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Full Length Research Paper

Influence of salicylic acid pre-treatment on cadmium tolerance and its relationship with non-protein thiol production in flax root

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Dose-dependent changes in cadmium (Cd) tolerance, non-protein thiol (NP-SH) production and their relationship were investigated in sixteen-day-old flax (*Linum usitatissimum* L.) seedlings derived from seeds pre-soaked with various salicylic acid (SA) doses and grown hydroponically under increased Cd concentrations (0, 50 and 100 µM $CdCl_2$). The results show that single Cd subjection decreased root elongation as expressed by tolerance index (TI). Moreover, an overproduction of NP-SH was detected in both roots and shoots. These Cd toxicity effects were directly related to the high levels of Cd amounts in flax tissues as expressed by root and shoot Cd bioaccumulation factors (BAF). In addition, Cd-tolerance of roots TI was negatively correlated with changes in root BAF but positively correlated with shoot BAF. However, positive correlation was illustrated between root TI and NP-SH contents. SA considerably reversed the Cd-induced decrease in root growth parameters and TI. Moreover, in Cd-treated plants, SA pre-soaking prevented Cd accumulation in the shoot as consequence of significant decreases in BAF of roots, Cd transport estimated by the translocation factor (TF) and shoot BAF, respectively. Interestingly, SA pre-treatment reduced BAF of roots and shoots, enhanced NP-SH production in roots and decreased it in leaves. These results suggest that SA might play a preventive role in Cd uptake, sequestration and translocation processes based primarily in roots where SA-enhanced NP-SH contribute to the improvement of flax tolerance to Cd stress.

Key words: Cadmium, salicylic acid, bioaccumulation, growth, non-protein thiols, *Linum usitatissimum.*

INTRODUCTION

Anthropogenic activities and particularly new industrial applications increased significantly Cd dissemination and intensified the worldwide problem of heavy metal environmental pollution (Butterman and Plachy, 2002). Use of phosphatic fertilizers, irrigation with wastewater and application of municipal based composts order to be the foremost sources of agricultural soil contaminations by Cd (Gupta et al., 2008; McGrath et al., 2001; Yang et al., 2004). In higher plants, Cd bioaccumulation can occur in edible parts of crop plants to a level which is potentially health-threatening for humans without manifestation of any phytotoxicity symptoms (Sanità di Toppi and Gabbrielli, 1999). Moreover, the degree of plant tolerance to Cd toxicity represents the first activator of the metal entry into the human food-chain (Hédiji et al., 2010; Obata and Umebayashi, 1997). In this context, flax can accumulate high levels of Cd and is considered as a tolerant species, with a TI estimated between 40 and 62% (Shi and Cai, 2009). Conversely, Cd hyper-accumulator plants have the potential to be used in special phytoremediation strategies of contaminated soils (Ma et al., 2001). Plants have adopted different strategies of tolerance to Cd stress (Hall, 2002). Some are able to avoid the toxicity effects through Cd-binding to the cell wall, by reducing its transport across the cell membrane and/or by active efflux (Cumming and Taylor, 1990; Douchiche et al., 2010; Hall, 2002; Li et al., 2002). Others can also tolerate the exposure to Cd by its chelation and compartmentalization into the vacuole (Briat and Lebrun,

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1999; Clemens, 2006; Djebali et al., 2002). However, Cd-binding represents a key mechanism, widely studied in the investigation of plant responses to Cd stress. Inside cells, Cd can be chelated by (i) organic acids such as citrate and malate (Clemens, 2001; Rauser, 1999), (ii) metal binding proteins (MBP) like metallothioneins and also (iii) metal binding complexes (MBC) for instance NP-SH (Cobbet, 2000).

