.1^{ab}
Se (IV)	94.8 ± 6.8^{a}	18.6 ± 1.6^{a}	48.4 ± 25.4^{a}	22.9 ± 3.7^{a}	33.0 ± 3.5^{a}

[a]Data are average values for 10 replicates ± standard deviation (SD). Different letters within each column indicate statistically significant differences (Tukey's test at $p < 0.05$). DW: dry weight.

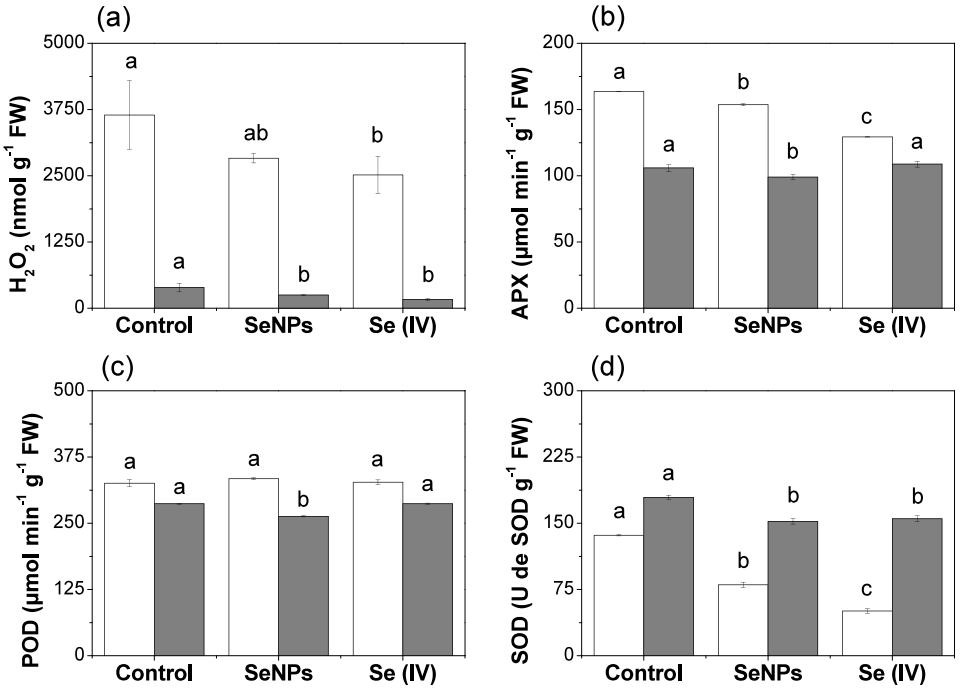

Figure 2. Effect of foliar application of SeNPs and Se (IV) on (a) hydrogen peroxide (H_2O_2) and on the antioxidants (b) ascorbate peroxidase (APX), (c) peroxidase (POD), and (d) superoxide dismutase (SOD) activities in rice leaves (white bars) and roots (gray bars). Data are average values for 3 replicates while error bars represent the standard deviation (SD). Different letters within each tissue indicate significant differences between groups by Turkey's test at $p < 0.05$. FW: fresh weight.

conditions, which may explain the lack of a pronounced growth-stimulatory effect of SeNPs.

Our results demonstrated a stimulatory effect of Se (IV) at the optimal concentration on rice growth and yield, whereas SeNPs showed neither stimulatory nor inhibitory effects on plant development. Therefore, regarding agronomic parameters, both Se (IV) and SeNPs can be considered safe and nontoxic Se fertilizers, although Se (IV) exhibited superior performance in terms of growth and productivity.

3.3. Effects of Different Se Forms on Plant Antioxidant System. The effects of different Se forms on the antioxidant system of rice plants were assessed by measuring the activities of selected enzymatic antioxidants and the H_2O_2 concentration in roots and leaves. The results are presented in Figure 2. Both Se treatments significantly reduced H_2O_2 levels in roots, with reductions of 37% for SeNPs and 58% for Se (IV). Additionally, Se (IV) decreased H_2O_2 levels in leaves by 31%, suggesting that both treatments enhanced the elimination of reactive oxygen species (ROS).

In general, higher enzymatic antioxidant activities were observed in the control group (without Se biofortification) in both roots and leaves. For APX activity, this difference was statistically significant ($p < 0.05$) in the roots and leaves of the SeNPs-treated plants and the leaves of the Se (IV)-treated plants. For POD activity, a significant reduction was observed only in the roots of SeNPs-treated plants compared to the control. In contrast, SOD activity significantly decreased in the roots and leaves of both Se-treated groups.

