

Cadmium accumulation in *Medicago sativa* seedlings treated with salicylic acid

G. DRAŽIĆ*, N. MIHAILOVIĆ and M. LOJIĆ

Institute for the Application of Nuclear Energy, 11080 Zemun, Serbia and Montenegro

Abstract

Growth parameters and cadmium accumulation were investigated in alfalfa seedlings treated with 10 μ M salicylic acid (SA) at the beginning of seed imbibition. Shoot and root growths were accelerated by SA treatment and suppressed by Cd both in presence and absence of SA. Cd accumulation was stimulated by SA in alfalfa seedlings in dependence of the treatment duration. K, Mg, Ca and Fe contents in roots are decreased in the presence of Cd alone, while SA induces a decrease of Mg, Ca and Fe. Shoot K, Mg and Ca concentrations are increased by Cd only in the absence of SA, while SA induces also an increase of these concentrations, but only in the absence of Cd. High negative correlation of Cd concentration with K and Ca concentrations in root indicates a competition for the same carrier not regulated by SA. Positive correlation between Cd and Mg concentrations in shoots, which is decreased by SA pre-treatment, together with the increase of positive correlation between Cd and Fe concentrations in shoots under the influence of SA, indicates a possible mechanism of SA action through maintenance of ionic homeostasis.

Additional key words: alfalfa, growth parameters, mineral nutrients, root, shoot.

Introduction

Cadmium induces complex changes in plants at genetic, biochemical and physiological levels. The most obvious are: *a*) the reduction of tissue and organ growth (Šottníková *et al.* 2003, Lunáčková *et al.* 2003/4, Liu *et al.* 2003/4), *b*) leaf chlorosis associated with changes of activity of enzymes involved in C, S and N metabolisms and in peroxidase activity (Astolfi *et al.* 2004), *c*) ethylene production caused by membrane degradation (Vassilev *et al.* 2004), and *d*) changes in the content of reactive oxygen species and activity of the antioxidant system (Skórzyńska-Polit *et al.* 2003/4). The principal mechanisms of plant response to cadmium stress include phytochelatin-based sequestration and compartmentalization processes, as well as additional defence mechanisms, based on cell wall immobilization, plasma membrane exclusion, induction of stress proteins, *etc.* The degree to which higher plants are able to take up Cd depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements. In particular, the uptake of Cd ions seems to be in competition for the same carrier

with nutrients, such as potassium, calcium, magnesium, iron, manganese, copper, zinc and nickel (Sanita di Toppi and Gabbriellini 1999).

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants (Raskin 1992). These include effects on ion uptake, membrane permeability, *etc.* (Barkosky and Einhelling 1993). In addition, SA interacts with other signalling pathways including those regulated by jasmonic acid and ethylene (Szalai *et al.* 2000, Ding and Wang 2003). SA induces an increase in the resistance of seedlings to osmotic stress (Borsani *et al.* 2001), low or high temperature by activation of glutathione reductase and guaiacol peroxidase (Kang and Saltveit 2002), and toxic action of heavy metals (Mazen 2004) by activation of systemic acquired resistance (Metraux *et al.* 1990). Cd exposure increased the free SA contents of barley roots approximately two times. Cultivation of dry barley caryopses presoaked in SA containing hydroponic solution for only 6 h partially protected the seedlings from Cd toxicity during following growth period (Metwally *et al.* 2003).

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Abbreviations: d.m. - dry mass; f.m. - fresh mass; SA - salicylic acid; SD - standard deviation.

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* Corresponding author; fax: (+381) 11 618 724, e-mail: gdrazic@inep.co.yu

In a previous paper, the effect of Cd (10, 100 and 200 μM) on tissue contents of macronutrients (N, P, K, Ca and Mg) and micronutrients (Fe, Zn, Cu, Mn) was investigated in hydroponically grown soybean seedlings. Concentration changes of analysed elements observed against increasing Cd accumulation indicated that acute Cd-phytotoxic effect monitored through chlorophyll content was not a consequence of nutrient deficiency (Dražić *et al.* 2004). It was also shown that the treatment of soybean seedlings with 6 mg dm^{-3} Cd during 72 h induced a slight growth inhibition in roots, stems and leaves and a significant desiccation of cotyledons and leaves with a decrease of chlorophyll content in leaves. Salicylic acid applied simultaneously at the concentrations of 1, 10 and 100 μM significantly alleviates the effect of Cd. SA does not decrease Cd uptake, but changes its distribution in plant organs

