Effect of salicylic acid pretreatment on cadmium toxicity in wheat

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Abstract

Cadmium (100, 400 and 1000 μM $CdCl_2$) treatments resulted in the inhibition of root dry biomass, root elongation and increased Cd accumulation in wheat (*Triticum aestivum* L.) roots. Further, these treatments decreased relative water content, chlorophyll content, $^{14}CO_2$-fixation, activities of phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase and abscisic acid content while increased malondialdehyde, hydrogen peroxide and free proline contents. Chloroplast and root ultrastructure was also changed. Pretreatment of seeds with SA (500 μM) for 20 h resulted in amelioration of these effects.

Additional key words: abscisic acid, chlorophyll, chloroplast, $^{14}CO_2$-fixation, H_2O_2, PEPC, proline, RuBPC, *Triticum aestivum.*

Introduction

Wheat (*Triticum aestivum* L.*)* is most widely grown crop in the world. The intensive use of high-phosphate fertilizers leads to an increased accumulation of metal ions, especially cadmium, in the soil (Taylor 1997). Cadmium is readily taken up by the cells of different plant species (Liu *et al.* 2007) and induces many morphological, physiological, biochemical and structural changes in plants, such as water imbalance, inhibition of seed germination, inhibition in photosynthesis, reduction of growth especially the root growth, disturbances in mineral nutrition, and sugar metabolism and therefore, strongly influences biomass production (Sanita di Toppi and Gabbrielli 1999, Moussa 2004) and finally can cause plant death (Kahle 1993). Cadmium produces alterations in the membranes by inducing changes in their lipid composition (Ouariti *et al.* 1997) and affects the activities of enzymes associated with membranes, such as that of H^+-ATPase (Fodor *et al.* 1995). Cd decreases photosynthetic rate due to reduced chlorophyll content and the enzymatic activity involved in CO_2 fixation (Greger and Ögren 1991). In many plants Cd enhances the level of lipid peroxidation and alteration in antioxidant systems (Somashekaraiah *et al.* 1992). Harmful effects produced by Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia *et al.* 2004).

Salicylic acid, commonly occurring in vascular plants, plays an essential role in regulation of plant growth, development and in plant response to environmental stress (Senaratana *et al.* 2000). Exogenous application of SA increased yield (Raskin 1992). Further, SA retarded ethylene synthesis, affected membrane depolarization, stimulated photosynthesis, protein synthesis and increased the content of chlorophyll (Khan *et al.* 2003, Shakirova *et al.* 2003). Salicylic acid mediates some acclimation responses to abiotic stresses, such as heavy metals, herbicides, low temperatures and salinity (Janda *et al.* 1999, Metwally *et al.* 2003). SA pretreatment alleviates Cd toxicity in barley (Metwally *et al.* 2003) and maize plants (Krantev *et al.* 2008). It has recently been found that SA treatment caused both ABA and proline accumulation in wheat and increased resistance to salinity (Shakirova *et al.* 2003). SA ameliorates the damaging effects of heavy metals, like lead and mercury (Mishra and Choudhuri 1999).

The present study investigated possible role of salicylic acid in alleviating cadmium toxicity. Root length, root dry mass, Cd content, relative water content (RWC), free proline level, abscisic acid (ABA) production, lipid peroxidation, H_2O_2 content, chlorophyll content, $^{14}CO_2$-fixation, the activities of the carboxylating enzymes (RuBPC and PEPC), and root cell ultrastructure were assessed to provide essential information on metal bioaccumulation and toxicity.

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Abbreviations: MDA - malondialdehyde; PEPC - phosphoenolpyruvate carboxylase; RuBPC - ribulose-1,5-bisphosphate carboxylase; RWC - relative water content; SA - salicylic acid.
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Materials and methods

