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Exogenous salicylic acid reduces cadmium content in spinach (*Spinacia oleracea* L.) shoots under cadmium stress

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Abstract

Background Consumption of leafy vegetables is a primary route of cadmium (Cd) exposure in the human body. Salicylic acid (SA) is a major stress signaling molecule that alleviates Cd toxicity in various plants. Our study aimed to investigate the effects of different SA concentrations on spinach growth, cadmium accumulation, and stress resistance physiology under cadmium stress (50 μmol/L).

Results Cd stress significantly markedly decreased spinach growth and biomass, reduced its photosynthetic efficiency, increased activities of antioxidative enzymes, and upregulated the relative expression of several genes involved in cadmium absorption and transport compared to the control. The exogenous application of SA mitigated the harmful effects of Cd in spinach. 0.8 and 1.6 mmol/L SA significantly increased spinach root length, plant height, and biomass and decreased the Cd content in shoots by 30.03 and 17.35% compared to the Cd-treated group. Moreover, SA alleviated the yellowing of leaves caused by Cd stress. Exogenous SA ameliorated Cd toxicity in spinach by reducing reactive oxygen species, malondialdehyde, proline, and soluble protein levels. Exogenous SA application reduced Cd absorption in spinach leaves by downregulating the expression of genes involved in Cd transport, such as *SoHMA4-like*, *SoNramp3.1-like*, *SoNramp6-like*, and *SoNramp7.2-like*. Principal component analysis and correlation analysis showed that exogenous SA application under Cd stress was correlated with plant Cd content, photosynthetic pigment content, and relative expression of Cd absorption and transportation-related genes.

Conclusions To summarize, these findings indicate that SA mitigates Cd toxicity in spinach by reversing the adverse effects of Cd stress on plant growth and reducing Cd accumulation in the shoots.

Keywords Spinach, Cadmium, Reactive oxygen species, Salicylic acid, Principal component analysis, Correlation analysis

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Introduction

The contamination of farmland with cadmium (Cd) is increasing due to intensified agricultural practices globally. Cadmium accumulation results in substantial adverse effects on animals and plants [1]. Elevated Cd levels affect crop yield and quality and can accumulate in the human body through the food chain, posing a risk to both agricultural soil ecology and health [2, 3]. Therefore, it is imperative to prevent Cd absorption in the human body through the consumption of contaminated crops or vegetables [4]. Numerous studies have demonstrated that high concentrations of Cd affect plant germination, inhibit plant growth, and promote yellowing of plant tissues and organs and leaf senescence, ultimately reducing plant yield [5–7]. Cd stress reduces the chlorophyll content and biomass of *Salix variegata* Franch by disrupting chloroplast structure [8]. Furthermore, prolonged exposure to Cd stress can result in flower abortion [9]. Cadmium is primarily absorbed and transported through transporters of essential plant nutrients such as zinc, iron, copper, and manganese into different cell compartments and plant tissues. These transport mechanisms are regulated by interactions with plant mineral nutrients and are modulated at transcriptional and post-translational levels [10]. Previous studies have indicated that exogenous plant hormones, such as salicylic acid, 5-aminolevulinic acid, jasmonic acid, tryptophan, and abscisic acid, activate plant defense mechanisms and reduce cadmium toxicity in plants [11–16].

Salicylic acid is an important defense-regulating hormone in plants [17]. The role of SA in local immunity, systemic acquired resistance, and responses to biotic and abiotic plant stress responses is associated with several protective properties against several plant diseases and stresses [18]. Ma et al. reported that exogenous SA enhanced salt stress tolerance in pepper by regulating ion absorption, gene expression, and post translational modifications [19]. However, previous studies have shown that SA-related signals are associated with plant adaptation to heavy-metal stress [20]. Exogenous SA treatment alleviated cadmium toxicity in potatoes by increasing their relative water content and SA content while reducing the contents of malondialdehyde and reactive oxygen species [11]. Moreover, SA reduced Cd accumulation in rice by regulating *OsHD-Zip*, and *OsLCD* expression [21]. At the same time, SA could enhance Cd resistance in rice by regulating the binding capacity of Cd to the cell wall [22]. However, there are few reports on the effects of SA on the transport and accumulation of Cd in vegetables under Cd stress.

Consumption of leafy vegetables is a primary route for Cd exposure in the body [23, 24]. Previous research demonstrated that leafy vegetables exhibited the highest heavy metal accumulation capacity, whereas melon vegetables had the lowest accumulation [25]. Liu et al. reported that edible leaves or stems of crops are markedly contaminated with Cd compared to seeds or fruits [26]. As a representative of leafy vegetables, Spinach is highly susceptible to Cd pollution compared to other crops, posing a risk to human health throughout the food chain [27]. Waheed et al. demonstrated that exposure to 50 μmol/L Cd stress significantly reduced the biomass, plant height, leaf area, and leaf length of spinach, consistent with our preliminary test results [28]. Therefore, we used 50 μmol/L Cd to induce Cd stress in this study. However, the precise physiological mechanisms of exogenous SA in reducing the detrimental effects of Cd on spinach growth and its accumulation in spinach have not been elucidated. Therefore, our study aimed to explore the effects of SA on spinach growth indicators, photosynthetic parameters, antioxidant enzyme activity, and cadmium content under cadmium stress conditions to provide a basis for safe vegetable production.

Materials and methods

Materials

The experimental material used in this study was the "Greenway TY771" spinach variety. Seeds were purchased from Chengdu Seed Station (Chengdu, China). The Hoagland nutrient solution was used as a nutrient source during spinach cultivation, and $CdCl_2 \cdot 2.5H_2O$ (analytical grade) was used as the Cd source. SA was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Experimental design

This study was conducted at the Chengdu Campus of Sichuan Agricultural University between August 2022 and December 2023. Uniform-sized healthy spinach seeds were washed thrice with ultrapure water, soaked with clean ultrapure water for 6 h, and evenly distributed in petri dishes containing wet filter paper. The petri dishes were then placed in a 24 ℃ artificial incubator for germination. Subsequently, the sprouted seeds were planted in a seedling tray containing perlite and vermiculite (v: v=1:1) and transferred to an artificial incubator. Half-strength Hoagland nutrient solution was added daily to the tray, and the incubator was set to 24/18 ℃ (day/night) and a 14/10 h (day/night) photoperiod under a 200 μmol/m^2/s light intensity. Seedlings exhibiting rapid and consistent growth at the four-true-leaf stage were selected and transplanted into 10×10 cm (depth × height) nutrient containers containing perlite. One plant was transplanted to each container, and the containers were transferred to a plastic dish (with a height of 8 cm) containing full-strength Hoagland's nutrient solution, which was replaced every 3 days.

Three days after the transplantation of the seedlings, the spinach leaves were sprayed with ultrapure water and

SA solutions at different concentrations (0.2 mmol/L, 0.4 mmol/L, 0.8 mmol/L, and 1.6 mmol/L). After 3 days of pretreatment, all treatment groups except the CK (control without Cd and SA spraying) were treated with a Hoagland nutrient solution containing 50 μmol/L Cd and different concentrations of an SA solution. The treatments were administered once every three days, for a total of three times. Different concentrations of the SA solution were sprayed four times (pretreatment once and treatment thrice after adding Cd) during the experiment. We ensured that at least 30% of the nutrient solution flowed out to prevent Cd accumulation in the nutrient bowl during nutrient solution replacement. The six treatments applied in this experiment were as follows: CK (the control without Cd and SA spraying), Cd (50 μmol/L Cd and without SA spraying), Cd+SA0.2 (50 μmol/L Cd+0.2 mmol/L SA), Cd+SA0.4 (50 μmol/L Cd+0.4 mmol/L SA), Cd+SA0.8 (50 μmol/L Cd+0.8 mmol/L SA) and Cd+SA1.6 (50 μmol/L Cd+1.6 mmol/L SA). The temperature was maintained at 24/18 ℃ (day/night), with a relative humidity of 75 – 80%, a light cycle of 14/10 h (day/night), and the strength of illumination in the artificial culture room was maintained at 300 μmol/m^2/s throughout the experiment. The pots were randomly rearranged regularly to reduce the impact of edge effects and effectively prevent and control pests and diseases.

Sample analysis

Spinach samples were collected 10 days after the final exogenous SA treatment, and the growth and morphological indicators of the entire plants were evaluated. Subsequently, fresh root and shoot samples were freeze-dried in liquid nitrogen and stored at -80 ℃ in an ultra-low temperature freezer to determine physiological and quality indicators. Shoot, and root samples were fixed in an oven at 105 ℃ for 15 min and then dried at 75 ℃ to a constant weight to determine Cd content.

Morphological observations and determination of plant growth and biomass

Ten days after the last spray application of SA, we performed a phenotypic assessment regarding the growth status and leaves of the spinach plants and recorded the observed phenotypic differences. The plant height and root length of spinach plants were measured to the nearest millimeter using a ruler. Subsequently, the spinach shoots and roots were washed with tap water and rinsed thrice with deionized water, followed by oven-drying at 105 ℃ for 15 min and 75 ℃ to constant weight for dry biomass determination.

Determination of photosynthetic pigment contents

The second and third functional leaves from the shoot tip (n=3) were harvested to determine the contents of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid) using the ethanol and acetone extraction methods described previously [28]. Briefly, the leaves were soaked in equal volumes of ethanol and acetone for 24 h until they were bleached. Then, the spectrophotometry values were measured in the ethanol and acetone solutions and used to calculate the photosynthetic pigment content.

Determination of photosynthetic parameters

The leaves used for the determination of photosynthetic pigment contents were used to determine the net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) using a LI-6400XT portable photosynthetic system (LI-COR Inc., Lincoln, NE). The photosynthetic parameters were manually set at 25 ℃, 1000 μmol/m^2/s light intensity, and a CO_2 concentration of 400 μmol/mol [28].

