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Foliar application with salicylic acid alleviates cadmium toxicity in chia (*Salvia hispanica* L.)

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ABSTRACT

Cadmium (Cd) accumulation is one of the serious global issues among the multitude of food security challenges. Their toxic effects are evident in different physiological and metabolic behaviors of plants. In this context, a potted study was conducted to investigate the effectiveness of salicylic acid (SA) spraying in alleviating Cd toxicity in chia (*Salvia hispanica*) seedlings. This pseudocereal as a superfood is characterized by high nutritional value and high therapeutic potential. We examined some growth traits, photosynthetic pigments, anthocyanin, antioxidant defense system, malondialdehyde (MDA), and certain osmoregulatory molecules. The results showed that the chia seedlings exposed to Cd showed a substantial decline in growth, especially in shoot and root length and number of leaves (25.5, 21.5, and 22.6%, respectively), whereas the aforesaid biochemical parameters were not significantly affected. However, SA treatment alleviated the Cd stress applied and markedly enhanced the stem and root growth, especially shoot length of about 32.3% compared to Cd treatment. Under combined treatment Cd+SA, we also recorded a significant increase in the accumulation of proline (85.3%) and anthocyanin (83.1%), and a significant enhancement in peroxidase activity (34.9%), compared to control. These responses exhibit the involvement of SA in mitigating Cd-induced oxidative damage and improving Cd tolerance in Chia. Principal component analysis and cluster analysis showed differences between individual and combined treatment. Based on these results, exogenous SA used in the right amounts could alleviate Cd-induced stress and boost chia seedlings' growth.

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Introduction

The toxicity of heavy metals or trace metal elements has emerged as one of the greatest risks to crop productivity that could get significantly worse over the next few decades [1]. The dispersion of heavy metals has been disrupted by increased anthropogenic activities such industrialization, intense use of groundwater for irrigation, modern agriculture practice, and sewage disposal, resulting in their accumulation at specific sites [2]). Human health is seriously threatened by the absorption of these heavy metals from polluted soil and their accumulation, particularly in food crops. Depending on their geological origin in the soil or human and industrial, the heavy metals concentration in the soil can range from 1 to 100,000 mg kg^{-1} [3].

Cadmium is among the most hazardous and toxic heavy metals due to its mobility in the soil-plant interface, bio-persistence and non-biodegradation in the environment, and toxicity to living organisms and humans even at low concentrations [4]. Being a non-essential toxic element, Cd can alter several physiological and morphological processes. Plant roots readily absorb Cd, which may then be loaded into xylem for transfer to other plant parts. Cd has an impact on plants by preventing the fixation of carbon, lowering chlorophyll levels, and reducing photosynthetic activity [1]. In higher plants, the photosynthetic machinery is one of the key factors that affect biomass production and is extremely sensitive to Cd. Additionally, soil exposure to Cd causes plant osmotic stress by decreasing stomatal conductance, relative water content, and transpiration, harming plants physiologically. As a consequence of these effects, an over-production of reactive oxygen species (ROSs) occurs, which damages plant membranes and destroys cellular organelles and biomolecules, such as DNA, RNA lipids, and proteins [1,5]. Regarding the soil-plant system, Cd interferes with the plant transport and absorption of certain elements, such as Fe, Zn, P, Mg, K, and Mn, resulting in leaf chlorosis and shunted growth, or even necrosis under higher Cd toxicity [1].

Salicylic acid (SA) is a recognized natural hormonal signaling molecule that is crucial for plant growth and development as well as participating in specific abiotic stress responses, such as heavy metal-induced plant responses [6,7]. Previous research has demonstrated that SA can mitigate Cd-induced damages in a variety of plant species, such as rice [8], wheat [9], mung bean [10], soybean [11], and maize [12]. Cd toxicity alleviation has been explained by various processes, including modulation of signaling pathway responsible for preventing Cd toxicity (e.g. chelation and sequestration), stimulation of protective compounds, interaction with other phytohormones, and induction of antioxidant defense mechanism [13].

Chia (*Salvia hispanica* L.) is a pseudocereal used widely in pre-Columbian Mesoamerica as a major commodity and its seeds highly prized for their great nutritional and medicinal benefits [14,15]. It was recently revived as a new crop that is highly distinguished from productive oilseeds by its high oil content and greatest Omega-3 fatty acid conentration [14]. Seeds of chia are also a rich source of proteins, dietary fiber, vitamins, carbohydrates, antioxidants, and nutraceuticals [16]. Given the chemical composition and health-promoting properties of chia seeds, there has recently been a sharp rise in interest in the potential application of chia seeds to the food industry and nutrition security [16,17]. However, although the inclusion of chia seeds as dietary supplements has increased in recent years in some countries [18], their toxic content of certain heavy metals including cadmium has been poorly studied.

The main objective of the current research is to examine how SA affects the antioxidant defense capacity and some important metabolites involved in the mechanisms of tolerance of chia seedlings grown under Cd stress. This will help to shed light on the harmful effects of Cd and test SA's capacity to protect against Cd toxicity.

Material and methods

Plant material and growth conditions

Seeds of *S. hispanica* (Oruro variety) were disinfected (5% sodium hypochlorite, 10 min), frequently washed with bidistilled water, and then sown directly in plastic bags (17 cm long, 11.5 cm wide, and 7 cm deep) containing a mixture (950 g) of sterilized sand (70%), clay (20), and peat (10%). The bags were irrigated and placed in a growth chamber (21±2 °C, 13/11 h photoperiod, and 70% relative humidity) for germination and growth for 12 days. After, the seedlings (about 6 to 8 cm height growth) were transferred to a greenhouse under controlled conditions (24±2 °C, 12 h of light per 24 h and 70–75% relative humidity). The different cadmium and salicylic acid treatments were applied after one week (acclimatization period of chia seedlings). The research was conducted at the Faculty of Science in Rabat (−6.83823880°/N, 34°00,796,730°/W, and 43.80 m a.s.l).