In plants, NP-SH compounds exist under various forms (i) the tripeptide: glutathione (γ-glutamyl cysteinyl glycine) and (ii) polymerized peptides (phytochelatins). These molecules are synthetized by enzymatic polymerization (γ-glutamyl-cysteine synthase) of glutathione which exist in the thiol-reduced and disulfide-oxidized forms (Gekeler et al., 1989). They play a key role in the regulation of redox balance and can be used as an indicator of oxidative stress (Rijstenbil and Wijnholds, 1996), in the detoxification of xenobiotics (Marrs, 1996) and heavy metals (Cobbett, 2000). The influence of NP-SH upon Cd is due to its extremely high affinity for the thiolic groups (Baudouin-Cornu and Labarre, 2006; Sun et al., 2005). For limiting the circulation of toxic free Cd ions inside the cytosol, NP-SH compounds form complexes with Cd which are accumulated in the vacuole through the activity of ABC-type transporters (Sanità di Toppi and Gabbrielli, 1999). SA is an important signaling molecule in plants and induces plant tolerance to various biotic and abiotic stresses (Horvath et al., 2007). It is reported that exogenous application of SA regulated various physiological activities in plants such as growth and development, ion uptake and transport and membrane permeability (Guo et al., 2009; Metraux et al., 1990; Raskin, 1992). SA-induced alleviation of salinity tolerance has been observed in tomato plants (Stevens et al., 2006). It has been revealed in previous studies that SA mitigates Cd negative effects on growth in barley (Metwally et al., 2003), soybean (Drazic and Mihailovic, 2005), rice (Guo et al., 2007), maize (Krantev et al., 2008) and pea (Popova et al., 2009). Ahmad et al. (2011) showed that exogenous application of SA can alleviate Cd toxicity in mustard by regulating the antioxidant defense system and metabolites involved in Cd tolerance. Thus, SA seems to play a supportive role in plant tolerance to Cd stress. In order to further establish the presumption that SA has a beneficial effect in plants toward Cd toxicity, the present study was undertaken to (i) investigate the uptake, accumulation and translocation of Cd under the influence of SA pre-treatment and (ii) examine the potential role of SA on NP-SH production to induce Cd detoxification and tolerance in flax plants.

MATERIALS AND METHODS

SA pre-treatment application and stress conditions

Flax seeds (*L. usitatissimum* L. var. Viking) were soaked during 8 h in 250 and 1000 µM SA solutions, as previously reported (Belkhadi et al., 2010). All of the seeds (pre-treated and control) were led to germinate on moistened filter paper for 4 days in the darkness at 25°C and then transferred to a growth chamber with a 16 h day (23°C)/ 8 h night (18°C) cycle, an irradiance of about 200 µmol photons m^{-2} s^{-1} and 75 to 80% humidity. At least, ten seeds were sown per pots. Each pot was continuously aerated and contained 6 liters of nutrient solution (pH 5.5) with the following composition: 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 µM ethylenediaminetetraacetic acid (EDTA)-Fe-K, 30 µM H_3BO_3, 10 µM $MnSO_4$, 1 µM $ZnSO_4$, 1 µM $CuSO_4$, 30 µM $(NH_4)_6Mo_7O_{24}$. Two days after transplanting, $CdCl_2$ was added to a fresh solution at 50 or 100 µM. Details of whole treatments per pots were finally as follows: 0 µM SA + 0 µM Cd, 0 µM SA + 50 µM Cd, 0 µM SA + 100 µM Cd, 250 µM SA + 0 µM Cd, 250 µM SA + 50 µM Cd, 250 µM SA + 100 µM Cd, 1000 µM SA + 0 µM Cd, 1000 µM SA + 50 µM Cd, 1000 µM SA + 100 µM Cd. After 10 days of Cd treatment, plant organs were collected to determine fresh weight (FW) and dry weight (DW, dried in oven at 60°C until constant weight).

Determination of Cd contents

Dried samples were digested in 3/1 ratio (v/v) $HNO_3/HClO_4$ mixture. Cd concentrations were determined by atomic absorption spectrometry (Perkin-Elmer, Analyst 300) using an air-acetylene flame.

Determination of total non-protein thiol contents

For the determination of total NP-SH, the frozen samples (200 mg FW) was homogenized in 100 mM HCl/1 mM EDTA solution and centrifuged at 12 000 g for 5 min at 4°C. Concentrations of NP-SH were determined as described by Noctor and Foyer (1988) using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) reagent and the absorbance was measured spectrophotometrically at 412 nm.

Data analysis

Cd content in roots and shoots was calculated using the BAF, which represents the ability of the plant to accumulate the metal in the considered tissue taking account its concentration in the medium (Zayed et al., 1998), calculated as follows: BAF = Cd concentration in plant tissue (mg kg^{-1}) at reap/Cd concentration added in the external nutrient solution (mg L^{-1}). The TF gives estimation about the plant aptitude to transport Cd into aerial parts and was defined as: TF = Cd concentration in shoots / Cd concentration in roots (that is, TF = shoots / roots BAF ratio). The TI of root and shoot was calculated according to the following equation: TI (%) = (organ length of Cd-treated plants / organ length of control) x 100

Statistical data

Each data point is the average of six replicates obtained from three independent experiments (n = 18). Statistical analyses were made with one-way analysis of variance (ANOVA) and Pearson correlation were performed by SPSS Version (17.0) as a function of SA pretreatments and exposure to Cd concentrations. When the differences were found, the Tukey's (HSD) test was used to establish the differences between specific treatments. Statistical differences were significant at $P \leq 0.05$.

