

Salicylic Acid Improves Root Antioxidant Defense System and Total Antioxidant Capacities of Flax Subjected to Cadmium

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Abstract

Cadmium (Cd) disrupts the normal growth and development of plants, depending on their tolerance to this toxic element. The present study was focused on the impacts of exogenous salicylic acid (SA) on the response and regulation of the antioxidant defense system and membrane lipids to 16-day-old flax plantlets under Cd stress. Exposure of flax to high Cd concentrations led to strong inhibition of root growth and enhanced lipid peroxides, membrane permeability, protein oxidation, and hydrogen peroxide (H₂O₂) production to varying degrees. Concomitantly, activities of the antioxidant enzymes catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11), and superoxide dismutase (SOD, EC 1.15.1.1), and the total antioxidant capacities (2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP)) were significantly altered by Cd. In contrast, exogenous SA greatly reduced the toxic effects of Cd on the root growth, antioxidant system, and membrane lipid content. The Cd-treated plantlets pre-soaked with SA exhibited less lipid and protein oxidation and membrane alteration, as well as a high level of total antioxidant capacities and increased activities of antioxidant enzymes except of CAT. These results may suggest that SA plays an important role in triggering the root antioxidant system, thereby preventing membrane damage as well as the denaturation of its components.

Introduction

ROOTS ARE THE FIRST PART of the plant to respond to metal stress. Cadmium (Cd) stress may disturb plant nutrient balance (Belkhadhi et al., 2010, Douchiche et al., 2010), inhibit growth (Hédiji et al., 2010, Gallego et al., 2012), and generate oxidative stress (Tamás et al., 2007). It is well known that Cd is a non-redox-active metal that does not trigger the direct formation of hydroxyl radicals (OH[•]) via Haber-Weiss and Fenton reactions, but indirectly, by disturbing the chloroplast and mitochondria electron transport rates (Hendry et al., 1992) and/or by inducing loss in enzymatic and nonenzymatic antioxidative system capacities (Gratão et al., 2005, Rodríguez-Serrano et al., 2009, Gallego et al., 2012).

The oxidative stress induced by Cd can also be correlated with damage to membrane lipids (Djebali et al., 2005; Belkhadhi et al., 2010), nucleic acids (Apel and Hirt, 2004, Gichner et al., 2004) and proteins (Djebali et al., 2008, Douchiche et al.,

2010). In response to reactive oxygen species (ROS), plants can induce a succession of detoxification reactions catalyzed by antioxidative enzymes (Gratão et al., 2012). However, in comparison of some species, Cd treatment can be seen to have opposite effects on certain antioxidative enzymes. In *Bacopa monnieri* L., Cd induced elevated superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (GPX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11), and glutathione reductase (GR, EC 1.6.4.2), but decreased catalase (CAT, EC 1.11.1.6) activities (Mishra et al., 2006). Severe diminution of peroxidase (POX, EC 1.11.1.7) and CAT activities were found in *Oryza sativa* roots, while in contrast to *B. monnieri*, SOD activity was also diminished (Guo et al., 2007). An increase in CAT activity under Cd stress has been found in other species (Balestrasse et al., 2001, Smeets et al., 2008).

Salicylic acid (SA) is a biomolecule that may be involved in several cell processes under normal and stress conditions. Under biotic and abiotic stress conditions, increasing evidence

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indicates that SA, a phytohormone that acts at low concentrations, is produced and accumulates inside cells to function as a signaling molecule in plants (Choi et al., 2012, Hao et al., 2012). SA is involved in gene expression in response to multiple stresses (Durrant and Dong, 2004, Fobert and Despres, 2005, Foyer and Noctor, 2005). When applied exogenously at appropriate concentrations, SA enhanced the efficiency of antioxidant system in plants (Radwan 2012, Saruhan et al., 2012). Furthermore, SA application has been reported to alleviate the symptoms of Cd toxicity observed in plants (Zawoznik et al., 2007; Zhang and Chen, 2011). Previous studies have shown that SA treatment mitigates the oxidative stress generated by Cd in different plant species such as barley (Metwally et al., 2003), soybean (Drazic and Mihailovic, 2005), rice (Guo et al., 2007), maize (Krantev et al., 2008), pea (Popova et al., 2009), and hemp (Shi et al., 2009). However, little has been reported about the role of SA in the increase of total antioxidant capacities under Cd stress. In the present study, we aim to characterize the mechanism by which SA protects plants against lipid and protein oxidative damage caused by Cd exposure. In roots, Zhang et al. (2011) have demonstrated that SA-induced Cd tolerances in *Phaseolus aureus* and *Vicia sativa* were related to increases in symplastic and apoplasmic antioxidant enzyme activities. Recent study also reported that the priming of seeds with lower concentrations of SA, before sowing, lowered the elevated levels of ROS due to Cd exposure and enhanced the activities of various antioxidant enzymes (CAT, GPX, GR, and SOD) in *Oryza sativa*, thereby protecting the plants from oxidative burst (Panda and Patra, 2007). However, contrary to this observation, Choudhury and Panda (2004) reported a decline in the activities of the antioxidant enzymes CAT, POX, SOD, and GR in rice following the pre-treatment with SA.

