Silicon nanoparticles alleviate cadmium toxicity in rice (*Oryza sativa* L.) by modulating the nutritional profile and triggering stress-responsive genetic mechanisms

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ARTICLE INFO

ABSTRACT

This study investigated the physiological and molecular responses of rice genotype '9311' to Cd stress and the mitigating effects of silicon oxide nanoparticles (SiO NPs). Cd exposure severely hindered plant growth, chlorophyll content, photosynthesis, and Cd accumulation. However, SiO NPs supplementation, particularly the SiONP100 treatment, significantly alleviated Cd-induced toxicity, mitigating the adverse effects on plant growth while maintaining chlorophyll content and photosynthetic attributes. The SiONP100 treatment also reduced Cd accumulation, indicating a preference for Si uptake in genotype 9311. Complex interactions among Cd, Si, Mg, Ca, and K were uncovered, with fluctuations in MDA and H_2O_2 contents. Distinct morphological changes in stomatal aperture and mesophyll cell structures were observed, including changes in starch granules, grana thylakoids, and osmophilic plastoglobuli. Moreover, following SiONP100 supplementation, genotype 9311 increased peroxidase, superoxide dismutase, and catalase activities by 56%, 44%, and 53% in shoots and 62%, 49%, and 65% in roots, respectively, indicating a robust defense mechanism against Cd stress. Notably, *OsNramp5*, *OsHMA3*, *OsSOD-Cu/Zn*, *OsCATA*, *OsCATB*, and *OsAPX1* showed significant expression after SiO NPs treatment, suggesting potential Cd translocation within rice tissues. Overall, SiO NPs supplementation holds promise for enhancing Cd tolerance in rice plants while maintaining essential physiological functions.

1. Introduction

Rapid industrialization and urbanization have significantly contributed to heavy metal (HM) accumulation in agricultural soils, threatening global food security (Zheng et al., 2023). Among these HMs, cadmium (Cd) ranks seventh on the US-EPA priority list (Li et al., 2022), as one of the most toxic and pervasive contaminants. Cd, a non-essential metal, can induce severe physiological and biochemical abnormalities in plants, including staple crops like rice (*Oryza sativa* L.) (Goncharuk and Zagoskina, 2023). It enters the soil primarily through anthropogenic

activities such as mining, industrial discharge, and agricultural practices involving Cd-containing fertilizers and pesticides (Dutta et al., 2020). Once in the soil, Cd exhibits a remarkable affinity for plant roots, leading to its uptake and subsequent accumulation in various plant tissues, including grain, affecting molecular, physiological, biochemical, and morphological processes and enzyme activities, including those essential for normal growth (Shahid et al., 2017; Salama et al., 2022; Sheteiwy et al., 2022). Cd-related physiological disorders within plants are primarily attributed to increased oxidative stress (Rizwan et al., 2016, 2017). In paddy fields, Cd contamination jeopardizes crop productivity and poses serious health risks to humans throughout the food chain (Zhao et al., 2023). Consequently, there is a pressing need for effective strategies to mitigate Cd toxicity in rice.

Silicon (Si) is the second most abundant element, comprising 27.7% after oxygen, constituting 47% of the Earth's crust (Brahma et al., 2020). While Si is not essential for land-dwelling higher plants, it contributes to several functions that promote plant development, particularly under biotic and abiotic stresses (Keller et al., 2015; Sarkar et al., 2022; Elekhtyar and AL-Huqail, 2023). However, Si is often found in inert forms in nature, making it unavailable to plants. Bioavailable and soluble forms of Si found in soil include monosilicic acid $[Si(OH)_4]$ and protosilicic acid $[H_4SiO_4]$, typically present in concentrations ranging from 0.1 to 0.6 mM. Plants exclusively absorb Si from the soil as H_4SiO_4 (Hou et al., 2023). Si application has enhanced photosynthetic capacity, carbon metabolism, and crop quality and production in rice, soybeans, and tomato (Chaiwong and Prom-u-thai, 2022; Hussain et al., 2021). The role of Si in enhancing Cd tolerance has been studied extensively, as evidenced by Ma et al. (2020). Previous research has demonstrated the significant contribution of Si in alleviating Cd-induced toxicity in rice (Cui et al., 2017). Supplementation of 0.6 mM sodium silicate to rice seedling roots significantly alleviated Cd-induced toxicity in hydroponic conditions (Farooq et al., 2016). Moreover, 1.0 mM silicic acid reduced Cd uptake and translocation by restricting the expression of Cd-responsive transporter genes in rice (Feng et al., 2017). Other studies have reported that adding available Si to soil significantly decreases Cd accumulation in rice (Wang et al., 2018; Cui et al., 2022).

In recent years, "nanotechnology has emerged as a promising tool in various fields, including agriculture, due to its unique physiochemical properties and potential applications (Ahmed et al., 2023a). Nanoparticles (NPs) have garnered particular interest as effective agents for enhancing plant growth, improving nutrient uptake, and mitigating the harmful effects of environmental stresses (Fincheira et al., 2021; Ahmed et al., 2023b). Among the diverse array of NPs, silicon oxide nanoparticles (SiO NPs) have demonstrated remarkable potential in promoting plant health and conferring tolerance to various abiotic stresses, including HM toxicity (De Sousa et al., 2019; Du et al., 2022). Due to their unique properties, including high surface area, large pore volume, and biocompatibility, SiO NPs have alleviated the toxic effects of HMs in various plant species (Emamverdian et al., 2020; Manzoor et al., 2022). The protecting properties of SiO NPs against Cd toxicity are multifaceted. SiO NPs primarily act as physical barriers, inhibiting Cd from entering root systems by adsorbing or sequestering the metal ions on their surfaces. In addition, SiO NPs can enhance antioxidant enzyme activities in plants, scavenging reactive oxygen species (ROS) produced under Cd stress and reducing oxidative damage (Memari-Tabrizi et al., 2021; Mostofa et al., 2021). Moreover, SiO NPs can regulate gene expression and modulate signaling pathways responsible for Cd detoxification, stimulating plant tolerance to Cd-induced stress (Riaz et al., 2022). Understanding the influence of SiO NPs on rice growth and development under Cd stress is crucial. Numerous studies have reported positive effects of SiO NPs on various agronomic traits, including plant height, biomass accumulation, root development, and chlorophyll content, even under Cd stress. These findings indicated the potential of SiO NPs to improve rice growth and productivity under Cd-contaminated conditions (Garg and Singh (2018). To our knowledge, little is known about the utilization of nanoparticles, specifically SiONPs, for enhancing the growth of Cd-stressed crop plants. It is noteworthy that nanoparticles can accumulate in soil and exert detrimental effects on plant health and physiology when present in high concentrations (El-Moneim et al., 2021). Therefore, the primary aim of this study was to investigate the influence of varying doses of SiO NPs on rice plants subjected to Cd stress. We assessed the following parameters: (1) the response of photosynthetic efficiency and nutrient uptake, (2) detoxification, ROS scavenging, and antioxidant activities, and (3) the expression of Cd transporter and antioxidant-responsive genes. This research offers an economical strategy for mitigating Cd stress in rice and extends our understanding of the mechanisms by which crop plants tolerate metal toxicity.

2. Material and methods

2.1. Characterization of SiO NPs

The SiO NPs (10–25 nm and 99% purity) were acquired from Xuzhou Jiachuang, Material Technology Co., Shanghai, China, and stored in an air-tight container at room temperature before use. "The size, phase purity, composition, and surface morphology of SiO NPs were assessed using transmission electron microscopy (TEM; JEM-1230, JEOL, Akishima, Japan) and scanning electron microscopy (SEM; TM-1000, Hitachi, Japan). The SEM and TEM samples were prepared according to Ahmed et al. (2021) on the aluminum stub and carbon-coated Cu grid. In addition, SEM was coupled with energy-dispersive X-ray (FESEM-EDX)" elemental composition analysis.