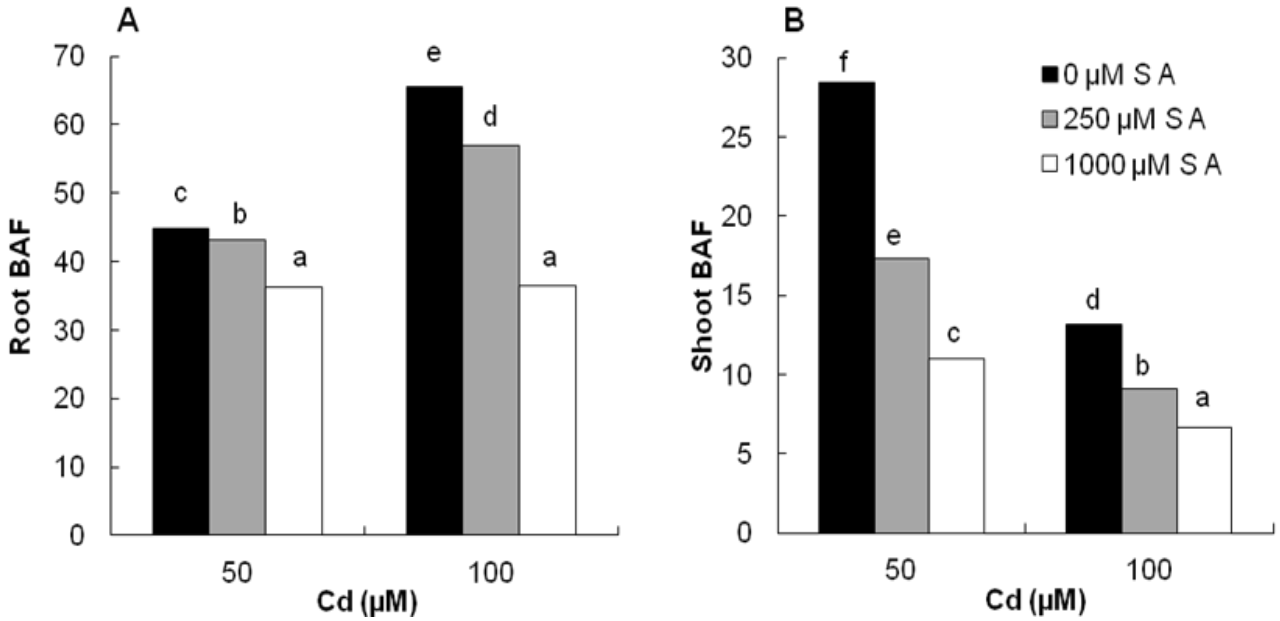

Figure 1. Effect of SA pre-treatment on Cd bioaccumulation factor (BAF) of roots (A) and shoots (B) of flax seedlings exposed during 10 days to increased levels of Cd concentrations. Data are means of three independent experiments (n=18). Changed letters indicates statistically different results at $P \leq 0.05$, according to Turkey's (HSD) test.

RESULTS

Effect of SA on Cd bioaccumulation and translocation

Cd bioaccumulation in roots and shoots of 16-day-old flax plants is shown in Figure 1. With increasing level of Cd concentration in the nutrient solution, there is mainly increase of Cd bioaccumulation in roots (BAF of roots varied from 44 to 65) and a significant decrease in shoots (BAF of shoots varied from 28 to 13). Cd bioaccumulation in roots was substantially higher than in shoots. Whatever was the concentration of Cd in the medium, SA decreased significantly BAF of roots and shoots. This decrease was dose and organ dependant. In roots, SA and Cd act antagonistly; at 50 and 100 µM Cd, respectively BAF decreased with increasing SA concentrations (Figure 1a). Similarly, in shoots, SA also decreased Cd bioaccumulation in a dose dependant manner but operated in synergy with Cd (Figure 1b). The translocation of Cd from roots to upper parts of flax seedlings can be expressed by the TF represented in Figure 2. Considering Cd-treated plants, the TF varied from 0.6 to 0.2, respectively at 50 and 100 µM Cd. Pre-treatment with increasing SA concentrations inhibited more the translocation of the metal to the aerial parts in a dose-dependent manner. In the presence of 50 µM Cd, pre-treatment with SA (1000 µM) had the lowest TF (0.3). However, at 100 µM Cd, SA (250 µM) represented the less significant TF (0.1). Hence, at the lowest and highest Cd-concentrations, SA induced inhibition of Cd translocation to shoots.