Determination of membrane peroxidation

The proline content in the plants was determined using the sulfosalicylic acid method, whereas the soluble protein and sugar contents were determined using the Coomassie brilliant blue G-250 method and anthrone-ethyl acetate methods, respectively. Malondialdehyde levels were determined using the thiobarbituric acid method. All assays were conducted according to the methods described by Tang et al. and Liang et al. [28, 29].

Determination of antioxidant enzyme activities and levels of reactive oxygen species

Antioxidant enzyme activity assays were conducted following previously described methods [29]. Superoxide dismutase (SOD) activity was evaluated using the nitro-blue tetrazolium method, peroxidase (POD) activity using the guaiacol method, and catalase (CAT) activity using the ultraviolet (UV) absorption method. The O_2^- and H_2O_2 contents were determined by the trace method using a reagent kit purchased from Beijing Solarbio Science &Technology Co., Ltd. (Beijing Solarbio Science &Technology Co., Ltd. Beijing, China).

Determination of cd content

Plant samples (0.5 g) were digested in a 4:1 nitric acid: perchloric acid solution (v: v) for 12 h to a clear solution, filtered and diluted to a volume of 50 mL. Subsequently, the Cd content was then determined using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA) [30]. The translocation factor (TF) was determined as the shoot Cd content divided by the root Cd content [31].

Table 1 Primers used for real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) validation

Gene	Gene ID	Forward primer (5′-3′)	Reverse Primer (5′-3′)
SoHMA3-like	LOC110778383	CAATGGCTAGCA GTTGCAGC	CTATAGTGCCA GCTTCGGTGT
SoHMA4-like	LOC110801776	GCATCAGAGAGG CTTCTGTCG	CCAATCCCCA TAAGGTGGA AGC
SoNramp3.1-like	LOC110798462	GGACCCTGAT GAAAATGAAG CAGA	TGCTTCCACC TCTATAACCC CATAT
SoNramp3.2-like	LOC110793365	CCACCAACCAAT TATTCGAGGATG	TGCTATGCTC ATCAGAAAC CCTG
SoNramp6-like	LOC110805077	ATGGCGACGTCA AATGAGCAG	AGACAAGGA ACCCAGGAC CCA
SoNramp7.2-like	LOC110801340	AGAAGGAATCGA GAAGGTCTGGA	AGTAGGAGCT TCATCAAAGT GTTTG
18 S		CCATAAACGATG CCGACCAG	AGCCTTGCGA CCATACTCCC

Determination of the relative expression of genes related to cd uptake and transport in spinach

Total RNA was extracted from all samples using an RNA prep pure plant kit (TIANGEN Biochemical Technology Co., Ltd, Beijing, China), according to the manufacturer's instructions. The RNA was reverse transcribed into cDNA using the TAKARA reverse transcription kit (TAKARA Bio Co., Ltd., Japan). All primers were synthesized by Shenggong Biotechnology Co., Ltd. (Shenggong Biotechnology Co., Ltd., Beijing, China), as shown in Table 1. Biomarker 2X SYBR Green Fast qPCR Mix fluorescent dyes were purchased from Yugong Biotechnology Co., Ltd (Yugong Biotechnology Co., Ltd., Lianyungang, China). The 18 S rRNA gene was used as an internal reference [32], and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of target genes.

Statistical analyses

Data were compiled and organized using Excel 2016 software (Microsoft Corp., Redmond, WA, USA), and statistical analyses were conducted using SPSS 25.0 statistical software (IBM, Armonk, NY, USA). Differences across the groups were determined by one-way ANOVA followed by Duncan's multiple range test at a $p<0.05$ significance level. Data are presented as the means of three biological replicates ± standard error (SE). Principal component and correlation analyses were performed to explore the relationship between various indicators. Figures were generated using Origin Pro 2021 software (Northampton, Massachusetts, USA).

Results

SA mitigates the impact of cd on plant biomass and growth

Cd stress significantly inhibited spinach growth compared with the control (Fig. 1A) and caused yellowing of leaf margins and accelerated leaf senescence (Fig. 1B). Spinach plants treated with Cd exhibited significantly reduced plant height, root length, shoot biomass, and root biomass (Fig. 1C, D, E, F). Application of 0.8 mmol/L SA alleviated the growth inhibition induced by

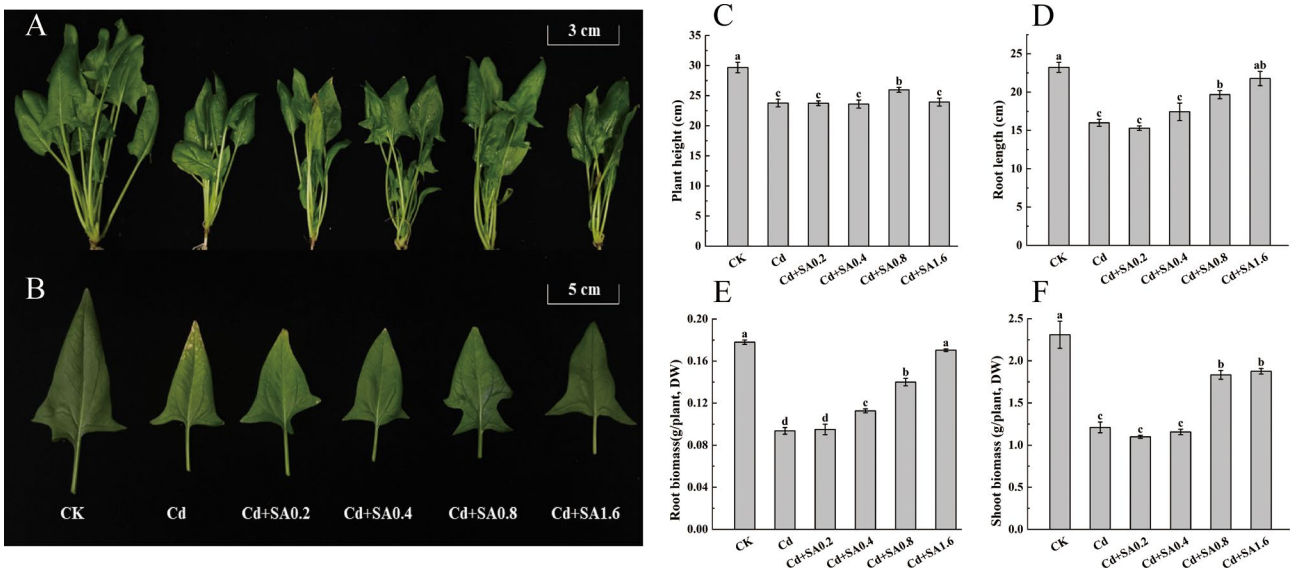

Fig. 1 Effects of Cd and SA on phenotype and growth of spinach plants under different treatment conditions. (**A**) Plant phenotype. (**B**) Leaf phenotype. (**C**) Plant height. (**D**) Root length. (**E**) Shoot biomass (DW). (**F**) Root biomass (DW). CK: the control without Cd and SA spraying; Cd: 50 μmol/L Cd and without SA spraying; Cd + SA0.2: Cd + 0.2 mmol/L SA; Cd + SA0.4: Cd + 0.4 mmol/L SA; Cd + SA0.8: Cd + 0.8 mmol/L SA; Cd + SA1.6: Cd + 1.6 mmol/L SA. Data are average values ± SE of 3 replicate samples. Values with different letters are significantly different ($p < 0.05$). Same as below

Cd on spinach and alleviated leaf senescence (Fig. 1A, B). Moreover, the addition of 0.8 mmol/L SA significantly increased spinach plant height by 9.26% compared to the group treated with Cd alone (Fig. 1C). Spinach root length and biomass proportionally increased with increased SA concentrations applied under Cd stress (Fig. 1D, E). Application of 1.6 mmol/L SA increased spinach's root length and root biomass by 36.04% and 81.85% compared to the group treated with Cd alone (Fig. 1D, F). These results indicate that exogenous SA can alleviate the growth inhibition of Cd on spinach by promoting its growth, abrogating leaf senescence, and increasing plant height, root length, shoot biomass, and root biomass.

Exogenously applied SA application increases photosynthetic parameters

Cd stress significantly reduced the Ci, Tr, Gs, and Pn parameters in spinach leaves by 17.80%, 38.01%, 45.03%, and 30.77%, respectively, compared to the CK group (Fig. 2). Ci, Tr, and Gs levels in spinach leaves under Cd stress conditions initially increased and then decreased with an increase in the concentration of applied SA (Fig. 2A, B, C). Application of 0.8 mmol/L SA increased Ci, Tr, and Gs in spinach leaves by 21.54%, 63.82%, and 95.43%, respectively, relative to the group only treated with Cd t (Fig. 2A, B, C). In addition, exogenous spraying of 0.8 mmol/L and 1.6 mmol/L SA under Cd stress increased the Pn of spinach leaves by 36.15% and 36.83%, respectively, compared to the group treated with Cd only (Fig. 2D). These results indicate that the exogenous application of SA could alleviate the damage caused by Cd stress to spinach by enhancing photosynthesis in leaves.

