

The dual effect of Selenium nanoparticles in rice seedlings: From increasing antioxidant activity to inducing oxidative stress

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ABSTRACT

Selenium deficiency has been a worldwide concern because it can pose a risk to human health. Selenium nanoparticles (SeNPs) are in general less toxic and more bioavailable than ionic Se and, therefore, their use for biofortification of food crops has attracted attention. Rice is a staple food consumed by half of the world's population. Indeed, studies on SeNPs' impacts on oxidative stress and antioxidant responses in rice seedlings are scarce. Herein, the effect of different concentrations of SeNPs on growth, antioxidant enzyme activity, reactive oxygen species, and nitric oxide metabolites in rice germinating seeds were investigated. Results revealed a dual effect of SeNPs on seedling growth. At low concentrations (0.5 mg L^{-1}), the nanoparticles did not affect seedling growth, while at higher concentrations root elongation was significantly decreased. Soaking rice seeds with SeNPs at 0.5 mg L^{-1} effectively improved the activity of ascorbate peroxidase, catalase, and superoxide dismutase enzymes. However, a decrease in antioxidant enzyme activity was observed with increasing SeNPs concentrations. All treatments decreased S-nitrosothiols and nitrite concentrations compared to the control. Hydrogen peroxide concentrations also decreased for concentrations higher than 5 mg L^{-1} , compared to the control. Our results suggest a beneficial effect of SeNPs at the optimal concentration of 0.5 mg L^{-1} on stimulating antioxidant metabolism and mitigating reactive oxygen species in plant tissues without affecting the physiological parameters.

1. Introduction

Selenium (Se) is a dietary micronutrient essential for human health. It is important to the maintenance of cellular metabolism and physiology and has a key role in antioxidant defense systems (Badgar, 2019; dos Reis et al., 2017; El-Ramady et al., 2014). Se deficiency is a human health concern worldwide since it is estimated that approximately one in seven people have a Se deficient diet (dos Reis et al., 2017; Jones et al., 2017). An insufficient Se intake increases the risk of several diseases such as some types of cancers, Keshan disease, heart muscle dysfunction, abnormal skin coloration, and fragile red blood cells (dos Reis et al., 2017; Kabata-Pendias and Mukherjee, 2007).

The concentration and bioavailability of Se in plant edible parts, which are sources of this nutrient for humans, can be influenced by various factors such as soil-plant interactions, plant type, soil selenium concentrations, and pH (dos Reis et al., 2017; Jones et al., 2017).

According to El-Ramady et al. (2014), intensive agriculture is contributing to the decrease of Se levels in foods. A projected decline of 8.4 to 8.7 % in soil Se concentrations is expected by 2080–2099, particularly in agricultural regions, potentially exacerbating Se deficiency (Jones et al., 2017).

Agronomic biofortification with inorganic Se, mainly selenite and selenate, is one of the strategies that have been used to increase Se content in the edible parts of crops, and therefore, Se intake by the population. Se-biofortification can be performed through soil fortification or foliar spraying in the leaves (dos Reis et al., 2017; Paniz et al., 2023). Although Se is not essential for higher plants, the application in small amounts can have beneficial effects, such as an increase in antioxidant activity (El-Ramady et al., 2014; Shanker, 2006). However, most plants do not tolerate high doses of this element. An excess of Se in plants can cause leaf chlorosis, and black spots, beyond decreased germination rate and growth (El-Ramady et al., 2014; White et al., 2004)

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Nanoparticles (NPs) have attracted attention in the agricultural field due to their unique properties related to their higher surface energy and surface-to-volume ratio (Badgar, 2019). In this sense, SeNPs may be an alternative for crop biofortification with Se, as they are more bioavailable and less toxic when compared to inorganic and organic forms (Badgar, 2019; El-Ramady et al., 2014). The size, shape, atomic arrangement, structure, and surface charge of SeNPs may influence their biological properties (Badgar, 2019). Studies have demonstrated antibacterial, antioxidant, and anticancer properties related to SeNPs (Boroumand et al., 2019; Freire et al., 2022; Yu et al., 2012).

In recent years, there has been increasing use of SeNPs as fertilizers in crop plants such as tomatoes (Morales-Espinoza et al., 2019; Neysanian et al., 2020), coffee (de Brito Mateus et al., 2021), strawberries (Zahedi et al., 2019), and rice (Badawy et al., 2021; Hussain et al., 2020; Wang et al., 2020, 2021). The application of SeNPs at low concentrations has been demonstrated to increase the Se content and bioavailability, as well as to reduce metal accumulation and improve physiological parameters. Some studies assessed the impact of SeNPs on antioxidant enzyme activity (de Brito Mateus et al., 2021; Morales-Espinoza et al., 2019; Neysanian et al., 2020; Zahedi et al., 2019) and oxidative markers (de Brito Mateus et al., 2021; Neysanian et al., 2020; Zahedi et al., 2019).

Given the increasing use of SeNPs in agriculture, studies involving the impact of them on all stages of plant growth are needed. The use of NPs as fertilizers may exert physical or chemical toxicity on plants, impair seed germination and seedling growth, and induce antioxidant changes (Boonyanitipong et al., 2011; Upadhyaya et al., 2017). Therefore, it is important to define the maximum concentration of NPs that plants can take without showing any signs of stress (Boonyanitipong et al., 2011).

Among food crops, rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. However, as far as we know, this is the first study to evaluate the impacts of SeNPs on antioxidant enzymes, reactive oxygen species (ROS), and nitric oxide (NO) metabolites in rice germinating seeds. This work aims to perform *in vitro* bioassays to assess the phytotoxicity of SeNPs applied at different concentrations (from 0 to 200 mg L⁻¹) and to determine the optimal concentration of SeNPs application for rice biofortification.

2. Material and methods

2.1. Chemical synthesis and characterization of SeNPs

The synthesis and characterization of the SeNPs used in this study were previously described (Freire et al., 2022). Briefly, the synthesis was performed via chemical reduction of Na₂SeO₃ (Sigma-Aldrich, St-Louis, USA) by ascorbic acid (Synth (Diadema, Brazil) using polyvinyl alcohol (PVA 30 000 to 70 000 MW, Sigma-Aldrich, St-Louis, USA) as a stabilizer. Transmission electron microscopy (TEM, JEM-2100 Plus, 200 kV, JEOL, USA) was used to investigate the SeNPs size distribution. For TEM analysis, diluted suspensions of SeNPs were drop-casted on carbon-coated grids and air-dried at room temperature (25 °C). The average size of the nanoparticles in the solid state was determined using ImageJ software. The obtained average diameter was 50.1 ± 5.6 nm. A representative TEM image of synthesized SeNPs is shown in Fig. 1.