Effect of SA on growth of Cd-treated plants

In response to Cd presence in the nutrient solution, total DW of flax plants are reduced significantly with increasing level of Cd stress (Table 1). At the highest Cd concentration, the DW of roots, stems and leaves were decreased by about 41, 47 and 50%, respectively. However, exogenous application of SA diminished this Cd-induced growth inhibition in a dose dependant manner. Furthermore, SA, at the concentration of 1000 µM, increased leaf area of Cd-stressed plants. Indeed, in the presence of 50 and 100 µM Cd, an enhancement by about 36 and 50% of the leaf area was noticed, respectively. Moreover, SA (250 + 1000 µM) in the occurrence of both Cd concentrations led to an increase in shoot/root ratio. This SA effect was more obvious at 100 µM Cd. Indeed, (1000 SA + 100 Cd) treated plants showed an increase in shoot/root ratio from 1.85 to 2.39 (Table 1). In flax plants, although Cd bioaccumulation was mainly in roots, these organs were less damaged in term of biomass than shoots particularly under the influence of SA. In fact, the degree of flax tolerance to Cd stress was investigated by monitoring TI of roots and shoots (Figure 3). Under Cd stress, TI-values of the roots were augmented with increasing levels of SA pre-treatment but in a dose dependant way (Figure 3a).

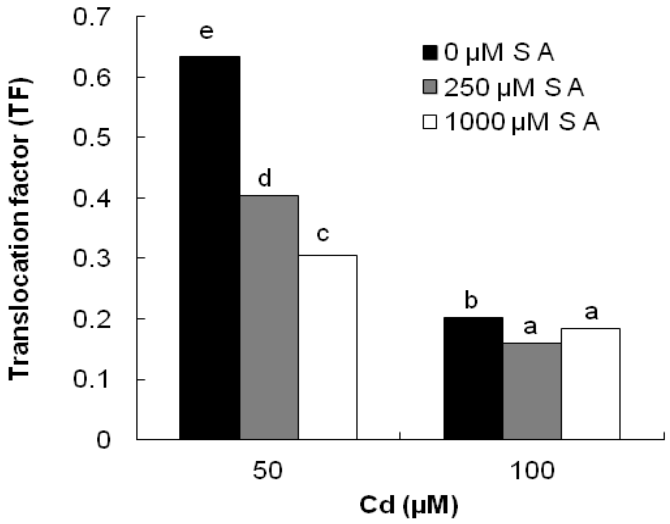

Figure 2. Effect of SA pre-treatment on Cd translocation factor (TF) of flax seedlings exposed during 10 days to different Cd concentrations. Data are means of three independent experiments (n=18). Changed letters indicates statistically different results at $P \leq 0.05$, according to Turkey's (HSD) test.

These results illustrate that SA pre-soaking with appropriate doses could enhance Cd-tolerance. Moreover, in the presence of Cd, plants pre-treated with 250 µM SA showed the highest root TI varied between 133 and 76%, at 50 and 100 µM Cd, respectively (Figure 3a). In shoots, SA pre-soaking induced a significant increase in TI of all Cd-treated plants (Figure 3b).

Effect of SA on total NP-SH compounds

After 10 days of Cd stress, the levels of NP-SH in roots and leaves of flax seedlings increased significantly according to the rise of Cd concentration in the medium (Figure 4a). Thus, this increase in both organs was also dominated by the pre-treatment with SA. In roots, SA enhanced significantly NP-SH content in a Cd-dose dependent mode (Figure 4a). In fact, under 100 µM Cd, an increase by about 1.3 and 1.2 times was noted in flax plants pre-soaked with 250 and 1000 µM SA, respectively in comparison with the control. However, an opposite effect of SA pre-treatment was marked in leaves resulting in a reduction in NP-SH content with or without Cd (Figure 4b). Data shown in Figure 5 indicated that there is a strong correlation between Cd bioaccumulation and NP-SH production in the root of flax seedlings. After Cd subjection, NP-SH accumulation in roots enhanced concomitantly with the increase in BAF values. Furthermore, the correlation also illustrated that SA-pretreated plants showed two similar behaviors in their response to Cd treatments: (i) a decrease in root BAF and (ii) an increase of NP-SH contents. This analysis suggests that the marked improvement of tolerance in SA-pretreated plants toward Cd is related to SA inference in Cd uptake and chelation processes.