depending on the concentration of added Cd. The obtained results indicate that the influence of SA on the alleviation of toxic effects of Cd is probably indirect, through a development of general anti-stress response of the seedlings which includes also the regulation of K and Mg distribution (Dražić and Mihailović 2005). For the present investigations alfalfa is chosen, being also a very important crop, phylogenetically close to soybean, but much more resistant to abiotic stresses. The experimental conditions were designed in such way to enable Cd-induced changes of growth parameters similar to those in soybean.

The aim of this study was to investigate the influence of SA pretreatment on Cd accumulation in underground and above-ground parts of alfalfa seedlings and its correlation with the content of the following nutrients: K, Mg, Ca and Fe.

Materials and methods

The seeds of alfalfa (*Medicago sativa* L. cv. Evropa, supplied by *Bioproduct*, Čačak, Serbia and Montenegro) were imbibed in distilled water or solution of 10 μM SA for 1, 3 and 6 h and then germinated on wet filter paper in the dark at 20 °C during 4 d. Seedlings of uniform size were transferred to experimental pots (100 plants per pot), each with 3 dm^3 of nutrient solution (Römheld and Marshner 1981), and were grown under controlled conditions: photosynthetic photon flux density 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf level, 12-h photoperiod, day/night temperature $20 \pm 2/12 \pm 2$ °C and relative humidity 85 %. The solutions were changed once a week. After 21 d, when the seedlings reached the stage of completely developed first trefoil leaf, they were transferred on the fresh nutrient solution with the addition of Cd (0, 3 and 5 mg dm^{-3}). After 7 d of growing under

the same conditions, the plants were harvested and roots and shoots were separated. Fresh masses and lengths were determined immediately after organ separation. Plant material was then dried at 40 °C until constant mass was reached. Dry plant material was powdered and mineralized in the presence of strong acids. The K, Ca, Mg, Fe and Cd contents were determined by atomic absorption spectrophotometry (*Pye Unicam 192*, Cambridge, England) using *Certipur* (*Merck*, Darmstadt, Germany) standards. All the experimental values reported in this article are means of at least three individual experiments. The significant differences were calculated using Student's *t*-test. Regression analysis was used to determine the dependence of Cd accumulation on SA pre-treatment and nutrient accumulation.

Results

Growth parameters: Cd in the used concentrations suppressed alfalfa seedlings development despite the SA pre-treatment. Cd alone did not exert a significant effect on root growth (Table 1), but induced a significant inhibition of fresh mass accumulation in shoots (about three times) together with a low inhibition of dry mass and length which are not statistically significant (Table 2). SA pre-treatment without Cd induced a significant increase of seedling growth. Root growth was not changed significantly, although a small stimulation of f.m. accumulation was observed, while the shoot growth was stimulated (f.m. about 3 times, and the length about 2 times). The shoot growth was more affected by Cd and SA than that of roots. SA pre-treatment alleviated Cd toxic effect on growth. In the roots of seedlings pre-treated with SA for 6 h Cd-induced growth inhibition was on the border of statistical significance (Table 1). SA pre-treatment and Cd act antagonistically on shoot

growth. Thus, Cd effect on f.m. increased with SA pre-treatment duration, while the effect on d.m. remained unchanged, and Cd annihilated SA-induced stimulation of shoot elongation (Table 2).

Cadmium accumulation: Cd uptake in alfalfa seedling was increased under the influence of SA pre-treatment, at the both applied Cd concentrations in the nutrient solution (Fig. 1). At the concentration of 5 mg(Cd) dm^{-3} , 1 h of SA pre-treatment was already sufficient to induce statistically significant stimulation of Cd uptake, while at the concentration of 3 mg(Cd) dm^{-3} , it needed 3 h of SA pre-treatment. Cd uptake was in high positive correlation with SA pre-treatment duration ($r = 0.928 - 0.993$).