Plants synthesize ROS as signaling molecules, which increase significantly under oxidative stress induced by factors such as salinity, cold, water deficit, or metal toxicity.[62,63] Overproduction of ROS can lead to oxidative damage, protein oxidation, nucleic acid damage, enzyme inhibition, and cell death.[64] Selenium plays a crucial role in controlling ROS production both directly and indirectly by regulating antioxidant activity.[62] Therefore, the observed reduction in H_2O_2 levels indicates the absence of oxidative stress. Consistent with our findings, Se (IV) application reduced ROS levels by 86.6% in rice suspension cells under Cd stress, alleviating Cd toxicity.[65] Similarly, seed priming with SeNPs at 50−100 mg L^{-1} reduced leaf H_2O_2 in tomato plants cultivated under both normal and water-stress conditions.[66]

Antioxidant enzymes are essential for mitigating oxidative stress by alleviating the effects of ROS on plants.[64] An increase in their activity is often indicative of oxidative stress.[51,67] Under such conditions, the plant antioxidant system is activated, with SOD serving as the first line of defense by converting O_2^- to O_2 and H_2O_2.[45] APX, as the initial enzyme

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

in the ascorbate-glutathione cycle, scavenges the H_2O_2 in plant tissues.[68] According to Feng, Wei, and Tu,[62] the response of SOD activity to Se exposure depends on the level of stress: at low stress levels, the plant's inherent antioxidative capacity may suffice, while under high stress, increased SOD activity is required to mitigate oxidative damage.

SeNPs are recognized as promising agents for enhancing the antioxidant defense system in plants, particularly under stress conditions.[66,69] However, the concentration of SeNPs is critical to their effectiveness. Khai et al.[69] reported that applying SeNPs at 0.1 mg L^{-1} to gerbera (*Gerbera jamesonii*) plants reduced the activities of antioxidant enzymes such as APX and SOD compared to the positive control, whereas higher SeNP concentrations increased their activities. In the present study, rice plants were cultivated under nonstress conditions, suggesting that increased antioxidant enzyme activity was not necessary to mitigate ROS. Furthermore, only a single SeNP concentration was tested, leaving open the possibility that higher concentrations might have stimulated the plants' antioxidant system. Our findings indicate that neither SeNPs nor Se(IV) induced oxidative stress in rice plants at the concentration tested, as evidenced by the reduction in antioxidant enzyme activities and ROS levels.

3.4. Selenium Uptake, Translocation, and Accumulation in Rice Plants. To investigate how Se content in rice tissues is influenced by the Se form, harvested rice plants were divided into four parts: roots, aerial parts (shoot + leaves), grains, and husks. Table 2 presents the total Se content in rice tissues for control and Se-biofortified groups. In the control group, some Se concentrations were below the limit of detection (LOD) (one replicate for grains and two for husks). In these cases, values were assigned as the detection limit divided by the square root of 2 (LOD/$\sqrt{2}$).[70,71] For aerial parts in the control group, five of the six replicates were below the LOD, making it impossible to calculate the mean, which was reported as < LOD.

Table 2. Total Se Content in Different Tissues of Rice Plants from the Control Group and Groups Cultivated under Foliar Applications of SeNPs and Se (IV)[a]

	Se concentration (μg kg^{-1} DW)			
Treatment	Root	Aerial part	Grain	Husk
Control	1655 ± 553[a]	<LOD[b]	228 ± 102[c]	169 ± 98[c]
SeNPs	1407 ± 239[ab]	867 ± 182[a]	462 ± 87[b]	418 ± 152[b]
Se (IV)	923 ± 196[b]	753 ± 316[a]	701 ± 11[a]	627 ± 154[a]

[a]Data are average values for 6 replicates ± standard deviation (SD). Different letters within each column indicate statistically significant differences (Tukey's test at $p < 0.05$). DW: dry weight.

In the control group, Se was detected in roots, grains, and husks, likely due to its natural presence in the soil. The bioavailability of Se in soil depends on both its concentration and chemical form. De Oliveira et al.[72] evaluated the mineral and toxic element content in rice grains cultivated in southern Brazil and found that Se accumulation varied with rice genotype and harvest season. For instance, certain rice genotypes accumulated up to 263 μg kg^{-1} of Se in grains. Consistent with our findings, the BRS PAMPA genotype accumulated 198 μg kg^{-1} of Se in grains.[72]

The application of Se, either as Se (IV) or SeNPs, significantly ($p < 0.05$) increased Se concentrations in aerial parts, grains, and husks compared to the control group. Regardless of the Se form, the pattern of Se accumulation in biofortified plants followed the sequence: husk < grains < aerial parts < roots. Notably, Se content in aerial parts was not influenced by the Se form, consistent with results from Yin et al.[73] for rice plants subjected to foliar application of different Se forms. Freire et al.[33] also reported no significant differences in leaf Se content between rice plants treated with foliar applications of Se (IV) or SeNPs, suggesting that both forms exhibit similar uptake patterns when applied via foliar spray.[33]

Selenium translocation from leaves to grains was evident in this study. Grain and husk Se content increased significantly with Se(IV) treatment, by 207% and 271%, respectively, compared to the control group. SeNP treatment also enhanced grain and husk Se content, by 103% and 147%, respectively, confirming the success of biofortification. Comparatively, Se(IV) treatment was more effective in promoting Se translocation and accumulation, increasing Se content in grains and husks by 52% and 50%, respectively, compared to SeNP treatment. This difference may reflect distinct transport mechanisms between Se (IV) and SeNPs. These results align with Freire et al.,[33] who found higher Se concentrations in grains following foliar application of Se(IV) than SeNPs. Interestingly, Se (IV) application reduced root Se content, whereas SeNPs had no significant effect. This suggests that foliar Se(IV) application might downregulate root Se uptake via unknown biochemical mechanisms, while SeNPs do not affect this process.