Determination of the threshold concentration of Cd

To determine the threshold concentration of Cd to be applied to chia seedlings, we carried out a preliminary germination test of chia seeds based on the relative water content (%) of seeds, as a physiological trait limiting germination and development. The preliminary germination test was carried out in glass Petri dishes. After disinfection, the chia seeds were sown on double Whatman paper soaked with aqueous solutions of cadmium sulfate ($CdSO_4$), and then the Petri dishes were kept in the dark for 4 days. The concentrations of the Cd solutions selected for this test were 0.5 mM, 0.7 mM, 0.9 mM, 1.1 mM and 1.3 mM.

Cd and sa treatments

Five different treatments, either individually or in combination, were applied to chia seedlings during a 16-day experimental period. Cadmium and salicylic acid treatment were carried out by soil irrigation and foliar spraying, respectively. The treatments were : (i) 1.2 mM $CdSO_4$ (Cd), (ii) 0.4 mM SA, (iii) 0.8 mM SA, (iv) Cd+SA 0.4 mM, and (v) Cd+SA 0.8 mM. Nonetheless, the control was sprayed with distilled water without applying cadmium stress. Cd and SA treatments were applied every four-day and once in 5 days, respectively, during the experimental period. Six bags were used for each treatment and each bag contained 5 to 6 chia seedlings.

Growth traits measurement

The number of leaves (NL) has been determined, and then chia seedlings were carefully removed from the soil and washed under running tap water to remove traces of the substrate to measure growth traits. The maximum length of shoots and roots (LS and LR) was recorded for each treatment, using a ruler graduated in centimeters (cm). The fresh and dry weight of shoots and roots (FWS, FWR, DWS, and DWR) was measured by weighing with a precision balance (mg). Dry weight is measured after drying fresh plant material in an oven at 80 °C for 4 h. We carried out 3–5 duplicates of each treatment (one plant per replicate).

Biochemical traits measurement

Liquid nitrogen was used to grind fresh leaf samples from control and treated chia seedlings into a fine powder, which was then put into storage at −20 °C for subsequent analysis. At 4 °C, all extraction steps were performed. The analytical-grade reagents required were all purchased from Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), and Sigma (St. Louis, USA). The experiment of each biochemical trait was repeated 3 times for each treatment (one plant per replicate).

Chlorophylls, carotenoids, and anthocyanins content quantification

From 100 mg of finely ground leaves, the content of chlorophylls (Chl) and carotenoids (Car) was measured spectrophotometrically in acetone (80%). To get rid of any leftover solid tissue, the extract was centrifuged at 10,000 × g for 1 min. Chlorophylls (Chl a and Chl b) and carotenoids (Car) were detected spectrophotometrically in the supernatant at 663, 647, and 470 nm, respectively. The Chl a, Chl b and carotenoids content was calculated using the following equations : Chl a = 0.999 × A663 - 0.0989 × A645, Chl b = - 0.328 × A663 + 1.77 × A645 and Car = (1000 × A470 - 3.27 × Chl a - 104 × Chl b) / 227.

Anthocyanin quantification was carried out according to Deikman and Hammer [19]. Preweighed finely ground powder of chia seedlings was homogenized with extraction buffer (81% water, 18% 1-propanol, and 1% HCl). The mixture was boiled for 3 min, then centrifuged at 10,000 × g for 5 min. The extracts' two absorbancies (A535 and A650) were determined spectrophotometrically. The content of anthocyanins was given as (A535-A650) mg g^{-1} dry weight (DW).

Soluble sugars and free proline content quantification

The finely crushed powder (100 mg) was homogenized with 1 mL of 95% ethanol and heated at 95 °C for 10 min. The residue was dissolved in 5 mL of distilled water, agitated, and then centrifuged at 10,000 × g for 10 min after the ethanol had evaporated. Soluble sugars and free proline concentration were measured in the resultant supernatant.

The concentration of total soluble sugars (TSS) was measured following the method of Dubois et al. [20]. The supernatant (1 mL) was homogenized with 5 mL sulfuric acid (98%) and 1 mL phenol 5%, boiled for 5 min, and left to cool to the ambient temperature for 30 min. After measuring the mixture's absorbance at 485 nm with a spectrophotometer, the concentration of soluble sugars was estimated using a standard curve that included glucose. The total soluble sugar concentration was given as mol g^{-1} DW.

Proline (Pro) was determined according to Bates et al. [21]. A voulme of 1 mL glacial acetic acid and 1 mL acid ninhydrin solution was added to the supernatant (1 mL), and the mixture was then heated for 1 hour in a boiling water bath. After adding 5 mL of toluene, the tubes were shaken and the absorbance at 528 nm was calculated using L-proline as a standard. Proline concentration was given as mol g^{-1} DW.

Activity measurement of antioxidant enzymes

Enzymes extraction

The antioxidant enzymes were extracted using a phosphate (NaH_2PO_4/Na_2HPO) buffer by homogenizing on ice 200 mg of finely ground powder of chia seedlings in 4 mL NaH_2PO_4/Na_2HPO (pH 7.8) with 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% (w/v) polyvinyl pyrrolidone (PVP). Using bovine serum albumin (BSA) as a standard, the total soluble protein content was calculated in accordance with Bradford's method [22] in order to establish the specific activity of the enzymes.