SA pre-treatment also increased Cd accumulation in alfalfa roots (Fig. 2A). High positive correlation ($r = 0.951, 0.991$) between SA treatment duration and Cd accumulation was found at the both external Cd

Table 1. The Cd-induced changes of root growth parameters of alfalfa seedlings pre-treated with 10 μ M SA. Mean values \pm SD are shown. *t*-test: *a* - different from 0 Cd (at the corresponding SA-treatment duration); *b* - different from 0 SA (at the corresponding Cd-concentration) at 0.05 level.

| Cd [mg dm ⁻³] | SA [h] | Root f.m. [mg plant ⁻¹] | Root d.m. [mg plant ⁻¹] | Root length [cm] |
|------------------------------|-----------|--|--|---------------------|
| 0 | 0 | 13.28 \pm 1.87 | 1.18 \pm 0.15 | 7.91 \pm 1.55 |
| 3 | 0 | 12.95 \pm 1.98 | 0.93 \pm 0.19 | 7.48 \pm 1.82 |
| 5 | 0 | 14.33 \pm 3.45 | 0.75 \pm 0.12 | 6.47 \pm 0.97 |
| 0 | 1 | 15.78 \pm 3.09 | 1.69 \pm 0.03 | 6.18 \pm 0.82 |
| 3 | 1 | 13.63 \pm 2.23 | 1.15 \pm 0.22 | 6.06 \pm 1.90 |
| 5 | 1 | 13.09 \pm 2.78 | 0.90 \pm 0.27 <i>a</i> | 5.90 \pm 0.96 |
| 0 | 3 | 18.95 \pm 3.34 | 1.68 \pm 0.08 | 9.28 \pm 2.20 |
| 3 | 3 | 15.18 \pm 2.28 | 1.23 \pm 0.16 | 9.03 \pm 2.55 |
| 5 | 3 | 15.86 \pm 3.27 | 1.06 \pm 0.15 <i>a</i> | 7.46 \pm 2.33 |
| 0 | 6 | 22.68 \pm 3.78 <i>b</i> | 1.96 \pm 0.22 | 10.36 \pm 2.49 |
| 3 | 6 | 16.27 \pm 3.55 | 1.22 \pm 0.25 | 9.91 \pm 2.45 |
| 5 | 6 | 13.52 \pm 2.98 | 0.88 \pm 0.23 <i>a</i> | 7.08 \pm 1.88 |

Table 2. The Cd-induced changes of shoot growth parameters of alfalfa seedlings pre-treated with 10 μ M SA. Mean values \pm SD are shown. *t*-test: *a* - different from 0 Cd (at the corresponding SA-treatment duration); *b* - different from 0 SA (at the corresponding Cd-concentration) at 0.05 level.

| Cd [mg dm ⁻³] | SA [h] | Shoot f.m. [mg plant ⁻¹] | Shoot d.m. [mg plant ⁻¹] | Shoot length [cm] |
|------------------------------|-----------|---|---|---------------------------|
| 0 | 0 | 4.07 \pm 1.72 | 2.32 \pm 0.12 | 2.69 \pm 0.28 |
| 3 | 0 | 7.45 \pm 1.56 <i>a</i> | 2.14 \pm 0.56 | 2.42 \pm 0.26 |
| 5 | 0 | 4.78 \pm 1.34 | 1.55 \pm 0.34 <i>a</i> | 2.03 \pm 0.30 |
| 0 | 1 | 17.26 \pm 3.07 | 2.56 \pm 0.10 | 3.15 \pm 0.42 |
| 3 | 1 | 7.30 \pm 1.67 <i>a</i> | 2.15 \pm 0.27 | 2.56 \pm 0.38 |
| 5 | 1 | 4.36 \pm 1.50 <i>a</i> | 1.50 \pm 0.11 <i>a</i> | 2.07 \pm 0.47 |
| 0 | 3 | 33.91 \pm 3.67 <i>b</i> | 3.81 \pm 0.35 <i>b</i> | 4.70 \pm 0.48 <i>b</i> |
| 3 | 3 | 8.35 \pm 1.88 <i>a</i> | 2.17 \pm 0.18 <i>a</i> | 3.41 \pm 0.52 |
| 5 | 3 | 6.13 \pm 1.57 <i>a</i> | 2.13 \pm 0.14 <i>a</i> | 2.58 \pm 0.41 <i>a</i> |
| 0 | 6 | 39.84 \pm 5.98 <i>b</i> | 4.52 \pm 0.24 <i>b</i> | 5.30 \pm 0.58 <i>b</i> |
| 3 | 6 | 11.11 \pm 2.04 <i>ab</i> | 2.72 \pm 0.30 <i>a</i> | 4.04 \pm 0.63 <i>b</i> |
| 5 | 6 | 7.65 \pm 1.75 <i>ab</i> | 2.29 \pm 0.37 <i>a</i> | 2.87 \pm 0.48 <i>ab</i> |