2.2. Plant material and growth conditions

Embrapa (Brazilian Agricultural Research Corporation) provided the rice seeds (*Oryza sativa* L. cv. BRS PAMPA) used in this study. To start the germination, the seeds were immersed in water for 48 h. Then the surface was sterilized with 2.5 % sodium hypochlorite (NaClO) for 10 min and washed thoroughly with ultrapure water. In the next step, the seeds were uniformly arranged in sterile disposable Petri dishes (90 mm x 15 mm) containing qualitative filter paper and 4 mL of sterile water and kept in a vertical incubator (Solab, Piracicaba, Brasil) at 32 °C in the

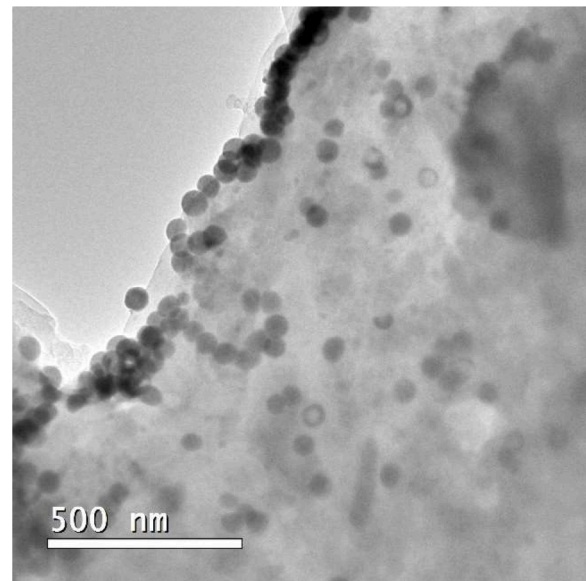


Fig. 1. Transmission electron microscopy (TEM) image of SeNPs showing spherical and well-dispersed nanoparticles. The white bar indicate 500 nm.

dark. After 48 h, the germinated seeds (radicle > 2 mm) were separated, and the treatments were applied as described below.

2.3. Plant treatments

The following concentrations of SeNPs were evaluated: 0, 0.5, 2.0, 5.0, 20, 50, and 200 mg L⁻¹. Germinated seeds were disposed in Petri dishes containing filter paper and 5 mL of sterile water or SeNPs suspension, with 6 seeds per dish and 6 dishes per concentration (36 seeds per treatment). The Petri dishes were closed, sealed with parafilm to avoid losses by evaporation, and placed in random positions inclined (45°) in an incubator at 32 °C for 72 h in the dark.

2.4. Growth measurements

At the end of the experiment, the numbers of roots (> 5 mm) were counted, and the root and shoot length ($n = 36$ biological replicates) were measured (Matrix®- MTX, ToolsWorld, China) (Boonyanitipong et al., 2011). Fresh weight was obtained, and seedlings were oven-dried at 45 °C until constant weight (SL-100, Solab, Piracicaba, Brasil) to estimate the dry weight ($n = 6$ biological replicates) of the whole germinating seeds (Upadhyaya et al., 2017).

2.5. Extraction and quantification of antioxidant enzyme activity

The quantification of the antioxidant enzymatic activity was performed by determining the activity of ascorbate peroxidase (APX), catalase (CAT), peroxidases (POD) and superoxide dismutase (SOD).

For the preparation of the enzyme extract, 100 mg of plant sample (wet mass) was weighed and frozen at -80 °C. Then, the sample was macerated with 1 mL of extraction buffer, which was composed of EDTA (1 mmol L⁻¹) potassium phosphate buffer (0.1 mol L⁻¹, pH 6.8) and PVPP (2% w/v) (Pelegriño et al., 2020). The extract was then sonicated (45 kHz) for 10 min and centrifuged for 10 min (12,100 rpm, Mini Spin, Eppendorf, Hamburg, Germany). The supernatant was collected and used for enzymatic determinations as described below. Due to the limited amount of sample, experiments were not performed in replicate. Each sample was analyzed three (APX and SOD) or two times (CAT and POD) and the activities were normalized to seed fresh weight and reaction duration (Pelegriño et al., 2020).

2.5.1. Ascorbate peroxidase (APX)

The APX activity was calculated based on the hydrogen peroxide (H_2O_2) dependent ascorbate oxidation (Shams et al., 2019). For this measurement, a 25 μL aliquot of the enzyme extract was mixed with 1.45 mL of an incubation buffer composed of EDTA (0.1 mmol L^{-1}), ascorbic acid (0.5 mmol L^{-1}) and potassium phosphate buffer (50 mmol L^{-1} , pH 7.0). Then, an aliquot of 25 μL of H_2O_2 (30 mmol L^{-1}) was added to the sample. The absorbance was measured after 2 min of reaction using a UV-visible spectrophotometer (Agilent 8454, Palo Alto, CA, USA) at 290 nm (Pelegriño et al., 2020). The blank was acquired using the same solution without ascorbic acid. Enzyme activity was calculated using Beer's law ($\epsilon = 2.8 \text{ L mmol}^{-1} \text{ cm}^{-1}$) (Nakano and Asada, 1981).

2.5.2. Catalase (CAT)

The activity of CAT was determined by monitoring the consumption of H_2O_2 at 240 nm for 3 min (Andrade et al., 2018; Upadhyaya et al., 2017). An aliquot of 50 μL of the enzyme extract was mixed with 950 μL of EDTA (0.1 mmol L^{-1}), and H_2O_2 (12.5 mmol L^{-1}) in potassium phosphate buffer (50 mmol L^{-1} , pH 7.0). The blank was obtained with the same solution without H_2O_2 . The decrease in absorbance was measured every 30 s (Pelegriño et al., 2020). Enzyme activity was calculated using Beer's law ($\epsilon = 36 \text{ L mmol}^{-1} \text{ cm}^{-1}$) (Anderson et al., 1995).

2.5.3. Peroxidases (POD)

A 10 μL aliquot of the extract was mixed with 1.79 mL of potassium phosphate buffer (20 mmol L^{-1} , pH 6.8) containing pyrogallol (20 mmol L^{-1}) and H_2O_2 (20 mmol L^{-1}). Absorbance was measured at 420 nm after 1 min of reaction (Pelegriño et al., 2020). Enzyme activity was calculated using Beer's law ($\epsilon = 2.47 \text{ L mmol}^{-1} \text{ cm}^{-1}$) (Anderson et al., 1995).

2.5.4. Superoxide dismutase (SOD)

Determination of SOD activity is based on the ability of this enzyme to inhibit the nitroblue tetrazolium (NBT) photochemical reduction (Anderson et al., 1995; Shams et al., 2019). Before SOD determination, a 40 μL aliquot of the extract was added to 1.96 mL of potassium phosphate buffer (50 mmol L^{-1} , pH 7.8) containing EDTA (0.1 mmol L^{-1}), riboflavin ($2 \mu\text{mol L}^{-1}$), methionine (13 mmol L^{-1}) and NBT ($75 \mu\text{mol L}^{-1}$) (Andrade et al., 2018; Liu et al., 2014). The reaction mixture was exposed to $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ of photosynthetic active radiation using a 60 W fluorescent lamp and the absorbance was measured at 560 nm after 10 min. An identical reaction mixture was kept in the dark and used as a blank for each sample. A mixture containing distilled water instead of extract was irradiated and used as a control. One unit of SOD was considered as the amount of enzyme that inhibited the reduction of NBT by 50 % (Liu et al., 2014; Shams et al., 2019).

2.6. Quantification of reactive oxygen species and No metabolites in germinating seeds

For the quantification of S-nitrosothiols (S-NO), nitrite (NO_2^-), and H_2O_2 , 100 mg of the germinating seed (wet mass) were initially weighed and frozen at -80°C . Then, samples were extracted by maceration with 1 mL of potassium phosphate buffer containing N-ethylmaleimide (NEM, 5 mmol L^{-1}). After that, samples were sonicated (45 kHz) for 10 min and centrifugated (12,100 rpm) for 10 min (Pelegriño et al., 2020, 2021).

Due to the limited amount of sample, experiments were not performed in replicate. Each sample was analyzed 2 times. An aliquot of 50 μL of the supernatant was used in the determinations, which were performed using the free radical analyzer WPI TBR4100/ 1025 Ameter (World Precision Instruments Inc., Sarasota FL, USA) with a NO (2 mm, ISO-NOP) or H_2O_2 (ISO-HPO-2) specific sensors. Solutions of CuCl_2 (100 mmol L^{-1} , 10 mL), $\text{KI}/\text{H}_2\text{SO}_4$ (100 mmol L^{-1} , 10 mL), and PBS

(100 mmol L^{-1} , 10 mL) were used for the quantification of S-NO, NO_2^- and H_2O_2 , respectively. The results were compared to standard curves obtained with GSNO for S-NO, NaNO_2 for NO_2^- and H_2O_2 for hydrogen peroxide (Oliveira et al., 2016; Pelegriño et al., 2020, 2021). Results were normalized against seed fresh weight.