2.2. Plant material and experimental conditions

The Indica rice genotype 9311, known for its high Cd accumulating ability (Wang et al., 2021), was used in this study. The experiments were conducted at the Advanced Seed Institute, Zhejiang University, Hangzhou, China. Seedlings were grown in plastic pots (5 plants per pot) under hydroponic conditions following the method of Jalil et al. (2023). Initially, the pots were filled with half-strength basic nutrient solution (BNS) with a pH adjusted to 6.5 for 7 days and then replaced with full-strength BNS. The BNS contained 1.0 mM K_2SO_4, 2.9 mM NH_4NO_3, 0.32 mM $NaH_2PO_4.2 H_2O$, 1.7 mM $MgSO_4.7 H_2O$, 1.0 mM $CaCl_2$, 18 μM H_3BO_3, 0.16 μM $CuSO_4.5 H_2O$, 9.1 μM $MnCl_2.4 H_2O$, 0.52 μM $(NH_4)_6Mo_7O_{24}.4 H_2O$, 0.15 μM $ZnSO_4.7 H_2O$, and 36 μM EDTA FeNa (Zeng et al., 2008). After 21 days, the rice seedlings were treated with cadmium ($CdN_2O_6.4 H_2O$, 20 μM L^{-1}) and silicon oxide nanoparticles (SiO NPs, 50 and 100 mg L^{-1}), alone and in combination with three replications of each treatment. Control seedlings were grown under BNS conditions with no NPs. The SiO NPs were sonicated for 30 min before being added to the BNS for better dispersion. The BNS was renewed every 3 days, and rice seedlings were sampled after 7 days of treatment.

2.3. Measurement of growth parameters

Rice seedling roots were thoroughly rinsed with distilled H_2O to move any external As deposits. Samples from each treatment were divided into roots and shoots to measure morphological parameters (lengths and dry weights). Seedling lengths were measured with a ruler, and dry weights were determined using a precision balance after oven-drying at 70 °C for 24 h. A subset of these samples ws stored at –80 °C for subsequent molecular and physiological analysis determination.

2.4. Measurement of chlorophyll contents, gas exchange parameters, and fluorescence

Gas exchange parameters, including stomatal conductance (gs), net photosynthetic rate (Pn), internal CO_2 concentration (Ci), and transpiration rate (E), were determined using an infrared gas analyzer (IRGA) within a portable photosynthetic system (LI-COR 6800, LICOR, Lincoln,

NE, USA). Measurement followed the methodology outlined by Faizan et al. (2020). SPAD values were measured using a portable chlorophyll meter (SPAD-502 +, Tokyo, Japan). Chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) were measured on fresh leaf samples placed in 10 mL plastic tubes containing a reaction solution (4.5: 4.5: 1 mL ethanol: acetone: deionized H_2O;) (O'Carrigan et al., 2014). The prepared samples were kept in the dark to remove the green color from the leaves. Chlorophyll a and b contents were determined by spectrophotometer readings at 663 nm and 645 nm, respectively, with the final calculation completed as described by Lichtenthaler and Buschmann (2001).

Chlorophyll fluorescence measurements were conducted by exposing intact leaves to darkness for 30 min before recording. Baseline fluorescence (F_0) was recorded under low light conditions (<0.05 $\mu M\ m^{-2}\ s^{-1}$ photosynthetically active radiation), while maximum fluorescence (Fm) was determined using a saturating light pulse (2500 $\mu M\ m^{-2}\ s^{-1}$ photosynthetically active radiation). Fluorescence variability was quantified as $Fv = Fm – F_0$, following the method outlined by Cai et al. (2020). The maximum quantum yield of PSII photochemistry (Fv/m) was also calculated. Color images of Fv/m values were generated using ImagingWin software, specifically the IMAGING-PAM system developed by Walz in Effeltrich, Germany.

2.5. Measurement of elemental and nutrients concentrations

To determine elemental (magnesium, potassium, calcium, silicon, and cadmium) concentrations, root samples were soaked in a 20 mM EDTA (ethylenediaminetetraacetic acid) solution for 30 min at room temperature to remove metal ions attached to root surfaces before washing with deionized H_2O. Dry shoot and root samples (0.2 g each) were digested with 5 mL concentrated nitric acid (HNO_3) at 120 ℃ for 1 h and then for 6 h at 140 ℃ in a dry thermos unit (DTU-2CN, Tokyo, Japan) following the method in Nazir et al. (2022). After digestion, the solutions were diluted with dd-H_2O to a final volume of 10 mL for further experimentation. Elemental concentrations were determined using a plasma mass spectrometer (ICP-MS, iCAP RQ, Thermo Scientific, USA).

2.6. Measurement of malondialdehyde, hydrogen peroxide, and histochemical analysis

We followed the methodology of Velikova et al. (2000) to quantify hydrogen peroxide (H_2O_2) levels in plant tissues. Approximately 200 mg of shoot or root sample was homogenized in a 0.1% trichloroacetic acid solution before centrifuging at 12,000 rpm for 20 min. The resulting supernatant (50 µL) was mixed with 100 µL of 1 M KI and 50 µL potassium phosphate buffer (10 mM, pH 7). The H_2O_2 content was determined using a microplate reader (Synergy H1 Bio-Tec) at 390 nm.

For malondialdehyde (MDA) content, we used a modified method based on Morales and Munné-Bosch (2019). Approximately 100 mg of leaf or root tissue was homogenized in a 65 mM potassium phosphate buffer (pH 7.8) before centrifuging at 12,000 rpm at 4 °C for 20 min. The "supernatant was mixed with a solution containing 5% trichloroacetic acid (TCA) and thiobarbituric acid and incubated at 95 °C for 25 min. The reaction was halted by cooling on ice, followed by centrifugation at 4600 rpm for 12 min. Absorbance values were read at 600 and 532 nm".

To detect the presence of H_2O_2 and $O_2^{\bullet-}$ accumulation in plant tissues, we used staining techniques with 3,3-diaminobenzidine (DAB) for leaves and nitro blue tetrazolium (NBT) for roots, following the methodology outlined in Romero-Puertas et al. (2004). Stained leaves and roots were photographed using a digital microscope (Leica MZ-g5, Germany).

2.7. Antioxidant assay

Shoot and root rice samples were crushed in a sodium phosphate buffer with pH 7.4 before centrifuging at 15,000x*g* for 30 min. The resulting supernatant was separated and stored at 4 ℃ for subsequent antioxidant enzyme activity determination using a spectrophotometer (UV–VIS 190, Japan) at 25 ℃. Superoxide dismutase (SOD) activity was assessed at 560 nm by evaluating its capacity to prevent a 50% photochemical reduction of NBT chloride, according to Zhang et al. (2008). Peroxidase (POD) activity was measured at 470 nm, with variations associated with guaiacol adjusted using a constant ($\varepsilon = 26.6\ mm\ cm^{-1}$), according to Zhou and Leul (1999). Catalase (CAT) activity was measured according to Aebi (1984).

2.8. Electron microscopy analysis of mesophyll cells and stomatal aperture

Leaf samples (without veins) were randomly collected and kept overnight in 2.5% glutaraldehyde before washing three times for 15 min with 0.1 M phosphate buffer at pH 7.0. Post-fixation of samples was performed using 1% OsO_4 for 2 h before washing three times for 15 min with 0.1 M phosphate buffer (pH 7.0). Next, the samples were dehydrated with a graded series of ethanol (30%, 50%, 70%, 80%, 90%, and 100%, respectively). "For TEM analysis, the samples were first immersed in 90% acetone for 15 min, followed by a subsequent immersion in 95% acetone for 15 min. Two rounds of dehydration followed before repeating this process using absolute acetone, with each wash taking 20 min. The samples were then fixed in Spurr's medium and divided into ultrathin slices using a LEICA EM UC7 ultratome, which were placed on copper grids for examination using a TEM (Hitachi Model H-7650).

For SEM analysis, the samples were subjected to two washes with absolute ethanol, each lasting 20 min, and then coated with a layer of gold-palladium using a Hitachi Model E-1010 ion sputter for 5 min. Finally, the samples were observed under an SEM (Hitachi Model SU-8010).