Pilon-Smits[23] suggested that the optimal Se concentration in plant tissues for promoting growth and stress resistance is 1 to 10 mg kg^{-1} DW, whith toxic effects occurring above 100 mg kg^{-1} DW. In this study, Se concentrations in all plant tissues were below this recommended range, except for roots in the control and SeNP groups, which fell within the range. For grains intended for human consumption, the recommended Se content is 0.1−0.2 mg kg^{-1} DW.[74] According to the *Codex Alimentarius*, Se-biofortified foods should not exceed 0.3 mg kg^{-1} of Se.[3] In the present study, biofortified rice grains exceeded this range, particularly in the Se(IV)-treated group.

Figure 3 ilustrates the TF for Se among different parts of the rice plants. Following foliar Se application, the TF was calculated from aerial parts to grains ($TF_{aerial\text{-}grains}$) and aerial parts to roots ($TF_{aerial\text{-}roots}$). As Se concentrations in the aerial parts of control plants were below the LOD, TF could not be calculated for this group.

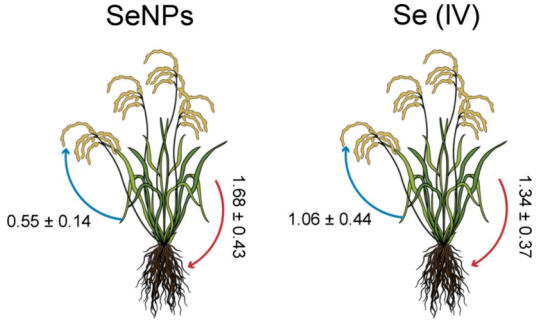

Figure 3. Selenium Transfer Factor (TF) values for rice plants cultivated under foliar applications of SeNPs or Se (IV). Data are average values for 6 replicates ± standard deviation (SD). The direction of the arrow indicates the direction of TF.

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

The Se average $TF_{aerial\text{-}grains}$ for Se (IV) group (1.06) was nearly double that of the SeNP group (0.55), indicating that Se (IV) application primarily promoted Se accumulation in grains, whereas SeNPs favored accumulation in aerial parts. Conversely, the $TF_{aerial\text{-}roots}$ was higher in the SeNP group (1.68) than in the Se (IV) group (1.34), consistent with total Se concentrations: grain Se content was higher in Se (IV)-treated plants, whereas root Se content was higher in SeNP-treated plants.

When applied to leaves, Se can be absorbed through stomata, trichomes, or cuticular pathways and subsequently transported to edible parts of the plant.[75,76] Se (IV) is internalized and transported via phosphate and silicon transporters,[4,77] facilitating its transport to aerial parts.[78,79] By contrast, SeNPs are absorbed by both roots and leaves and transported to other plant parts, but their uptake and translocation rates appear lower than those of Se(IV).[31,33] Differences in uptake, accumulation, and translocation between Se forms have also been observed in other plant.[80] Therefore, these results highlight the higher efficiency of foliar Se(IV) application for grain biofortification compared to SeNPs.

3.5. Estimated Daily Intake of Se through Biofortified Rice Consumption. In this study, the influence of Se biofortification on the EDI of Se through rice consumption was investigated. The amount of essential nutrients considered adequate for human health is referred to as DRI. For Se, the DRI established by the IOM is 55 μg day^{-1} for adults.[16] As discussed in the previous section, Se application in the form of either SeNPs or Se (IV) significantly increased Se concentrations in rice grains (Table 2). This increase notably impacted the EDI of Se from the consumption of biofortified rice (Figure 4).

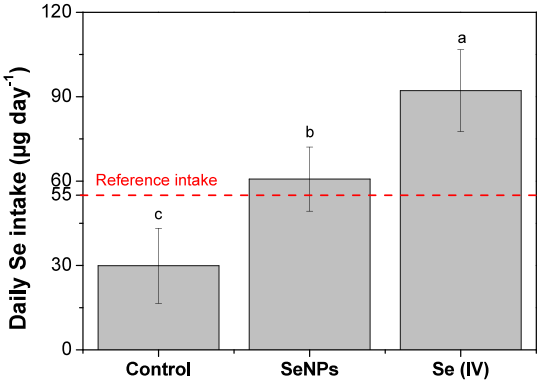

Figure 4. Daily Se intake (μg day^{-1}) from the rice grains of the control group and groups cultivated under foliar applications of SeNPs and Se (IV). Data are average values for 6 replicates while error bars represent the standard deviation (SD). Different letters within each tissue indicate significant differences between groups by Turkey's test at $p < 0.05$.

The calculated EDI ranged from 29.9 μg day^{-1} for nonbiofortified rice to 92.2 μg day^{-1} for Se (IV)-biofortified rice, representing an increase from 54% (control plants) to 168% (Se (IV)-biofortified plants) of the Se DRI of 55 μg day^{-1}. The Se content in control plants was insufficient to meet the DRI, while Se biofortification significantly improved the EDI. Se (IV) application resulted in a maximum EDI of 92.2 μg day^{-1}, exceeding the recommended DRI of 55 μg day^{-1}. In contrast, SeNP-biofortified rice resulted in an EDI of 60.7 μg day^{-1}, meeting 110% of the Se DRI. Importantly, neither treatment exceeded the UL of 255 μg day^{-1},[17] indicating that consuming biofortified grains poses no risk to human health.