Taken together, this work provides novel avenues toward understanding the mechanisms of plant enhancement of tolerance to Cd-mediated oxidative stress by using SA priming event and its possible function in the restoration and maintenance of the cell membrane integrity in roots. To test this possibility, SA-related changes in hydrogen peroxide (H_2O_2) production, membrane integrity, antioxidant capacities, and membrane lipid content and fatty acid profiles have been studied in roots of Cd-treated flax plantlets.

Materials and Methods

Flax seeds (cv. Viking) were soaked for 8 h in SA solutions as previously shown in Belkhadi et al. (2010). They were then germinated for 4 days at 25°C in the dark. Uniform plantlets were transferred to a continuously aerated nutrient solution (pH 5.5) containing 1 mM $MgSO_4$, 2.5 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4 , 2 mM KNO_3 , 2 mM NH_4Cl , 50 mM EDTA-Fe-K, 30 mM H_3BO_3 , 10 mM $MnSO_4$, 1 mM $ZnSO_4$, 1 mM $CuSO_4$ and 30 mM $(NH_4)_6Mo_7O_{24}$. The nutrient solution was buffered with HCl/KOH and changed twice per week. After growing for 2 days, plantlets were subjected during 10 days to $CdCl_2$ appropriately from moderate to high concentrations (50 to 100 μM). Five replicates were in individual 6 l plastic beakers made for control and Cd treatments. Plantlets were grown in a growth chamber at a day/night cycle of 16h/8 h, at 23°C/18°C, respectively, a relative humidity close to 75% and a light intensity of 200 μmol photons $m^{-2} s^{-1}$. Roots were then detached, washed with deionized water and immedi-

ately used or frozen at $-80^\circ C$. Three independent culture experiments were performed.

Roots of the harvested plantlets were washed carefully using distilled water to eliminate any contamination. Root diameter, surface area, and volume were recorded by THSCSA Image Tool (IT) Version 3.0. Three plantlets from each replication of all treatments were selected for data collection.

The level of lipid peroxidation in roots was determined as 2-thiobarbituric acid (TBA) reactive metabolites, chiefly malondialdehyde (MDA), as described previously by Buege and Aust (1972). Membrane permeability of roots was determined as follows. Root tissue (0.1 g) was vibrated for 30 min in deionized water, followed by measurement of conductivity of bathing medium (EC1). Then, the samples were boiled for 15 min and the final conductivity (EC2) of the medium was measured using a conductivity meter (Consort C832).

Root samples were homogenized in 0.2N perchloric acid (pH 7.5). The flax H_2O_2 content in roots was determined as described by O'Kane et al. (1996). Protein-bound carbonyl content was determined by using the dinitrophenylhydrazine (DNPH) method, according to Reznick and Packer (1994). The level of carbonyl groups was estimated using an extinction coefficient of 22,000 $M^{-1} cm^{-1}$.

Total SOD activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition in the reduction of NBT as monitored at 560 nm. For the assay of APX activity, 2 mM ascorbate was added in the extraction medium. The reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.1% H_2O_2 , 0.5 mM ascorbate (extinction coefficient 2.8 $mM^{-1} cm^{-1}$) and root extract induced a linear decrease in absorbance at 290 nm for 25 s (Nakano and Asada, 1981). GPX activity was measured according to Landberg and Greger (2002) following the change in absorption at 470 nm. CAT activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 $mM^{-1} cm^{-1}$) at 240 nm for 30 s (Aeby, 1984). Soluble protein contents in enzyme extracts were determined by the method of Bradford (1976).

For antioxidant potential analysis, extracts were prepared following Ferreres et al. (2007). 20 mg of powder material was suspended in 2 mL of Milli-Q water, then boiled for 1 h at 100°C. Suspension was filtered and supernatant lyophilized (BETA 2-8 LD plus, Christ). The dry material was weighed and dissolved in Milli-Q water to reach a concentration of 10 mg mL^{-1} . Antioxidant potential of the samples was determined spectrophotometrically by monitoring the disappearance of radical DPPH according to the method of Brand-Williams et al. (1995). Readings were taken at 517 nm after 1 h of incubation at room temperature in a microplate reader (Spectra MR, DYNEX Technologies). After subtracting the value of the blank from each sample, absorbance was plotted against concentration of samples, EC50 values (concentrations which produced 50% inhibition) were computed for each extract and were used to compare among samples. Antioxidant potential of the samples was also measured by the FRAP assay developed by Benzie et al. (1996). Readings were taken at 593 nm after 1 h at room temperature. Sample absorbance was plotted against concentration of samples; the concentration which produced an absorbance of 1.00 was