2.9. Measurement of gene expression analysis

Rice seedling roots were collected to measure expression levels of genes related to Cd transport and antioxidant enzyme activities (Table S1). "Total RNA was extracted using a TRIeasyTM RNA extraction kit (Yeasen Biotechnology Co., Ltd. Shanghai, China) according to the manufacturer's instrucons. The quality and quantity of extracted RNA were assessed using % agarose gel and Nanodrop, respectively. Subsequently, 2.0 µg RNA samples were used as a template to synthesize cDNA using a Hifair® III 1st Strand cDNA Synthesis SuperMix kit (Yeasen Biotechnology Co., Ltd. Shanghai, China) with gDNA digester plus. The qRT-PCR was performed with the obtained cDNA using SYBER green (BioRad, USA) following the manufacturer's instructions. We used the $2^{-\Delta\Delta Ct}$ method to analyze the relative expression of genes (Livak and Schmittgen, 2001). Each PCR reaction (20.0 µL) contained 1.0 µL cDNA template, 2.5 µL of 10 × buffer, 1.0 µL of each forward and reverse primer (10 µmol/L), 10.0 µL of 2 × SYBR Green Mix, and ddH2O for a final volume of 20.0 µL. The PCR reaction had the following cycling conditions: 95 °C for 3 min, 30 cycles of 95 °C for 15 s, 58 °C for 20 s, and 72 °C for 20 s. The internal reference for this analysis was the rice actin gene (*OsActin*). The specific gene primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/) (Table S1)". Each sample had three independent replicates.

2.10. Statistical analysis

The mean values and standard error of measured data were calculated using three independent replications. Statistics 8.1, a statistical software program, was used to analyze the data in a one-way analysis of variance (ANOVA). Bar graphs illustrating each parameter's mean and SE were generated using Graph Pad Prism (version 8.0.2). Significant differences between treatments were determined using an LSD test at a 95% probability level.

3. Results

3.1. Characterization of SiO NPs

Silicon dioxide nanoparticles (SiO NPs) were characterized using SEM and TEM, revealing predominantly spherical morphology with dimensions ranging from 10 to 25 nm (Fig. 1A, B). However, SiO NPs showed poor dispersion and tended to cluster. The energy-dispersive X-ray spectroscopy (EDS) analysis revealed the elemental composition of SiO NPs, with oxygen (O) being the predominant element (59.96%), followed by silicon (39.70%), phosphorus (0.28%), iron (0.13%), and potassium (0.06%) (Fig. 1C).

3.2. Silicon enhanced rice plant growth

Cd stress alone significantly reduced shoot length (SL), root length (RL), shoot dry weight (SDW), and root dry weight (RDW) in rice seedlings by 33%, 42%, 37%, and 51%, respectively, compared to the control (Fig. 2A–D). However, SiO NPs supplementation, alone or combined with Cd stress, significantly enhanced these growth parameters, partially alleviating Cd-induced toxicity. The SiONP50 and SiONP100 treatments under Cd stress increased SL by 12% and 27%, RL by 17% and 35%, SDW by 14% and 30%, and RDW by 22% and 44%, respectively, compared to plants treated with Cd stress alone (Fig. 2A–D).

3.3. Silicon counteracts Cd-induced changes in gas exchange parameters and chlorophyll

Cd stress decreased the performance of gas exchange parameters, with 37%, 22%, 44%, and 52% reductions in net photosynthesis (Pn), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci), respectively. The SiONP50 and SiONP100 treatments combined with Cd stress increased Pn by 21% and 35%, E by 10% and 19%, gs by 22% and 32%, and Ci by 34% and 41%, respectively, compared to plants treated with Cd alone (Table 1). Cd stress alone also suppressed Chla by 64%, Chlb by 32%, total Chl (Chla+b) by 55%, carotenoids by 36%, SPAD values by 38%, and fluorescence by 49%
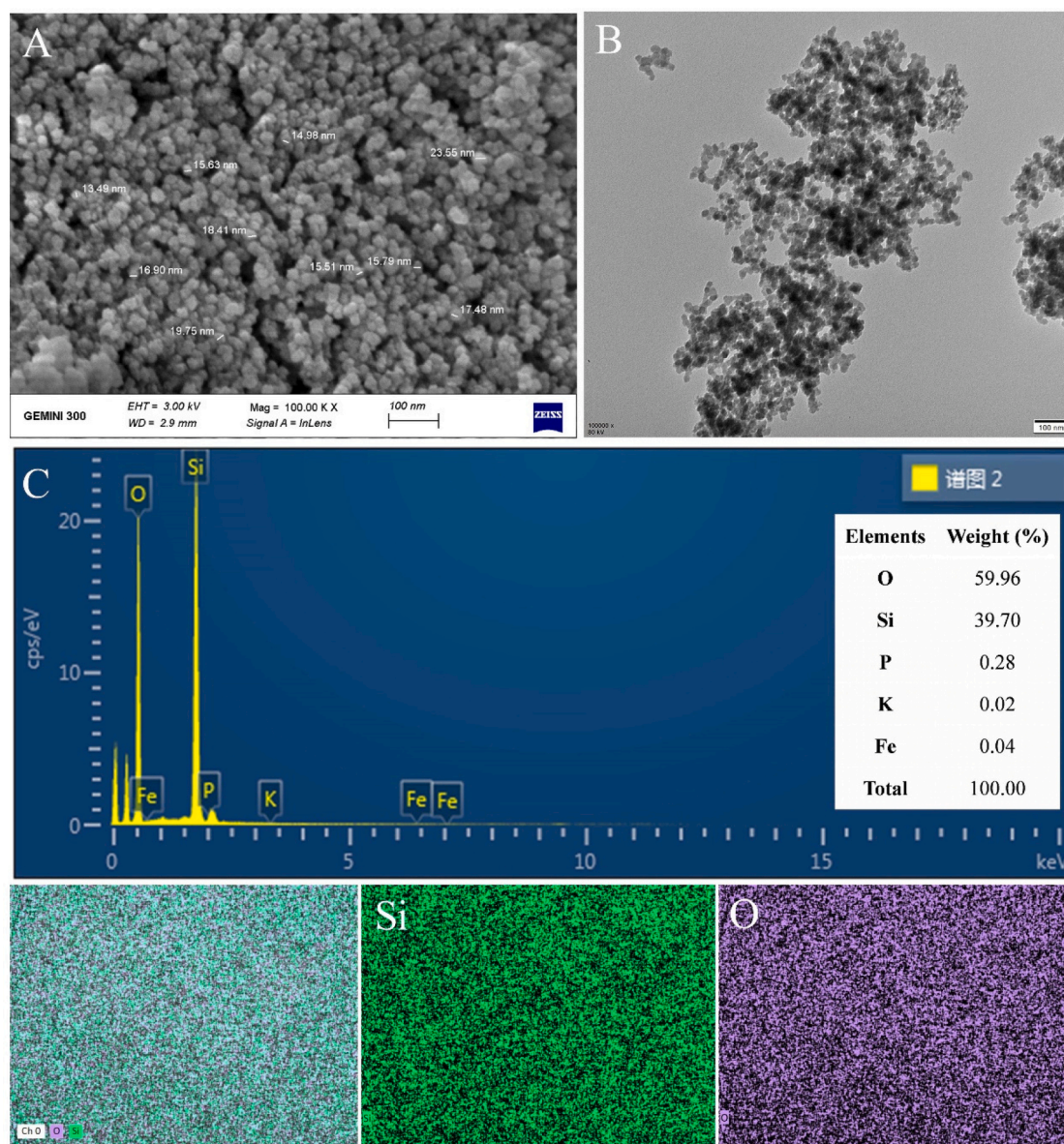