The present study provides valuable insights into the use of SeNPs for agronomic biofortification and their efficacy in increasing Se content in edible plant parts, which could benefit human health. Previous studies have also demonstrated the feasibility of Se biofortification in enhancing the Se EDI in populations. For instance, rice biofortification with 25 g Se ha^{-1}, applied as Se (VI), increased the Se EDI to 24.7 μg day^{-1}, while the consumption of nonbiofortified rice provided only 3.72% of the daily Se requirement.[81] Similarly, biofortification of adzuki bean with Se (IV) or Se (VI) has been shown to produce Se-enriched sprouts, as the consumption of 2−22 g exceeded the Se DRI.[80]

However, the Se form and application dose can significantly affect the outcomes of biofortification. In some cases, Se fertilizers may lead to grains with Se concentrations exceeding recommended limits. Paniz et al.[55] reported that Se biofortification of two Brazilian rice varieties with 5 mg L^{-1} of Se (VI) applied to the soil resulted in rice grains that exceeded the IOM tolerable intake level of 400 μg day^{-1} by 37−73%. Yuan et al.[29] observed that foliar application of Se (IV) was more effective in increasing the Se EDI from rice consumption compared to organic Se fertilizers, providing 28−82% of the DRI. The findings of the present study suggest that SeNPs may be a safer option for rice biofortification compared to inorganic Se. SeNPs provide a more controlled release of Se, reducing the risk of exposure to excessively high doses of this element.

3.6. Selenium Speciation in Rice Grains. Despite the essential role of Se for humans, the differences in bioactivity, bioavailability, and toxicity among Se compounds highlight the importance of not only analyzing the total Se concentration in the edible parts of plants but also considering Se speciation when evaluating the nutritional and toxicological properties of foods like cereals.[29,31,82] Identifying the different Se species is essential for ensuring food safety and maximizing Se absorption from biofortified foods. It is well-established that inorganic Se species are the most toxic to human health, whereas organic species are more bioavailable.[29,73,83] Rice plants can accumulate both inorganic and organic forms of Se.[73,84]

Se (IV) and Se (VI) are commonly used as inorganic Se fertilizers in biofortification programs. However, safer and more efficient Se fertilizers are needed.[29] In this context, SeNPs represent a promising alternative for rice biofortification. Nonetheless, Se speciation depends on the exogenous Se form applied, and the biotransformation of SeNPs in plants remains incompletely understood.[31,85] Since rice grains are the edible part of the plant, husked rice was chosen for Se speciation analysis.

Using multispecies calibration solutions, four Se compounds were successfully separated: $SeCys_2$, Se^{4+}, SeMet, and Se^{6+}, with retention times of 4.1, 6.2, 12.1, and 16.8 min, respectively. All calibration curves exhibited good linearity ($R^2 \geq 0.9987$). The four Se species used for preparing calibration standards were also identified in the SRM NIST 1568b (rice flour), where organic species predominated (data not shown).

Following the cultivation of rice plants under different treatments, Se species were extracted from rice grains through enzymatic hydrolysis and subsequently detected. Figure 5

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

presents representative chromatograms obtained for each treatment group.

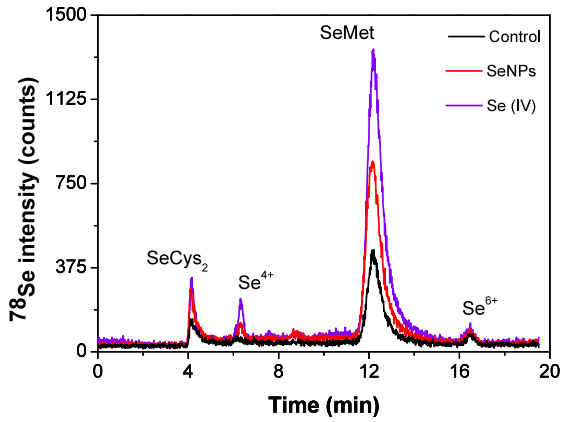

Figure 5. Examples of HPLC-ICP-MS chromatograms of Se species extracted from rice grains from the control group (dark line) and grains cultivated under foliar applications selenium nanoparticles (SeNPs, red line) or sodium selenite (Se (IV), purple line). Identified species were: $SeCys_2$, selenocystine; Se^{4+}, selenite; SeMet, selenomethionine; Se^{6+}, selenate.