concentrations. The shortest treatment inducing statistically significant increase of Cd accumulation was 3 h. Cadmium accumulation in shoots was also increased with SA pre-treatment duration (Fig. 2B). They were in high mutual positive correlation ($r = 0.979$), except at 6 h SA + 5 mg(Cd) dm⁻³. Prolongation of SA pre-treatment from 3 to 6 h did not further increase of Cd accumulation in shoots at external Cd concentration of 5 mg dm⁻³. The shortest SA pre-treatment inducing statistically significant effect was 6 h at 3 mg(Cd) dm⁻³ and 3 h at 5 mg(Cd) dm⁻³.

Nutrient contents: Potassium content in roots was decreased under the influence of Cd independently of SA,

while in shoots it was increased, but the effect was lost at SA pre-treatment longer than 1 h. Cadmium stimulated Mg transport from roots to shoots, but this effect was lost completely at 6 h SA pre-treatment. Cd and SA applied individually caused decrease in Ca content in roots and its increase in shoots. Fe content was decreased in roots under the influences of Cd and SA alone, but at SA pre-

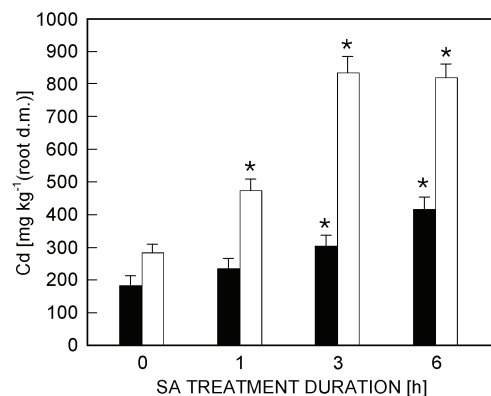


Fig. 1. The effect of SA pre-treatment on Cd uptake in alfalfa seedlings treated 72 h with 3 mg dm⁻³ (black columns) or 5 mg dm⁻³ (empty columns) Cd in nutrient medium. Mean values of 3 biological and 2 chemical repetitions are showed; bars represent SD; * - differences to 0 SA significant at 0.05 level.

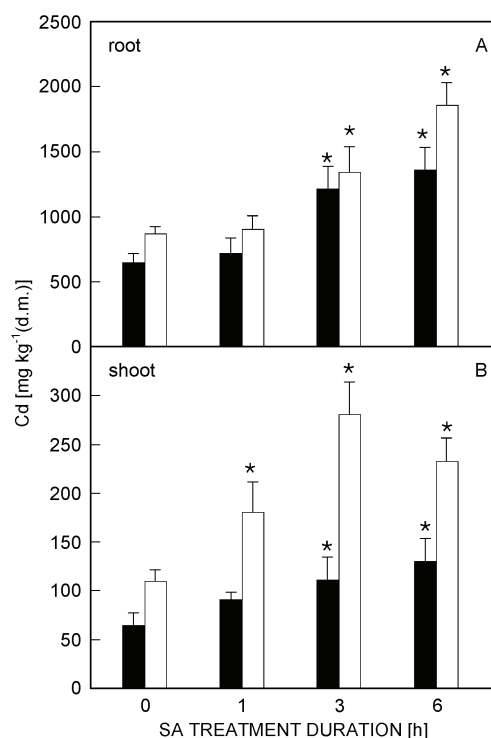


Fig. 2. The effect of SA pretreatment on Cd accumulation in roots (A) and shoots (B) of alfalfa seedlings treated 72 h with 3 mg dm⁻³ (black columns) or 5 mg dm⁻³ (empty columns) Cd in nutrient medium. Mean values of 3 biological and 2 chemical repetitions are showed; bars represent SD; * - differences to 0 SA significant at 0.05 level.

treatments for 3 and 6 h, Cd did not exert a significant effect. In shoots, Fe content changes were much less pronounced and only at SA pre-treatment for 6 h a significant increase of Fe concentration was found as a function of external Cd concentration increase (Table 3).