2.7. Statistical analysis

The Kolmogorov–Smirnov test was used to analyze the normality of the data, and a normal distribution was found. The data obtained for each variable were subjected to the one-way analysis of variance (ANOVA). Tukey's post-hoc test ($p < 0.05$) was used to assess significantly differences between groups. The software Statistica 8.0 (StatSoft, Tulsa, USA) was used.

3. Results and discussion

3.1. Effect of SeNPs on the growth of germinating rice seeds

The effects of SeNPs (ranging from 0, 0.5, 2, 5, 20, 50, and 200 mg L^{-1}) were studied on rice seedling growth. Fig. 2 shows representative images of each treatment with SeNPs applied to rice seeds, after the end of the experiment. The exposure of rice seeds to SeNPs showed a clear dose-dependent inhibitory effect on seedling growth. A visible decrease in the radicle length was observed with the increase of SeNPs concentrations, mainly for concentrations higher than 20 mg L^{-1} (Fig. 2E). No visible changes in seedling growth were observed at concentrations below 5 mg L^{-1} (Fig. 2D).

Fig. 3 shows the growth parameters measured on germinating rice seeds. There was a statistically significant difference ($p < 0.05$) between the groups concerning root length (Fig. 3A), shoot length (Fig. 3B), fresh weight (Fig. 3C) and number of radicles $> 5 \text{ mm}$ (Fig. 3E). A general trend of decrease in plant parameters was noticed with the increasing SeNPs concentrations. Concentrations of 50 and 200 mg L^{-1} presented significant root growth and number of roots inhibition. By comparing with the control group, the root length was decreased by 64–73 % and the number of roots was decreased by 48–79 % with 50–200 mg L^{-1} SeNPs, respectively. The shoot growth was significantly inhibited at 200 mg L^{-1} of SeNPs compared to the control. That was interpreted as a possible toxic effect of SeNPs above 50 mg L^{-1} .

The more pronounced effect of the toxicity of SeNPs in the roots in comparison with other parts of the seedling can be explained by the rice seed coat, which can act as a protector for the embryo, but cannot protect the whole seed (Boonyanitipong et al., 2011). Concerning dry mass (Fig. 3D), there was no significant difference between the studied groups, showing that SeNPs did not adversely affect rice seedling mass.

Various studies have reported a dual effect of Se on plants. Over a narrow range, Se effect on plant growth changes from beneficial to toxic (Feng et al., 2013; Gupta and Gupta, 2017), which corroborates with the results observed in the present study. At low doses, Se protects plants from a range of abiotic stresses including cold, drought, and metal stress (Gupta and Gupta, 2017). The two main causes of the phytotoxic effect related to high Se doses on plants are the Se induced oxidative stress and distorted protein structure and function (Gupta and Gupta, 2017). The first occurs because Se, at high doses, acts as pro-oxidant and generates ROS. The second occurs through the incorporation of Se-amino acids into proteins (Eustice et al., 1981; Gupta and Gupta, 2017). Other toxic effects of Se in plants include enhancement of ethylene formation (Konze et al., 1978), replacement of sulfur in cellular constituents (Stadtman, 1974), and the decrease in thermostability of glutelin (Ei et al., 2020; Hussain et al., 2018).

Similarly to our results, Thuesombat et al. (2014) observed an inhibitory effect of silver NPs (AgNPs) in the germination and growth of rice seeds with increasing sizes and concentrations of the NPs. Wang et al. (2012) observed that rice seedlings treated with sodium selenite between 2 and 6 mg L^{-1} showed enhanced growth, while higher

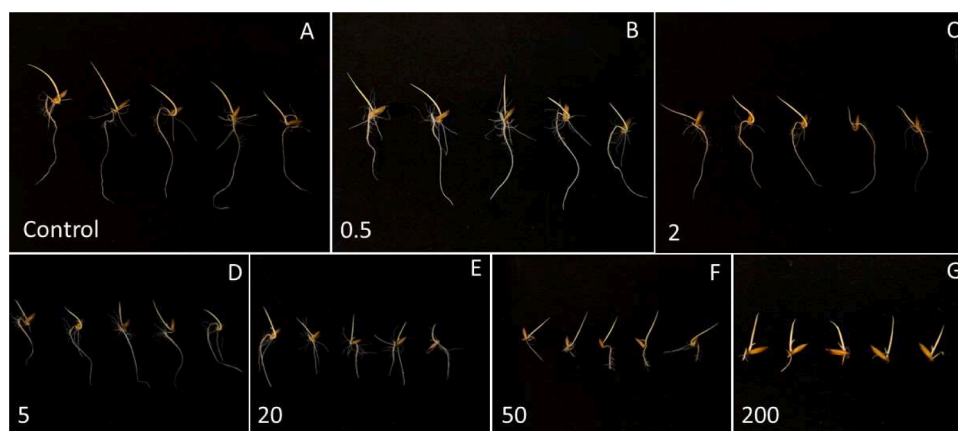


Fig. 2. Representative images of germinating rice seeds treated with control (A) and SeNPs at the concentrations: 0.5 (B), 2 (C), 5 (D), 20 (E), 50 (F), and 200 mg L⁻¹ (G) at the end of the experiment.