TABLE 1. EFFECT OF SA ON ROOT GROWTH OF 16-DAY-OLD Cd-TREATED FLAX PLANTLETS

SA (μM)	Cd (μM)	Root				
		Dry weight (mg plant ⁻¹)	Length (cm)	Diameter (mm)	Surface (dm ² plant ⁻¹)	Volume (cm ³ plant ⁻¹)
0	0	5.51 ± 0.27 ^e	31.78 ± 1.81 ^{bc}	2.44 ± 0.15 ^c	14.16 ± 1.19 ^d	19.34 ± 1.17 ^d
0	50	4.34 ± 0.15 ^{bc}	16.09 ± 0.8 ^e	1.33 ± 0.03 ^{ab}	3.83 ± 0.21 ^b	2.70 ± 0.3 ^b
0	100	3.21 ± 0.13 ^a	12.05 ± 1.13 ^e	1.04 ± 0.02 ^a	1.63 ± 0.06 ^a	1.19 ± 0.05 ^a
250	0	5.21 ± 0.1 ^{cd}	45.69 ± 2.3 ^{cd}	3.16 ± 0.2 ^{cd}	13.39 ± 0.7 ^d	51.78 ± 2.3 ^g
250	50	5.55 ± 0.2 ^{ef}	31.82 ± 1.5 ^{bc}	2.69 ± 0.11 ^b	7.08 ± 0.26 ^c	21.38 ± 1.5 ^e
250	100	4.26 ± 0.11 ^{ab}	18.23 ± 0.13 ^a	1.16 ± 0.1 ^a	2.94 ± 0.02 ^{ab}	3.03 ± 0.3 ^{bc}
1000	0	5.59 ± 0.21 ^{ef}	40.90 ± 1.7 ^d	3.57 ± 0.25 ^d	16.01 ± 1.22 ^e	46.88 ± 2.1 ^f
1000	50	4.75 ± 0.25 ^{cd}	25.80 ± 2.31 ^b	1.41 ± 0.03 ^{ab}	4.13 ± 0.13 ^{bc}	7.37 ± 0.24 ^c
1000	100	3.75 ± 0.07 ^a	14.20 ± 0.7 ^e	1.28 ± 0.13 ^{ab}	1.81 ± 0.02 ^a	2.03 ± 0.19 ^{ab}

^aData are means of three independent experiments (\pm SE). ^bMeans with different letters indicate statistically different results at $p \leq 0.05$, according to Tukey's (HSD) test.

computed and was used to compare among samples. For both assays, three replications were analyzed for each sample and standard prepared with different concentrations of Trolox® were also measured. The results were expressed in μmol Trolox g^{-1} dry weight (DW).

Lipids in roots were extracted according to Garcés and Mancha (1993) using 30 mg plant tissue. Fatty acids were identified by comparing the retention times of flax root methyl esters with those of known mixtures of standard fatty acids (Sigma) run on the same column under the same conditions.

Statistical calculations were performed with SPSS-17 statistical software. Mean difference comparison among different treatments was done by ANOVA and Tukey's (HSD) test at a 0.05 probability level.

Results

When Cd was present in the nutrient solution, flax plantlets exhibited reduced root growth. Root dry weight, length, diameter, surface, and volume decreased proportionally with increasing concentrations of Cd (Table 1). In contrast, pre-soaking with SA for 8 h led to an increase in the root growth parameters in a Cd-dependent manner. As a result, already after 10 days exposure to Cd, the whole root system appeared less short and thick (Fig. 1).

In roots, Cd treatments showed a significant increase of H_2O_2 content compared with control (Fig. 2A). It increased by about 37% over control at 50 μM Cd and was further enhanced

to nearly 62% more over control at 100 μM Cd. However, upon the 8-h SA pretreatment, there was no significant decrease in the H_2O_2 content in flax roots compared to those with Cd only (Fig. 2A).

The damage by Cd to membranes was investigated by monitoring MDA content and electrolyte leakage (Fig. 2B and C). Relative to the control, Cd-treated flax plantlets exhibited a higher rate of lipid peroxidation. However, it was observed that 8-h SA pretreatment exhibited a significant reduction of Cd-increased MDA content (Fig. 2B). Electrolyte leakage was also altered by the 8-h SA pretreatment, but the extent of change was not as great as the change in the MDA level (Fig. 2C). Only at 100 μM Cd, the electrolyte leakage level in roots increased significantly by 1.6-fold as compared with the control. 8-h SA pretreatment counteracted the Cd-induced loss of membrane permeability. At the highest metal concentration, the most prominent effect was at 1000 μM SA; a nearly 46% decrease was noticed (Fig. 2C). Significant increases in total protein content were observed between treated and control flax plantlets irrespective of the Cd concentration, (Fig. 2D). Within both Cd concentrations (50 and 100 μM), 8-h SA pretreatment led to restoration of the protein content and that in a dose dependent way. Thus, at 100 μM Cd-treated plantlets, SA (250 or 1000 μM) inhibited the amount of protein oxidation in roots by about 2.4- and 1.4-fold, respectively, compared to control (Fig. 2E).