Exogenously applied SA increases the content of photosynthetic pigment constituents

Cd stress significantly reduced the contents of chlorophyll and carotenoid in spinach leaves relative to the CK group (Fig. 3). Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in spinach leaves initially increased and then decreased with an increase in the concentration of exogenously applied SA under Cd stress

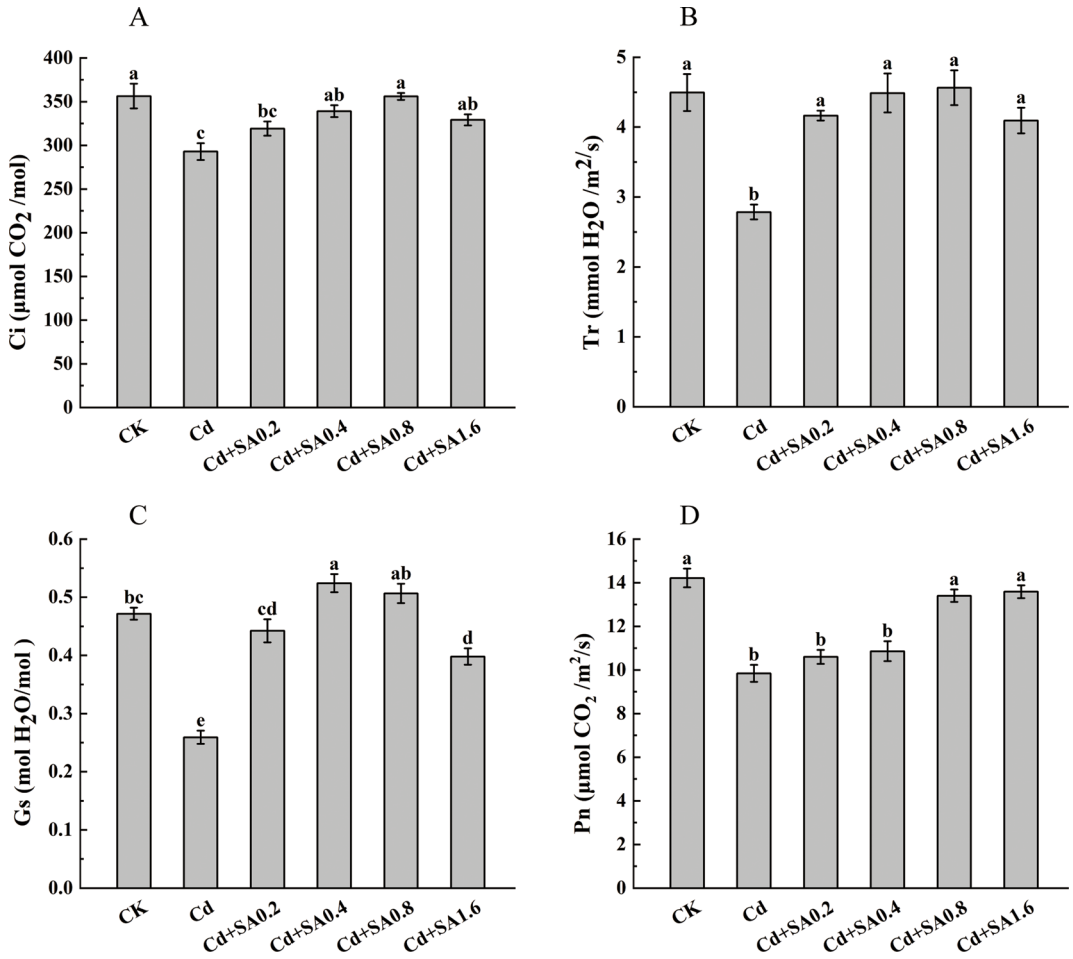

Fig. 2 Effects of Cd and SA on photosynthesis of spinach plants under different treatment conditions. (**A**) Ci, intercellular CO_2 concentration; (**B**) Tr, transpiration rate; (**C**) Gs, stomatal conductance; (**D**) Pn, net photosynthetic rate

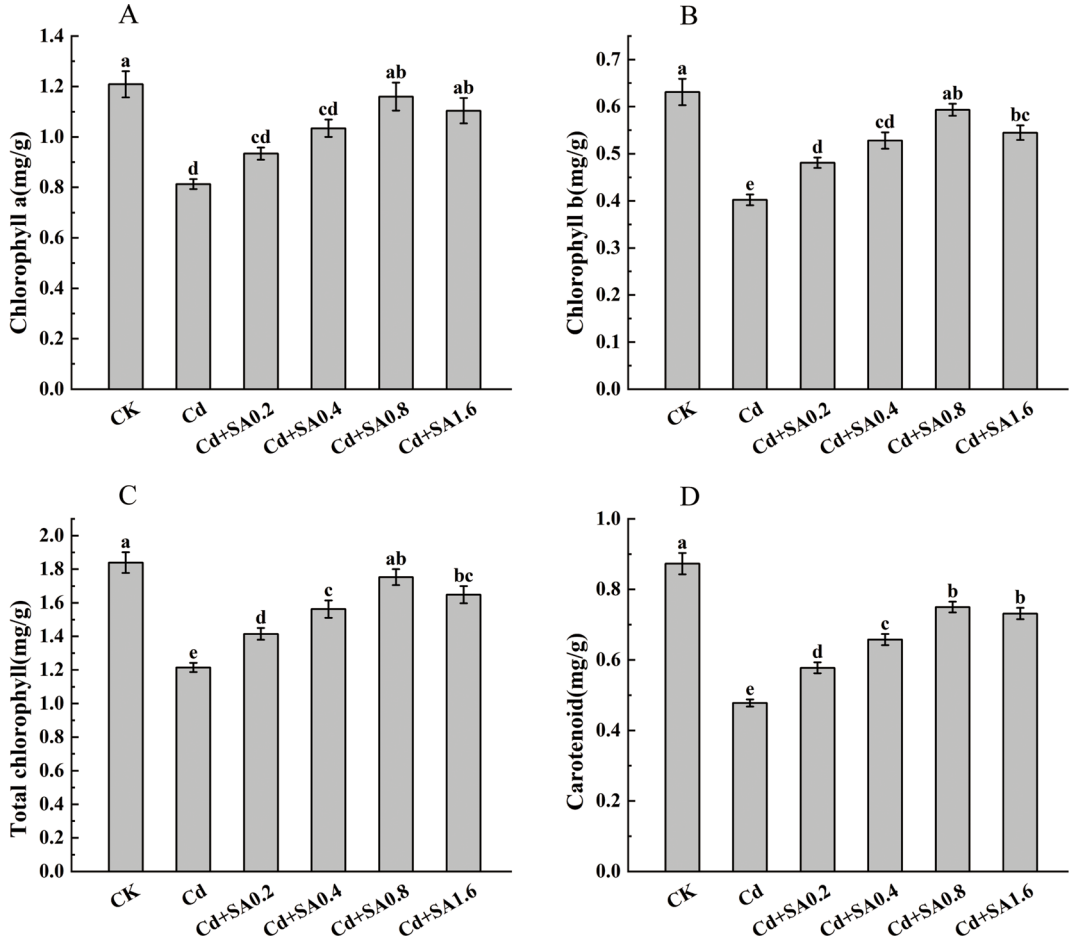

Fig. 3 Effects of Cd and SA on photosynthetic pigment content of spinach plants under different treatment conditions. (**A**) Chlorophyll a content; (**B**) Chlorophyll b content; (**C**) Total chlorophyll content; (**D**) Carotenoid content

(Fig. 3). Spray application of 0.8 mmol/L SA increased the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid in spinach leaves by 42.64%, 47.61%, 44.28%, and 56.97%, respectively, compared with the CK group (Fig. 3). These results indicated that the application of exogenous SA abrogated the Cd-mediated photosynthetic inhibition in spinach by increasing the content of photosynthetic pigments in spinach leaves, with 0.8 mmol/L SA identified as the optimal concentration.

Exogenously applied SA alleviates membrane lipid peroxidation and modulates the levels of osmoregulatory compounds

Plants treated with Cd alone exhibited a significant increase in the content of proline, malondialdehyde, soluble sugar, and soluble proteins by 52.26%, 34.28%, 26.56%, and 98.42%, respectively, compared with the CK group (Fig. 4). Malondialdehyde, soluble sugar, and soluble protein contents in the leaves of spinach subjected to Cd stress decreased gradually with an increase in the concentration of exogenously applied SA (Fig. 4B, C, D). Treatment of the plants with 1.6 mmol/L SA decreased the contents of proline, malondialdehyde, soluble sugars, and soluble proteins in spinach leaves by 23.49%, 20.70%, 37.19%, and 24.07%, respectively, compared to plants treated with Cd alone (Fig. 4). Therefore, exogenous SA could alleviate the toxicity of Cd to spinach by reducing the levels of osmoregulatory and lipid peroxidation compounds that accumulated under Cd stress.

Exogenously applied SA modulates the contents of reactive oxygen species

Cd stress markedly increased the levels of O_2^- and H_2O_2 in spinach by 48.68% and 40.29%, respectively, compared with the CK group (Fig. 5A, B). Increased exogenously applied SA concentration decreased the $O_2^{\cdot}$- content in plants under Cd stress. Treatment of spinach plants with 0.8 mmol/L SA and 1.6 mmol/L SA decreased the $O_2^{\cdot-}$ content by 18.58% and 24.93% compared to the group treated with Cd alone, respectively (Fig. 5A). Similarly, the H_2O_2 content decreased with an increase in SA concentration. Application of 0.4 mmol/L, 0.8 mmol/L, and