A high negative correlation was found between Cd

and K, and between Cd and Ca in roots, which was not changed under the influence of SA. Correlation between Cd and Fe in roots and shoots was positive; it was significant without SA and at 1 h SA in roots, and at 3 and 6 h SA in shoots (Table 4).

Table 3. The Cd-induced changes of root and shoot nutrient contents of alfalfa seedlings pretreated with 10 μ M SA. Means \pm SD are shown. *t*-test: *a* - different from 0 Cd (at the corresponding SA-treatment duration); *b* - different from 0 SA (at the corresponding Cd-concentration) at 0.05 level.

| Organ | Cd [mg dm ⁻³] | SA [h] | K content [mg g ⁻¹ (d.m.)] | Mg content [mg g ⁻¹ (d.m.)] | Ca content [mg g ⁻¹ (d.m.)] | Fe content [mg g ⁻¹ (d.m.)] |
|-------|------------------------------|-----------|--|---|---|---|
| Root | 0 | 0 | 42.4 \pm 7.54 | 3.96 \pm 0.65 | 4.83 \pm 0.65 | 9.58 \pm 3.88 |
| | 3 | 0 | 24.0 \pm 5.34 | 5.51 \pm 0.88 | 2.89 \pm 0.43 | 5.62 \pm 1.56 <i>a</i> |
| | 5 | 0 | 14.4 \pm 4.27 <i>a</i> | 2.57 \pm 0.56 | 0.84 \pm 0.30 | 3.76 \pm 1.02 <i>a</i> |
| | 0 | 1 | 35.1 \pm 4.54 | 4.72 \pm 0.56 | 3.64 \pm 0.45 | 13.25 \pm 3.04 |
| | 3 | 1 | 22.8 \pm 4.76 | 5.45 \pm 0.72 | 4.00 \pm 0.46 | 6.00 \pm 1.42 <i>a</i> |
| | 5 | 1 | 16.0 \pm 3.22 <i>a</i> | 1.17 \pm 0.45 <i>a</i> | 0.36 \pm 0.12 <i>a</i> | 3.95 \pm 1.08 <i>a</i> |
| | 0 | 3 | 42.4 \pm 5.34 | 2.77 \pm 0.42 | 1.64 \pm 0.45 <i>b</i> | 5.52 \pm 1.24 <i>b</i> |
| | 3 | 3 | 17.4 \pm 3.16 <i>a</i> | 4.67 \pm 0.75 | 0.97 \pm 0.22 <i>b</i> | 4.43 \pm 1.44 |
| | 5 | 3 | 15.3 \pm 2.87 <i>a</i> | 1.14 \pm 0.32 | 0.60 \pm 0.24 <i>a</i> | 5.09 \pm 1.58 |
| | 0 | 6 | 43.6 \pm 7.44 | 1.59 \pm 0.24 <i>b</i> | 1.57 \pm 0.26 <i>b</i> | 4.73 \pm 0.63 <i>b</i> |
| | 3 | 6 | 19.3 \pm 5.12 <i>a</i> | 1.25 \pm 0.28 <i>b</i> | 0.63 \pm 0.20 <i>a,b</i> | 4.08 \pm 0.65 |
| | 5 | 6 | 11.4 \pm 2.78 <i>a</i> | 1.09 \pm 0.32 | 0.80 \pm 0.12 <i>a</i> | 4.63 \pm 0.60 |
| Shoot | 0 | 0 | 33.4 \pm 5.75 | 2.44 \pm 0.52 | 0.79 \pm 0.23 | 0.71 \pm 0.12 |
| | 3 | 0 | 38.9 \pm 4.93 | 5.65 \pm 0.56 <i>a</i> | 1.56 \pm 0.35 <i>a</i> | 1.75 \pm 0.24 <i>a</i> |
| | 5 | 0 | 56.1 \pm 6.88 <i>a</i> | 6.03 \pm 0.70 <i>a</i> | 1.47 \pm 0.42 <i>a</i> | 1.73 \pm 0.22 <i>a</i> |
| | 0 | 1 | 37.2 \pm 6.32 | 5.02 \pm 0.74 <i>b</i> | 2.33 \pm 0.35 <i>b</i> | 0.96 \pm 0.23 |
| | 3 | 1 | 41.0 \pm 4.34 | 4.44 \pm 0.70 | 1.39 \pm 0.38 | 1.01 \pm 0.27 |
| | 5 | 1 | 48.7 \pm 7.03 | 5.76 \pm 0.75 | 1.75 \pm 0.36 | 0.99 \pm 0.29 |
| | 0 | 3 | 65.8 \pm 8.92 <i>b</i> | 5.53 \pm 0.76 <i>b</i> | 2.83 \pm 0.46 <i>b</i> | 0.60 \pm 0.12 |
| | 3 | 3 | 61.8 \pm 8.56 <i>b</i> | 7.10 \pm 0.88 | 3.65 \pm 0.59 <i>b</i> | 1.57 \pm 0.18 <i>a</i> |
| | 5 | 3 | 66.4 \pm 8.14 | 5.93 \pm 0.82 | 1.88 \pm 0.34 | 1.03 \pm 0.16 |
| | 0 | 6 | 61.8 \pm 9.56 <i>b</i> | 5.13 \pm 0.91 <i>b</i> | 2.61 \pm 0.34 <i>b</i> | 0.52 \pm 0.15 |
| | 3 | 6 | 65.2 \pm 9.34 <i>b</i> | 5.79 \pm 1.42 | 2.62 \pm 0.32 <i>b</i> | 1.42 \pm 0.26 <i>a</i> |
| | 5 | 6 | 65.7 \pm 10.22 | 5.50 \pm 1.24 | 2.30 \pm 0.38 | 2.45 \pm 0.32 <i>a</i> |