Fig. 1. Characterization of SiO NPs. (A) Scanning electron microscopy-SEM (Scale bar = 500 nm); (B) Transmission electron microscopy-TEM (Scale bar = 100 nm); (C) Energy-dispersive X-ray analysis (EDAX).
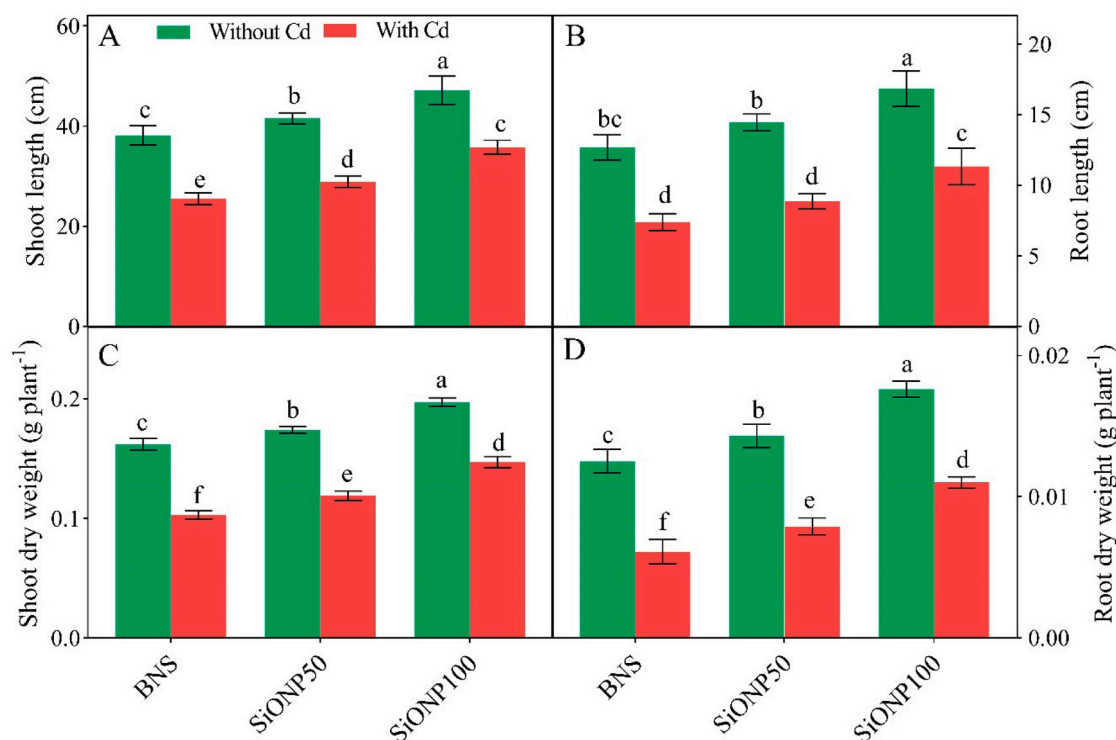
Fig. 2. Effects of SiO NPs on plant growth in rice seedlings under cadmium stress. (A,B) Length of shoots and roots; (C,D) Dry weight of shoot and roots. Vertical bars represent the mean $\pm$SD of three independent replicates. Different letters above error bars indicate the significant difference between treatments at $p \leq 0.05$. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 μM L^{-1}); **Cd+ SiONP50** (20 μM L^{-1} + 50 mg L^{-1}) and **Cd+ SiONP100** (20 μM L^{-1} + 100 mg L^{-1}).

Table 1
Effects of Si-NPs applications on chlorophyll pigments and gas exchange parameters in leaves of rice seedlings under Cd stress.

Treatments / variables	Chl a	Chl b	Chl a+b	Car		Pn	E	gs	Ci
			mg g^{-1} FW			μmol m^{-2} s^{-1}	mmol H$_2$O m^{-2} s^{-1}	mmol CO$_2$ m^{-2} s^{-1}	μmol mol^{-1}
BNS	5.29[b]	2.24[c]	7.53[c]	0.67[c]		14.07[b]	4.42[b]	430.44[a]	187.65[a]
Cd	1.91[e]	1.51[d]	3.42[e]	0.43[d]		8.80[d]	3.44[d]	269.31[d]	89.55[c]
SiON50	5.68[b]	2.57[b]	8.25[b]	0.81[b]		14.66[ab]	4.53[b]	439.28[a]	188.90[a]
Cd+SiONP50	3.23[d]	2.11[c]	5.34[d]	0.64[c]		11.16[c]	3.84[cd]	306.76[c]	142.19[b]
SiONP100	6.89[a]	3.47[a]	10.36[a]	0.97[a]		16.03[a]	5.20[a]	444.08[a]	19133[a]
Cd+SiONP100	4.67[c]	2.53[b]	7.20[c]	0.85[b]		13.49[b]	4.25[bc]	352.78[b]	157.32[b]

Note: Each value represents mean of three replicates ± SD (n = 3). Different letters above values indicate the significant difference between treatments at p ≤ 0.05. **Chl a**– chlorophyll a; **Chl b**: chlorophyll b; **Chl a+b**: total chlorophyll contents; **Car**: carotenoids; **Pn**: Net photosynthetic rate; **E**: transpiration rate; **gs**: stomatal conductance; **Ci**: intercellular CO$_2$ concentrations. Treatments: **BNS**-Control; **Cd** (20 μM); **SiONP50** (50 mg); **SiONP100** (100 mg); **Cd+ SiONP50** (20 μM + 50 mg) and **Cd+ SiONP100** (20 μM + 100 mg).

compared to the control group without Cd stress. Conversely, the maximum increase was recorded in SiONP100 treatment Chla by 59%, Chlb by 40%, Total Chl by 53%, and carotenoids (by 49%) under Cd stress (Table 1). Additionally, the SiONP100 treatment combined with Cd stress enhanced SPAD and Fv/m values by 31% and 46%, respectively, compared to Cd stress alone (Fig. 3A, B). Furthermore, visual evidence from false-color leaf images revealed a color shift from purple to light blue/green due to decreased Fv/m ratios under Cd stress. The SiONP50 and SiONP100 treatments combined with Cd stress significantly restored leaf colors (Fig. 3C).

3.4. Silicon improves nutrient profiling and discourages Cd uptake

Cd stress alone significantly decreased essential nutrient levels in rice seedling shoots and roots, including Mg by 40% and 48%, Ca by 49% and 58%, K by 36% and 26%, and Si by 31% and 34%, respectively, compared to the control (Table 2). The SiONP100 treatment combined

with Cd stress restored nutrient uptake, with Mg, Ca, and K reaching their control levels. This treatment also decreased Cd content by 28% in shoots and 48% in roots and increased Si content by 82% in shoots and 93% in roots compared to plants treated with Cd alone. Furthermore, the SiONP100 treatment combined with Cd stress significantly enhanced Mg and Ca concentrations by 44% and 37% in shoots and 41% and 49% in roots compared to Cd stress alone. Notably, rice seedlings in the SiONP50 and SiONP100 treatments alone had no Cd detected in shoots or roots.

3.5. Silicon dissuades Cd-induced MDA and oxidative burst (H$_2$O$_2$)

Cd stress increased MDA contents by 64% and 70% and H$_2$O$_2$ production by 60% and 68% in shoots and roots, respectively, relative to the control. The SiONP50 and SiONP100 treatments combined with Cd stress decreased MDA contents by 21% and 43% in roots and 26% and 48% in shoots, respectively, compared to Cd stress alone. However,
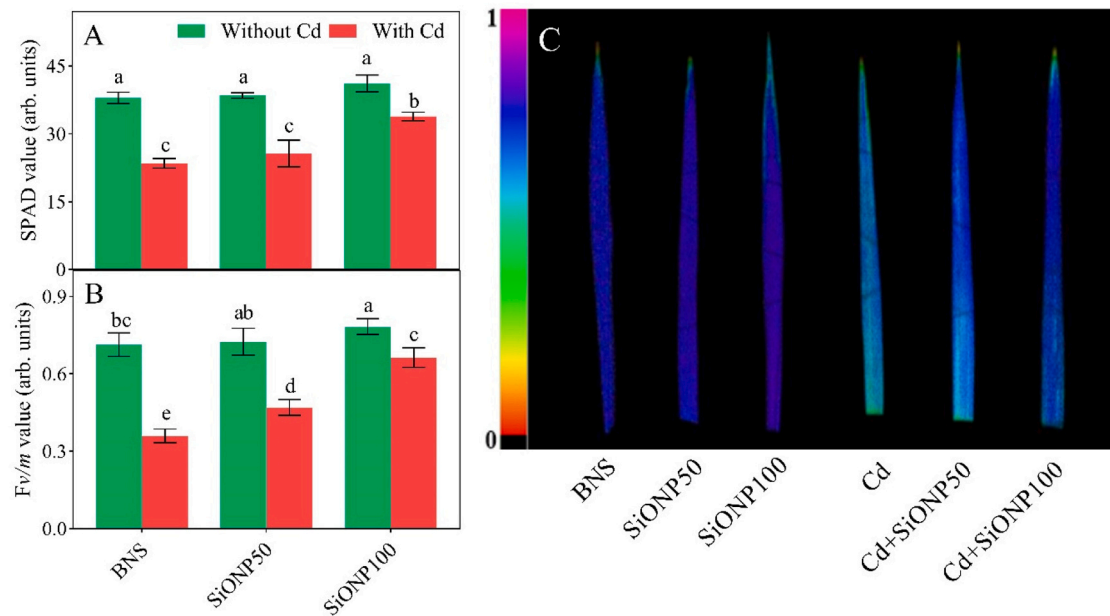
Fig. 3. Effects of SiO NPs on, chlorophyll contents and fluorescence in seedlings under cadmium stress. (A) SPAD value; (B) Fv/m value; (C) False color images of Fv/m. Vertical bars represent the mean $\pm$SD of three independent replicates. Different letters above error bars indicate the significant difference between treatments at $p \leq 0.05$. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 μM L^{-1}); **Cd+ SiONP50** (20 μM L^{-1} + 50 mg L^{-1}) and **Cd+ SiONP100** (20 μM L^{-1} + 100 mg L^{-1}).