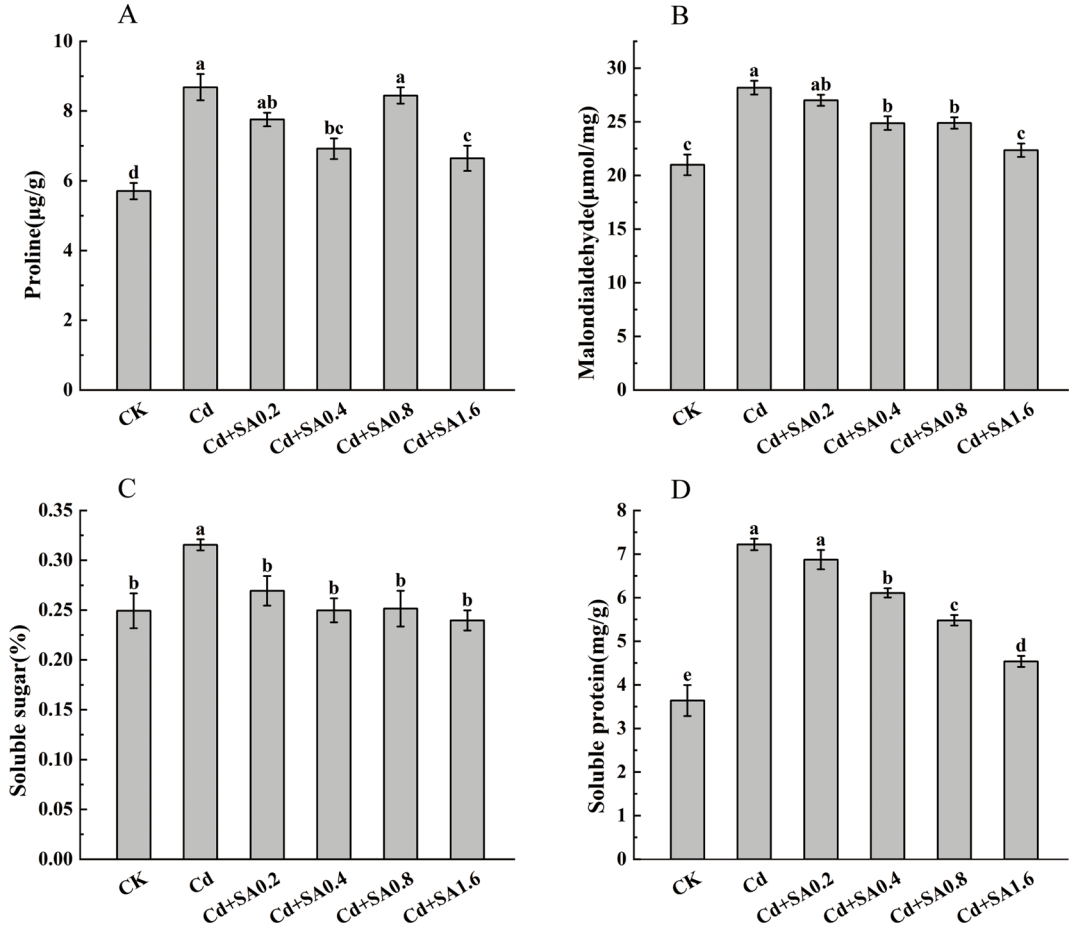

Fig. 4 Effects of Cd and SA on the osmotic adjustment substance of spinach plants under different treatment conditions. (**A**) Proline; (**B**) Malondialdehyde; (**C**) Soluble sugar; (**D**) Soluble protein

1.6 mmol/L SA decreased the H_2O_2 content by 13.15%, 16.41%, and 17.81% compared to the group only treated with Cd, respectively (Fig. 5B). These results indicate that SA could alleviate the oxidative damage caused by Cd in spinach leaves by reducing the levels of O_2^- and H_2O_2 under Cd stress conditions.

Exogenously applied SA modulated the activities of antioxidant enzymes to alleviate oxidative stress induced by Cd stress

Spinach leaves exhibited significantly increased POD, SOD, and CAT activities after treatment with Cd by 93.65%, 4.24%, and 39.93%, respectively, compared with the CK group (Fig. 5C, D, E). Notably, SA significantly reduced the CAT activity of spinach leaves subjected to Cd stress. The CAT activity was reduced by 22.60% and 15.56% after treatment with 0.8 mmol/L and 1.6 mmol/L SA, respectively, compared with the Cd-treated group (Fig. 5E). Moreover, the application of 0.4 mmol/L SA reduced the POD activity of spinach leaves under Cd stress by 3.44% compared with the Cd-treated group (Fig. 5C). SA enhanced the SOD activity of spinach under Cd stress, with 0.8 mmol/L and 1.6 mmol/L SA resulting in a 31.00% and 27.99% increase, respectively, compared with the group only treated with Cd (Fig. 5D). These results indicated that SA could decrease the oxidative damage to spinach caused by Cd by modulating the activities of antioxidant enzymes.

SA reduces cd accumulation in spinach shoots

Initially, the Cd content in shoots and the translocation factor of spinach treated with Cd decreased, but it subsequently increased in response to an increase in SA concentration (Fig. 6A, C). In contrast, no significant changes were observed in the Cd content of the root (Fig. 6B). Under Cd stress, the application of 0.4 mmol/L, 0.8 mmol/L, and 1.6 mmol/L SA resulted in a decrease in Cd content in spinach shoots by 10.77%, 30.03%, and 17.35%, respectively, compared to Cd treatment alone (Fig. 6A). Application of 0.8 and 1.6 mmol/L SA to spinach plants subjected to Cd stress decreased Cd translocation by 31.79% and 21.96%, respectively (Fig. 6C). These findings indicated that exogenous SA could reduce

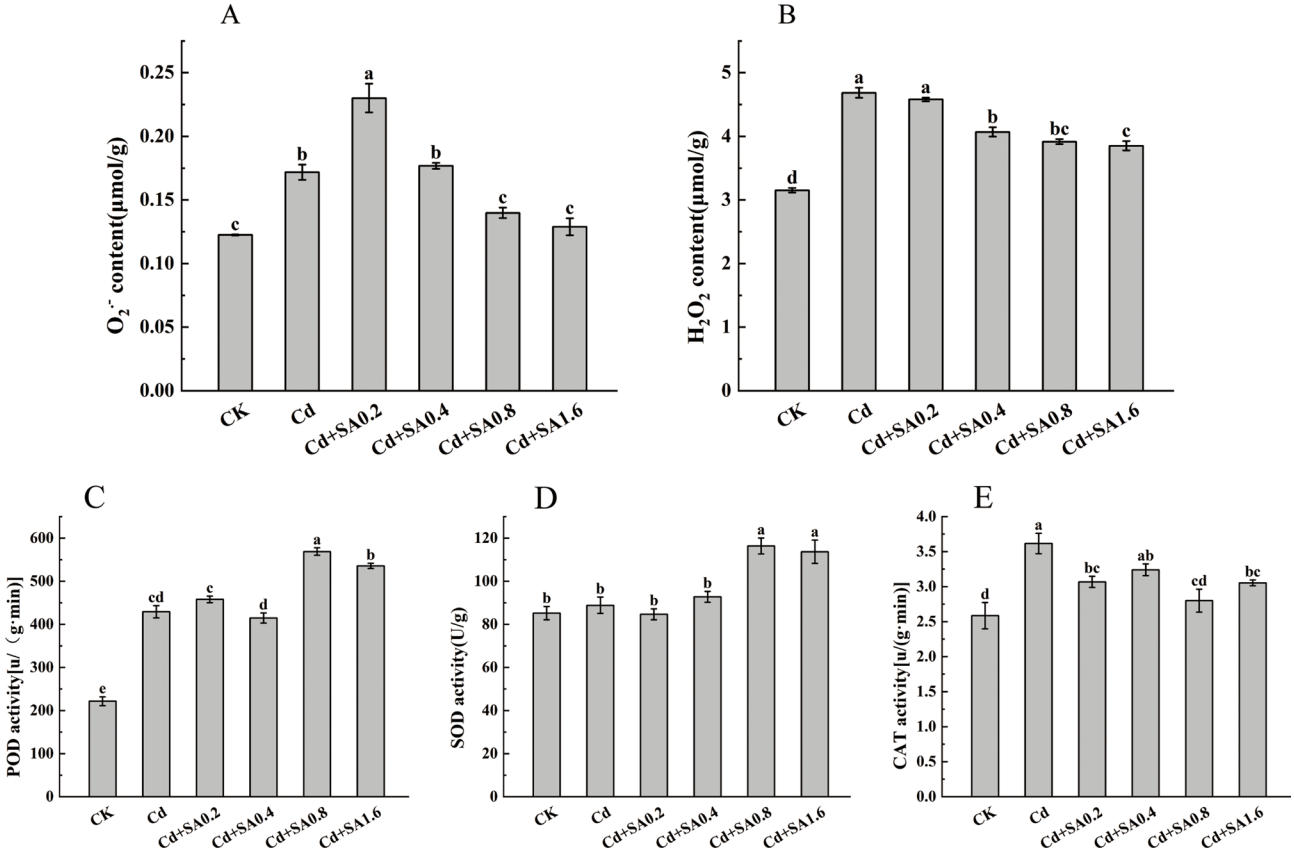

Fig. 5 Effects of Cd and SA on reactive oxygen content and activity of antioxidant enzymes in spinach plants under different treatment conditions. (**A**) $O_2^{\cdot-}$ content; (**B**)H_2O_2 content, (**C**) Peroxidase (POD) activity, (**D**) Superoxide dismutase (SOD) activity, (**E**) Catalase (CAT) activity

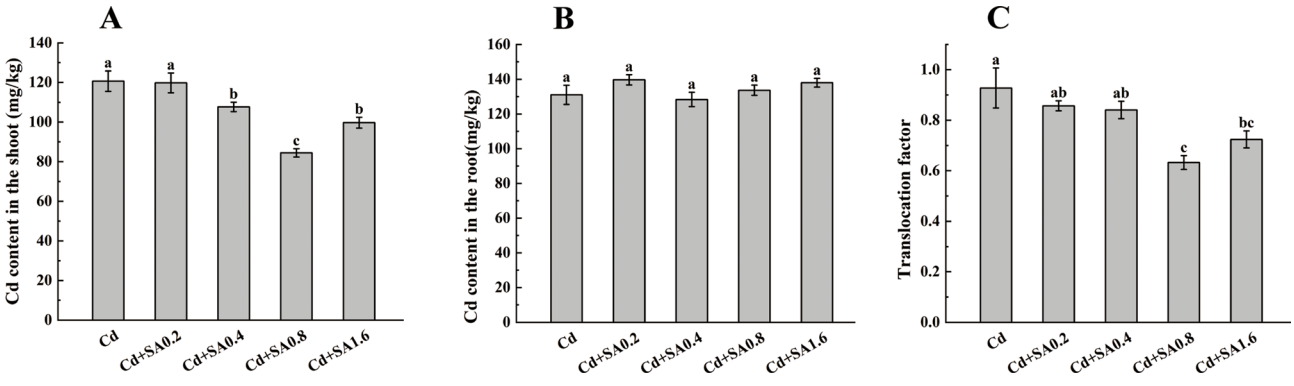

Fig. 6 Effects of Cd and SA on cadmium (Cd) content and Cd translocation factor. (**A**) Cd content in the shoot, (**B**) Cd content in the root, (**C**) Translocation factor

the Cd content in spinach shoots by reducing Cd translocation, with the most optimal effects observed at 0.8 mmol/L SA.