Table 1
Effect of different Cd concentrations on the relative water content (%) of *S. hispanica* seeds after 4 days of germination (preliminary test).

Cd	Control	0.5 mM	0.7 mM	0.9 mM	1.1 mM	1.3 mM
	94.91±1.80a	92.91±0.60ab	92.69±1.42ab	92.20±0.73ab	85.02±4.01b	84.34±8.49b

Values are means ± standard deviation, $n = 5$. Means in the line with different letters are significantly different according to Tukey's test ($p \leq 0.05$).

Catalase (CAT) and peroxidase (POD) activity

CAT activity was assessed using Aebi's method [23]. The test mixture consisted of 1 mL of the reaction solution (7.5 μl of 30% H_2O_2 in 100 mM Na-phosphate buffer (pH 7.8)), and enzyme extract (50 μl). Furthermore, according to the method of Chen and Zhang [24], total POD activity was measured. The reaction solution (28 μl of 0.2% guaiacol, 19 μl of 30% H_2O_2 in 100 mM phosphate buffer (pH 7.8)), and enzyme extract (50 μl) made up the assay combination (1 mL). A spectrophotometer was used to measure the drop in absorbance caused by the breakdown of H_2O_2 molecules every 15 s (for a total of 1 min), and the activities of CAT and POD were expressed as Unit mg-1 protein.

Malondialdehyde content quantification

According to Chen and Zhang [24], the frozen powder (100 mg) was homogenized in 1 mL of 100 mM phosphate buffer (pH 7.8). After centrifugation (10,000 × g for 20 min), the supernatant (100 µl) and 1 mL of 0.25% (w/v) thiobarbituric acid (TBA) were homogenized. This reaction mixture was heated for 15 min at 90 °C, then rapidly cooled on an ice bath for 5 min. In order to adjust for nonspecific turbidity, the absorbance was then measured at 532 nm and 600 nm. According to its extinction coefficient of 155 mM^{-1} cm^{-1}, the MDA concentration was determined and reported as nmol mg^{-1} DW.

Statistical analysis

All data were tested by the analysis of variance (ANOVA) to investigate the effect of treatment on the growth and biochemical traits studied. Using Tukey's post hoc test, means were compared at the 5% significant level. Principal component analysis (PCA) and cluster analysis were carried out to determine and distinguish the different treatments studied by referring to the discriminating variables. SPSS, version 25 for Microsoft Windows, was used to perform the statistical analyses.

Results

Preliminary test

Based on statistical analysis (ANOVA) of the preliminary test results (Table 1), when comparing the different concentrations of the cadmium treatment with the control, we recorded a significant decrease ($p \leq 0.05$) in relative water content (%) of chia seeds from the 1.1 mM concentration. Nonetheless, the statistical difference between both concentrations of 1.1 mM and 1.3 mM was not significant ($p \leq 0.05$). Indeed, we retained the intermediate concentration of 1.2 mM as the threshold concentration to be applied to chia seedlings in our experiment.

Growth traits

The effect of SA on growth and biochemical mechanisms of tolerance in *S. hispanica* under cadmium stress conditions was evaluated to test the degree of Cd stress tolerance of this pseudocereal. The results of vegetative growth traits showed a significant difference between control, Cd stressed, and SA-sprayed seedlings under cadmium stress (Fig. 1A-D). Cd significantly decreased shoot length and leaf number in *S. hispanica* seedlings after 16 days of metal treatment compared to control chia seedlings ($p \leq 0.05$) (Fig. 1A and D). The significant decrease in these traits was about 25.5%, 21.5%, and 22.6% for the shoot and root length, and the number of leaves, respectively. Thus, stem and root dry weight and stem fresh weight were reduced by 42.9%, 23.6%, and 27.14% in Cd-stressed seedlings compared to the control, respectively (Figs. 2 and 3). However, no significant statistical difference was recorded for fresh root weight ($p = 0.99$) (Fig. 1B).

As for the effect of SA spraying, we revealed that SA significantly improved vegetative growth, especially at 0.8 mM SA (Fig. 1A-C). The comparison of the results showed a non-significant increase in *S. hispanica* seedlings grown under SA treatment at 0.8 mM, mainly in root length, stem fresh weight, and stem and root dry weight, compared to control (Fig. 1A-C). Combined treatment (Cd+SA) results on *S. hispanica* seedlings revealed a significant difference compared to seedlings grown under Cd stress conditions ($p \leq 0.05$) (Fig. 1A). This statistical difference was mainly recorded for shoot length in seedlings under combined treatment (Cd+SA at 0.8 mM) with a clear increase of about 32.3%, compared to seedlings subjected to Cd treatment only. However, root fresh weight did not change significantly between the different treatments ($p \leq 0.05$) (Fig. 1B).