As shown in Figure 5, all four Se species were identified and quantified in rice grains from all treatment groups. SeMet was the predominant species, followed by $SeCys_2$, with concentration increasing from the control group to the SeNP-treated group and further to the Se (IV)-treated group. Previous studies have similarly shown that organic Se, particularly SeMet, is the most prevalent and nutritionally significant Se species in cereal grains.[73,82,85]

In contrast with our results, Wang et al.[31] observed that different Se treatments resulted in distinct Se species in the roots and shoots of rice seedlings cultivated hydroponically with SeNPs, Se (IV), or Se (VI). However, SeMet was still the predominant species in rice shoots treated with SeNPs or Se (IV). These differences may be attributed to variations in Se uptake and biotransformation mechanisms under different application methods, such as foliar spraying versus hydroponic systems. Yin et al.[73] investigated the effects of different Se forms (organic and inorganic species) and application methods (root irrigation versus foliar spraying) on Se accumulation and speciation in rice. Thew found that both Se forms and application methods influenced the accumulation of specific Se species.[73] However, SeNPs were not included in that study.

In the control group, the proportion of Se species followed the order: SeMet (70%) > $SeCys_2$ (15%) > and Se^{6+} (10%) > Se^{4+} (5%). Se biofortification altered these proportions. In SeNP-treated plants, the proportions were: SeMet (84%) > $SeCys_2$ (10%) > Se^{4+} (3%) = Se^{6+} (3%). In Se (IV)-treated plants, the proportions were: SeMet (87%) > $SeCys_2$ (8%) > Se^{4+} (3%) > Se^{6+} (2%).

The proportion of organic Se increased in Se-biofortified groups from 85% in the control group to 94−95%. SeMet content rose from 70% to 84−87%, while $SeCys_2$ decreased from 15% to 8−10%. Yuan et al.[29] reported no significant differences between organic-Se fertilizer and Se (IV) fertilizer in terms of organic Se proportions in brown rice. In their study, organic Se accounted for 85% of total Se in treatment groups, compared to 78% in the control group.[29] Similarly, in rice shoots from SeNP- or Se (IV)-treated plants, SeMet was the dominant species, accounting for 95% and 102% of total Se, respectively, demonstrating the bioavailability of SeNPs.[31] Organic Se also predominated in Se-biofortified wheat grains, comprising 93−100% of total Se, with SeMet representing 87−96% and $SeCys_2$ comprising 4−13%.[85]

For inorganic Se, the proportion of Se^{6+} decreased from 10% in the control group to 2−3% in biofortified grains, while Se^{4+} showed a slight reduction from 5% to 3%. These results align with a previous study with rice seedlings, which found that when rice plants were supplied with Se (IV), Se^{4+} constituted 4.29% of total Se in rice shoots.[31] In biofortified wheat grains, Se^{6+} was the predominant inorganic species, ranging from 1 to 6%.[85] Additionally, rice biofortification with SeNPs decreased the proportion of inorganic Se from 40% to 25−35%.[86] Yuan et al.[29] observed an increase in SeMet proportion and a decrease in $SeCys_2$ and Se^{4+} proportions in brown rice following Se biofortification. The increase in organic Se proportions, coupled with the decrease in inorganic Se proportions, may represent an enhancement in the nutritional quality of biofortified grains. Organic Se species, particularly SeMet, have higher nutritional value and bioavailability (>90%) and are the most desirable Se species for dietary intake.[82] Thus, the proposed biofortification protocol could effectively improve the EDI of Se.

Consistent with the total Se results, the concentration of organic Se species ($SeCys_2$ and SeMet) in rice grains also increased significantly with Se application compared to the control group (Table 3). These findings align with those of Hussain et al.,[86] who reported increased organic Se content and proportions in rice grains following foliar SeNP treatments. In the present study, Se (IV) was more effective than SeNPs in increasing SeMet concentrations, achieving a 363% rise compared to the control group.

In this study, the extractable Se content accounted for approximately 56% of the total Se in the samples. This low recovery may be attributed to the presence of other Se species that were not extracted or quantified using the method

Table 3. Selenium Species Concentration in Rice Grains of Plants from the Control Group and Groups Cultivated under Foliar Applications of SeNPs and Se (IV)[a]

	Se species concentration ($\mu g\ kg^{-1}$ DW)				
Treatment	$SeCys_2$	Se^{4+}	SeMet	Se^{6+}	Sum
Control	11.3 ± 1.9[b]	4.6 ± 2.9[b]	85.1 ± 24.3[c]	7.1 ± 4.4[a]	108 ± 24
SeNPs	19.6 ± 2.5[a]	6.5 ± 1.3[b]	265.3 ± 28.1[b]	5.0 ± 0.8[a]	296 ± 32
Se (IV)	22.1 ± 3.8[a]	11.1 ± 2.1[a]	394.3 ± 20.0[a]	4.9 ± 1.1[a]	432 ± 19

[a]Data are average values for 4 replicates ± standard deviation (SD). Different letters within each column indicate statistically significant differences by Turkey's test at $p < 0.05$. Identified species were: $SeCys_2$, selenocystine; Se^{4+}, selenite; SeMet, selenomethionine; Se^{6+}, selenate. DW: dry weight.