Exogenously applied SA modulated the expression of genes implicated in cd uptake and transport

Cd stress significantly increased the relative expression levels of *SoHMA3-like*, *SoHMA4-like*, *SoNramp3.2-like*, and *SoNramp6-like* genes by 114.40%, 341.83%, 194.87%, and 258.22%, respectively compared to CK (Fig. 7A, B, C, D). Conversely, Cd stress down-regulated the relative expression of *SoNramp3.1-like* and *SoNramp7.2-like* compared to CK (Fig. 7F, I). Exogenous SA increased *SoHMA3-like* expression. Compared to Cd alone, *SoHMA3-like* expression increased by 38.45% and 49.69% after application of 0.8 and 1.6 mmol/L SA (Fig. 7D). On the contrary, exogenous SA application downregulated the expression of *SoHMA4-like*, *SoNramp3.1-like*,

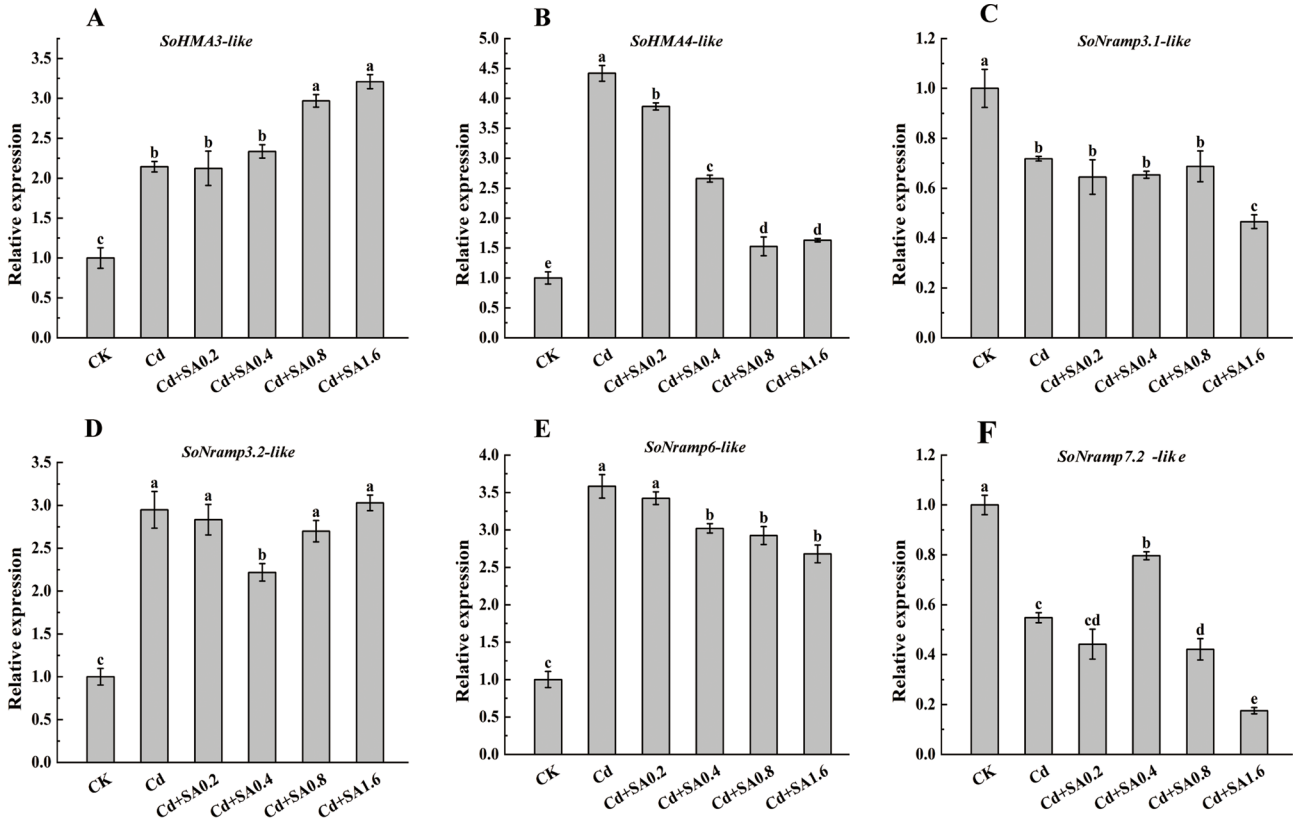

Fig. 7 Effects of different exogenous SA on gene expression of *SoHMA3-like* (**A**), *SoHMA4-like* (**B**), *SoNramp3.1-like* (**C**), *SoNramp3.2-like* (**D**), *SoNramp6* (**E**), *SoNramp7.2-like* (**F**) in spinach under cadmium (Cd) stress

SoNramp6-like, and *SoNramp7.2-like* compared with the group treated with Cd only. The expression of *SoHMA4-like*, *SoNramp3.1-like*, *SoNramp6-like*, and *SoNramp7.2-like* decreased by 63.10%, 35.15%, 25.20%, and 68.03% after treatment with 1.6 mmol/L SA compared to the group treated solely with Cd (Fig. 7E, F, H, I). Based on these findings, SA could alleviate Cd stress on spinach by regulating the expression levels of genes involved in Cd absorption and transport.

Principal component (PCA) and correlation analysis

PCA was performed to explore the combined impact of the variation in various physiological indicators and the expression of genes involved in Cd uptake and transport in spinach responses under Cd stress after treatment with different concentrations of SA. PC1 and PC2 explained 61.1% and 18.4% of the variance, respectively. The different treatments were distinctly clustered, with insignificant differences observed between 0.8 mmol/L and 1.6 mmol/L SA. However, the other treatments exhibited significantly different clustering along the PCA axis (Fig. 8A). A significant negative correlation was observed between the CK and Cd treatments in PC1. Additionally, the 0.8 mmol/L and 1.6 mmol/L SA treatments exhibited significant negative correlations with the Cd treatment in PC1 and PC2. Most indicators lay on the negative side of PC1 and, at the same time, on the positive side of PC2 (Fig. 8B). The Cd content in spinach, translocation factor, and expression of genes associated with Cd uptake and transport (*SoHMA3-like*, *SoNramp3.2-like*, and *SoNramp6-like*) were positively related with PC1 and PC2 (Fig. 8B). The PCA results showed that the impact of SA could be explained by changes in Cd content, photosynthetic pigment content, and relative expression levels of genes involved in Cd uptake and transport.

Correlation analysis was performed to further comprehensively visualize the relationship between the various indicators of Cd stress, with the results presented as a heat map (Fig. 8C). Plant growth and biomass were positively correlated with the contents of photosynthetic pigments ($p \leq 0.01$) (Fig. 8C). Conversely, plant growth and biomass were significantly negatively correlated with CAT activity levels, reactive oxygen species content (H_2O_2 and $O_2^{\cdot-}$), Cd content, and the expression of *SoHMA4-like* and *SoNramp6-like* ($p \leq 0.01$) (Fig. 8C). The Cd content was significantly positively correlated with the levels of proline, malondialdehyde, soluble proteins, POD, CAT activities, and H_2O_2 and $O_2^{\cdot-}$levels (Fig. 8C). In addition, POD and SOD activities and the expression of *SoNramp3.1-like* and *SoNramp7.2-like* exhibited