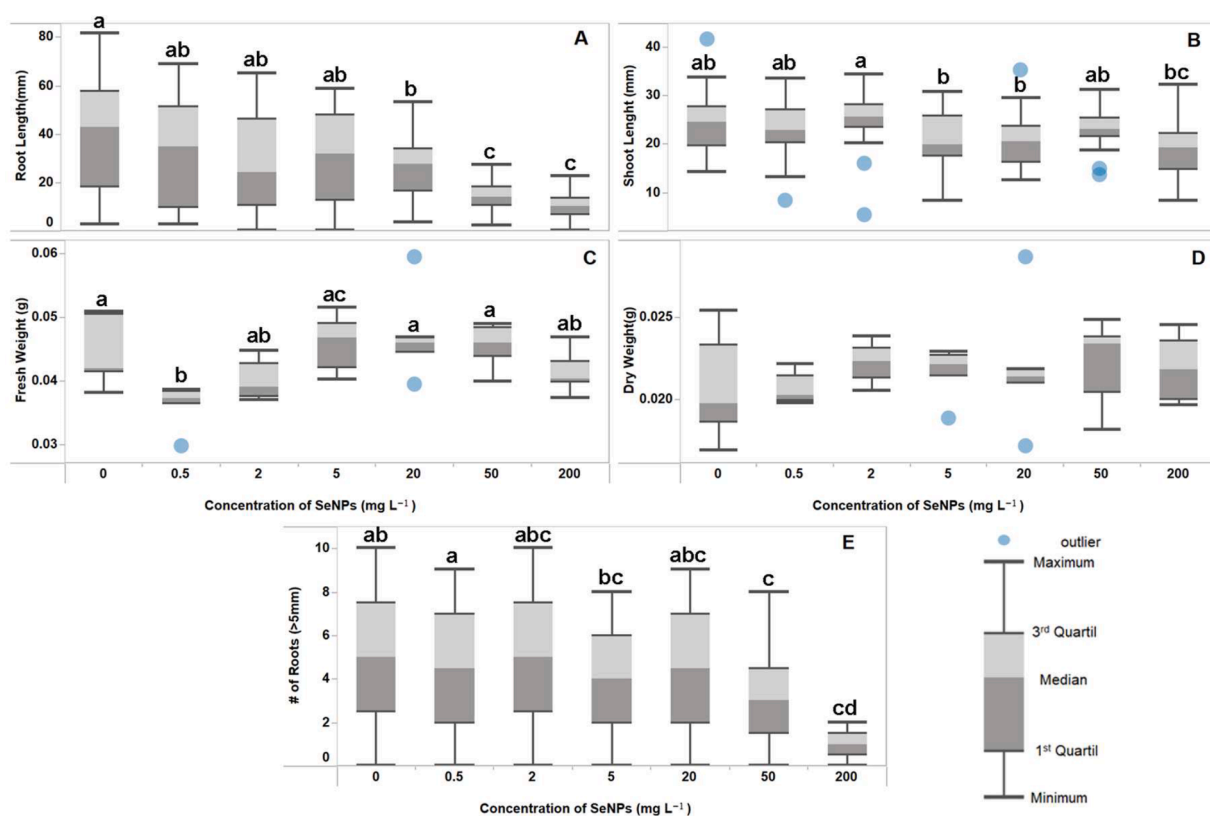


Fig. 3. Effect of SeNPs application rates on root (A) and shoot length (B), fresh (C) and dry weight (D), and number of roots > 5 mm (E) in germinating rice seeds. Different lower-case letters indicate significant difference between groups by the Tukey test ($p < 0.05$).

concentrations ($>6 \text{ mg L}^{-1}$) suppressed seedling growth. [Ei et al. \(2020\)](#) observed that Se and Zn concentrations in rice seeds significantly influenced the germination rate and seedling growth (root and shoot length). In the above-mentioned study, the seeds were obtained from plants treated with Zn, Se, and with both elements combined. Similar to our results, the authors did not observe differences in seedling dry mass between treatments ([Ei et al., 2020](#)). In another study, foliar application of SeNPs alone or combined with silicon NPs improved rice growth and quality by enhancing grain yield, rice biomass, and Se contents ([Hussain et al., 2020](#)).

It is worth mentioning that the application of SeNPs as fertilizer can have different effects when plants are grown under stress. [Zhao et al. \(2020\)](#) reported that low doses of SeNPs (0.01 to 10 mg L^{-1}) were not

toxic to garlic plants, while the exposure dose of 50 mg L^{-1} inhibited seedling growth. On the other hand, under Hg stress, the increasing SeNPs doses mitigated the inhibitive effect of Hg ([Zhao et al., 2020](#)). A recent study showed that soaking rice grains in SeNPs at 6.25 mg L^{-1} before sowing enhanced plant growth in plants cultivated under saline stress. Plants treated with SeNPs presented increased root thickness and volume, relative water content, dry matter, and grain yield ([Badawy et al., 2021](#)). The authors attributed these favorable effects of SeNPs to some factors, including the activation of an anti-oxidative system to scavenge ROS and the accumulation of biochemical compounds, such as total phenolic and antioxidant capacity ([Badawy et al., 2021](#)).

The results suggest that the application of SeNPs can significantly influence the physiological characteristics of rice plants, especially at

high concentrations, when plants are grown without any type of stress.

3.2. Effect of SeNPs on antioxidant enzyme activities

The effect of SeNPs concentration on antioxidant enzymatic activities was studied. Statistically significant differences ($p < 0.05$) between groups were observed for all enzymes, indicating that the application of SeNPs induced changes in the antioxidant system of rice seedlings. In general, a trend of increase in the antioxidant enzyme activities was observed for the lowest SeNPs concentrations, followed by a decrease with increasing SeNPs concentrations (Fig. 4).

Antioxidant enzymes are the first line of plant antioxidant system defense, responsible for complex protection mechanisms against the damage caused by ROS (Upadhyaya et al., 2017). The production of ROS tends to increase in response to abiotic stress, causing cellular damage. An increase in antioxidant levels increases the ability to scavenge ROS at the cellular level, conferring tolerance against stress (Upadhyaya et al., 2017). Antioxidants can react directly or indirectly with ROS (Feng et al., 2013).

3.2.1. APX activity

APX is the first enzyme of the ascorbate-glutathione cycle, which scavenges the H_2O_2 in plant tissues (Thuesombat et al., 2016). In the present study, APX activity in germinating rice seeds enhanced in response to the application of SeNPs at all concentrations compared to the control, as shown in Fig. 4A. The observed increase in the APX activity can contribute to a decrease in H_2O_2 levels, as this enzyme acts in the removal of ROS (Thuesombat et al., 2016). However, comparing the groups of treatment, it was observed a tendency of decrease in the APX activity from 0.5 mg L^{-1} of SeNPs onwards, followed by a new increase from 50 mg L^{-1} . These results can be interpreted as a maximum stimulation of the antioxidant system of rice seedlings at the lowest SeNPs concentration, which then decreases with increasing SeNPs concentrations. However, at very high concentrations (50 and 200 mg L^{-1}), SeNPs may be generating a lot of stress for the plant, which makes enzymatic activity to increase again to combat this oxidative stress.

Thuesombat et al. (2016) observed that the application of AgNPs induced an increase in APX activity in rice seedlings in response to saline stress.

3.2.2. CAT activity

CAT is one of the main antioxidant enzymes related to the control of ROS. This enzyme acts by converting excessive H_2O_2 in water and oxygen (Thuesombat et al., 2016). Relative to the control, a significant increase in CAT activity was observed when rice seedlings were exposed to the lowest concentration of SeNPs (0.5 mg L^{-1}), followed by a return to normal levels and a decrease in concentrations higher than 20 mg L^{-1} (Fig. 4B). This confirm that the lowest concentration is the optimum for stimulating rice antioxidant system. The observed increase in CAT activity can accelerate the elimination of H_2O_2 by the plant, reducing oxidative stress. An increase in CAT activity was also observed in rice seeds treated with zinc NPs (ZnNPs) (Upadhyaya et al., 2017), and in rice seedlings after treatment with AgNPs (Thuesombat et al., 2016). The application of selenite or selenate in rice plants exposed to Cd increased CAT activity in the roots, according to Wan et al. (2019).

3.2.3. POD activity

For POD, although an increasing trend was observed at the concentration of 0.5 mg L^{-1} , it was not significant compared to the control group. No treatment group showed significant variation in relation to the control (Fig. 4C). Comparing the groups of treatments, the application of 0.5 mg L^{-1} of SeNPs enhanced POD activity compared to 2 and 50 mg L^{-1} . POD also helps to minimize oxidative stress through the removal of ROS.