The effect of Cd on the activities of antioxidative enzymes (Fig. 3A–D) was significant in most Cd-exposed roots. CAT activity was significantly increased in Cd-treated roots while,

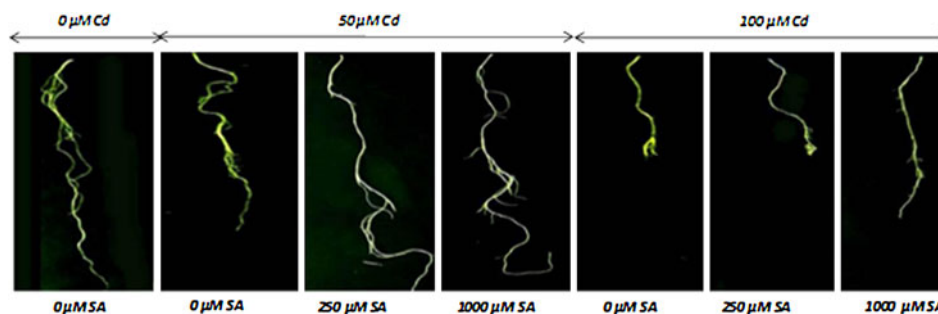


FIG. 1. Root morphological aspects of SA-pretreated flax plantlets grown in hydroponics culture and subjected for 10 days to increased CdCl_2 concentrations.

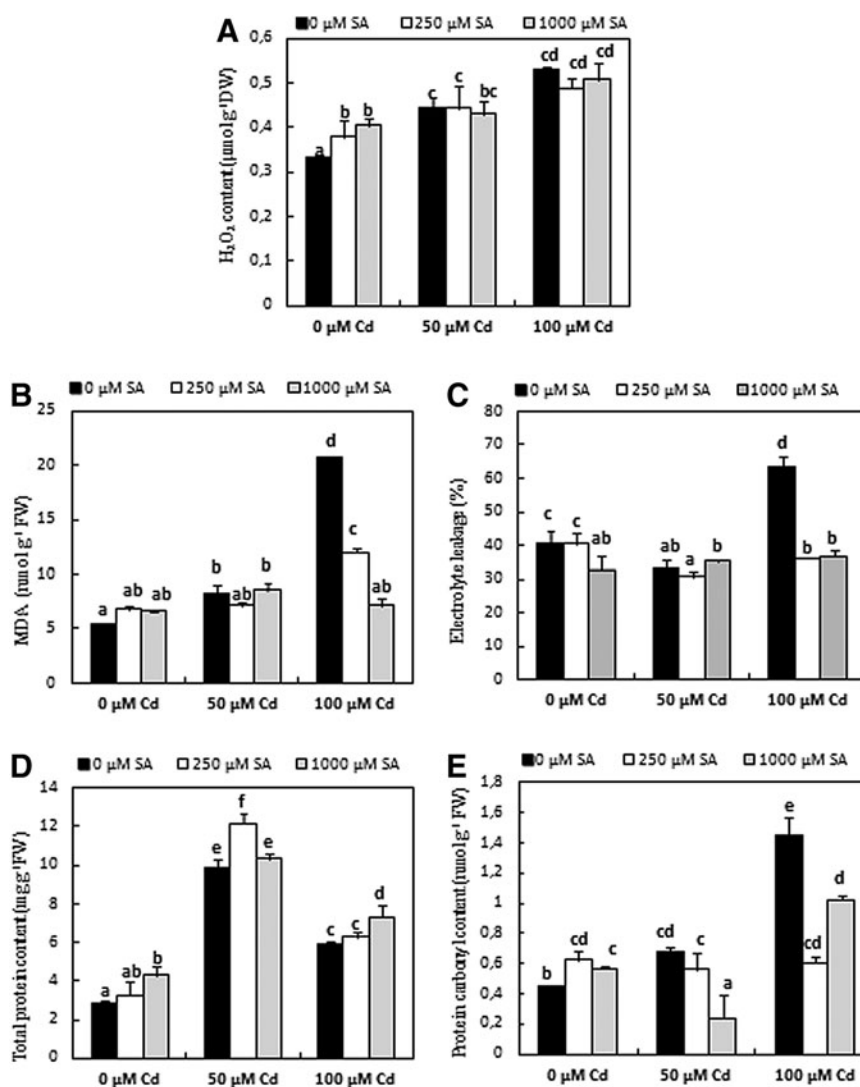


FIG. 2. The effects of salicylic acid (SA) on hydrogen peroxide content (A), lipid peroxidation (B), electrolyte leakage (C), total protein (D), and protein carbonyl (E) contents in roots of flax plantlets under cadmium (Cd) stress. Values are the means of 5 replicate experiments \pm SE. Bars with different letters are statistically different at $p \leq 0.05$.