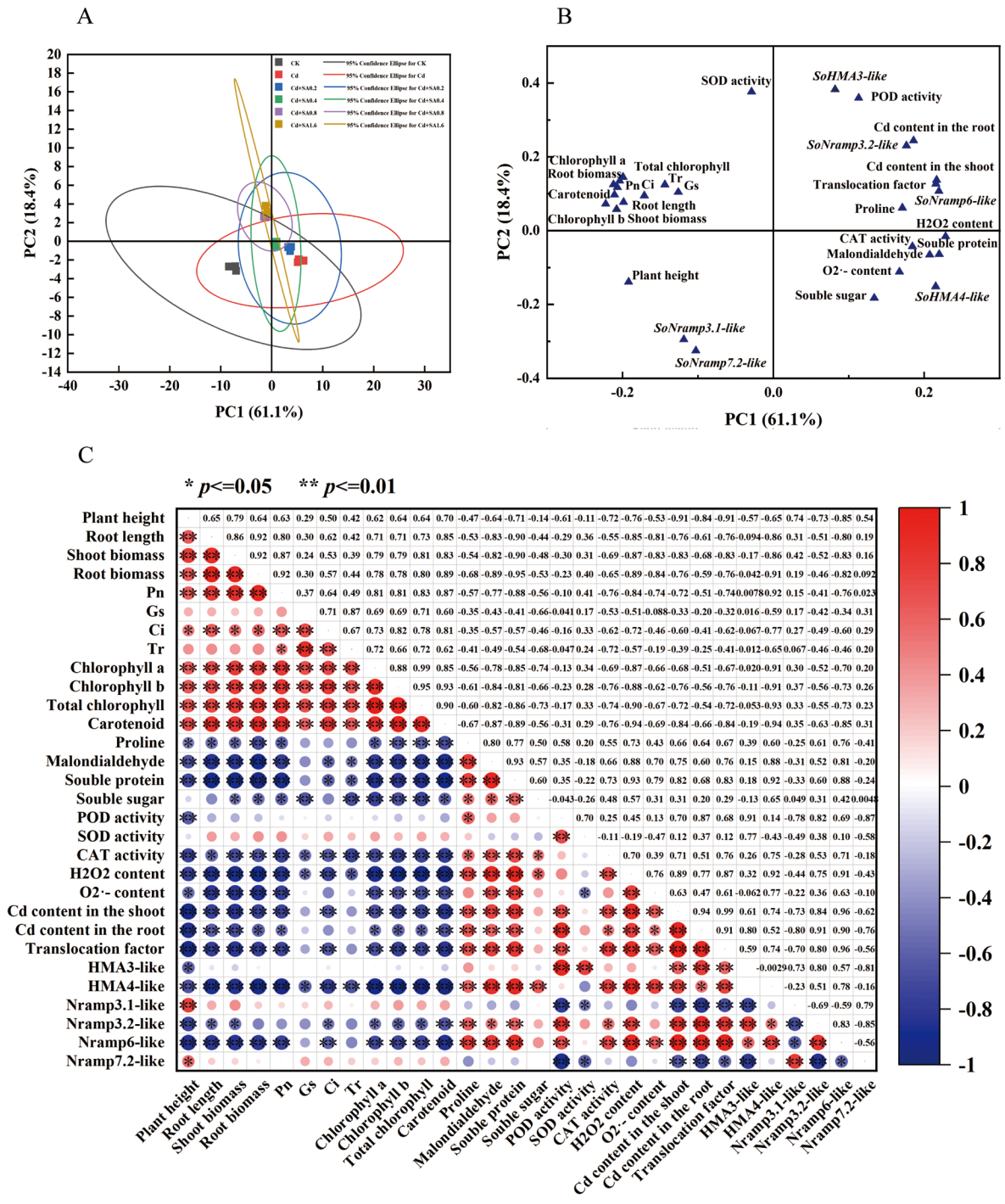

Fig. 8 PCA analysis and correlation analysis. (**A**) PCA score plot; (**B**) PCA loading plot, (**C**) Correlation heat map

insignificant correlation with most indicators ($p \leq 0.01$) (Fig. 8C).

Discussion

Crop yield can be majorly affected by the excessive accumulation of heavy metals in the soil. Numerous studies have demonstrated that the exogenous application of different compounds can reduce heavy metal accumulation in vegetables [33]. The results of our study indicated that cadmium stress negatively affected the growth of spinach, significantly reducing plant biomass and height and accelerating leaf senescence and yellowing at the leaf edges. Cd stress markedly affected wheat growth, causing a significant decrease in biomass accumulation, consistent with the present results [34]. Recent studies reported the role of salicylic acid as a protective agent against

abiotic (cold, heat, salt, heavy metal) stress and biotic stress in plants [35]. Notably, the application of salicylic acid could mitigate the adverse effects of high temperatures on rapeseed by increasing the activity of antioxidant enzymes [36]. Salicylic acid conferred cadmium tolerance on soybean and rice seedlings [37, 38]. Li et al. demonstrated that the Cd-stress-mediated growth inhibition in potatoes was significantly alleviated after exogenous SA application [11]. Similarly, the present study demonstrated that the growth and biomass of spinach subjected to Cd stress significantly increased after treatment with 0.8 and 1.6 mmol/L SA compared to plants subjected to Cd stress alone (Fig. 1). These findings indicate that SA alleviates the growth inhibition effect induced by Cd stress in spinach.

The photosynthesis process is extremely sensitive to changes in the environment. Cd stress affects plant photosynthesis through two processes. Firstly, Cd causes stomatal limitations, which limits the entry of CO_2 into stomata or the release of O_2, thereby affecting photosynthesis [39]. Secondly, Cd affects the process through non-stomatal limitations by reducing chlorophyll content through the disruption of the thylakoid structure in chloroplasts, ultimately inhibiting photosynthesis [40]. In our study, cadmium stress substantially reduced the levels of photosynthetic parameters and contents of photosynthetic pigments. As demonstrated in a previous study, exogenous SA reversed the reduction in plant photochemical efficiency induced by Cd stress and alleviated Cd toxicity in *Arabidopsis thaliana* [17]. In addition, Obono et al. reported that under cadmium stress conditions, exogenously applied SA significantly increased the chlorophyll content in Chinese cabbage, thereby alleviating Cd toxicity in the plants [41]. In the current study, 0.8 and 1.6 mmol/L SA significantly enhanced the photosynthetic rate of spinach under cadmium stress conditions and increased the contents of photosynthetic pigments, consistent with previous findings. These observations can be attributed to the effects of SA in alleviating Cd stress in plants by increasing the contents of photosynthetic pigments, such as chlorophyll, and enhancing carbamylating enzyme activities, ultimately increasing the rate of photosynthesis [42–44].

Cadmium stress promotes excessive production of H_2O_2 and O_2^- in plants, which can damage proteins, lipids, and other biomolecules, exacerbating the Cd toxicity in plants [45]. Malondialdehyde is a critical indicator of cellular lipid peroxidation, offering insights into the degree of damage to plant cell membranes [46]. Su et al. observed that Cd stress significantly increased H_2O_2 and $O_2^{\cdot-}$ and proline contents in tobacco leaves and roots [40]. In the current study, Cd stress significantly increased the content of reactive oxygen species, antioxidant enzyme activities, and the levels of proline, malondialdehyde, soluble proteins, and soluble sugars in spinach leaves. Previous studies reported that the application of SA could alleviate Cd stress by enhancing antioxidant enzyme activities and inhibiting the production of excessive H_2O_2 and $O_2^{\cdot-}$ in plants [17, 47]. Moreover, Guo et al. observed that exogenous application of SA in tomato subjected to Cd stress reduced malondialdehyde content and increased CAT activity [48]. SA can act directly as an antioxidant, scavenging excessive H_2O_2 produced in peas under Cd stress conditions [49]. A study by Lu et al. demonstrated that SA significantly reversed the increase in SOD, POD, and CAT activities induced by Cd stress in duckweed leaves [50]. Similarly, we observed that exogenous SA alleviated Cd toxicity in spinach by reducing the contents of reactive oxygen species, malondialdehyde, soluble sugars and soluble proteins, and CAT activity.

Application of SA to plants can inhibit the transport of Cd from the roots to the aboveground parts. For example, a study by Raza et al. showed that SA markedly reduced Cd uptake and ameliorated Cd-induced inhibition of radish root growth [51]. Similar results were observed in wheat, ryegrass, and rice [52–54]. The results of this study indicate that SA reduced the Cd content and translocation factor in the aboveground organs of spinach, which is consistent with previous research findings. Research has demonstrated that plants lack transport proteins specifically designated for Cd transport in plants. Cd is transported through ion transport proteins, such as Nramp and HMA family proteins [55, 56]. Majumdar et al. demonstrated that the application of SA in rice seedlings subjected to Cd stress upregulated *OsHMA3* and *OsPCS1* genes and downregulated *OsNRAMP2* [57]. Moreover, Huang et al. reported that SA could alleviate the toxicity of Cd on rice by modulating *OsNRAMP5* and *OsHMA3* gene expression [38]. Our research results indicate that SA potentially reduced Cd absorption in spinach leaves by downregulating the relative expression of *SoHMA4-like*, *SoNramp3.1-like*, *SoNamp6-like*, and *SoNramp7.2-like* genes. Thus, exogenous SA can reduce spinach shoot Cd uptake. SA can mitigate cadmium toxicity in spinach by reducing the content of reactive oxygen species, promoting photosynthesis, and downregulating the expression of genes involved in Cd transport (Fig. 9). PCA analysis and correlation analysis indicated that the Cd content was significantly positively correlated with the proline levels, malondialdehyde, soluble proteins, POD activity, CAT activity, and reactive oxygen species content. However, the mechanisms underlying these results should be further studied.

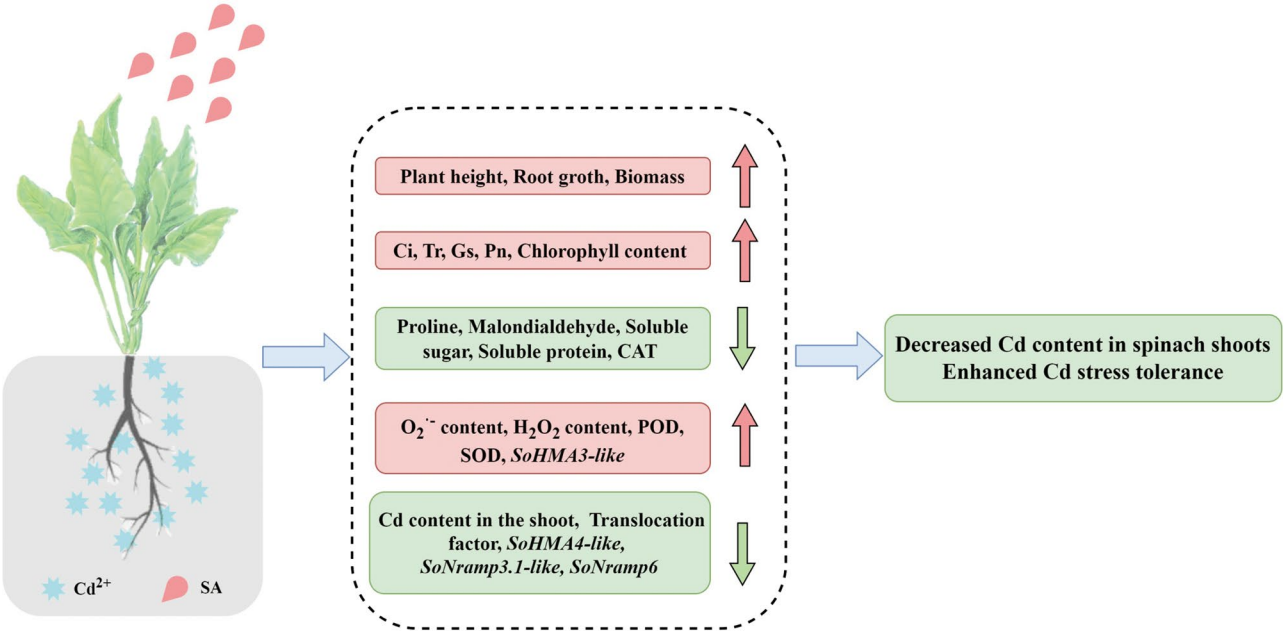

Fig. 9 Schematic diagram of the mechanisms underlying Cd toxicity alleviation by exogenously applied SA to spinach plants