3.2.4. SOD activity

SOD is the most effective intracellular enzymatic antioxidant and the first line of antioxidative defense, that acts in the regulation of the cellular concentration of O_2^- , converting it into H_2O_2 (Thuesombat et al., 2016; Upadhyaya et al., 2017). In the present study, the effects of SeNPs on SOD activity in rice seedlings were similar to those observed for CAT activity. Compared to the control, an increase in SOD activity was

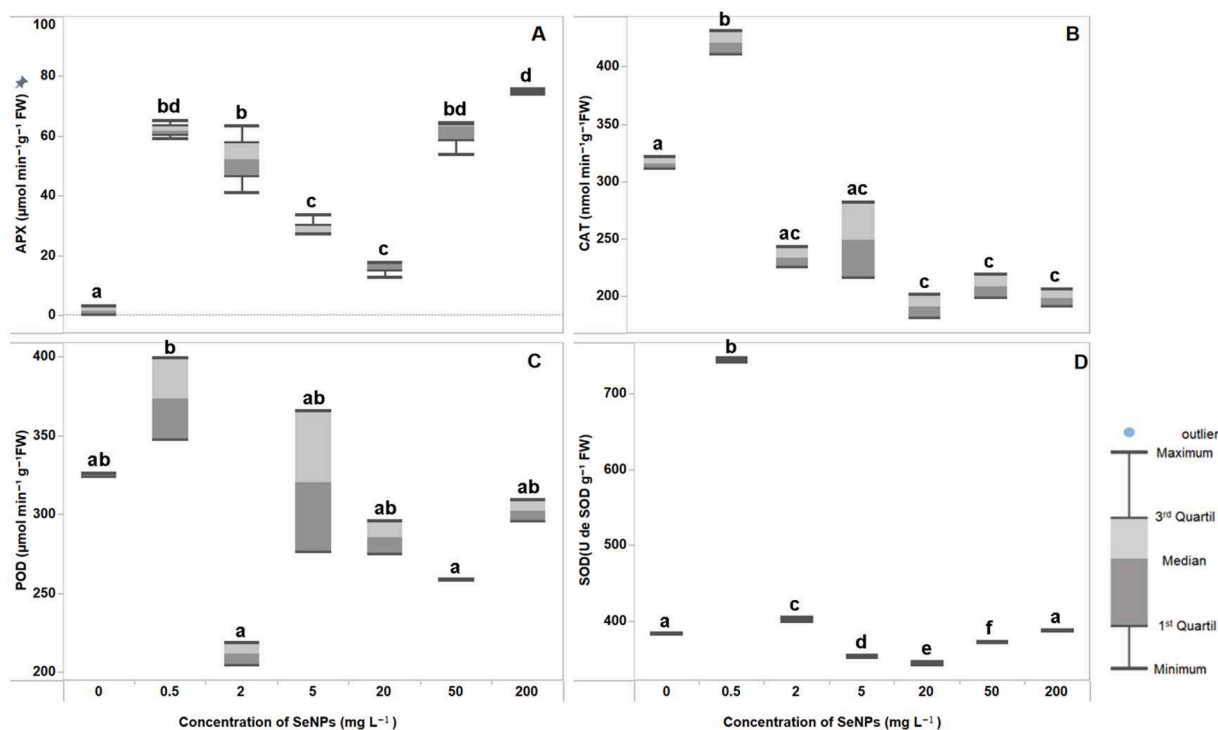


Fig. 4. Effect of SeNPs application rates on Ascorbate Peroxidase (APX) (A), Catalase (CAT) (B), Peroxidases (POD) (C), and Superoxide Dismutase (SOD) (D) activities of germinating rice seeds. Different lower-case letters indicate significant difference between groups by the Tukey test ($p < 0.05$).

observed for the lowest concentrations of SeNPs (0.5 and 2 mg L^{-1}), followed by a decrease from 5 mg L^{-1} and a return to control levels at 200 mg L^{-1} (Fig. 4D). This increase in SOD activity at lower concentrations of SeNPs may indicate an adaptation of the plant to increase growth during germination through regulation of ROS levels (Upadhyaya et al., 2017).

Several studies have suggested that Se may be directly involved in the extinction of ROS such as O_2^- and OH^- through increasing SOD activity (Andrade et al., 2018; Nawaz et al., 2016). It is known that Se increases activity levels and gene expression of SOD. In a previous study, the application of 0.5 mg kg^{-1} of Se promoted an increase in SOD activity in rice plants (Andrade et al., 2018). According to Feng et al. (2013), three main factors control SOD activity in plants exposed to Se. The first is the level of stress. While at low stress levels, the plant antioxidant capacity is sufficient, at high stresses, an enhancement in SOD activity can be required, which could be achieved by Se addition. The second factor that influences SOD activity is the Se dose. Finally, the third factor is related to the concentrations of SOD cofactors, such as Fe, Mn, Cu, and Zn (Feng et al., 2013).

The results of antioxidant enzyme activity suggest that SeNPs, when applied at low concentrations (0.5 mg L^{-1}), stimulated the antioxidant system of rice seedlings. On the other hand, higher SeNPs concentrations (>2 mg L^{-1}) may have a toxic effect on rice germinating seeds. Similar to our results, Chen et al. (2014) observed that CAT, SOD, and POD activities first increased and then decreased in wheat seedlings with increasing Cd concentrations. Analogously to what happens with the Cd, exposure to SeNPs at low doses could stimulate the antioxidant enzymes and enhance their activities. However, at high levels, the application of SeNPs may induce stress and disturb enzyme synthesis, as reported for Cd (Lin et al., 2007; Vögeli-Lange and Wagner, 1990). Other studies showed that the application of Se at low concentrations has beneficial effects on plants, stimulating seedling growth, participating in the antioxidant defense system, and increasing tolerance against abiotic stress (Andrade et al., 2018; Yao et al., 2010). However, in the present study, the plants were grown free from any type of stress, therefore, it is not possible to state that the effect of SeNPs under stress conditions would be the same.

3.3. Effect of SeNPs on S-nitrosothiols, nitrite, and hydrogen peroxide contents

Significant changes were observed in S-NO, NO_2^- and H_2O_2 concentrations in germinating rice seeds treated with SeNPs (Fig. 5). There was a decrease in S-NO concentration following the application of 0.5 mg L^{-1}

of SeNPs and this specie was not detected in samples from the other groups (Fig. 5A). The S-NO is a metabolite of NO in plants and is related to the level of stress. The reduction observed in S-NO content may be related to its consumption, as these molecules act to reduce oxidative stress species, promoting antioxidant metabolism (Nabi et al., 2019).

S-nitrosated (or S-nitrosylated) proteins can be directly correlated to the NO activity in molecular levels. Numerous S-nitrosated proteins are related to plant immunity such as the NADPH oxidase, which generates superoxide (Yun et al., 2011), NPR1, which is a major transcription coactivator (Lindermayr et al., 2010), and the peroxynitrite reductase peroxiredoxin E (Romero-Puertas et al., 2007) and the chaperone-like protein CDC48 involved in the control quality of proteins. Moreover, plant cellular levels of NO and S-nitrosoglutathione (GSNO), a low molecular weight S-nitrosothiol, are controlled by the enzyme S-nitrosoglutathione reductase (GSNOR) (Jahnová et al., 2019; Li et al., 2021). In fact, it has been demonstrated that NO derived from nitrate assimilation by plants is able to suppress GSNOR by S-nitrosation reaction, a covalent bond of NO to cysteine moieties leading to S-nitrosothiols (S-NO), preserving GSNO (a known bio-reservoir of NO in cells) (Fruntillo et al., 2014). Thus, NO signaling, and nitrate assimilation are connected allowing plants to tune NO scavenging and generation.

An increase in S-NO concentration may indicate toxicity after exposure to high concentrations of NPs, as observed by Pelegrino et al. (2020) in bioassays with lettuce seeds exposed to CuO NPs. However, different from this study, Pelegrino et al. (2020) observed a direct, linear response of S-NO to CuO NPs concentrations. In another study with lettuce plants, Pelegrino et al. (2021) observed a decrease in S-NO levels in lettuce leaves treated with CuO NPs combined with GSNO, while the administration of CuO NPs or GSNO separated significantly increased S-NO levels.