Table 2

Effects of SiONPs application on accumulation of microelement concentrations of rice seedlings under Cadmium (Cd) stress.

Tissues	Treatments / variables	Magnesium (Mg)	Calcium (Ca)	Potassium (K)	Silicon (Si)	Cadmium (Cd)
						mg g^{-1} DW
Shoots	**BNS**	5.94[c]	11.48[ab]	35.20[b]	0.59[e]	0.00[d]
	Cd	3.64[e]	5.81[d]	22.40[d]	0.37[f]	2.58[a]
	SiONP50	6.82[b]	12.00[ab]	36.74[a]	2.56[c]	0.00[d]
	Cd+SiONP50	5.32[d]	7.86[cd]	26.45[c]	1.92[d]	1.72[b]
	SiONP100	8.84[a]	12.65[a]	34.90[ab]	4.30[a]	0.00[d]
	Cd+SiONP100	6.47[b]	9.92[bc]	27.78[c]	2.70[b]	0.94[c]
Roots	**BNS**	14.30[c]	17.56[a]	59.19[bc]	0.80[c]	0.00[d]
	Cd	7.47[f]	6.92[c]	43.83[e]	0.53[d]	7.90[a]
	SiONP50	16.17[b]	17.95[a]	61.46[ab]	4.83[b]	0.00[d]
	Cd+SiONP50	10.18[e]	11.43[b]	53.80[d]	3.56[c]	5.95[b]
	SiONP100	19.41[a]	18.11[a]	65.36[a]	7.24[a]	0.00[d]
	Cd+SiONP100	11.92[d]	14.06[b]	55.44[cd]	4.35[bc]	4.88[c]

Note: Each value represents mean of three replicates $\pm$ SD (n = 3). Different letters above values indicate the significant difference between treatments at p $\leq$ 0.05. Treatments: **BNS**-Control; **Cd** (20 μM); **SiONP50** (50 mg); **SiONP100** (100 mg); **Cd+ SiONP50** (20 μM + 50 mg) and **Cd+ SiONP100** (20 μM + 100 mg).

H_2O_2 production in roots and shoots decreased by 19% and 16% in the SiONP50 treatment and 41% and 54% in the SiONP100 treatment, respectively (Fig. 3A–D). Histochemical staining (NBT and DAB) of leaves confirmed these findings; Cd-treated leaves had considerable dark brown and dark blue staining, while the SiO NPs treatments combined with Cd stress decreased the intensity of these stains, more so with SiONP100 than SiONP50, compared to Cd stress alone (Fig. 3E, F).

3.6. Silicon strengthens the antioxidant defense system

Cd stress significantly increased POD, SOD, and CAT activities by 56%, 44%, and 53% in shoots and 62%, 49%, and 65% in roots, respectively, relative to the control (Fig. 4). Antioxidant enzyme activities in the SiO NPs treatments did not significantly differ from Cd stress alone. The SiO NPs treatments combined with Cd stress significantly improved POD, SOD, and CAT activities, compared to Cd stress alone, particularly in the SiONP100 treatment, increasing upto 30%, 34%, and 40% in shoots, and 26%, 28%, and 31% in roots, respectively (Fig. 4).

3.7. Silicon rescues mesophyll cell damage and stomatal aperture

Electron microscopy revealed structural abnormalities in cell organelles within mesophyll cells under Cd stress, including chloroplasts, nucleoli, starch granules, grana thylakoids, and osmophilia plastoglobuli (Fig. 5A–F). Mesophyll cells exposed to Cd stress displayed aberrations in their nuclei, characterized by disrupted nucleoli, indistinct nuclear membranes, and disrupted grana thylakoids. The chloroplasts within these cells exhibited an absence of osmophilia plastoglobuli. SiO NPs supplementation completely reversed these aberrations. Stomatal closure occurred under Cd stress alone. The SiO NPs treatments alone had greater stomatal opening than the control, while the SiO NPs treatments combined with Cd stress slightly reduced stomatal aperture compared to Cd stress alone (Fig. 5G–L).
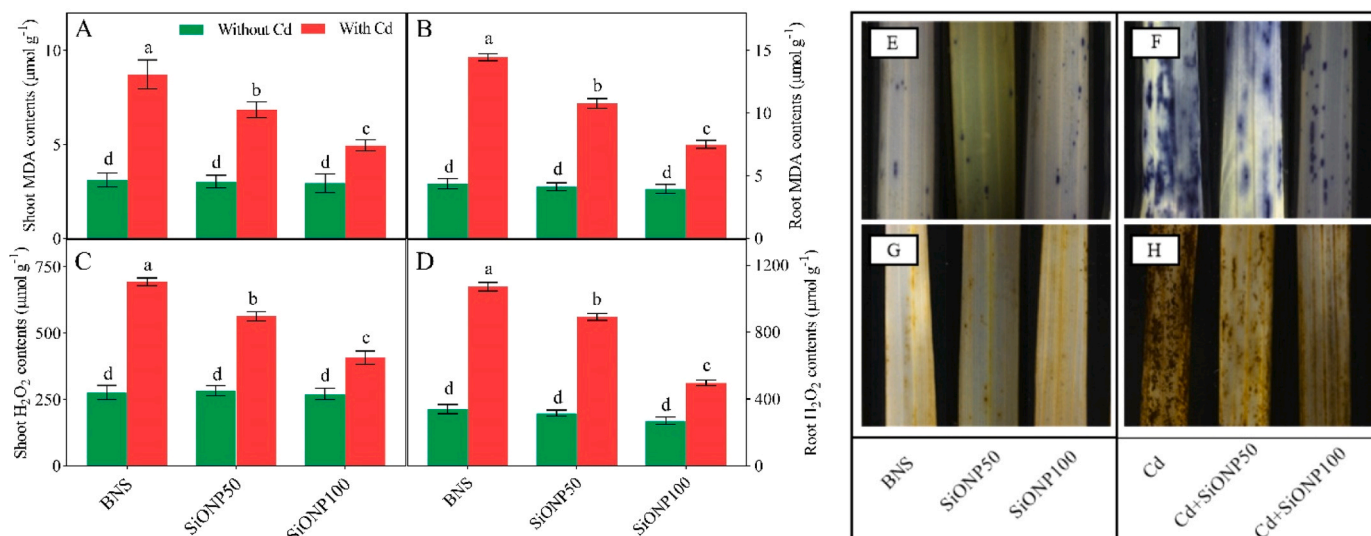
Fig. 4. Effects of SiO NPs on malondialdehyde and hydrogen peroxide in shoots and roots of rice seedlings and histochemical staining of leaves to identify the accumulation of superoxide (O^{2-}) through NBT: nitro blue tetrazolium and hydrogen peroxide (H_2O_2) through DAB: 3,3-diaminobenzidine under cadmium stress. (A, B) MDA contents; (C,D) H_2O_2 contents; (E,F) NBT; (G,H) DAB. Vertical bars represent the mean $\pm$SD of three independent replicates. Different letters above error bars indicate the significant difference between treatments at $p \leq 0.05$. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 μM L^{-1}); **Cd+ SiONP50** (20 μM L^{-1} + 50 mg L^{-1}) and **Cd+ SiONP100** (20 μM L^{-1} + 100 mg L^{-1}).