8-h SA pretreatment alone (1000 μ M) decreased it, and the effect was similar with the addition of Cd (50 or 100 μ M) (Fig. 3A). After 10 days of Cd stress, root GPX and APX showed significantly higher activities levels than control, especially at 100 μ M Cd. A significantly enhanced response in both enzymes activities occurred by 8-h SA pretreatment followed by Cd exposure (Fig. 3B and C). In contrast, SOD activity was suppressed by Cd addition in the medium. Cd treatment caused an increase of 28% and 50% upon exposure to 50 and 100 μ M Cd, respectively (Fig. 3D). However, SA (250 or 1000 μ M) inhibited Cd-decreased activity of SOD (Fig. 3D).

At 50 and 100 μ M Cd, the total antioxidant capacities increased with respect to the control plantlets (Fig. 4A and B). These Cd-induced increases were further improved by the 8-h SA pretreatment but in a dose-dependent manner. In terms of 100 μ M Cd, the maximum values have been registered at 250 μ M SA for both analyzed tests (Fig. 4A and B).

The amount of total lipid (TL) evaluated from the amount of total fatty acids was changed in Cd-treated roots and highly dependent on the metal concentration in the medium (Fig. 5).

At 50 and 100 μ M Cd, TL content of roots was 40.5% and 79% reduced, respectively, compared to control. Only at the highest Cd dose did 8-h SA pretreatment significantly enhance TL content in roots of flax plantlets (Fig. 5). In controls, the fatty acids composition of TL in flax roots contained mostly linolenic (C18:3), linoleic (C18:2), and palmitic (C16:0) acids (about 87% of the total fatty acids). CdCl₂ (50 and 100 μ M) showed a slight statistically significant increase in the degree of fatty acid unsaturation in the roots (Table 2). The impact of Cd on the fatty acid composition of root cell membranes became more evident at higher concentration. The SA-induced change in the lipid unsaturation was similar to that caused by Cd. Interestingly, when Cd was added in the medium (50 or 100 μ M), 8-h pretreatment with SA (250 μ M) significantly increased the total extent of fatty acid unsaturation (Table 2).

Discussion

In this work, we investigate the potential defense mechanisms enabling primed plantlets to overcome Cd-induced