A similar tendency was observed for NO_2^- , as all treatments decreased its concentration compared to the control group (Fig. 5B). For the treatment with SeNPs at 0.5 mg L^{-1} , the results were below the detection limit. Comparing the groups, it was observed a tendency of increasing in the NO_2^- levels from 0.5 to 20 mg L^{-1} of SeNPs, which may be related to the observed decrease in enzymatic activity (mainly APX, CAT, and SOD) at 20 mg L^{-1} of SeNPs. This increase is followed by a decrease from 50 mg L^{-1} . Interestingly, this is an opposite trend to that observed for APX activity. This may indicate that from 0.5 mg L^{-1} onwards, the increase in SeNPs concentrations begins to cause stress in the plant, which could increase the synthesis of NO from nitrite. However, at 50 – 200 mg L^{-1} of SeNPs the stress can be so high that it causes a disturbance in the antioxidant system and reduces nitrite production. Pelegrino et al. (2020) observed an increase in the level of NO_2^- in lettuce

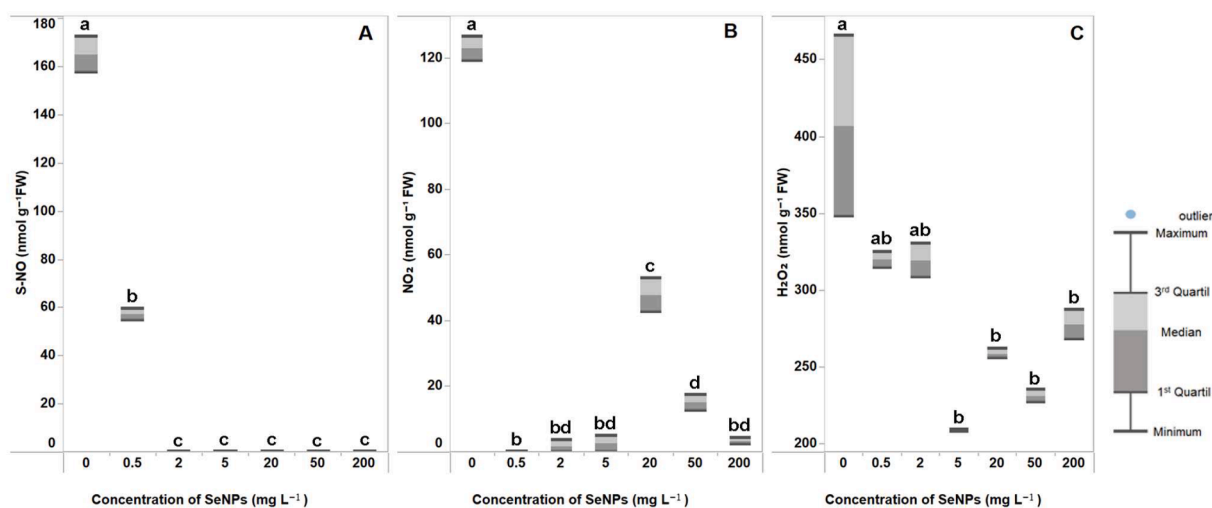


Fig. 5. Effect of SeNPs application rates on S-nitrosothiols (S-NO) (A), nitrite (NO_2^-) (B), and hydrogen peroxide (H_2O_2) (C) in germinating rice seeds. Different lower-case letters indicate significant difference between groups by the Tukey test ($p < 0.05$).

radicals with increasing concentrations of CuO NPs, which may indicate toxicity after seed exposure to a high concentration of NPs.

Recently, the role of NO in several plant physiological processes has gained importance and recognition. Although recent progress in the clarification of NO functions in plants, the exact mechanisms of its generation in plant tissues is still a challenge. In plants, nitrate assimilation pathway is responsible for nitrite production. In this sense, NO generation is linked to the enzymatic activity of nitrate reductase, which converts nitrate into nitrite, which can be further reduced to NO (Pieretti et al., 2023; Santolini et al., 2017).

A tendency of decrease in H₂O₂ levels was observed by comparing with the control group for all SeNPs concentrations (Fig. 5C), but this decrease was only significant from 5 mg L⁻¹. This is probably due to the activation of the anti-oxidative system of rice seedlings to scavenge ROS. Under normal conditions, plants produce ROS at low levels (Feng et al., 2013; Wan et al., 2019). It is known that accumulation of ROS, such as H₂O₂, is indicative of oxidative stress, and may be related to heavy metal toxicity, salinity, cold, water excess, or deficit (Feng et al., 2013). In a study with rice plants, Wan et al. (2019) observed that stress conditions due to exposure to Cd increased the H₂O₂ content by 23 % in the roots, whereas the co-addition of selenite or selenate caused significant decreases (59–78 %) in the levels of this molecule. Andrade et al. (2018) reported an increased concentration of H₂O₂ in the leaves of rice plants subjected to water deficit compared to those irrigated. In the same study, the application of Se in concentrations up to 0.5 mg kg⁻¹ of soil caused a reduction in the H₂O₂ content in leaves, associated with an increase in SOD activity, showing a protective effect of this element (Andrade et al., 2018).

The observed decrease in the concentrations of H₂O₂, NO₂⁻ and S-NO may indicate that the application of SeNPs at low doses does not cause stress to the plants, on the contrary, it decreases the ROS levels in the rice plant, possibly due to the regulation of the antioxidant system. Other studies have reported a similar trend for ionic Se. This element was shown to control the production of ROS directly and indirectly via the regulation of antioxidants (Feng et al., 2013). A minor addition of Se could reduce ROS generation (especially O₂⁻ and H₂O₂) mainly in stressed plants, through a disturbance of the ROS reaction chain, reducing the damage to lipids of the cell membrane. On the other hand, Se in excess triggers the accumulation of ROS in plant tissues, probably due to an imbalance in levels of thiols, GSH, NADPH, and ferredoxins, molecules related to both Se assimilation and ROS quenching (Feng et al., 2013; Filek et al., 2008; Freeman et al., 2010; Kumar et al., 2012; Mroczek-Zdyrska and Wójcik, 2012).

4. Conclusions

This study brings important findings on the dual effect of SeNPs on rice germinating seeds. As evidenced by the results, soaking rice seeds in a SeNPs suspension at low concentrations (0.5 mg L⁻¹) did not affect seedling growth, but enhanced antioxidant enzyme activities and reduced S-NO and NO₂⁻ formation. These results confirm the hypothesis that treatment with SeNPs has a protective effect on rice plants, stimulating the antioxidant system and scavenging ROS. Meanwhile, with increasing SeNPs concentrations, seedling growth was strongly inhibited, and there was a decrease in antioxidant enzyme activity, suggesting a toxic effect related to the excess of SeNPs. Based on these findings, this study highlights the efficacy of SeNPs at the optimum concentration of 0.5 mg L⁻¹ in enhancing the antioxidant activity of rice seedlings and the ROS scavenging system without affecting the physiological parameters of the plant. Therefore, it is reasonable to consider the use of SeNPs as a fertilizer to produce Se-biofortified rice as a strategy to mitigate Se deficiency. Future long-term studies are needed to evaluate the response of this treatment on Se concentrations in rice tissues and the effects of SeNPs in soil-plant systems, as well as in rice plants cultivated until grain maturation.

CRedit authorship contribution statement

Bruna Moreira Freire: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Conceptualization. **Camila Neves Lange:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Yasmin Tavares Cavalcanti:** Writing – original draft, Investigation, Visualization, Formal analysis. **Joana Claudio Pieretti:** Writing – review & editing, Methodology, Investigation. **Amedea Barozzi Seabra:** Writing – review & editing, Supervision, Resources. **Bruno Lemos Batista:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.stress.2024.100372](https://doi.org/10.1016/j.stress.2024.100372).