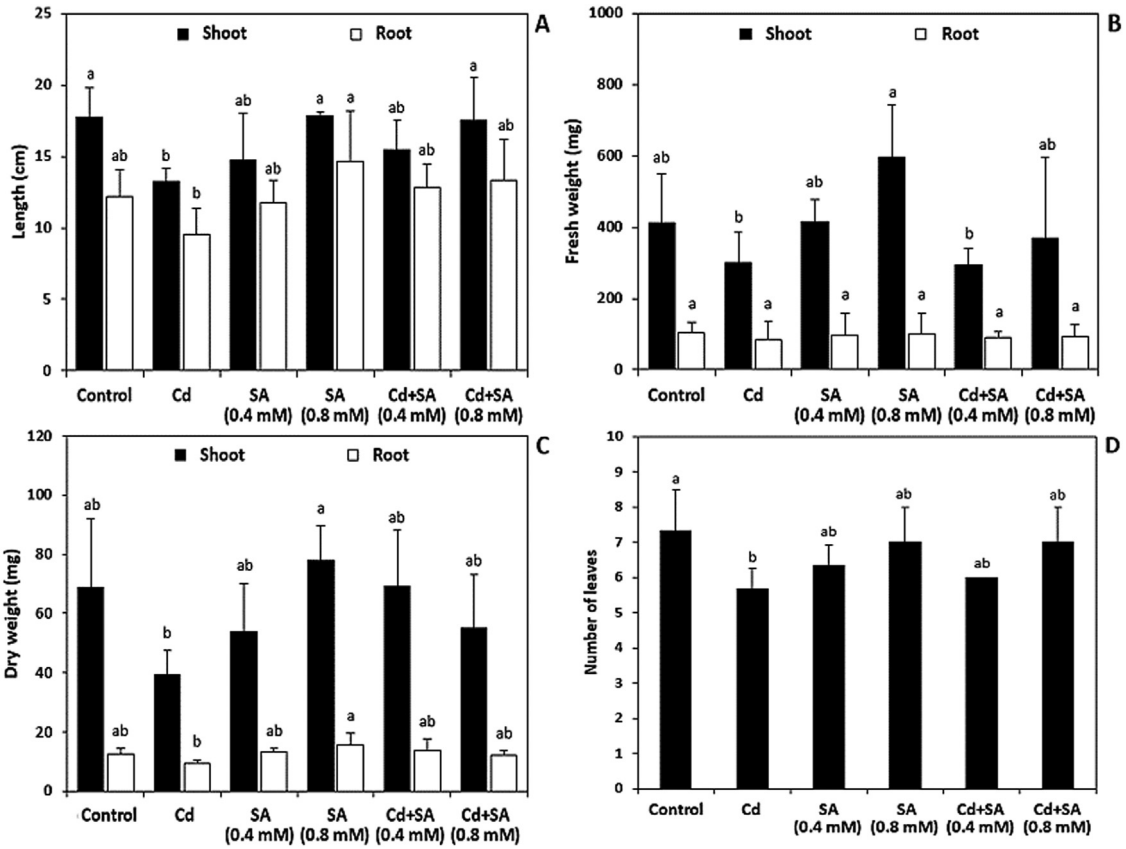


Fig. 1. Effect of SA on the length (A), the fresh weight (B), the dry weight (C) of stem and root, and the number of leaves (D) in *S. hispanica* seedlings grown under Cd stress. Means with different lowercase letters are significantly different at 5%.

Table 2
Effect of SA on chlorophyll, carotenoid, and anthocyanin content in *S. hispanica* seedlings grown under Cd stress.

Treatment	Chl a (mg/g)	Chl b (mg/g)	Chl a/b	Chl $a + b$ (mg/g)	Carotenoid (mg/g)	Anthocyanin (mg/g)
Control	8,44±1,17a	11,43±1,92a	0,74±0,02b	19,87±3,09a	49,60±4,88ab	0,71±0,25b
Cd	7,73±2,20ab	10,14±3,35ab	0,77±0,04ab	17,87±5,55ab	38,56±10,34bc	0,93±0,07ab
SA (0.4 mM)	8,07±2,09ab	11,14±3,31a	0,73±0,03b	19,21±5,40a	55,03±13,17a	0,99±0,24ab
SA (0.8 mM)	7,50±0,64ab	9,77±0,97ab	0,77±0,01ab	17,28±1,61ab	49,33±3,77ab	0,99±0,13ab
Cd+SA (0.4 mM)	4,44±0,83c	5,64±1,03c	0,79±0,02a	10,07±1,86c	31,19±6,68c	1,30±0,20a
Cd+SA (0.8 mM)	5,42±0,77bc	6,72±0,94bc	0,81±0,01a	12,14±1,70bc	32,86±4,33c	0,99±0,23ab
Treatment effect	$P \leq 0,05$	$P \leq 0,05$	$P \leq 0,05$	$P \leq 0,05$	$P \leq 0,05$	$P \leq 0,05$

Values are means ± standard deviation, $n = 3$. Means in the same column with different letters are significantly different according to Tukey's test ($p \leq 0.05$).
Values are means ± standard deviation, $n = 3$. Means in the same column with different letters are significantly different according to Tukey's test ($p \leq 0.05$).

Changes in chlorophylls, carotenoids, and anthocyanins content

Results of chlorophyll, carotenoid, and anthocyanin contents in chia seedlings grown under Cd stress conditions are shown in Table 2. The analysis of variance revealed a significant difference between the different treatments ($p \leq 0.05$). Compared to control, a significant decrease in Chl a and Chl b of about 47.4% and 50.7%, respectively were recorded in seedlings treated with Cd+SA 0.4 mM and about 35.8% (Chl a) and 41.2% (Chl b) in Cd+SA 0.8 mM. However, However, the Cd and SA treatments applied separately did not significantly affect the Chl a and b content in comparison with the control. A similar result is recorded for total chlorophyll content (Chl $a + b$) (Table 2). As for the Chl a/b ratio, our results exhibited a significant increase in its value in chia seedlings under combined treatments compared to control ($p \leq 0.05$). This increase is approximately 6.8% and 9.5% under Cd+SA at 0.4 mM and Cd+SA at 0.8 mM treatments, respectively. Thus, similarly to the effects noted on chlorophylls, carotenoids are significantly reduced in seedlings grown under combined treatments in comparison with control ($p \leq 0.05$). Carotenoid content was reduced by approximately 22.3% in Cd-stressed chia seedlings,