DISCUSSION

In flax plants, our data showed an increase in BAF of roots, closely proportional to the Cd concentration in the medium. The assessment of the consequences of Cd bioaccumulation revealed that due to their aptitude to accumulate Cd in the root, flax seedlings manifested only a slender chlorosis which meant that root system may constitute an effective barrier against the transport of the metal into the upper parts in order to lessen its damaging effects on photosynthetic organs. In fact, in previous studies, a significant decrease in chlorophyll content has been reported in flax seedlings exposed to Cd stress (Belkhadi et al., 2010; Douchiche et al., 2010). On the other hand, under our experimental conditions and in the presence of Cd, BAF of roots was strongly higher than BAF of shoots resulting in a weak TF (<1) observed almost in all treatments. Also, the root Cd-tolerance indicated high values of TI varied from 67 to 40% in Cd-treated plants. Besides, it is well known that plants which accumulate at least 100 ppm Cd in shoot are considered as Cd hyperaccumulator but to be selected as a candidate for phytoremediation of polluted soils, an important production of biomass must be noticed (Kramer et al., 2000). However, in addition to the shoot Cd amounts and growth performance, TF > 1 is another criterion that should also be considered when evaluating

Table 1. Effect of Cd (50 and 100 µM) and SA (250 and 1000 µM) on root stem and leaf growth parameters.

Treatment	Root DW (mg plant^{-1})	Stem DW (mg plant^{-1})	Leaf DW (mg plant^{-1})	Total DW (mg plant^{-1})	Shoot/Root ratio	Leaf area (cm^2)
0 SA + 0 Cd	5.51 e	4.15 b	7.45 d	17.11 f	2.11 d	7.86 e
0 SA + 50 Cd	4.34 bc	2.91 a	4.58 b	11.83 b	1.73 a	4.52 b
0 SA + 100 Cd	3.21 a	2.19 a	3.74 a	9.14 a	1.85 b	3.58 a
250 SA + 0 Cd	5.21 cd	6.22 cd	6.99 cd	18.42 h	2.54 i	10.62 f
250 SA + 50 Cd	5.55 ef	7.20 e	5.05 ab	17.80 g	2.21 e	5.33 cd
250 SA + 100 Cd	4.26 ab	5.82 c	4.86 ab	14.94 e	2.51 h	4.32 bc
1000 SA + 0 Cd	5.59 ef	6.85 d	6.57 c	19.01 i	2.40 g	12.14g
1000 SA + 50 Cd	4.75 cd	5.07 bc	4.41 ab	14.23 d	2.00 c	6.18 d
1000 SA + 100 Cd	3.75 a	4.68 bc	4.28 ab	12.71 c	2.39 f	5.40 d

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

Table 2. Interrelationships (coefficients of correlation, r) established between Cd bioaccumulation and physiological and biochemical characters of SA-presoaked flax plants in response to Cd stress.

Character	Root BAF	Shoot BAF	TF	Root TI (%)	NP-SH in leaves (µmol g^{-1} FW)	NP-SH in roots (µmol g^{-1} FW)
Root BAF	1					
Shoot BAF	0.051	1				
TF	0.031	0.998**	1			
Root Ti (%)	-0.063	0.773**	0.765**	1		
NP-SH in leaves (µmol g^{-1} FW)	0.851**	-0.415**	-0.441**	-0.400**	1	
NP-SH in roots (µmol g^{-1} FW)	0.517**	-0.709**	0.267*	-0.681**	0.697**	1

[a] **Correlation is significant at the 0.01 level. BAF, Bioaccumulation factor; TF, translation factor; TI, tolerance index; NNP-SH, non protein thiol.

hyper-accumulation, measuring the ability of the plant to translocate Cd from roots to shoots (Ma et al., 2001).

Based on these data, even if flax is demonstrated as a tolerant plant, it cannot be considered as hyperaccumulator since the criteria of high growth performance and TF > 1 are inappropriate. Conversely, our plant may constitute an interesting material for studying Cd accumulation and tolerance mechanism in polluted soils. Our experiments illustrated that the pre-soaking of flax seeds during 8 h with a range of SA concentrations (0, 250 and 1000 µM) reduced both BAF of shoots and roots, which could be due to the fact that SA reduced the uptake of Cd and its translocation to shoot throughout xylem vessel. Cd accumulation in cells is dependent on membrane integrity (Djebali et al., 2005) and cation transporter activity (Yazaki et al., 2006). In fact, it is well known that Cd entry via Zn, Fe, and Ca transporters is the basis process of its uptake by root cells (Hall and Williams, 2003; Welch and Norvell, 1999). This absorption and transport of Cd happens because of the similarity in the electron structure between Cd and essential cations (Cosio et al., 2004). However, the beneficial effect of SA on Cd bioaccumulation could be credited to the enhancement of cation uptakes (Ca, Mg and Fe) in Cd-treated plants (Belkhadi et al., 2010). Similarly, it had been reported that supply of essential cations such as Zn (Aravind and Prasad, 2005), Ca (Boulila et al., 2006) and Fe (Sharma et al., 2004) had protective effect against Cd toxicity. Correspondingly, the pre-treatment with SA significantly lessens growth inhibition and enhanced root tolerance assessed by the TI. Because plant roots are first to face toxic metal and play a key role in the determination of the translocation rate to the upper parts of the plant, the measurement of processes happening in roots could clearly identify how plants respond to toxic metals. Furthermore, in earlier studies, NP-SH production has been considered as an indicator of Cd stress and had as a function the Cd detoxification, chelation and binding in the cytosol before its sequestration in vacuoles (Vögeli-Lange and Wagner, 1990). Besides, in plants not considered as hyper-accumulator but tolerant, roots had been deemed as the major site of NP-SH synthesis owing to the excessive internal accumulation of free Cd ions inside cells (Clemens, 2006).