References

- Anderson, M.D., Prasad, T.K., Stewart, C.R., 1995. Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiol* 109 (4), 1247–1257.
- Andrade, F.R., da Silva, G.N., Guimarães, K.C., Barreto, H.B.F., de Souza, K.R.D., Guilherme, L.R.G., Faquin, V., Dos Reis, A.R., 2018. Selenium protects rice plants from water deficit stress. *Ecotoxicol. Environ. Saf.* 164, 562–570.
- Badawy, S.A., Zayed, B.A., Bassiouni, S.M., Mahdi, A.H., Majrashi, A., Ali, E.F., Seleiman, M.F., 2021. Influence of nano silicon and nano selenium on root characters, growth, ion selectivity, yield, and yield components of rice (*Oryza sativa* L.) under salinity conditions. *Plants* 10 (8), 1657.
- Badgar, K., 2019. The synthesis of selenium nanoparticle (SeNPs)–Review. *Acta agrar. Debr.* (1), 5–8.
- Boonyanitipong, P., Kositsup, B., Kumar, P., Baruah, S., Dutta, J., 2011. Toxicity of ZnO and TiO₂ nanoparticles on germinating rice seed *Oryza sativa* L. *Int. J. Biosci. Biochem. Bioinforma.* 1 (4), 282.
- Boroumand, S., Safari, M., Shaabani, E., Shirzad, M., Faridi-Majidi, R., 2019. Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. *Mater. Res. Express* 6 (8), 0850d8.
- Chen, C., Zhou, Q., Cai, Z., 2014. Effect of soil HHCB on cadmium accumulation and phytotoxicity in wheat seedlings. *Ecotoxicol.* 23 (10), 1996–2004.
- de Brito Mateus, M.P., Tavanti, R.F.R., Tavanti, T.R., Santos, E.F., Jalal, A., Dos Reis, A. R., 2021. Selenium biofortification enhances ROS scavenge system increasing yield of coffee plants. *Ecotoxicol. Environ. Saf.* 209, 111772.
- dos Reis, A.R., El-Ramady, H., Santos, E.F., Gratão, P.L., Schomburg, L., 2017. Overview of selenium deficiency and toxicity worldwide: affected areas, selenium-related health issues, and case studies. In: Pilon-Smits, E.A.H., Winkel, L.H.E., Lin, Z.Q. (Eds.), *Selenium in plants: molecular, physiological, Ecological and Evolutionary Aspects*. Springer, Cham, Switzerland, pp. 209–230.
- Ei, H.H., Zheng, T., Farooq, M.U., Zeng, R., Su, Y., Zhang, Y., Liang, Y., Tang, Z., Ye, X., Jia, X., Zhu, J., 2020. Impact of selenium, zinc and their interaction on key enzymes,