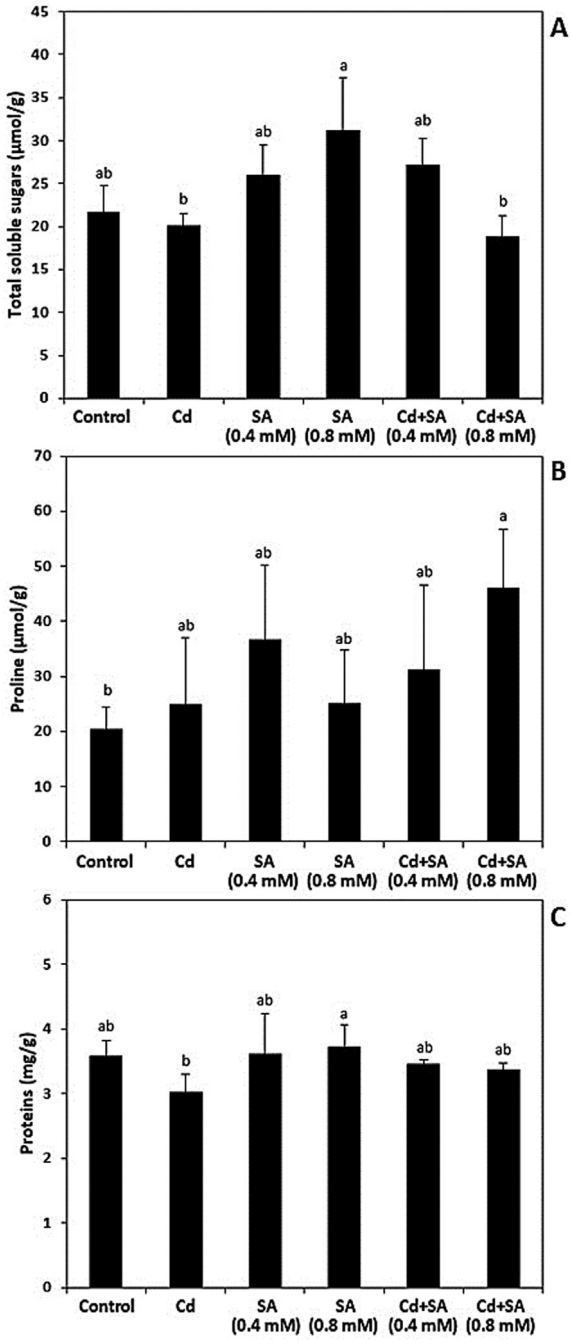


Fig. 2. Effect of SA on the leaf content of total soluble sugars (A), proline (B), and total proteins in *S. hispanica* seedlings grown under Cd stress. Means with different lowercase letters are significantly different at 5%.

while SA at 0.4 mM increased it by approximately 10.9% compared to the control (Table 2). Moreover, a high significant anthocyanin concentration was found in chia seedlings grown under Cd+SA treatment at 0.4 mM ($p \leq 0.05$). Compared to the control, this significant difference was about 83.1% (Table 2).

Soluble sugars and proline accumulation

The results of total soluble sugars, free proline, and proteins revealed a significant difference between both Cd and SA treatments ($p \leq 0.05$) (Fig. 3A-C). Following the ANOVA analysis, we only recorded a significant difference between Cd and SA at 0.8 mM treatments ($p \leq 0.05$) for total soluble sugars and protein content (Fig. 3A and C). However, no significant