Conclusions

Based on the results of our study, 0.8 and 1.6 mmol/L of exogenously applied SA alleviated the inhibition of spinach growth induced by Cd stress. Furthermore, SA enhanced photosynthesis and increased the levels of photosynthetic pigments in spinach under Cd stress. Moreover, SA could abrogate the oxidative damage induced by Cd by reducing the contents of reactive oxygen species and osmoregulatory substances. In addition, 0.8 and 1.6 mmol/L SA application decreased Cd absorption in spinach leaves by downregulating the relative expression of *SoHMA4-like*, *SoNramp3.1-like*, *SoNramp6-like*, and *SoNramp7.2-like* genes, resulting in reduced Cd content in spinach shoots. In summary, the application of 0.8 and 1.6 mmol/L SA could effectively mitigate the toxicity of Cd in spinach.

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Author contributions
Wen Tang: Investigation, data curation, writing-original draft. Le Liang: Investigation, data curation. Haixing Yang: Data curation. Xuena Yu: Data curation. Xudong Ye: Investigation, data curation. Yongdong Xie: Investigation. Rulong Li: Investigation. Lijin Lin: Investigation. Zhi Huang: Investigation. Bo Sun: Investigation. Guochao Sun: Investigation. Li Liu: Investigation. Huanxiu Li: Conceptualization. Yi Tang: Conceptualization, writing-review and editing.

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Data availability
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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References

1. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang M, Q. Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and Human Health implications. Toxics. 2021;9(3):42. https://doi.org/10.3390/toxics9030042.
2. Carvalho MEA, Castro PRC, Azevedo RA. Hormesis in plants under cd exposure: from toxic to beneficial element? J Hazard Mater. 2020;384:121434. https://doi.org/10.1016/j.jhazmat.2019.121434.
3. Wang M, Chen Z, Song W, Hong D, Huang L, Li Y. A review on Cadmium exposure in the Population and intervention strategies against Cadmium Toxicity. Bull Environ Contam Toxicol. 2021;106(1):65–74. https://doi.org/10.1007/s00128-020-03088-1.
4. Xiang M, Li Y, Yang J, Lei K, Li Y, Li F, et al. Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. Environ Pollut. 2021;278:116911. https://doi.org/10.1016/j.envpol.2021.116911.
5. Tai Z, Yin X, Fang Z, Shi G, Lou L, Cai Q. Exogenous GR24 alleviates Cadmium Toxicity by reducing Cadmium Uptake in Switchgrass (*Panicum virgatum*) Seedlings. Int J Environ Res Public Health. 2017;14(8):852. https://doi.org/10.3390/ijerph14080852.
6. Shen C, Fu HL, Liao Q, Huang B, Fan X, Liu XY, Xin JL, Huang YY. Transcriptome analysis and physiological indicators reveal the role of sulfur in cadmium accumulation and transportation in water spinach (*Ipomoea aquatica* Forsk). Ecotoxicol Environ Saf. 2021;225:112787. https://doi.org/10.1016/j.ecoenv.2021.112787.