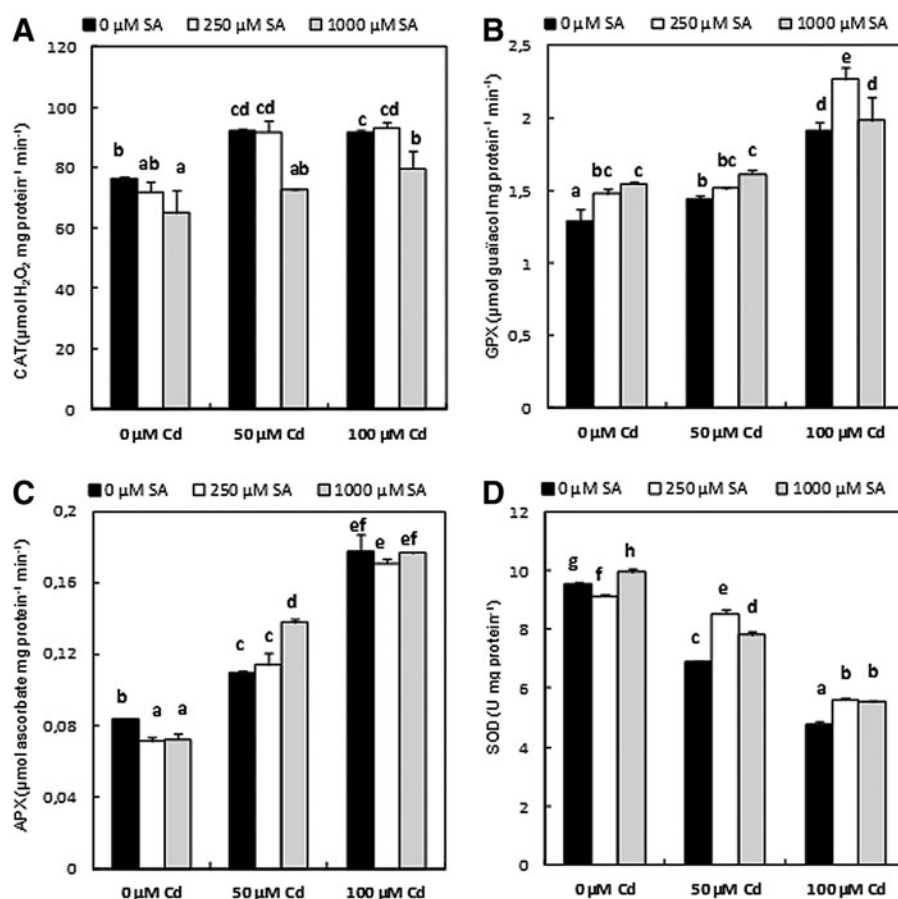


FIG. 3. The effects of salicylic acid (SA) on the activities of catalase (CAT) (A), guaiacol peroxidase (GPX) (B), ascorbate peroxidase (APX) (C), and superoxide dismutase (SOD) (D) in roots of flax plantlets under cadmium (Cd) stress. Values are the means of 5 replicate experiments \pm SE. Bars with different letters are statistically different at $p \leq 0.05$.

oxidative damage to proteins and membranes (oxidation, loss of membrane fluidity and integrity, and the degradation of its components). Flax roots exposed for 10 days to 50 and 100 μM CdCl_2 had reduced dry weight and the morphological characters such as surface, volume, and diameter (Table 1). These alterations observed at the root level could be a consequence

of Cd interference in plant metabolism (Liu et al., 2007), as well as modification in antioxidant enzyme activities (Rodríguez-Serrano et al., 2009).

The results showed that Cd decreased the biomass and induced the generation of H_2O_2 (Fig. 2A), which agrees with previous reports by Schützendübel and Polle (2002) for Scots

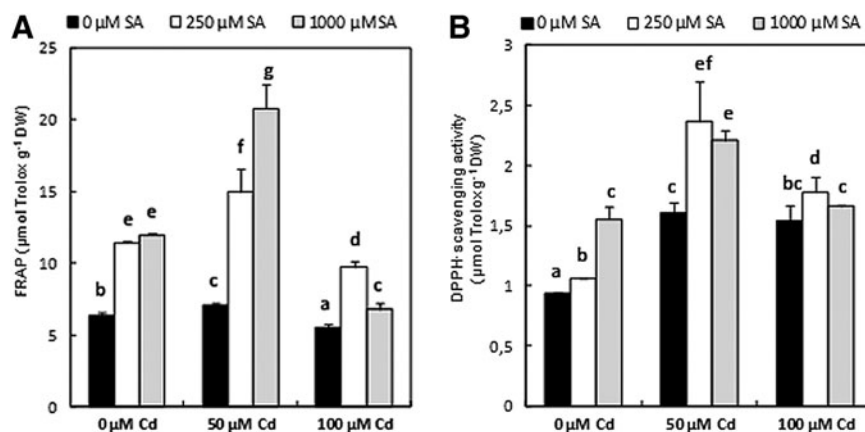


FIG. 4. The effects of salicylic acid (SA) on ferric reducing antioxidant power (FRAP) (A) and DPPH scavenging activity (B) in roots of flax plantlets under cadmium (Cd) stress. Values are the means of 5 replicate experiments \pm SE. Bars with different letters are statistically different at $p \leq 0.05$.

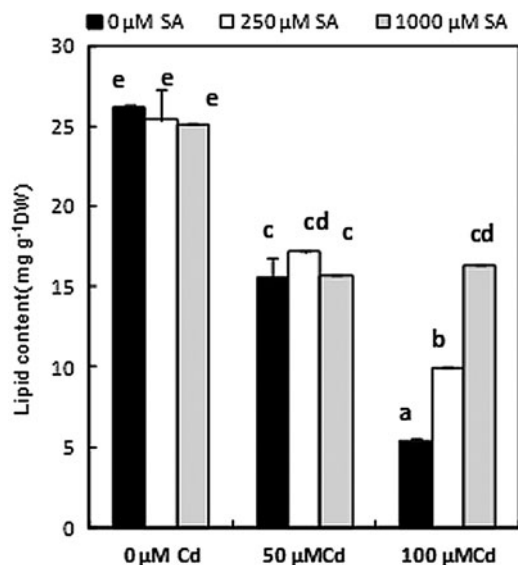


FIG. 5. The effects of salicylic acid (SA) on total lipid content in roots of flax plantlets under cadmium (Cd) stress. Values are the means of 5 replicate experiments \pm SE. Bars with different letters are statistically different at $p \leq 0.05$.

pine, Ortega-Villasante et al. (2007) for alfalfa, Liu et al. (2008) for tomato, and Gonçalves et al. (2009) for potato. Cd-increased H_2O_2 concentration led to lipid peroxidation, causing membrane damage and leakage of electrolytes (Schützendübel and Polle 2002). However, in this experiment, the reduction in biomass, induction of electrolyte leakage, lipid peroxidation, and protein carbonylation were partially alleviated by applying SA to Cd-treated flax plantlets (Table 1 and Fig. 2B-E). This is linked to the SA-increased levels of GPX, APX and SOD activities under Cd stress (Fig. 3B, C, and D).