A homogenous lot of wheat seeds *(Triticum aestivum* L.*)* cv. Giza 155 was obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. They were surface sterilized in 0.1 % (m/v) sodium dodecylsulphate solution and then thoroughly rinsed with sterile deionized water. Seeds were presoaked for 20 h, either in 500 μM SA or distilled H_2O as a control. SA was initially dissolved in few drops of dimethylsulfoxide and the final volume was reached using distilled water. After that they were germinated in the dark at 24 °C for 5 d. Seedlings of equal size and vigor were transplanted to black polyethylene pots containing continuously aerated full-strength Hoagland's nutrient solution (Rafi and Epstein 1999) which contained four Cd concentrations (0, 100, 400 and 1000 μM $CdCl_2.2.5\,H_2O$). The plants were grown in a controlled growth chamber under 15-h photo-period, 65 - 75 % relative humidity, day/night temperature of 22/20 °C and the photosynthetic photon flux density at maximum plant height about 440 μmol m^{-2} s^{-1}. Five seedlings were planted in each pot. Each treatment was replicated four times and each replicate included four pots. All plants were harvested one month after Cd treatments and separated into leaves, stem, and root. For proline level determination, leaves were stored at -20 °C prior to analyses.

Root length was measured using a centimeter scale. For dry biomass measurement, roots were dried at 70 °C for 2 d. The dried roots were digested in glass tubes containing 5 cm^3 concentrated nitric acid at 100 °C until the solution turned clear. The final volume was adjusted to 20 cm^3 with distilled water. Total Cd content was measured using atomic absorption Spectrometer (*Perkin-Elmer 3110*).

The leaf relative water content was determined by the method of Bandurska (1991). Photosynthetic activity ($^{14}CO_2$-fixation) was measured in the Atomic Energy Authority Radioisotope Department, Cairo, Egypt, with the method published previously (Moussa 2008). One pot from each treatment was placed under a bell jar and $^{14}CO_2$ was generated inside this chamber by a reaction between 10 % HCl and 100 mg Na_2CO_3 (as a carrier of $NaH^{14}CO_3$ (1.87×10^6 Bq). Then the samples were illuminated with a tungsten lamp (440 μmol m^{-2} s^{-1}). After 30 min exposure, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80 % hot ethanol. The ^{14}C was assayed in the ethanolic extracts using a Bray Cocktail (Bray 1960) and a Liquid Scintillation Counter (*LSC2-Scaler Ratemeter SR7, Nuclear Enterprises*).

Chlorophyll content was quantified according to Porra *et al.* (1989). Free proline was determined according to the method described by Bates *et al.* (1973). The activity of phosphoenol pyruvate carboxylase was determined as described by Blanke *et al.* (1986) and the activity of ribulose1,5-bisphosphate carboxylase was determined according to Warren *et al.* (2000). Lipid peroxidation was measured in terms of malondialdehyde content using the thiobarbituric acid reaction as described by Madhava Rao and Sresty (2000). The contents of hydrogen peroxide were measured according to Patterson *et al.* (1984). The isolation and estimation of ABA by high-performance liquid chromatography (HPLC) were performed according to Bandurska and Stroinski (2003).

For transmission electron microscopy the roots were harvested after 15 d of culture. The root tips (0 - 1 mm), and segments (2 - 3 mm) and small segments of leaf (70 - 90 nm in thickness) of control and treated plants were fixed in 5 % glutaraldehyde in phosphate buffer (pH 7) for 4 h and then thoroughly washed with the same buffer for three times. This was followed by post-fixation with 2 % osmium tetroxide in the same buffer for 2 h. They were dehydrated in acetone series and embedded in cycloaliphatic epoxide resin *ERL 4221*. Ultrathin sections of 80 nm were cut on an ultramicrotome (*Ultracut E*, *Leica Microsystems,* Bensheim, Germany) with a diamond knife, and were picked on copper grids. The sections were stained with 1 % uranyl acetate for 1 h and lead citrate for 15 min. They were examined on and photographed with transmission electron microscopy (TEM, *Jeol Jem 1200 EX II*, Tokyo, Japan).

All the experiments were repeated at least four times and data presented are means of four separate experiments.