In addition, it has been showed that 90% of NP-SH compounds induced by Cd in wheat roots were accounted for by phytochelatins (Maier et al., 2003). These authors

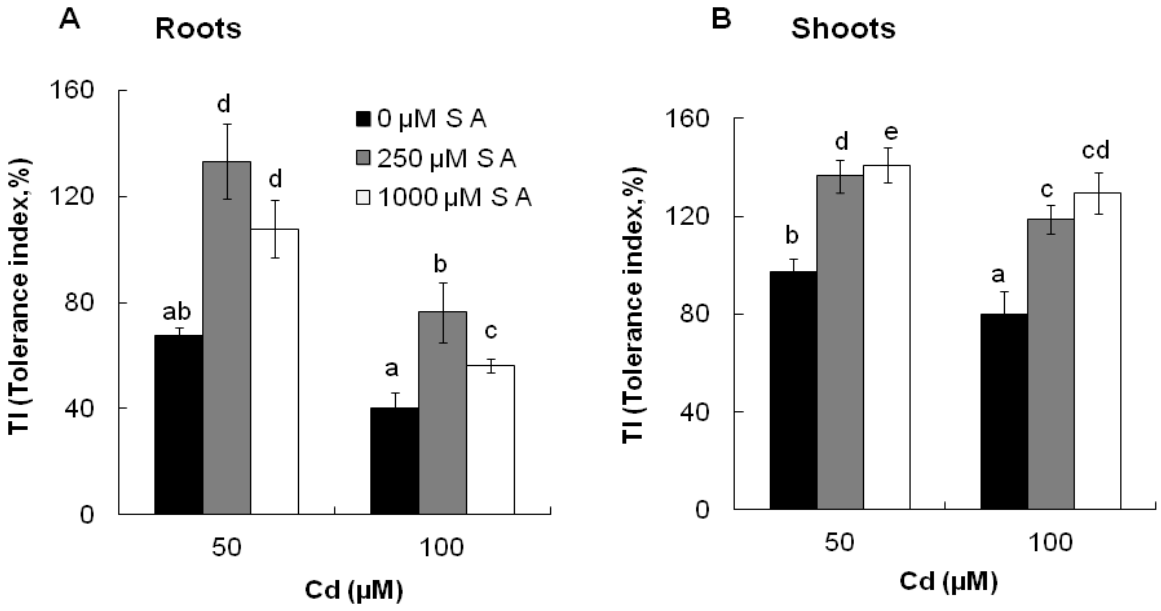

Figure 3. Effect of SA pre-treatment on tolerance index in roots (A) and shoots (B) of flax seedlings treated during 10 days with increasing Cd levels. Data are means of three independent experiments (n=18). Changed letters indicates statistically different results at $P \leq 0.05$, according to Turkey's (HSD) test.

also agree with the hypothesis that aside from detoxification, NP-SH plays an important role in intracellular metal homeostasis (Grill et al., 1989; Thumann et al., 1991). Besides, under our present experiments, Cd was found to induce significantly NP-SH synthesis in both roots and shoots. This increase was Cd-concentration dependent. However, under the influence of Cd, roots exhibited higher amounts of NP-SH than shoots. Correspondingly, it was also reported that intracellular NP-SH production is directly related to free cytosolic Cd ion concentrations (Ahner et al., 1994). Based on these experiments, the present study provides that there is a dependent relationship between Cd bioaccumulation in the tissue (BAF of shoots and roots) and the corresponding NP-SH amounts. In parallel, we suggest that pre-treatment with SA significantly improves plant tolerance to Cd stress by the enhancement of NP-SH amounts in roots. This could be due to the SA role in alleviating metal-induced oxidative stress (Guo et al., 2007). In fact, the antioxidant property of NP-SH depends on the oxidation of –SH group of the tripeptide to disulfide form (Meister, 1995; Noctor and Foyer, 1998). Generally, the plant exhibition of high amount of NP-SH during Cd stress indicates its ability to tolerate cellular metal load. The increased level of NP-SH may also be due to the stimulation of sulfate reduction pathway enzymes such as Adenosine-5'-phosphosulfate (APS) reductase and serine acetyltransferase (Freeman et al., 2004); while decreases observed at shoot level could possibly due to NP-SH consumption for glutathione (GSH) and phytochelatins (PCs) synthesis (Seth et al., 2007).