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

Table 4. Concentrations of Macro, Micronutrients, and Potentially Toxic Elements in Different Tissues of Rice Plants from the Control Group and Groups Cultivated under Foliar Applications of SeNPs and Se (IV)[a]

Element (conc.)	Tissue	Control	SeNPs	Se (IV)
Na (mg kg^{-1})	Grain	94.4 ± 67.4^{a}	58.9 ± 40.9^{a}	79.4 ± 80.1^{a}
Na (g kg^{-1})	Husk	1.87 ± 1.12^{a}	1.19 ± 0.78^{a}	1.51 ± 1.20^{a}
Na (g kg^{-1})	Aerial part	1.01 ± 0.63^{a}	1.25 ± 0.75^{a}	1.28 ± 0.89^{a}
Na (mg kg^{-1})	Root	804 ± 417^{a}	673 ± 390^{a}	857 ± 263^{a}
Mg (g kg^{-1})	Grain	1.84 ± 0.16^{a}	1.78 ± 0.06^{a}	1.87 ± 0.18^{a}
Mg (mg kg^{-1})	Husk	689 ± 241^{a}	744 ± 192^{a}	843 ± 125^{a}
Mg (g kg^{-1})	Aerial part	4.31 ± 0.66^{a}	4.15 ± 1.03^{a}	4.96 ± 1.13^{a}
Mg (mg kg^{-1})	Root	865 ± 50^{a}	717 ± 188^{a}	754 ± 197^{a}
P (g kg^{-1})	Grain	4.42 ± 0.34^{a}	4.20 ± 0.23^{a}	4.26 ± 0.47^{a}
P (mg kg^{-1})	Husk	741 ± 290^{a}	726 ± 240^{a}	736 ± 185^{a}
P (mg kg^{-1})	Aerial part	670 ± 174^{a}	411 ± 138^{a}	399 ± 232^{a}
P (mg kg^{-1})	Root	598 ± 142^{a}	514 ± 136^{ab}	404 ± 77^{b}
K (g kg^{-1})	Grain	3.11 ± 0.17^{a}	2.93 ± 0.14^{a}	3.06 ± 0.32^{a}
K (g kg^{-1})	Husk	7.32 ± 0.70^{a}	7.19 ± 0.81^{a}	8.30 ± 1.28^{a}
K (g kg^{-1})	Aerial part	20.4 ± 2.2^{a}	23.1 ± 2.7^{a}	21.3 ± 3.3^{a}
K (mg kg^{-1})	Root	970 ± 382^{a}	654 ± 211^{a}	629 ± 154^{a}
Ca (mg kg^{-1})	Grain	107 ± 8^{a}	117 ± 10^{a}	117 ± 13^{a}
Ca (mg kg^{-1})	Husk	720 ± 101^{a}	908 ± 228^{a}	949 ± 213^{a}
Ca (g kg^{-1})	Aerial part	4.99 ± 0.87^{a}	4.01 ± 0.84^{a}	4.48 ± 1.13^{a}
Ca (g kg^{-1})	Root	3.86 ± 0.39^{a}	3.97 ± 0.98^{a}	3.83 ± 0.70^{a}
Mn (mg kg^{-1})	Grain	15.6 ± 1.7^{a}	16.0 ± 2.1^{a}	15.1 ± 2.7^{a}
Mn (mg kg^{-1})	Husk	69.7 ± 18.2^{a}	67.7 ± 20.8^{a}	64.7 ± 19.1^{a}
Mn (mg kg^{-1})	Aerial part	228 ± 62^{a}	150 ± 52^{a}	170 ± 59^{a}
Mn (mg kg^{-1})	Root	122 ± 42^{a}	121 ± 39^{a}	80 ± 32^{a}
Co (μg kg^{-1})	Grain	11.9 ± 1.7^{a}	10.0 ± 4.1^{a}	10.7 ± 1.4^{a}
Co (μg kg^{-1})	Husk	37.5 ± 10.9^{a}	45.3 ± 7.2^{a}	42.5 ± 9.6^{a}
Co (μg kg^{-1})	Aerial part	52.4 ± 22.7^{a}	57.9 ± 28.3^{a}	50.3 ± 16.7^{a}
Co (mg kg^{-1})	Root	1.95 ± 0.26^{a}	1.48 ± 0.29^{b}	1.08 ± 0.28^{b}
Ni (μg kg^{-1})	Grain	189 ± 45^{a}	134 ± 56^{a}	118 ± 57^{a}
Ni (mg kg^{-1})	Husk	1.20 ± 0.58^{a}	1.83 ± 0.42^{a}	1.35 ± 0.31^{a}
Ni (μg kg^{-1})	Aerial part	581 ± 228^{a}	376 ± 120^{a}	374 ± 194^{a}
Ni (mg kg^{-1})	Root	4.51 ± 1.54^{a}	2.82 ± 0.29^{b}	2.72 ± 0.58^{b}
Cu (mg kg^{-1})	Grain	3.90 ± 1.50^{a}	2.51 ± 0.48^{a}	2.53 ± 0.94^{a}
Cu (mg kg^{-1})	Husk	5.03 ± 1.80^{a}	4.90 ± 1.42^{ab}	2.88 ± 0.68^{b}
Cu (μg kg^{-1})	Aerial part	756 ± 191^{a}	710 ± 254^{a}	667 ± 415^{a}
Cu (mg kg^{-1})	Root	5.92 ± 1.71^{a}	4.69 ± 0.70^{ab}	4.02 ± 0.96^{b}
Zn (mg kg^{-1})	Grain	38.1 ± 4.9^{a}	34.5 ± 1.5^{ab}	33.2 ± 1.3^{b}
Zn (mg kg^{-1})	Husk	24.0 ± 7.6^{a}	22.8 ± 2.5^{a}	22.8 ± 4.7^{a}
Zn (mg kg^{-1})	Aerial part	31.2 ± 3.0^{a}	38.0 ± 13.4^{a}	31.3 ± 5.1^{a}
Zn (mg kg^{-1})	Root	71.5 ± 11.7^{a}	49.0 ± 7.4^{b}	50.0 ± 12.3^{b}
As (μg kg^{-1})	Grain	175 ± 28^{b}	210 ± 22^{a}	177 ± 17^{ab}
As (μg kg^{-1})	Husk	189 ± 50^{a}	232 ± 31^{a}	197 ± 46^{a}
As (μg kg^{-1})	Aerial part	600 ± 114^{a}	643 ± 125^{a}	496 ± 77^{a}
As (mg kg^{-1})	Root	22.5 ± 3.2^{a}	21.4 ± 4.2^{a}	14.5 ± 2.3^{b}
Cd (μg kg^{-1})	Grain	60 ± 29^{a}	34 ± 9^{a}	37 ± 19^{a}
Cd (μg kg^{-1})	Husk	16 ± 8^{a}	14 ± 7^{a}	17 ± 12^{a}
Cd (μg kg^{-1})	Aerial part	23 ± 9^{a}	31 ± 16^{a}	31 ± 16^{a}
Cd (μg kg^{-1})	Root	557 ± 468^{a}	260 ± 172^{a}	246 ± 167^{a}