Discussion

Response to Cd stress in higher plants is a complex phenomenon. Cd evokes a response of parallel and/or consecutive events, rapid physiological and slow morphological processes, in which every mechanism could be at the same time cause and effect of metabolic changes, directly or indirectly related to the management of Cd stress. The response may be represented by “fan-shaped” multicomponent model which takes into consideration the first line of defence, directly related with Cd stress (phytochelatins and vacuolar compartmentalization) and the second line which is less specific (stress proteins, stress ethylene and peroxylases) (Sanita di Toppi and Gabrielli 1999). Cadmium at the concentrations applied in our experiment (3 and 5 mg dm⁻³) and short exposure time (7 d) induce acute Cd stress and rapidly full “fan-shaped” response in order to

Table 4. Correlation coefficient (*r*) between the accumulated Cd and nutrient contents in roots and shoots of alfalfa under the influence of SA pre-treatment. Statistical significance at *P* < 0.05: 0.754, at *P* < 0.01: 0.874.

| Organ | Nutrient | SA 0 h | SA 1 h | SA 3 h | SA 5 h |
|-------|----------|--------|--------|--------|--------|
| Root | K | -0.972 | -0.991 | -0.998 | -0.898 |
| | Mg | -0.237 | -0.108 | -0.575 | -0.591 |
| | Ca | -0.912 | -0.935 | -0.997 | -0.836 |
| | Fe | +0.832 | +0.910 | +0.420 | +0.593 |
| Shoot | K | +0.487 | +0.197 | +0.887 | +0.916 |
| | Mg | +0.861 | +0.982 | +0.262 | +0.456 |
| | Ca | -0.896 | -0.993 | -0.437 | -0.225 |
| | Fe | +0.732 | +0.693 | +0.802 | +0.991 |

detoxify Cd ions and repair Cd damage. SA pre-treatment attenuates growth inhibition induced by Cd, especially in shoots (Tab. 2), and in the absence of Cd it induces shoot growth stimulation. SA-induced growth stimulation had also been noticed in other plant species (Sharikova *et al.* 2003), as well as the effect in suppressing the effects of biotic (Alvarez *et al.* 2000) and abiotic stresses (Tissa *et al.* 2000). Protective action of SA in the presence of heavy metals is tied with cell membrane stabilization (Mishra and Choudhuri 1999), change of hormonal balance (Sharikova *et al.* 2003) and Cd ion inactivation (Metwally *et al.* 2003). SA significantly decreases lipid peroxidation and delays senescence in Cd-treated *Arabidopsis*. These effects of SA might be achieved by SA-induced protein synthesis and stimulation of antioxidants (Mazen 2004).