3.8. Silicon positively influenced the expression of cadmium transport and antioxidant-related genes

We analyzed the relative expression levels of two cadmium transporter and four antioxidant-related genes, i.e., *OsNramp5*, *OsHMA3*, *OsSOD-Cu/Zn*, *OsCATA*, *OsCATB*, and *OsAPX1* in rice seedlings roots exposed to SiO NPs and Cd stress (Fig. 6). The SiONP50 and SiONP100 alone treatments did not significantly affect the expression levels of *OsNramp5* or *OsHMA3* but expressed significantly compared to the BNS (control) treatment. The SiONP50 and SiONP100 treatments combined with Cd stress significantly decreased *OsNramp5* expression levels, while only the SiONP100 treatment decreased the expression of *OsHMA3*. The SiO NPs treatments alone did not significantly affect the expression levels of the four antioxidant-related genes compared to the control, whereas Cd stress alone substantially upregulated these genes. However, the SiONP50 and SiONP100 treatments combined with Cd stress enhanced these gene expression levels by 2.4- and 1.6-fold for *OsNramp5*, 1.1- and 1.4-fold for *OsHMA3*, 1.8- and 2.4-fold for *OsSOD-Cu/Zn*, 1.3- and 1.7-fold for *OsCATA*, 2.0- and 2.1-fold for *OsCATB*, and 1.7- and 2.3-fold for *OsAPX1*, respectively, compared to Cd stress alone, with no significant differences compared to the control (Fig. 7).

4. Discussion

Nanoparticles (NPs) have emerged as pivotal players in addressing environmental problems, such as mitigating HM stress in plants (Manzoor et al., 2022). Among these NPs, SiO NPs have garnered significant attention for their ability to alleviate Cd stress in crop plants. Cadmium contamination threatens agricultural ecosystems by impairing crop growth and contaminating food supplies, endangering global food security (Ahmed et al., 2023). Recent studies have demonstrated the effectiveness of exogenously applied NPs, including TiO, CuO, FeO, CaO, and ZnO NPs, in mitigating Cd toxicity in important food crops such as barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and rice (Noman et al., 2020). Among the different NPs, SiO NPs can stimulate various aspects of plant development, including root development, growth rates, and crop yields. Moreover, judicious SiO NPs application offers a potent solution by efficiently reducing Cd-induced oxidative stress and enhancing the overall resilience of stressed plants (Yadav et al., 2023).

Growth inhibition is a prominent and easily observable symptom in plants exposed to HMs (Al-Huqail et al., 2017; Gong et al., 2019). Our study revealed that Cd stress restricted rice seedling growth, including shoot and root lengths and dry weights (Fig. 2), aligning with previous findings (You et al., 2021). Notably, SiO NPs exhibited efficacy in enhancing plant growth compared to Cd stress alone. Moreover, our study highlights the significance of assessing plant fitness, particularly under challenging environmental conditions, by examining photosynthetic pigments and gas exchange attributes. SiO NPs played a pivotal role in enhancing chlorophyll content (Chla, Chlb, Chla+b, carotenoids, and SPAD value) and gas exchange parameters (Pn, E, gs, and Ci) in rice leaves (Table 2). Similarly, TiO$_2$ NPs augmented Pn in soybean plants exposed to Cd toxicity (Ji et al., 2017). While Cd stress alone significantly reduced chlorophyll content in rice seedlings, as evidenced by decreased SPAD and Fv/m, SiO NPs supplementation to rice seedlings alleviated these detrimental effects (Fig. 3). Similarly, Nazir et al. (2022) and Fatemi et al. (2020), who reported adverse effects of Cd and Pb exposure on photosynthetic activity in barley and coriander plants, respectively, showcased the ameliorative potential of CaO NPs and SiO NPs in countering these adverse effects.

Elevated Cd concentrations adversely affect a plant's capacity to uptake and transport essential nutrients, disrupting mineral metabolism and leading to nutrient deficiency (Luyckx et al., 2021). Cadmium competes with vital mineral nutrient ions for the same transport mechanisms, resulting in an inadequate supply of nutrients crucial for optimal plant growth and development (Rizwan et al., 2019; Rahman et al., 2021). Our study revealed that SiO NPs played a pivotal role in enhancing the acquisition and utilization of essential plant nutrients (P, K, and Mg) while substantially decreasing Cd accumulation (Table 2). This facet is likely a key factor behind Si's ability to promote plant growth even under Cd-induced toxicity. Previous studies support our findings, emphasizing the role of exogenously applied SiO NPs in facilitating the uptake of essential nutrients by plant roots, including critical mineral elements like Zn, Fe, Mn, Ca, Mg, P, and K (Koleva et al., 2022; Hou et al., 2023). Consequently, SiO NPs supplementation counteracts the nutrient deficiency triggered by Cd, fostering overall plant health.