[a]Data are average values for 6 replicates ± standard deviation (SD). Different letters within each column indicate statistically significant differences (Tukey's test at $p < 0.05$).

employed, such as selenomethylselenocysteine (MeSeCys), which is known to occur in Se-enriched plants. Palomo-Siguero et al.[87] reported MeSeCys proportions as high as 64% in Se-enriched radishes.

Interestingly, the application of SeNPs did not significantly alter the concentrations of inorganic Se species (Se^{4+} and Se^{6+}), whereas Se (IV) application increased Se^{4+} content by 2.4-fold compared to control plants. These findings indicate that in rice tissues, SeNPs are primarily biotransformed into organic Se species such as SeMet and $SeCys_2$. By contrast, following Se (IV) treatment, a fraction of the applied Se remains as Se^{4+}, while another portion is converted into organic Se species.

It is well-established that once absorbed by plants, Se (IV) is readily metabolized into organic Se, primarily SeMet and $SeCys_2$, making it a commonly used fertilizer for producing Se-

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

enriched crops. Organic Se species can be incorporated into selenoproteins, promoting plant growth development. However, due to the chemical similarity between Se and sulfur (S), Se can also replace S in proteins, a mechanism contributing to Se toxicity in plants.[58,80,88−90] In contrast, the biotransformation of SeNPs in plant tissues is not yet fully understood. Wang et al.[31] demonstrated in hydroponic experiments that SeNPs can be converted into Se^{4+} and organic Se. In this study, no significant differences were observed in Se^{4+} concentrations between the control and SeNP-treated groups, possibly due to differing biotransformation mechanisms arising from the method of application. Given that inorganic Se species are the most toxic for humans, while organic Se species are more bioavailable and nutritionally valuable,[29,73,83] the foliar application of SeNPs appears to be a promising approach for producing Se-biofortified rice for human consumption.

3.7. Effects of Different Se Formulations on Elemental Uptake and Accumulation. The effects of Se biofortification on the uptake and accumulation of mineral elements and PTEs by plants were evaluated. Table 4 shows the concentrations of Na, Mg, P, K, Ca, Mn, Co, Ni, Cu, Zn, As, and Cd in rice tissues from the control and Se-enriched groups. For certain elements, such as Na and Cd, high standard deviations were observed, hampering the identification of significant differences between groups. This high variability reflects the natural plant-to-plant heterogeneity commonly observed in crop plants.[91]

As shown in Table 4, statistical analysis indicates that Se biofortification did not influence the concentrations of Na, Mg, K, Ca, Mn, and Cd in rice tissues. However, Se (IV) application significantly inhibited nutrient uptake by rice roots. The concentrations of P, Co, Ni, Cu, and Zn in the roots of Se (IV)-treated plants were lower than those in the control group, with reductions ranging from 30% (Zn) to 45% (Co). These decreases negatively impacted Cu accumulation in husks (−43%) and Zn in grains (−13%). SeNP treatment had a lesser effect on nutrient uptake and accumulation, reducing Co, Ni, and Zn concentrations in roots by 24%, 37%, and 31%, respectively. Importantly, grain nutrient concentrations were unaffected by SeNP treatment.