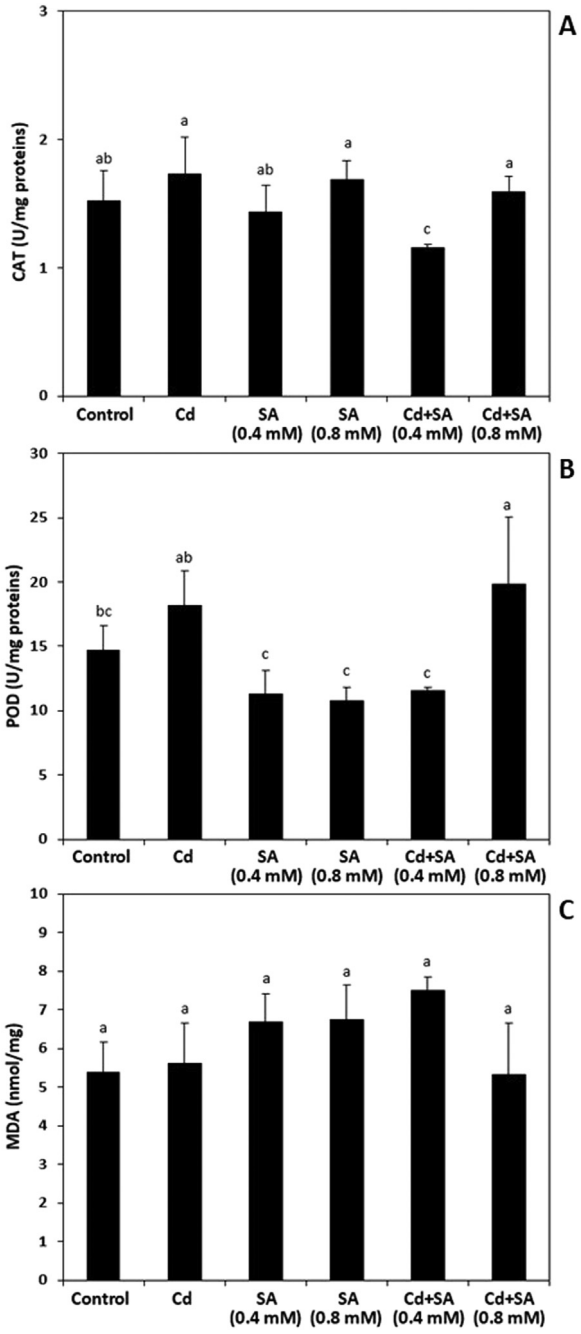


Fig. 3. Effect of SA on the activity of CAT (A), POD (B), and MDA accumulation (C) in *S. hispanica* seedlings grown under Cd stress. Means with different lowercase letters are significantly different at 5%.

statistical differences were noted between all treatments and the control ($p \leq 0.05$). The proline results obtained showed that Cd+SA treatment induced a significant proline accumulation in chia seedlings of about 85.3% compared to the control (Fig. 3B). As for comparing the effect of SA concentration (0.4 and 0.8 mM), there were no significant differences between individual and combined treatments.

Antioxidant defense system

Stimulation of the enzymatic antioxidant defense system in *S. hispanica* seedlings was evaluated by measuring the CAT and POD. The results showed a significant difference among the different treatments including the control ($p \leq 0.05$)

Table 3
Matrix of components 1 and 2 for all parameters studied under different treatments of SA and Cd.

Parameters	Principal Components (PC)	
	PC 1	PC 2
Number of leaves (NL)	0,08	0,49
Length of shoots (LS)	−0,16	0,47
Length of roots (LR)	−0,02	0,80
Fresh weight of shoots (FWS)	0,37	0,61
Fresh weight of roots (FWR)	0,40	0,43
Dry weight of shoots (DWS)	0,04	0,80
Dry weight of roots (DWR)	0,05	0,84
Total soluble sugars (TSS)	0,25	0,47
Proline (Pro)	−0,45	−0,11
Proteins	0,40	0,61
Catalase (CAT)	0,43	−0,27
Peroxidase (POD)	−0,29	−0,65
Malondialdehyde (MDA)	−0,11	0,27
Anthocyanin (Ant)	−0,48	0,32
Carotenoids (Car)	0,95	−0,10
Chl a	0,96	−0,22
Chl b	0,97	−0,20
Chl a + *b*	0,97	−0,21
Chl a/b	−0,86	0,01

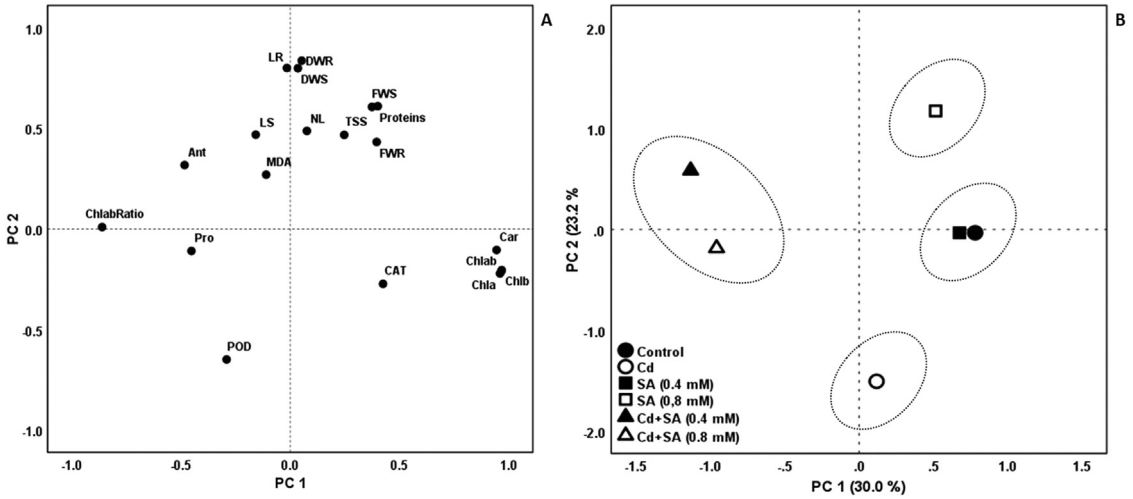


Fig. 4. 2D scatterplots showing the distribution of all studied parameters (A) and treatments (B) according to the two main principal components obtained by PCA in chia seedlings.

(Fig. 3A-B). The high significant CAT activity ($p \leq 0.05$) was recorded in chia seedlings grown under Cd (1.74 U mg^{-1} proteins), SA 0.8 mM (1.69 U mg^{-1} proteins) and Cd+SA 0.8 mM treatments (1.59 U mg^{-1} proteins); while the highest POD activity was noted under the combined treatment Cd+SA at 0.8 mM (19.82 U mg^{-1} proteins). Under these conditions, POD activity was significantly increased of about 43.9% in comparison to control. However, the lowest enzymatic activity was noted in chia seedlings under Cd+SA 0.4 mM treatment for CAT and SA treatments (0.4 mM and 0.8 mM) and Cd+SA 0.4 mM for POD (Fig. 3A- B). The MDA content was determined to assess the oxidative damage likely to be caused by metallic stress and the results obtained are presented in Fig. 3C. Following the analysis of the variance, no significant differences in MDA content were recorded between the different treatments ($p \leq 0.05$).