Although it is not possible to establish the mechanism of protective SA action as a part of alfalfa response to stress on the basis of our experiment, antagonistic influences of Cd and SA indicate that there occurs activation of some basic signal necessary for homeostasis maintenance. The suggestion is supported by short duration of SA pre-treatment (1 to 6 h) and its comparatively low concentration. The fact that alfalfa seed was treated by SA at the start of germination shows that it is more likely that there occurs SA transport into the seed where some process has been induced which permanently influences the seedling development and Cd resistance, than that there is a direct interaction of Cd with SA (Nigam *et al.* 2001). A convenient candidate for such signal is activation of aldose/aldehyde reductase (ALR; E.C. 1.1.1.21.). ALR from alfalfa was found to be active with 4-hydroxynon-2-enal (HNE) (Oberschall *et al.* 2000) which is a highly toxic lipid peroxide degradation product (Bartels 2001). It has been shown recently that tobacco plants overproducing alfalfa ALR show higher tolerance to cadmium stress than wild type lines (Hegedus *et al.* 2004), through a restricted oxidative damage. This could also be the explanation of the more intensive action of SA and Cd (in spite of higher root Cd accumulation) in shoots (consisting mostly of leaves) than in roots (Tables 1, 2) because it had been shown that high Cd concentrations in soil (14, 28 and 42 mg kg⁻¹) provoked serious disturbances of the barley chloroplast membranes by a peroxidation of C_{18:3} fatty acid (Vassilev *et al.* 2004).

In roots of alfalfa seedlings Cd is accumulated in dependence of external concentration of the added Cd, reaching 7 to 11 times higher values than in shoots (Fig. 2A). An increase in Cd accumulation found in shoots and roots is correlated with the treatment duration and concentration of the added Cd. The shortest treatment

inducing any significant effect was 1 h and 3 h for shoot and root, respectively. Similar to the results of this paper, SA-stimulated Cd uptake in leaves in the presence of SA (200 µM) in the growth medium significantly reduced the metal-alone-retarding effect on growth in *Corchorus olitorius* (Mazen 2004). In barley seedlings it had been shown that SA alleviated Cd toxicity, but not due to a decreased accumulation of Cd in roots and shoots, although the stimulation of Cd uptake had not been found (Metwally *et al.* 2003). The effect of SA on Cd accumulation is most probably indirect (since SA and Cd are not simultaneously present in the nutrient solution). Possible causes of SA-stimulated Cd accumulation include: *a*) activation of some divalent cation transporter capable to bind Cd (Hall and Williams 2003), *b*) changes of enzyme activities in S metabolism (Mazen 2004), and *c*) enhanced Cd ion immobilization in leaf, and especially root, intercellular spaces (Kevrešan *et al.* 2003) or in the vacuole (Rausser 1995). High negative Cd-K and Cd-Ca correlations in roots, which are independent of SA pre-treatment indicate that Cd and K ions, as well as Cd and Ca ions compete for the same transporters (Kim *et al.* 2002), the activity of which is SA-independent (Table 4). Cooperative absorption between Cd and Fe, Mn, Cu in rice plants is shown through correlations of these element contents (Liu *et al.* 2003). However, correlations between nutrient contents, Cd accumulation and Cd toxic effect differ significantly in dependence of plant species and cultivar, developmental stage and the experimental method.

The prevailing part of cell Cd is bound to phytochelatins (PC) and transported into vacuole as PC-Cd-S complex (Hall 2002) through Fe-dependent transporters (Hall and Williams 2003). High positive correlation between Cd and Fe in shoots after SA pre-treatment (Table 4) indicate possible SA action in Cd deposition out of important metabolic processes (in vacuoles) which may explain the decrease of Cd phytotoxic influence in spite of increased Cd accumulation.

It may be concluded that SA significantly increases Cd accumulation in alfalfa seedlings with simultaneous decrease of Cd phytotoxic effect through some process activated at the very initiation of germination (3 h imbibition), and which involves transporters participating in nutrient transport. For further studying of this process, detailed investigations of phytochelatin and lipid peroxidation are necessary. The obtained results may be a basis for applied investigations in the field of phytoremediation, because they offer a possibility of increased accumulation of a heavy metal (Cd) in above-ground parts, with an increased heavy metal resistance.

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