Superoxide radicals generated by oxidative metabolism were dismutated into H_2O_2 and O_2 by SOD which acts as a first line of defense response *in planta* (Gratão et al., 2012, Radwan, 2012). The increased SOD activity due to SA might protect biomolecules from being attacked by superoxide radicals. It has been shown that exogenous SA application

resulted in the alleviation of Cd-induced ROS overproduction in *Arabidopsis thaliana* (Zhang and Chen, 2011) and maize seedlings (Radwan, 2012). Consequently, the lower level of lipid peroxidation, and thus the lower degree of membrane damage, might result from the SA-increased activity of SOD enzyme in flax roots (Fig. 3D).

Protein carbonylation may occur due to direct oxidation of amino acid side chains (e.g., proline, arginine, lysine, and threonine) by ROS and/or by protein reactions with lipid peroxidation products, such as 4-hydroxy-2-nonenal (HNE). Numerous studies have shown that Cd bioaccumulation lead to the oxidation of proteins in plant tissues (Romero-Puertas et al., 2002, Djebali et al., 2008, Douchiche et al., 2010), which is in agreement with our present findings (Fig. 2E). On the other hand, SA-decrease of lipid peroxidation may partially contribute to the Cd alteration effect on protein content in roots (Fig. 2B). In fact, several studies reported the increase in total protein content and formation of new proteins in pea due to SA treatment (Katoch, 2007, Çağ et al., 2009).

Plant membranes are the first functional structure to come into contact with toxic metals; it is considered to play a critical role in metal tolerance of plants. The presence of polyunsaturated fatty acids in the plasma membrane results in increased membrane permeability to ions (Hanzel and Williams, 1990). The depletion of unsaturated fatty acids may also indicate lipid peroxidation elicited by Cd (Ben youssef et al., 2005; Djebali et al., 2005). In a study of pea roots, Hernández and Cooke (1997) reported a similar decrease in the fatty acid saturation in response to Cd stress. SA acts in response to the Cd-induced changes in fatty acid saturation, [i.e., the increased unsaturation in the roots (Table 2)]. Moreover, it can be assumed that the defensive role of SA against Cd toxicity might be credited to its function in regulating root membrane permeability.

Other mechanisms involved in the prevention of Cd-induced membrane damage require the synthesis of antioxidant enzymes. In fact, an increase in antioxidant enzyme activities after Cd treatment has been detected in *Hordeum vulgare* (Guo et al., 2004); *O. sativa* (Hsu and Kao, 2004); *Triticum aestivum* (Khan et al., 2007); *Brassica juncea* (Mobin and Khan, 2007); *Vigna mungo* (Singh et al., 2008); *C. arietinum* (Hassan et al., 2008). In this study, Cd affected SOD, CAT, APX, and GPX

TABLE 2. EFFECT OF SA ON FATTY ACID OF TOTAL LIPIDS COMPOSITION IN ROOT OF 16-DAY-OLD FLAX PLANTLETS SUBJECTED DURING 10 DAYS TO Cd TOXICITY

Fatty acids (%TL)		Roots						
SA (μ M)	Cd (μ M)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Unsaturation
0	0	30.22 \pm 1.68 ^{ab}	Tr	5.96 \pm 0.60 ^a	6.62 \pm 0.82 ^{ab}	43.78 \pm 0.58 ^{bc}	13.40 \pm 0.33 ^{ab}	69.77 \pm 1.66 ^a
0	50	29.75 \pm 0.15 ^c	Tr	6.88 \pm 0.34 ^{ab}	6.74 \pm 0.24 ^{ab}	42.08 \pm 0.18 ^b	14.55 \pm 0.52 ^b	70.25 \pm 0.13 ^a
0	100	27.13 \pm 0.83 ^a	Tr	8.19 \pm 0.29 ^{bc}	8.15 \pm 0.32 ^{bc}	42.51 \pm 0.54 ^b	13.94 \pm 0.62 ^{ab}	72.78 \pm 0.54 ^b
250	0	30.02 \pm 0.73 ^c	Tr	6.04 \pm 0.90 ^a	6.21 \pm 0.77 ^a	43.55 \pm 2.29 ^{bc}	14.18 \pm 0.12 ^b	69.99 \pm 0.74 ^a
250	50	27.45 \pm 1.00 ^a	Tr	7.63 \pm 0.13 ^b	7.73 \pm 0.04 ^b	41.97 \pm 1.30 ^b	15.21 \pm 2.48 ^c	72.55 \pm 1.00 ^b
250	100	28.43 \pm 0.73 ^{ab}	Tr	8.11 \pm 0.06 ^{bc}	8.14 \pm 0.09 ^{bc}	39.87 \pm 0.47 ^a	15.43 \pm 0.12 ^c	71.55 \pm 0.73 ^{ab}
1000	0	29.72 \pm 0.15 ^c	Tr	6.02 \pm 0.67 ^a	5.85 \pm 0.45 ^a	44.66 \pm 3.20 ^c	13.74 \pm 2.23 ^{ab}	70.28 \pm 0.15 ^a
1000	50	30.07 \pm 1.16 ^c	Tr	7.22 \pm 0.12 ^b	7.52 \pm 0.19 ^{bc}	42.52 \pm 0.4 ^b	12.70 \pm 0.67 ^a	69.94 \pm 1.15 ^a
1000	100	28.41 \pm 0.45 ^{ab}	Tr	8.02 \pm 0.35 ^{bc}	8.09 \pm 0.28 ^{bc}	41.51 \pm 1.00 ^b	13.98 \pm 0.04 ^{ab}	71.59 \pm 0.41 ^{ab}