Previous studies have demonstrated that Se enrichment can exert stimulatory or inhibitory effects on essential element content in various plant species,[40,73,80,81,92] likely due to interactions between Se and other elements or their transporters. In a prior study on rice seedlings, foliar application of either SeNPs or Se (IV) did not affect the concentrations of Na, Mg, Cu, Ca, K, and Zn in roots or Na, Mg, Mn, Cu, and Zn in leaves.[40] Additionally, both SeNPs and Se (IV) were shown to reduce Co uptake by rice roots,[40] which is in good agreement with the findings of the present study. Similarly, de Oliveira, Nomura, and Naozuka[80] observed that Se (IV) enrichment reduced in Cu and Zn concentrations in the roots of adzuki bean sprouts. In contrast, Reis et al.[81] found that Se (VI) application combined with N fertilization increased rice grain concentrations of P, K, Ca, Cu, and Zn, while Mg and Mn contents remained unchanged. Such discrepancies across studies may be attributed to differences in plant species, developmental stages, Se concentration, source, and application methods.

Regarding PTEs, Se (IV) treatment reduced root As uptake by 36%, whereas SeNP treatment increased grain As concentration by 20% compared to the control group. Se is known to protect plants from heavy metal stress, including As and Cd toxicity, but further research is needed to understand the role of SeNPs in mitigating heavy metal stress.[4] Hussain et al.[86] reported that foliar application of SeNPs and SiNPs decreased Cd accumulation in rice grains grown in Cd-contaminated soils. There is also evidence that foliar application of SeNPs mitigates Cd stress in tomatoes.[93] In the present study, Cd concentrations in grains decreased by 38−43% following Se treatments, although the reduction was not statistically significant ($p > 0.05$). It is worth mentioning that the plants in this study were cultivated under nonstress conditions, which may influence the results. Consistent with our findings, foliar application of SeNPs or Se (IV) in rice seedlings cultivated without metal stress had no significant impact on Cd concentrations in roots or leaves.[40]

The observed reduction in root As concentration in Se (IV)-treated plants may be attributed to the antagonism between As and Se.[94] This antagonism occurs because both arsenate and Se (IV) are taken up via the phosphate transport pathway, leading to competition for binding sites.[90,94,95] These results can be related to the observed decrease in root P uptake following Se (IV) treatment, which indicates a competitive inhibition between Se (IV) and phosphate in plant metabolism.[4,77] Additionally, arsenite and Se (IV) share the same Si transporter,[96,97] which may further explain the observed antagonistic effect. Previous studies have also shown that Se inhibits As uptake or translocation in rice.[40,98−100]

The *Codex* Committee on Contaminants in Foods has established a maximum limit of 350 $\mu g\ kg^{-1}$ for As for husked rice.[101] Although grain As concentrations increased following SeNP treatment, all Se-enriched grains remained below this limit, regardless of the Se form applied, indicating that consumption of these grains is safe in terms of As exposure.

The results suggest that foliar application of Se, whether in ionic or nanoparticulate form, impacts the uptake and accumulation of both nutrients and PTEs in rice plants. In grains, the edible parts of rice, Se treatments did not significantly alter the concentrations of nutrients and PTEs, except for an increase in As content in the SeNP group and a slight decrease in Zn content in the Se (IV) group. This indicates that Se-enriched rice is not nutritionally superior to nonbiofortified rice (apart from Se content) but remains safe for consumption regarding PTEs exposure.

In conclusion, the distribution, biotransformation, and accumulation of Se in rice plants differed between the two evaluated Se fertilizers. Regarding agronomic parameters, both Se (IV) and SeNPs were found to be nontoxic Se fertilizers under the evaluated conditions. Moreover, no evidence of oxidative stress was observed in plants upon SeNPs or Se (IV) treatment, as both treatments reduced H_2O_2 levels in rice tissues. The total Se determination demonstrated that foliar application of Se (IV) was more efficient for grain biofortification compared to SeNPs. However, SeNPs provided a more controlled release of Se, potentially reducing the risks associated with exposure to high doses of this element. Se speciation revealed distinct behaviors between ionic and nanoparticulate forms of Se. Although organic Se, mainly SeMet, was the predominant species in rice grains for both treatments (accounting for 94−95% of total Se), Se (IV) application resulted in higher selenite concentrations in grains. Elemental determination confirmed that the use of SeNPs for rice biofortification does not significantly impact the nutritional quality of grains, and the consumption of Se-enriched

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ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

grains is safe with respect to PTE exposure. The findings of this study represent a significant advancement in the agronomic biofortification of rice to address global Se deficiency. The results highlight that while both Se fertilizers have distinct advantages and disadvantages, they each hold potential for producing Se-enriched rice for human consumption.

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ABBREVIATIONS

APX Ascorbate peroxidase
CRC Collision reaction cell
DRI Dietary reference intake
DW Dry weight
EDI Estimated daily intake
HPLC High-performance liquid chromatography
ICP-MS Inductively coupled plasma mass spectrometry
LoD Limit of detection
NBT Nitroblue tetrazolium
POD Peroxidase
ROS Reactive oxygen species
Se (IV) Sodium selenite
Se (VI) Sodium selenate
$SeCys_2$ Seleno-L-cystine
SD Standard deviation
SeMet Selenomethionine
SeNPs Selenium nanoparticles
SOD Superoxide dismutase
SRM Standard reference materials
TF Translocation factor

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