Multivariate statistical analysis

Based on PCA we selected the first two principal components, which represent the major part of the cumulative variance, about 53.2% of the total variance (PC1: 30% and PC2: 23.2%). Regarding the matrix of components (Table 3) and the bi-dimensional space (Fig. 4A), PC1 is strongly correlated with Chlb (0.97), Chlab (0.97), Chla (0.96), and Car (0.94) in the positive part, and Chla/b ratio (0.86) in the negative part, whereas PC2 has strong correlations with a dry weight of roots

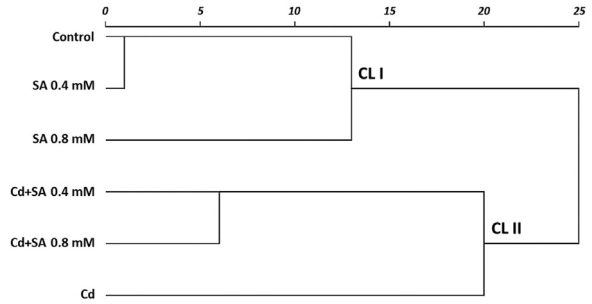


Fig. 5. Hierarchical clustering of the treatments applied to *S. hispanica* seedlings based on the principal components approach.

and shoots (0.84 and 0.80, respectively), LR (0.80), FWS (0.61) and proteins (0.61) in the positive part and POD (0.65) in the negative part. Furthermore, Fig. 5 shows the generated clusters that are the result of the Hierarchical Clustering of Principal Components. Both figures made it possible to separate and distinguish between the different treatments applied. Two main clusters were distinguished (Fig. 5), each encompassing two sub-clusters that are represented as groups on the PCA space (Fig. 4B). The four identified treatment groups (Grp) were: Grp1 grouping treatments: Control and SA 0.4 mM and according to CP2, it is clearly separated from Grp2 (SA 0.8 mM treatment) by a slight decrease in LR, DWS, and DWR and from Grp 3 (Cd treatment) by the low enzymatic activity of POD. Nonetheless, the Grp 4 (Cd+SA 0.4 mM and Cd+SA 0.4 mM treatments) is clearly distinguished by a higher Chla/b ratio compared to other groups.

Discussion

Cadmium as one of the most hazardous heavy metals, its uptake by plants leads to growth inhibition and reduction of biomass production [4]. Cd exposure inhibits plant growth causing morphological changes in leaves and roots. The study of the effect of this metallic element on *S. hispanica* showed a significant inhibitory effect on its vegetative growth. Compared to control, Cd significantly reduced the length of the shoot and the number of leaves. Similar results were observed in *Vigna radiata*, where Cd significantly inhibited shoot length [10] and contrary to our results, the same study reported that Cd stress also significantly reduced other growth-related attributes, such as root length, fresh and dry biomass. Our result can be explained by the transport of Cd from contaminated soil to aerial parts by way of the roots and its accumulation in chia seedlings may directly interfere with the shoot metabolism, resulting in a reduction in height similar to that seen in several cereals including maize [25], rice [26] and wheat [27]. Growth inhibition by cadmium may potentially have resulted from the irreversible inhibition of the proton pump, which is involved in regulating cell division and cell elongation rate. In addition, the Cd-induced growth inhibition could be at least partially due to the induction of lipid peroxidation, disruption of metabolism, and inhibition of CO_2 fixation enzymes, as well as a decrease in net photosynthetic rate [6]. These damaging effects result in growth retardation, reduced nutrient uptake, chlorosis and leaf necrosis, ionic imbalance, inhibition of stomatal opening, reduction in biomass production, and can lead to cell death [1,5,7]. However, a large number of studies have shown that SA can reduce the negative impact of Cd stress and enhance plant resistance to this metallic element [6,7]. Based on our results, the improvement of parameters related to the growth of *S. hispanica* seedlings under Cd stress can be explained by the involvement of SA in the maximum induction of indole acetic acid (IAA) activity, which promotes rapid cell division in the apical meristematic region [10]. Available results in the literature reported that exogenous SA treatment improved vegetative growth under Cd stress conditions in rice [8] and mung bean [10], indicating that SA reduced Cd-induced toxicity during the vegetative growth phase by improving nutrient absorption, water relations and photosynthesis through detoxification processes [28].

Results of chlorophyll pigment content in chia seedlings showed that Cd did not cause their degradation compared to controls (normal appearance of leaves that remained green without chlorosis). In contrast, other studies have reported that Cd causes chlorophyll degradation, resulting in yellowing and chlorosis of leaves in rice [8], mung bean [10], maize [12], and rapeseed [28]. For carotenoid and anthocyanin contents, no significant differences were noted between Cd-stressed seedlings and control. However, combined treatment (Cd+SA) significantly decreased chlorophyll and carotenoid content compared to both control and Cd treatment. This result can be explained by the effect of SA foliar spraying which seems to cause a stomatal limitation in chia seedlings. Under Cd stress, Liu et al. [29] reported that the exogenous application of SA on castor bean seedlings resulted in a decrease in photosynthetic pigment content (chlorophylls and carotenoids), as well as a reduction in the rate of photosynthesis. However, some studies reported that SA significantly reduced the Cd-induced chlorophyll decline in rice [8] and rapeseed leaves [28]. As for anthocyanins, our results showed a significant increase in their content under Cd+SA 0.4 mM treatment in comparison to the control. This could result from both Cd-induced stress and SA-induced induction. Anthocyanins are pigments produced in response to various types of stress including metallic stress [30], and the effect of certain phytohormones such as SA on the anthocyanin biosynthesis in plants exposed to heavy metals has been demonstrated [13]. Many studies have documented the role of exogenous treatment by SA in enhancing the concentration of some classes of polyphenols, such as anthocyanins, under metallic stress conditions [13]. Foliar SA spraying of Cd-treated

chia seedlings could help protect the functioning of the photosynthetic system by increasing anthocyanin phenolic compounds that are considered antioxidants to mitigate the toxic effects of reactive oxygen species produced during oxidative stress [31].