- grain yield, selenium, zinc concentrations, and seedling vigor of biofortified rice. *Environ. Sci. Pollut. Res.* 27 (14), 16940–16949.
- El-Ramady, H.R., Domokos-Szabolcsy, É., Abdalla, N.A., Alshaal, T.A., Shalaby, T.A., Sztirik, A., Prokisch, J., Fári, M., 2014. Selenium and nano-selenium in agroecosystems. *Environ. Chem. Lett.* 12, 495–510.
- Eustice, D.C., Kull, F.J., Shrift, A., 1981. Selenium toxicity: aminoacylation and peptide bond formation with selenomethionine. *Plant Physiol* 67 (5), 1054–1058.
- Feng, R., Wei, C., Tu, S., 2013. The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.* 87, 58–68.
- Filek, M., Keskinen, R., Hartikainen, H., Szarejko, I., Janiak, A., Miszalski, Z., Golda, A., 2008. The protective role of selenium in rape seedlings subjected to cadmium stress. *J. Plant Physiol.* 165 (8), 833–844.
- Freeman, J.L., Tamaoki, M., Stushnoff, C., Quinn, C.F., Cappa, J.J., Devonshire, J., Fakra, S.C., Marcus, M.A., McGrath, S.P., Hoewyk, D.V., Pilon-Smits, E.A.H., 2010. Molecular mechanisms of selenium tolerance and hyperaccumulation in *Stanleya pinnata*. *Plant Physiol* 153 (4), 1630–1652.
- Freire, B.M., Cavalcanti, Y.T., Lange, C.N., Pieretti, J.C., Pereira, R.M., Gonçalves, M.C., Nakazato, G., Seabra, A.B., Batista, B.L., 2022. Evaluation of collision/reaction gases in single-particle ICP-MS for sizing selenium nanoparticles and assessment of their antibacterial activity. *Nanotechnol* 33 (35), 355702.
- Frunghillo, L., Skelly, M.J., Loake, G.J., Spoel, S.H., Salgado, I., 2014. S-nitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. *Nat. Commun.* 5 (1), 5401.
- Gupta, M., Gupta, S., 2017. An overview of selenium uptake, metabolism, and toxicity in plants. *Front. Plant Sci.* 7, 2074.
- Hussain, S.T., Hussain, A., Dar, S.A., 2018. Biofortification benefits of zinc application to rice genotypes. *J. Pharmacogn. Phytochem.* 7 (1), 1555–1558.
- Hussain, B., Lin, Q., Hamid, Y., Sanaulah, M., Di, L., Hashmi, M.L.R., Khan, M.B., He, Z., Yang, X., 2020. Foliage application of selenium and silicon nanoparticles alleviates Cd and Pb toxicity in rice (*Oryza sativa* L.). *Sci. Total Environ* 712, 136497.
- Jahnová, J., Luhová, L., Petřivalský, M., 2019. S-nitrosoglutathione reductase—the master regulator of protein S-nitrosation in plant NO signaling. *Plants* 8 (2), 48.
- Jones, G.D., Droz, B., Greve, P., Gottschalk, P., Poffet, D., McGrath, S.P., Seneviratne, S. I., Smith, P., Winkler, L.H., 2017. Selenium deficiency risk predicted to increase under future climate change. *Proc. Natl. Acad. Sci. U.S.A.* 114 (11), 2848–2853.
- Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements from Soil to Human. Springer, Berlin/Heidelberg, Germany.
- Konze, J.R., Schilling, N., Kende, H., 1978. Enhancement of ethylene formation by selenoamino acids. *Plant Physiol* 62 (3), 397–401.
- Kumar, M., Bijo, A.J., Baghel, R.S., Reddy, C.R.K., Jha, B., 2012. Selenium and spermine alleviate cadmium induced toxicity in the red seaweed *Gracilaria dura* by regulating antioxidants and DNA methylation. *Plant Physiol. Biochem.* 51, 129–138.
- Li, B., Sun, C., Lin, X., Busch, W., 2021. The emerging role of GSNOR in oxidative stress regulation. *Trends Plant Sci* 26 (2), 156–168.
- Lin, R., Wang, X., Luo, Y., Du, W., Guo, H., Yin, D., 2007. Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere* 69 (1), 89–98.
- Lindermayr, C., Sell, S., Müller, B., Leister, D., Durner, J., 2010. Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. *Plant Cell* 22 (8), 2894–2907.
- Liu, S., Dong, Y., Xu, L., Kong, J., 2014. Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regul* 73 (1), 67–78.
- Morales-Espinoza, M.C., Cadenas-Pilego, G., Pérez-Alvarez, M., Hernández-Fuentes, A.D., Cabrera de la Fuente, M., Benavides-Mendoza, A., Valdés-Reyna, J., Juárez-Maldonado, A., 2019. Se nanoparticles induce changes in the growth, antioxidant responses, and fruit quality of tomato developed under NaCl stress. *Molecules* 24 (17), 3030.
- Mroczek-Zdyrska, M., Wójcik, M., 2012. The influence of selenium on root growth and oxidative stress induced by lead in *Vicia faba* L. minor plants. *Biol. Trace Elem. Res.* 147 (1), 320–328.
- Nabi, R.B.S., Tayade, R., Hussain, A., Kulkarni, K.P., Imran, Q.M., Mun, B.G., Yun, B.W., 2019. Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. *Environ. Exp. Bot.* 161, 120–133.
- Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22 (5), 867–880.
- Nawaz, F., Naeem, M., Ashraf, M.Y., Tahir, M.N., Zulfiqar, B., Salahuddin, M., Shabbir, R. N., Aslam, M., 2016. Selenium supplementation affects physiological and biochemical processes to improve fodder yield and quality of maize (*Zea mays* L.) under water deficit conditions. *Front. Plant Sci.* 7, 1438.
- Neysanian, M., Iranbakhsh, A., Ahmadvand, R., Oraghi Ardebili, Z., Ebadi, M., 2020. Comparative efficacy of selenate and selenium nanoparticles for improving growth, productivity, fruit quality, and postharvest longevity through modifying nutrition, metabolism, and gene expression in tomato; potential benefits and risk assessment. *PLoS ONE* 15 (12), e0244207.
- Oliveira, H.C., Gomes, B.C., Pelegrino, M.T., Seabra, A.B., 2016. Nitric oxide-releasing chitosan nanoparticles alleviate the effects of salt stress in maize plants. *Nitric Oxide* 61, 10–19.
- Paniz, F.P., Pedron, T., Procópio, V.A., Lange, C.N., Freire, B.M., Batista, B.L., 2023. Selenium biofortification enhanced grain yield and alleviated the risk of arsenic and cadmium toxicity in rice for human consumption. *Toxics* 11 (4), 362.
- Pelegrino, M.T., Kohatsu, M.Y., Seabra, A.B., Monteiro, L.R., Gomes, D.G., Oliveira, H.C., Rolim, W.S., Jesus, T.A., Batista, B.L., Lange, C.N., 2020. Effects of copper oxide nanoparticles on growth of lettuce (*Lactuca sativa* L.) seedlings and possible implications of nitric oxide in their antioxidative defense. *Environ. Monit. Assess.* 192 (4), 1–14.
- Pelegrino, M.T., Pieretti, J.C., Lange, C.N., Kohatsu, M.Y., Freire, B.M., Batista, B.L., Fincheira, P., Tortella, G.R., Rubilar, O., Seabra, A.B., 2021. Foliar spray application of CuO nanoparticles (NPs) and S-nitrosoglutathione enhances productivity, physiological and biochemical parameters of lettuce plants. *J. Chem. Technol. Biotechnol.* 96 (8), 2185–2196.
- Pieretti, J.C., Pelegrino, M.T., Silveira, N.M., Rodrigues, M.G., Seabra, A.B., 2023. State-of-the-art and perspectives for nanomaterials combined with nitric oxide donors: from biomedical to agricultural applications. *ACS Appl. Nano Mater.*
- Romero-Puertas, M.C., Laxa, M., Matte, A., Zaninotto, F., Finkemeier, I., Jones, A.M.E., Perazzolli, M., Vandelle, E., Dietz, K.J., Delledonne, M., 2007. S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. *Plant Cell* 19 (12), 4120–4130.
- Santolini, J., André, F., Jeandroz, S., Wendehenne, D., 2017. Nitric oxide synthase in plants: where do we stand? *Nitric Oxide* 63, 30–38.
- Shams, M., Ekinici, M., Turan, M., Dursun, A., Kul, R., Yildirim, E., 2019. Growth, nutrient uptake and enzyme activity response of Lettuce (*Lactuca sativa* L.) to excess copper. *Environ Sustain* 2 (1), 67–73.
- Shanker, A.K., 2006. Countering UV-B stress in plants: does selenium have a role? *Plant Soil* 282 (1–2), 21–26.
- Stadtman, T.C., 1974. Selenium Biochemistry: proteins containing selenium are essential components of certain bacterial and mammalian enzyme systems. *Science* 183 (4128), 915–922.
- Thuesombat, P., Hannongbua, S., Akasit, S., Chadchawan, S., 2014. Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol. Environ. Saf.* 104, 302–309.
- Thuesombat, P., Hannongbua, S., Ekgasit, S., Chadchawan, S., 2016. Effects of silver nanoparticles on hydrogen peroxide generation and antioxidant enzyme responses in rice. *J. Nanosci. Nanotechnol.* 16 (8), 8030–8043.
- Upadhyaya, H., Roy, H., Shome, S., Tewari, S., Bhattacharya, M.K., Panda, S.K., 2017. Physiological impact of Zinc nanoparticle on germination of rice (*Oryza sativa* L.) seed. *J. Plant Sci. Phytopathol* 1, 062–070.
- Vögelli-Lange, R., Wagner, G.J., 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves: implication of a transport function for cadmium-binding peptides. *Plant Physiol. (Bethesda)* 92 (4), 1086–1093.
- Wan, Y., Wang, K., Liu, Z., Yu, Y., Wang, Q., Li, H., 2019. Effect of selenium on the subcellular distribution of cadmium and oxidative stress induced by cadmium in rice (*Oryza sativa* L.). *Environ. Sci. Pollut. Res.* 26 (16), 16220–16228.
- Wang, Y.D., Wang, X., Wong, Y.S., 2012. Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. *J. Proteom.* 75 (6), 1849–1866.
- Wang, K., Wang, Y., Li, K., Wan, Y., Wang, Q., Zhuang, Z., Guo, Y., Li, H., 2020. Uptake, translocation and biotransformation of selenium nanoparticles in rice seedlings (*Oryza sativa* L.). *J. Nanobiotechnol* 18 (1), 1–15.
- Wang, K., Wang, Y., Wan, Y., Mi, Z., Wang, Q., Wang, Q., Li, H., 2021. The fate of arsenic in rice plants (*Oryza sativa* L.): influence of different forms of selenium. *Chemosphere* 264, 128417.
- White, P.J., Bowen, H.C., Parmaguru, P., Fritz, M., Spracklen, W.P., Spiby, R.E., Meacham, M.C., Mead, A., Harriman, M., Trueman, L.J., Smith, B.M., Thomas, B., Broadley, M.R., 2004. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana*. *J. Exp. Bot.* 55 (404), 1927–1937.
- Yao, X., Chu, J., Ba, C., 2010. Antioxidant responses of wheat seedlings to exogenous selenium supply under enhanced ultraviolet-B. *Biol. Trace Elem. Res.* 136 (1), 96–105.
- Yu, B., Zhang, Y., Zheng, W., Fan, C., Chen, T., 2012. Positive surface charge enhances selective cellular uptake and anticancer efficacy of selenium nanoparticles. *Inorg. Chem.* 51 (16), 8956–8963.
- Yun, B.W., Feechan, A., Yin, M., Saidi, N.B., Le Bihan, T., Yu, M., Moore, J.W., Kang, J.G., Kwon, E., Spoel, S.H., Pallas, J.A., Loake, G.J., 2011. S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature* 478 (7368), 264–268.
- Zahedi, S.M., Abdelrahman, M., Hosseini, M.S., Hoveizeh, N.F., Tran, L.S.P., 2019. Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium-nanoparticles. *Environ. Pollut.* 253, 246–258.
- Zhao, J., Liang, X., Zhu, N., Wang, L., Li, Y., Li, Y.F., Zheng, L., Zhang, Z., Gao, Y., Chai, Z., 2020. Immobilization of mercury by nano-elemental selenium and the underlying mechanisms in hydroponic-cultured garlic plant. *Environ. Sci. Nano* 7 (4), 1115–1125.