Another significant factor in ameliorating Cd stress in rice crops is the capacity of SiO NPs to reduce oxidative stress by enhancing antioxidative capabilities within plant tissues. Cd stress in plants initiates ROS accumulation, including MDA and H$_2$O$_2$, ultimately leading to
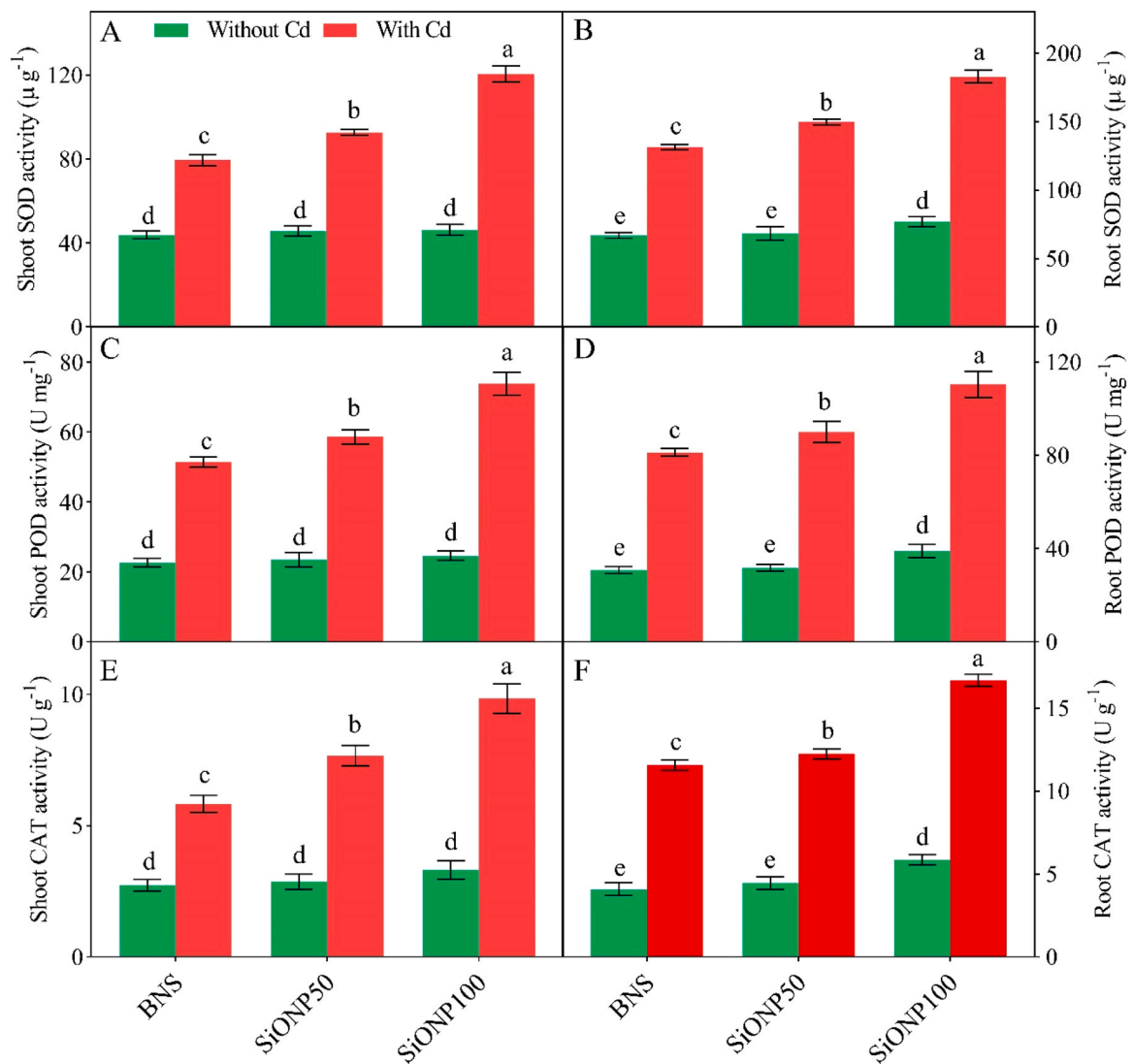
Fig. 5. Effects of SiO NPs on antioxidant enzyme activities in shoots and roots of rice seedlings under cadmium stress. (A,B) superoxide dismutase (SOD); (C,D) peroxidase (POD); (E,F) catalase (CAT). Vertical bars represent the mean $\pm$SD of three independent replicates. Different letters above error bars indicate the significant difference between treatments at $p \leq 0.05$. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 µM L^{-1}); **Cd+ SiONP50** (20 µM L^{-1} + 50 mg L^{-1}) and **Cd+ SiONP100** (20 µM L^{-1} + 100 mg L^{-1}).
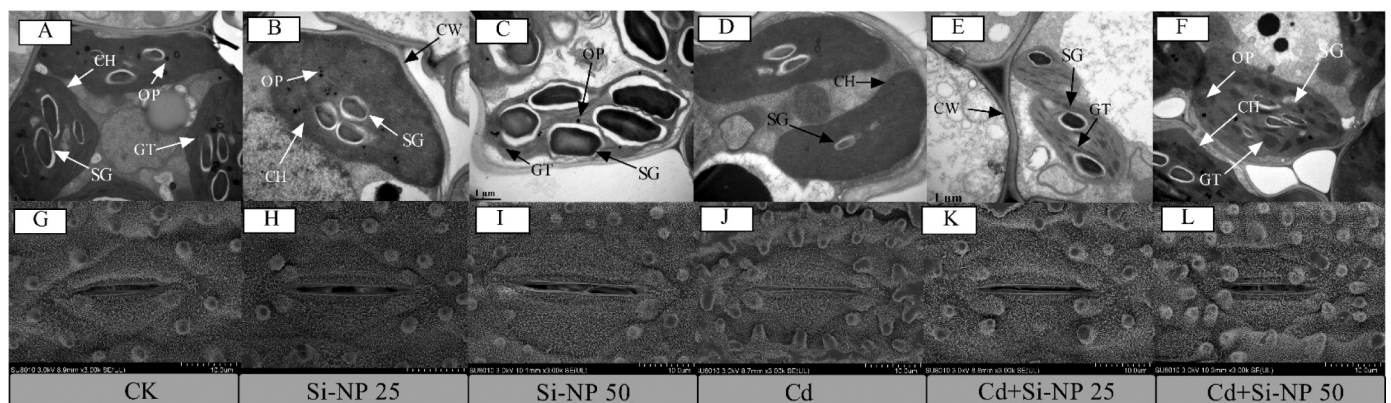
Fig. 6. Ultrastructural changes of mesophyll cells and opening of stomatal apperture. The observation of ultra-structure of mesophyll cells of leaves from plants treated alone and in combination with SiO NPs and Cadmium. **(A-F)** TEM view of mesophyll cells (bar = 0.5 µm); (G-L) SEM view of stomatal aperture (bar = 1 µm). CW-cell wall; CH-chloroplast; M-mitochondria; N-nucleus; Nue-nucleolus; GT-grana thylakoids; SG-starch granules and OP-osmophilic plastoglobuli. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 µM L^{-1}); **Cd+ SiONP50** (20 µM L^{-1} + 50 mg L^{-1}) and **Cd+ SiONP100** (20 µM L^{-1} + 100 mg L^{-1}).
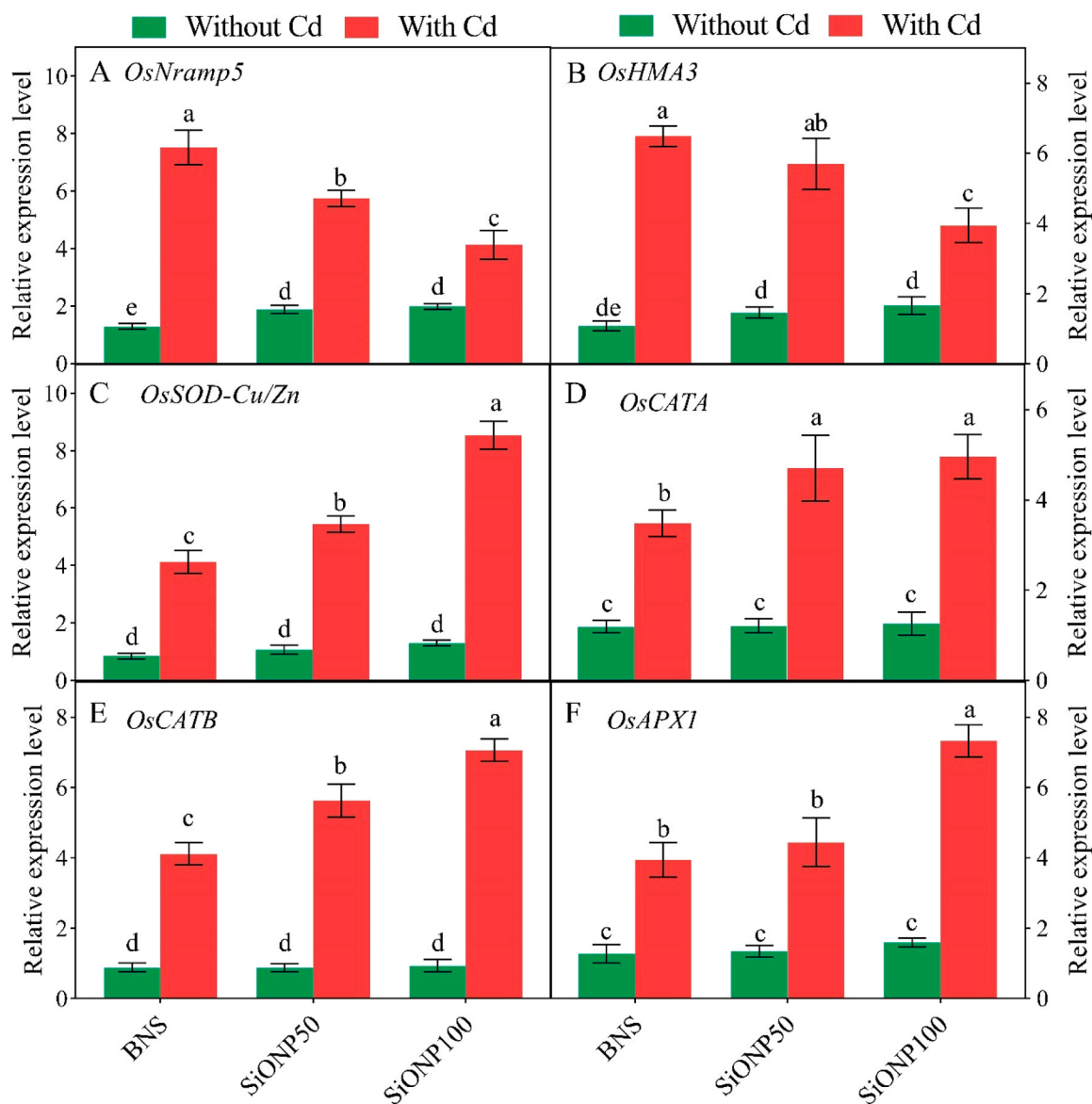
Fig. 7. Effects of SiO-NPs application on expression of cadmium transporter and antioxidant related genes in roots of rice seedlings after 7 days of Cd exposure. Treatments: **BNS**-Control; **SiONP50** (50 mg L^{-1}); **SiONP100** (100 mg L^{-1}); **Cd** (20 μM L^{-1}); **Cd**+ **SiONP50** (20 μM L^{-1} + 50 mg L^{-1}) and **Cd**+ **SiONP100** (20 μM L^{-1} + 100 mg L^{-1}).

oxidative stress. ROS production, in turn, activates antioxidant mechanisms such as SOD, APX, POD, and CAT to mitigate the detrimental consequences of lipid peroxidation and H$_2$O$_2$ buildup (Sachdev et al., 2021). Our research findings highlight the connection between Cd accumulation in rice and severe ultrastructural damage, alongside elevated MDA and H$_2$O$_2$ levels. Importantly, our study demonstrated that SiO NPs supplementation significantly reduced oxidative stress in rice through improved antioxidant activity (CAT, POD, and SOD) (Fig. 5) and reduced oxidative stress markers (MDA and H$_2$O$_2$) (Fig. 4). These results align with prior research, including Riaz et al. (2023), who showcased substantial improvements in SOD and POD activities, accompanied by a decline in oxidative stress marker levels, in Cd-stressed rice plants following SiO NPs application. Similarly, Brasili et al. (2020) reported a remarkable alleviation of Cr-induced stress, marked by increased SOD, APX, and POD activities, after applying Fe NPs to tomato plants exposed to elevated Cr concentrations.