Cd stress is known to disrupt metabolic pathways leading to accelerate lipid peroxidation and senescence and processes by increasing the production of ROS and free radicals that damage tissue [32]. Nonetheless, plants have evolved many defense mechanisms, including enzymatic and non-enzymatic ones, to scavenge ROS and alleviate their harmful effects [33]. In response to Cd stress, *S. hispanica* seedlings produced some compatible solutes including proline and soluble sugars and activated antioxidative enzymes such as CAT and POD. These biochemical responses have been improved in SA-treated seedlings. Through the activation of antioxidant responses, SA can directly scavenge ROS and/or indirectly modulate redox balance [9]. SA is known to reduce the accumulation of several heavy metals, including Cd [13]. Li et al. [34] reported that foliar application of SA reduces cadmium toxicity in potatoes by inducing a significant accumulation of proline. This non-enzymatic antioxidant acts as a chelator, shielding enzymes from Cd-induced inhibition, and forming a Cd-Pro-complex that is nontoxic to the plant [13,32]. In addition to Cd sequestration in plants, proline could reduce Cd-induced damage by maintaining a strict reducing environment inside the plant cell [32]. By comparing chia Cd-treated seedlings and seedlings grown under combined treatment (Cd+SA at 0.4 mM), the latter accumulated more total soluble sugars and proteins. These compounds are important osmotic regulators and under metal stress, their contents were considered a good indicator of oxidative damage. Under Cd stress conditions, soluble protein and soluble sugar contents are impacted and consequently reduced [35]. While SA at 0.4 mM enhanced the amounts of soluble protein and sugar in Cd-stressed chia seedlings. These findings suggest that SA may regulate the homeostasis of plant cells, mitigating the hazardous effect of Cd on chia seedlings. This corroborates with the findings of other authors who have highlighted that the exogenous SA supply improved the accumulation of soluble protein and soluble sugar in metal-stressed plants such as rice under chromium stress [36], mung bean under Cd stress [10], fenugreek under arsenic and zinc stress [37], and sunflower under Cu stress [38]. SA plays a key role in improving plant tolerance to metal stress thereby inducing the synthesis of soluble sugar and soluble protein and its regulation ability depends on its concentration [13].

In addition, the activation of enzymatic defense systems allows plants to control ROS production and reduce oxidative damage [33]. In the present study, Cd stress significantly induced the enzymatic defense system (CAT and POD) of chia seedlings, which was enhanced by the exogenous application of SA (combined treatment Cd+SA at 0.8 mM). A similar result was reported in mung bean seedlings in which SA improved the activity of the enzymatic defense system by increasing the activity of CAT, POD, and APX under cadmium stress conditions [10]. Increased enzymatic antioxidant activity with higher proline amount speed up H_2O_2 detoxification, thus alleviating oxidative damage [33,39]. SA also lessens the effects of oxidative stress brought on by Cd by further accelerating the antioxidant machinery in plants [10,39]. It improved the tolerance of chia seedlings under Cd stress by protecting them against oxidative damage, as indicated by the results of MDA content. Several authors reported evidence that SA mitigated Cd-induced damage by lowering ROS levels and bolstering the antioxidant defense systems in plants, including rice [8], maize [12], brown mustard [39], and safflower [40]. Overall, our results suggest that SA exogenous application by foliar spray, at early stages of chia development, at 0.8 mM may have protective effects on cadmium-induced oxidative damage by enhancing antioxidant defense and osmoprotectant capacity.

Conclusion

This study has shown that Cd has an inhibitory and toxic effect on the growth of chia seedlings. A significant decrease in LS and LR, as well as the number of leaves, was recorded. At the metabolic level, chia seedlings under Cd stress showed some tolerance by activating its antioxidant defense system and accumulating proline. These responses were improved by foliar spraying of chia seedlings by SA to mitigate the toxic effects of Cd. Under Cd stress conditions, SA treatment allowed chia seedlings to maintain CAT and POD enzyme activity by limiting lipid peroxidation. The accumulation of free proline and anthocyanin was improved in the SA-treated Cd stressed seedlings. Furthermore, 0.8 mM of SA had the greatest potential to alleviate the harmful effects of Cd stress in chia seedlings. Nevertheless, extensive and complementary studies focusing on various other tolerance mechanisms seem necessary to characterize the degree of Cd tolerance of chia and to identify the dual role of SA in growth and heavy metal stress response in *S. hispanica*.

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Authors' contributions

Conceptualization, S.R., and A.C.; methodology, S.R., and B.S.; software, S.R., and A.C.; validation, C.T., Z.E.A.T., A.S., M.L., A.F.M. and C.E.M.; formal analysis, A.C.; writing-original draft preparation, S.R.; writing-review and editing, A.C., C.T., and Z.E.A.T.; supervision, A.C. The author(s) read and approved the final manuscript.

Declaration of Competing Interest

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

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