Greater effort has been made to correlate internal Cd accumulation in plant and their response to the toxic effects of the metal. However, our results clearly showed that the evaluation of Cd enhancement of tolerance in flax was consistent by evaluating the effects of SA on Cd bioaccumulation and NP-SH production in plant tissues. Besides, our results showed that SA pre-treatment with proper doses significantly decreased Cd bioaccumulation in flax roots and consequently enhanced root tolerance and Cd-induced NP-SH production. Under Cd stress, linear relationships between metal toxicity, measured as growth inhibition, and expression of NP-SH have been found in *Silene vulgaris, Zea mays, Tristicum aestivum* and *microalga Stichococcus bacillaris* (De Knecht et al., 1995; Schat and Kalff, 1992; Sneller et al., 1999). However, present results were further in favor of a potential role for NP-SH related to metal tolerance. In SA pre-treated plants, the degree of root tolerance to Cd stress was positively correlated with Cd-induced NP-SH amounts. SA application enhanced Cd tolerance in flax seedlings by increasing NP-SH production by roots. Considering this relationship between NP-SH and Cd tolerance, several studies have been carried out to relate metal toxicity to the level of NP-SH in plant. For example, according to Metwally et al. (2005) pea genotypes responded to Cd toxicity with a significant increase in sulphydryl groups (NP-SH) that was not correlated with their tolerance to Cd. Previously, Nussbaum et al. (1988) reported that the enhancement of cysteine levels and NP-

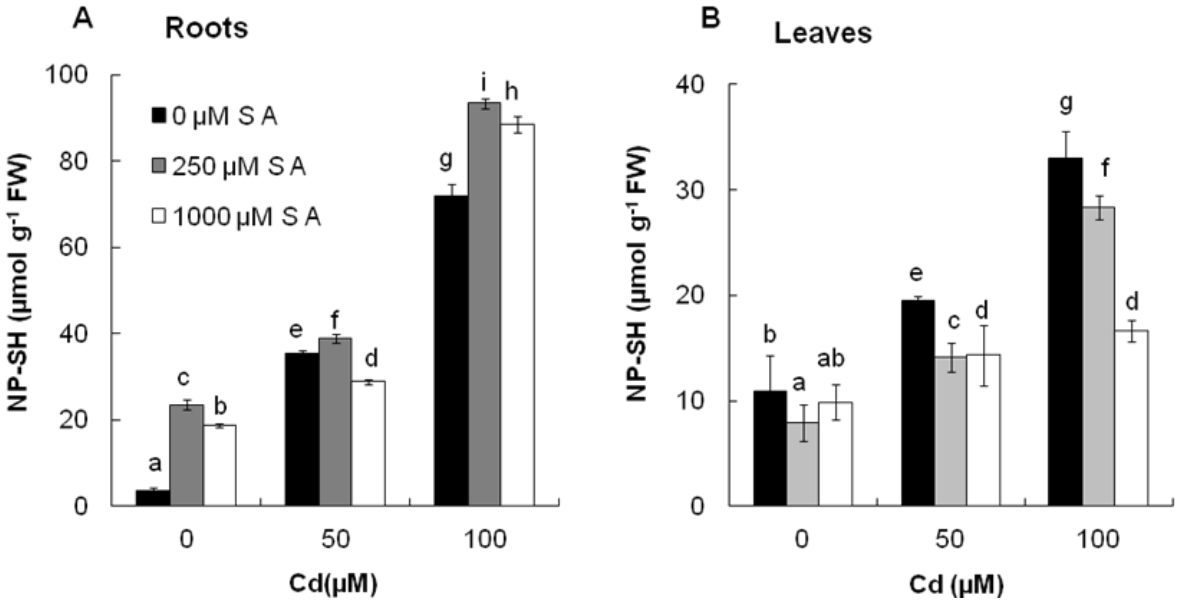

Figure 4. Effect of SA pre-treatment on non-protein thiol (NP-SH) content in roots (A) and leaves (B) of flax seedlings exposed during 10 days to increased Cd levels. Data are means of three independent experiments (n=18). Changed letters indicates statistically different results at $P \leq 0.05$, according to Turkey's (HSD) test.