The "ultrastructure of chloroplasts in rice plants has been investigated in multiple research programs addressing their exposure to Cd and/or treatment with SiO NPs (Manzoor et al., 2022). One study

reported that rice plants subjected to Cd stress exhibited notable ultrastructural alterations in chloroplasts, including thylakoid stacking disorders, reduced grana number and size, and changes in the chloroplast envelope (Liu et al., 2020). We found that rice plants under combined Cd stress and SiO NP supplementation exhibited significantly less damage to chloroplasts and cellular organelles than those exposed to Cd stress alone (Fig. 5). Chloroplasts in SiO NPs-treated plants without stress had a more organized grana thylakoid structure, more stacked grana, and more osmiophilic plastoglubuli, indicating that SiO NPs played a pivotal role in preserving the structural integrity of chloroplasts even in the presence of Cd stress. Moreover, stomata serve as vital conduits for gas and water exchange between plants and their surroundings, making them pivotal in understanding how environmental stresses affect crop development (Zhu et al., 2020). In our study, Cd stress substantially damaged stomatal apertures compared to other treatments (Fig. 5), which was directly linked to the toxic effects of Cd on rice plants. In another study, the distortion in guard cell morphology under As stress was attributed to the suppression of specific metabolic pathways responsible for maintaining turgor pressure in guard cells" (Liu et al.,

2020; Abeed and Dawood, 2020).

Beyond its role in forming physical barriers within cell walls, Si also influences the expression of genes associated with Cd uptake and transport and defense-related antioxidant activities (Alshegaihi et al., 2023; Hou et al., 2023). In our study, the expression of Cd transporter genes (*OsNramp5* and *OsHMA3*) significantly decreased in Cd-stressed rice seedlings after the exogenous application of SiO NPs, while the expression of antioxidant-related genes (*OsSOD-Cu/Zn*, *OsCATA*, *OsCATB*, and *OsAPX1*) significantly increased (Fig. 7), consistent with the findings of Cui et al. (2017). Consequently, SiO NPs emerge as promising agrichemicals for promoting plant development and reducing tissue Cd concentration in rice, with the proposed mechanism illustrated in Fig. 8. Furthermore, SiO NPs supplementation has the potential to minimize Cd accumulation in rice. Specifically, an optimum dose of 100 mg L^{-1} SiO NPs is recommended for rice seedlings exposed to Cd toxicity to decrease Cd uptake and alleviate phytotoxicity during early growth. This beneficial effect is attributed to the modulation of the antioxidant defense system, efficient ROS scavenging, and restricted Cd accumulation in plant tissues.

5. Conclusion

This study highlighted the significant role of SiO NPs in enhancing the growth and productivity of rice plants cultivated in a Cd-contaminated environment. The deleterious effects of Cd toxicity stem from ROS generationultimately impairing plant growth with reduced biomass and imbalacing the nutrients uptake which resulted in low production. SiO NPs act as ROS scavengers, mitigating the oxidative stress imposed by Cd on rice plants. Our study provided compelling evidence that SiO NPs, particularly at 50 and 100 mg L^{-1}, ameliorated Cd toxicity in rice plants in a dose-dependent manner through ROS suppression and enhancednutrient (Mg, Ca, K, and Si) uptake, photosynthesis and antioxidant enzyme activities. Furthermore, we observed distinct morphological changes in stomatal aperture and mesophyll cell structure, including alterations in starch granules, grana thylakoids, and osmophilic plastoglobuli. The relative expression of Cd transporter and antioxidant-responsive genes supported the genetic basis for these observed phenotypic variations. In summary, SiO NP supplementation holds promise in conferring Cd stress tolerance in rice plants by preserving vital physiological functions. This research offers valuable insights into the complex interactions between Cd, Si, and various other elements and the adaptive responses of crop genotypes, paving the way for sustainable strategies to mitigate heavy metal contamination in agriculture.

CRediT authorship contribution statement

S.J., and M.M.N.: conceived, designed and performed the experiment, and wrote the original draft; M.A.U.A., and A.A.A.: investigation, funding, formal data analysis; B.A., F.Z., N.O., M.A.E., and H.A.M.: wrote, reviewed, and edited the manuscript; B.A, R.N.Q., and J.W.H.Y.: wrote, reviewed, and edited the manuscript; X.J.: supervised the study, and wrote, reviewed, and edited the manuscript.

Declaration of Competing Interest

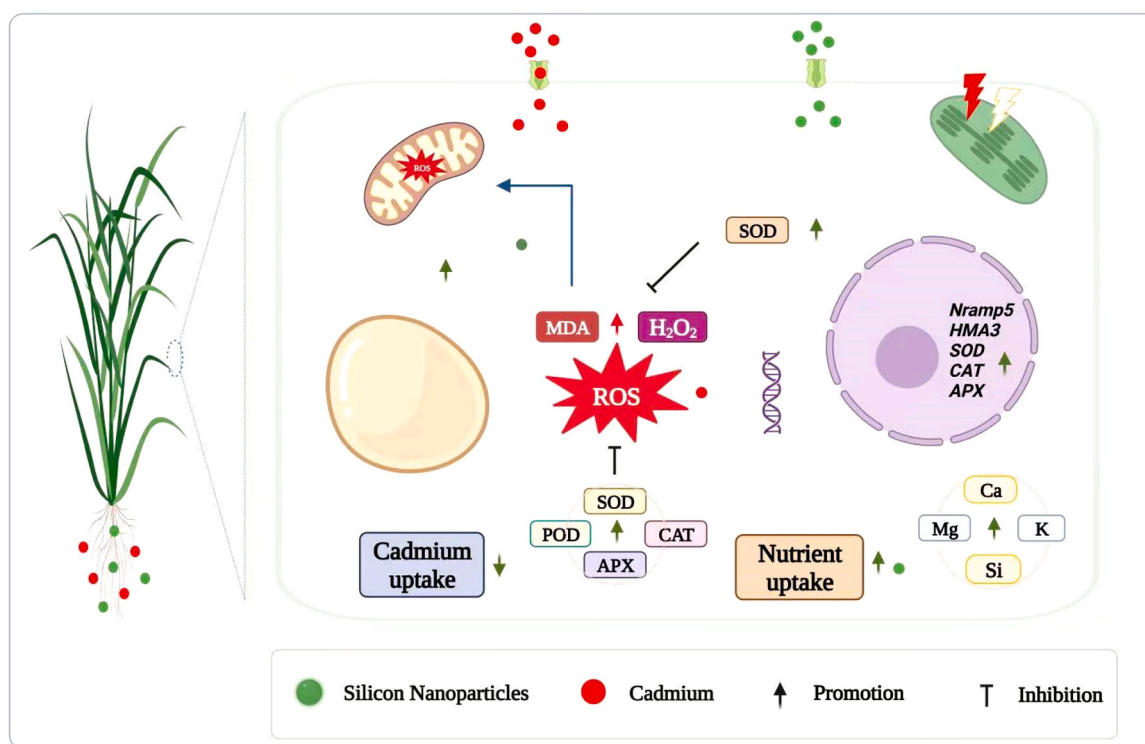The authors declare that they have no known competing financial

Fig. 8. Working model showed mitigation mechanism of SiONPs supplementation to reduce Cd-induced toxicity in rice seedlings. Initially, SiONPs after entering plant body, activate antioxidant enzymes (i.e., SOD, POD, and CAT), which in turn help rice seedlings to manage metal-induced oxidative stress. Further, SiONPs application supports plant growth by improving Zn^{2+} ions homeostasis, photosynthesis positively altering the gene expression levels responsive to Cd transport and antioxidant activities.

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.

Acknowledgment

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115699.

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