7. Zhu T, Li L, Duan Q, Liu X, Chen M. Progress in our understanding of plant responses to the stress of heavy metal cadmium. Plant Signal Behav. 2021;16(1):1836884. https://doi.org/10.1080/15592324.2020.1836884.
8. Chen HC, Zhang SL, Wu KJ, Li R, He XR, He DN, Huang C, Wei H. The effects of exogenous organic acids on the growth, photosynthesis and cellular ultrastructure of *Salix variegata* Franch. Under cd stress. Ecotoxicol Environ Saf. 2020;187:109790. https://doi.org/10.1016/j.ecoenv.2019.109790.
9. Hédiji H, Djebali W, Belkadhi A, Cabasson C, Moing A, Rolin D, Brouquisse R, Gallusci P, Chaïbi W. Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. South Afr J Bot. 2015;97:176–81. https://doi.org/10.1016/j.sajb.2015.01.010.
10. Zhao FJ, Tang Z, Song JJ, Huang XY, Wang P. Toxic metals and metalloids: Uptake, transport, detoxification, phytoremediation, and crop improvement for safer food. Mol Plant. 2022;15(1):27–44. https://doi.org/10.1016/j.molp.2021.09.016.
11. Li Q, Wang G, Wang Y, Yang D, Guan C, Ji J. Foliar application of salicylic acid alleviate the cadmium toxicity by modulation the reactive oxygen species in potato. Ecotoxicol Environ Saf. 2019;172:317–25. https://doi.org/10.1016/j.ecoenv.2019.01.078.
12. Lei GJ, Sun L, Sun Y, Zhu XF, Li GX, Zheng SJ. Jasmonic acid alleviates cadmium toxicity in Arabidopsis via suppression of cadmium uptake and translocation. J Integr Plant Biol. 2020;62(2):218–27. https://doi.org/10.1111/jipb.12801.
13. Jiang J, Wang Z, Kong X, Chen Y, Li J. Exogenous tryptophan application improves cadmium tolerance and inhibits cadmium upward transport in broccoli (*Brassica oleracea* var. *italica*). Front Plant Sci. 2022;13:969675. https://doi.org/10.3389/fpls.2022.969675.
14. Zhao Y, Wang J, Huang W, Zhang D, Wu J, Li B, Li M, Liu L, Yan M. Abscisic-acid-regulated responses to Alleviate Cadmium toxicity in plants. Plants (Basel). 2023;12(5):1023. https://doi.org/10.3390/plants12051023.
15. Yang L, Wu Y, Wang X, Lv J, Tang Z, Hu L, Luo S, Wang R, Ali B, Yu J. Physiological mechanism of exogenous 5-Aminolevulinic Acid Improved the Tolerance of Chinese Cabbage (*Brassica pekinensis* L.) to cadmium stress. Front Plant Sci. 2022;13:845396. https://doi.org/10.3389/fpls.2022.845396.
16. Zhang D, Wang H, Zhang Y, Su Z, Hu T, Liu J, Ding Q, Niu N, Ma L. Methyl jasmonate enhances the safe production ability of Cd-stressed wheat by regulating the antioxidant capacity, cd absorption, and distribution in wheat. Plant Physiol Biochem. 2024;212:108788. https://doi.org/10.1016/j.plaphy.2024.108788.
17. Zhang W, Chen W. Role of salicylic acid in alleviating photochemical damage and autophagic cell death induction of cadmium stress in *Arabidopsis thaliana*. Photochem Photobiol Sci. 2011;10(6):947–55. https://doi.org/10.1039/c0pp00305k.
18. Wani AB, Chadar H, Wani AH, Singh S, Upadhyay N. Salicylic acid to decrease plant stress. Environ Chem Lett. 2016;15(1):101–23. https://doi.org/10.1007/s10311-016-0584-0.
19. Ma J, Wang Y, Wang LY, Lin D, Yang Y. Transcriptomic analysis reveals the mechanism of the alleviation of salt stress by salicylic acid in pepper (*Capsicum annuum* L). Mol Biol Rep. 2023;50(4):3593–606. https://doi.org/10.1007/s11033-022-08064-y.
20. Kovács V, Gondor OK, Szalai G, Darkó E, Majláth I, Janda T, Pál M. Synthesis and role of salicylic acid in wheat varieties with different levels of cadmium tolerance. J Hazard Mater. 2014;280:12–9. https://doi.org/10.1016/j.jhazmat.2014.07.048.
21. Wang F, Tan H, Huang L, Cai C, Ding Y, Bao H, Chen Z, Zhu C. Application of exogenous salicylic acid reduces cd toxicity and cd accumulation in rice. Ecotoxicol Environ Saf. 2021a;207:111198. https://doi.org/10.1016/j.ecoenv.2020.111198.
22. Chen ZJ, Huang J, Li S, Shao JF, Shen RF, Zhu XF. Salylic acid minimize cadmium accumulation in rice through regulating the fixation capacity of the cell wall to cadmium. Plant Sci. 2023;336:111839. https://doi.org/10.1016/j.plantsci.2023.111839.
23. Huang Y, He C, Shen C, Guo J, Mubeen S, Yuan J, Yang Z. Toxicity of cadmium and its health risks from leafy vegetable consumption. Food Funct. 2017;8(4):1373–401. https://doi.org/10.1039/c6fo01580h.
24. Huang Y, Chen J, Sun Y, Wang H, Zhan J, Huang Y, Zou J, Wang L, Su N, Cui J. Mechanisms of calcium sulfate in alleviating cadmium toxicity and accumulation in *pak choi* seedlings. Sci Total Environ. 2022b;805:150115. https://doi.org/10.1016/j.scitotenv.2021.150115.
25. Zhou H, Yang WT, Zhou X, Liu L, Gu JF, Wang WL, Zou JL, Tian T, Peng PQ, Liao BH. Accumulation of Heavy metals in Vegetable Species Planted in Contaminated soils and the Health Risk Assessment. Int J Environ Res Public Health. 2016;13(3):289. https://doi.org/10.3390/ijerph13030289.
26. Liu H, Probst A, Liao B. Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China). Sci Total Environ. 2005;339(1–3). 153 – 66https://doi.org/10.1016/j.scitotenv.2004.07.030.
27. Nafees M, Sehrish AK, Alomrani SO, Qiu L, Saeed A, Ahmad S, Ali S, Guo H. Mechanism and synergistic effect of sulfadiazine (SDZ) and cadmium toxicity in spinach (*Spinacia oleracea* L.) and its alleviation through zinc fortification. J Hazard Mater. 2024;464:132903. https://doi.org/10.1016/j.jhazmat.2023.132903.
28. Tang W, Liang L, Li RL, Xie YD, Li XM, Li HX, Lin LJ, Huang Z, Sun B, Sun GC, Tu LH, Tang Y. Effects of exogenous melatonin on the growth and cadmium accumulation of lettuce under cadmium-stress conditions. Environ Prog Sustain Energy. 2023;42(2):e14014. https://doi.org/10.1002/ep.14014.
29. Waheed S, Ahmad R, Irshad M, Khan SA, Mahmood Q, Shahzad M. Ca_2SiO_4 chemigation reduces cadmium localization in the subcellular leaf fractions of spinach (*Spinacia oleracea* L.) under cadmium stress. Ecotoxicol Environ Saf. 2021;207:111230. https://doi.org/10.1016/j.ecoenv.2020.111230.
30. Liang L, Tang W, Lian H, Sun B, Huang Z, Sun G, Li X, Tu L, Li H, Tang Y. Grafting promoted antioxidant capacity and carbon and nitrogen metabolism of bitter gourd seedlings under heat stress. Front Plant Sci. 2022;13:1074889. https://doi.org/10.3389/fpls.2022.1074889.
31. Tang Y, Wang LM, Xie YD, Yu XN, Li HX, Lin LJ, Liao MA, Wang ZH, Sun GC, Wang X, Liang D, Xia H, Tu LH. Effects of exogenous abscisic acid on the growth and cadmium accumulation of lettuce under cadmium-stress conditions. INT J ENVIRON CH. 2019;85:1–12. https://doi.org/10.1080/03067319.2019.1639686.
32. Rastmanesh F, Moore F, Keshavarzi B. Speciation and phytoavailability of heavy metals in contaminated soils in Sarcheshmeh area, Kerman Province, Iran. Bull Environ Contam Toxicol. 2010;85(5):515–9. https://doi.org/10.1007/s00128-010-0149-z.
33. Pan X, Sun F, Ge CH, Wang QH, Wang XL. Identification and Analysis of Spinach Transcription Factor *bZIP* Gene Family [J]. Mol Plant Breed. 2024;1–12. http://kns.cnki.net/kcms/detail/46.1068.S.20230531.1135.014.html
34. Muhammad AG, Basharat A, Rukhsanda A, Rashad WKQ, Anam N, Muhammad A, Saleem B, Muhammad HS, Muyassar HA, Aishah A, Shafaqat A. Alleviating role of Gibberellic Acid in Enhancing Plant Growth and Stimulating Phenolic compounds in Carrot (*Daucus carota* L.) under lead stress. Sustainability, 2021a;13(21):12329. https://doi.org/10.3390/su132112329
35. Ni J, Wang Q, Shah FA, Liu W, Wang D, Huang S, Fu S, Wu L. Exogenous melatonin confers Cadmium Tolerance by counterbalancing the Hydrogen Peroxide Homeostasis in Wheat Seedlings. Molecules. 2018;23(4):799. https://doi.org/10.3390/molecules23040799. Published 2018 Mar 30.
36. Asgher M, Khan MI, Anjum NA, Khan NA. Minimising toxicity of cadmium in plants–role of plant growth regulators. Protoplasma. 2015;252(2):399–413. https://doi.org/10.1007/s00709-014-0710-4.
37. Muhammad AG, Muhammad MA, Basharat A, Khurram Z, Muhammad A, Romana A, et al. Role of salicylic acid in heat stress tolerance in tri-genomic *Brassica napus* L. Bioagro. 2021;33(1):13–20. https://www.researchgate.net/publication/347584414.
38. Noriega G, Caggiano E, Lecube ML, Cruz DS, Batlle A, Tomaro M, Balestrasse KB. The role of salicylic acid in the prevention of oxidative stress elicited by cadmium in soybean plants. Biometals. 2012;25(6):1155–65. https://doi.org/10.1007/s10534-012-9577-z.
39. Huang Q, Xu R, Zhang Y, Yan ZX, Chen HW, Shao GS. Salicylic acid ameliorates Cadmium Toxicity by increasing nutrients uptake and upregulating antioxidant enzyme activity and Uptake/Transport-Related genes in *Oryza sativa* L. indica. J Plant Growth Regul. 2022a;42:1158–70. https://doi.org/10.1007/s00344-022-10620-6.
40. Wang M, Duan S, Zhou Z, Chen S, Wang D. Foliar spraying of melatonin confers cadmium tolerance in *Nicotiana tabacum* L. Ecotoxicol Environ Saf. 2019;170:68–76. https://doi.org/10.1016/j.ecoenv.2018.11.127.
41. Su Y, Qin C, Begum N, Ashraf M, Zhang L. Acetylcholine ameliorates the adverse effects of cadmium stress through mediating growth, photosynthetic activity and subcellular distribution of cadmium in tobacco (*Nicotiana Benthamiana*). Ecotoxicol Environ Saf. 2020;198:110671. https://doi.org/10.1016/j.ecoenv.2020.110671.
42. Obono MF, Xiong ZT, Qiu HJ. Salicylic acid alleviates the cadmium toxicity in Chinese cabbages (*Brassica chinensis*). Pak J Biol Sci. 2007;10(18):3065–71. https://doi.org/10.3923/pjbs.2007.3065.3071.

43. Liu Z, Ding Y, Wang F, Ye Y, Zhu C. Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Rep. 2016;35(4):719–31. https://doi.org/10.1007/s00299-015-1925-3.
44. Xu LL, Fan ZY, Dong YJ, Kong J, Bai XY. Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of two peanut cultivars under cadmium stress. Biol Plant. 2015;59:171–82. https://doi.org/10.1007/s10535-014-0475-9.
45. Shi GR, Cai QS, Liu QQ, Wu L. Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes. Acta Physiol Plant. 2009;31(5):969–77. https://doi.org/10.1007/s11738-009-0312-5.
46. Nahar K, Rahman M, Hasanuzzaman M, Alam MM, Rahman A, Suzuki T, Fujita M. Physiological and biochemical mechanisms of spermine-induced cadmium stress tolerance in mung bean (*Vigna radiata* L.) seedlings. Environ Sci Pollut Res Int. 2016;23(21):21206–18. https://doi.org/10.1007/s11356-016-7295-8.
47. Rizwan M, Ali S, Adrees M, Rizvi H, Zia-Ur-Rehman M, Hannan F, Qayyum MF, Hafeez F, Ok YS. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. Environ Sci Pollut Res Int. 2016;23(18):17859–79. https://doi.org/10.1007/s11356-016-6436-4.
48. Guo B, Liu C, Liang Y, Li N, Fu Q. Salicylic acid signals Plant Defence against Cadmium Toxicity. Int J Mol Sci. 2019;20(12):2960. https://doi.org/10.3390/ijms20122960.
49. Guo J, Zhou R, Ren X, Jia H, Hua L, Xu H, Lv X, Zhao J, Wei T. Effects of salicylic acid, Epi-Brassinolide and calcium on stress alleviation and cd accumulation in tomato plants. Ecotoxicol Environ Saf. 2018;157:491–6. https://doi.org/10.1016/j.ecoenv.2018.04.010.
50. Popova LP, Maslenkova LT, Yordanova RY, Ivanova AP, Krantev AP, Szalai G, Janda T. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. Plant Physiol Biochem. 2009;47(3):224–31. https://doi.org/10.1016/j.plaphy.2008.11.007.
51. Lu Q, Zhang T, Zhang W, Su C, Yang Y, Hu D, Xu Q. Alleviation of cadmium toxicity in *Lemna minor* by exogenous salicylic acid. Ecotoxicol Environ Saf. 2018;147:500–8. https://doi.org/10.1016/j.ecoenv.2017.09.015.
52. Raza SH, Shafiq F. Exploring the role of salicylic acid to Attenuate Cadmium Accumulation in Radish (*Raphanus sativus*). Int J Agric Biol. 2013;15(3):547–52. https://doi.org/10.1071/AN12292.
53. Majumdar S, Sachdev S, Kundu R. Salicylic acid mediated reduction in grain cadmium accumulation and amelioration of toxicity in *Oryza sativa* L. Cv Bandana. Ecotoxicol Environ Saf. 2020;205:111167. https://doi.org/10.1016/j.ecoenv.2020.111167.
54. Agami RA, Mohamed GF. Exogenous treatment with indole-3-acetic acid and salicylic acid alleviates cadmium toxicity in wheat seedlings. Ecotoxicol Environ Saf. 2013;94:164–71. https://doi.org/10.1016/j.ecoenv.2013.04.013.
55. Bai XY, Dong YJ, Kong J, Xu LL, Liu SA. Effects of application of salicylic acid alleviates cadmium toxicity in *perennial ryegrass*. Plant Growth Regul. 2015;75:695–706. https://doi.org/10.1007/s10725-014-9971-3.
56. Bozzi AT, Gaudet R. Molecular mechanism of Nramp-Family Transition Metal Transport. J Mol Biol. 2021;433(16):166991. https://doi.org/10.1016/j.jmb.2021.166991.
57. Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P. *AtHMA3*, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol. 2009;149(2):894–904. https://doi.org/10.1104/pp.108.130294.

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