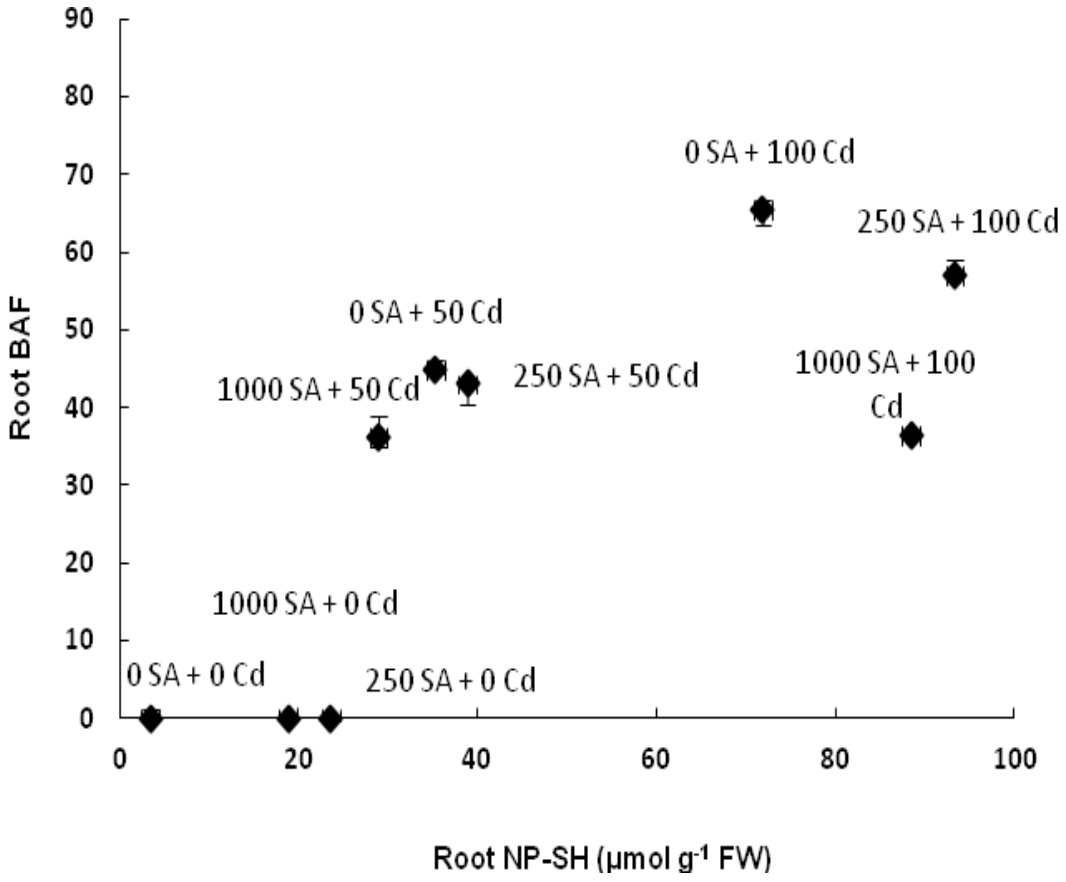

Figure 5. Relationship between the bioaccumulation of Cd and non-protein thiols (NP-SH) content in the root system.

SH contents in *B. monnieri* under Cd toxicity suggested their active participation in the detoxification of oxygen species and free radicals. However, the decrease in cysteine content in *B. monnieri* at higher concentrations of Cd might be due to decreased activities of sulphate reduction enzymes, ATP-sulphurylase and adenosine 5-phosphosulphate sulphotransferase. Globally, our findings reveal that the positive effect of the pre-treatment with SA on Cd tolerance led to its principal role at root level, which consisted on preventing Cd accumulation in roots (decrease in BAF of roots), increase root tolerance (TI) to Cd by the enhancement of NP-SH amounts.

In conclusion, it was suggested that SA and Cd had conflicting effects in Cd toxicity. Furthermore, use of SA-induced priming event by pre-soaking the seeds appears to be a promising strategy for the enhancement of plant tolerance to Cd stress. As SA can decrease Cd bioaccumulation in flax tissues, it may play an important role on reducing the accumulation of Cd in crops growing in contaminated soils.

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Abbreviations

Cd, Cadmium; **NP-SH,** non-protein thiol; **SA,** salicylic acid; **TI,** tolerance index; **BAF,** bioaccumulation factors; **TF,** translocation factor; **MBP,** metal binding proteins; **MBC,** metal binding complexes; **FW,** fresh weight; **DW,** dry weight; **EDTA,** ethylenediaminetetraacetic acid; **PCs,** phytochelatins; **DTNB,** 5, 5'-dithiobis-2-nitrobenzoic acid; **APS,** adenosine-5'-phosphosulfate; **GSH,** glutathione.

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