^aData are means of three independent experiments (\pm SE). ^bMeans with different letters indicate statistically different results at $p \leq 0.05$, according to Tukey's (HSD) test, (Tr, trace).

activities in flax plantlets, with their impact being higher at 100 μ M Cd (Fig. 3). In the roots, Cd, especially at this concentration, tended to increase their activities (with the exception of SOD). The severe Cd-decrease in SOD activity might be caused by the continuous competition between Zn and Cd for the same sites in Cu/Zn SOD. Similarly, Rodriguez-Serrano et al. (2009) showed that Cd-decrease in Ca lead to Cu/Zn SOD downregulation, which resulted in the overproduction of superoxide radicals in *Pisum sativum*.

However, our results showed that the 8-h SA pretreatment caused a significant increase in GPX, APX, and SOD, and a decrease in CAT activity in Cd-treated plantlets (Fig. 3A-D). In barley seedlings treated with Cd, Metwally et al. (2003) reported that total root APX activity reflected the cytosolic isoforms and the Cd response was fully suppressed by SA. Conversely, the same authors reported that CAT activity and expression level decreased upon SA pretreatment. According to Janda et al. (1999), a new peroxidase isoform was detected in maize treated with SA, even the total activity did not increase significantly.

The effect of SA on the activation of SOD may facilitate the integrity of membrane structures of root cells, because SOD is involved in the processes of lipid peroxidation deactivation (Zenkov et al., 2001). But, the significance of catalase inhibition could be partly due to the possible binding of SA to CAT. In fact, SA has proved capable of binding directly to CAT enzyme (Chen et al., 1993), isolated from tobacco, inhibiting its activity (Conrath et al., 1995). The *in vitro* SA catalase-inhibiting effect has also been demonstrated in many other plant species, such as *Arabidopsis*, tomato, cucumber (Sánchez-Casas and Klessig, 1994), and tobacco (Horváth et al., 1998).

Numerous studies showed that exogenous application of SA can influence the antioxidant capacity of plant cells (Janda et al., 2003, Ananieva et al., 2004, Radwan, 2012). Besides, since adaptation to oxidative stress involves not only the regulation of the synthesis and repair of proteins but also enhanced antioxidant capacity (Ananieva et al., 2004), two different tests were used in this work to confirm the SA-induced changes in antioxidant activities of Cd-treated plantlets (Fig. 4A and B). In roots, Cd stress significantly increased FRAP and DPPH-radical scavenging activity. This effect was improved by SA in Cd-stressed plantlets.

Conclusion

To summarize, it was demonstrated that the exogenous 8-h SA pretreatment may improve the tolerance of flax roots to Cd-induced oxidative stress. Moreover, SA-enhanced antioxidant enzymes SOD, APX, and GPX maintained ROS such as superoxide radicals at minimum levels with respect to 100 μ M Cd treatment. Therefore, SA-increase of the total antioxidant capacities in Cd-treated flax roots avoids its deleterious effects in cell membranes. For these reasons, we can conclude that due to Cd high toxicity and its potent free-radical scavenging ability, SA-induced priming could be used as a potential preventive action taken to limit oxidative damages.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

APX = ascorbate peroxidase
 CAT = catalase
 Cd = cadmium
 DPPH = 2,2'-diphenyl-1-picrylhydrazyl
 DW = dry weight
 FRAP = ferric reducing antioxidant power
 GPX = guaiacol peroxidase
 H₂O₂ = hydrogen peroxide
 MDA = malondialdehyde
 SA = salicylic acid
 SOD = superoxide dismutase (SOD)
 TL = total lipid