Results

SA priming resulted in initial increase in root length and dry mass in control plants (Table 1) and Cd decreased both parameters less in SA treated plants. In the controls, no Cd was detected. Cd accumulation with increased Cd concentration in nutrient solution was also lower in SA primed ones (Table 1). Exposure of plants to Cd^{2+} led to a decrease in leaf RWC from 94.7 to 75.8 % and from 96.1 to 83.7 % in SA non-treated and treated plants, repectively. (Table 1). We demonstrated that Cd-induced growth inhibition was accompanied by a significant decrease in the chlorophyll content, the rate of $^{14}CO_2$-fixation and activities of RuBPC and PEPC. Pretreatment with SA before exposure to Cd alleviated the inhibitory effects of Cd on these parameters (Table 2). The contents of H_2O_2, MDA and free proline increased significantly with increasing Cd concentration, the prominent effect was observed at 1000 μM Cd (nearly three fold rises in free proline content compared to the control). A less increase in these parameters was observed after SA pretreatment (Tables 1,2). ABA content decreased with increasing Cd concentration but less in SA treated plants (Table 2).

Root tip cells of wheat control plants had a typical ultrastructure with highly condensed cytoplasm

Table 1. Root length [cm], root dry mass [g plant^{-1}], Cd content in root [mg g^{-1}(d.m.)], leaf relative water content [%], MDA [μmol g^{-1}(f.m.)] and H_2O_2 [μmol g^{-1}(f.m.)] in wheat plants treated with Cd at different concentrations and pre-treated or not with 500 μM SA. Data are the means of four separate experiments ± SE. ** and * denote significant differences at 0.01 and 0.05 % levels, respectively.

Treatment	Root length	Root dry mass	Cd content	RWC	MDA	H_2O_2
Control	12.9 ± 0.38	0.23 ± 0.002	0.0 ± 0.0	94.7 ± 5.7	388 ± 23.3	99 ± 5.9
100 μM Cd	11.2 ± 0.22	0.20 ± 0.010	3.9 ± 0.234*	91.3 ± 7.3	413 ± 33.1	138 ± 5.5*
400 μM Cd	9.4 ± 0.41*	0.17 ± 0.007*	6.8 ± 0.544*	87.1 ± 2.6	497 ± 44.7*	167 ± 11.7*
1000 μM Cd	7.2 ± 0.36**	0.10 ± 0.006**	17.5 ± 1.963**	75.8 ± 3.8**	563 ± 56.3**	183 ± 12.5*
Control + SA	15.9 ± 0.85	0.32 ± 0.028	0.0 ± 0.0	96.1 ± 6.7	281 ± 16.9	84 ± 1.7
100 μM Cd + SA	13.6 ± 0.95	0.27 ± 0.011	1.8 ± 0.126*	94.2 ± 10.3	319 ± 19.1	93 ± 8.4
400 μM Cd + SA	11.9 ± 0.47	0.22 ± 0.009	4.3 ± 0.602*	90.6 ± 4.5	290 ± 14.3**	110 ± 6.6*
1000 μM Cd + SA	9.9 ± 0.29*	0.16 ± 0.008**	7.4 ± 0.731**	83.7 ± 3.1**	185 ± 7.4**	125 ± 8.7*

Table 2. Chlorophyll *a*+*b* content [μg g^{-1}(f.m.)], $^{14}CO_2$ fixation [kBq mg^{-1}(f.m.)], PEPC [μmol(NADH) mg^{-1}(protein) min^{-1})] and RuBPC [μmol(RuBP) mg^{-1}(protein) min^{-1})] activities, free proline content [μmol g^{-1}(f.m.)] and ABA content [nmol g^{-1}(f.m.)] in plants treated with Cd at different concentrations and pre-treated or not with 500 μM SA. Means of four separate experiments ± SE. ** and * denote significant differences at 0.01 and 0.05 % levels, respectively.

Treatment	Chlorophyll	$^{14}CO_2$ fixation	PEPC	RuBPC	Free proline	ABA
Control	3.74 ± 0.18	18673 ± 933	25 ± 1.7	78 ± 4.7	250 ± 15	0.695 ± 0.061
100 μM Cd	3.42 ± 0.20*	17125 ± 685	21 ± 1.6*	73 ± 2.9*	384 ± 46**	0.718 ± 0.072
400 μM Cd	3.00 ± 0.21**	12378 ± 1237*	13 ± 0.9*	52 ± 3.1*	508 ± 43**	0.501 ± 0.031*
1000 μM Cd	1.78 ± 0.23	5986 ± 239**	6 ± 0.2**	28 ± 2.8**	722 ± 52**	0.208 ± 0.023**
Control + SA	3.86 ± 0.23	20721 ± 1243	27 ± 2.3	82 ± 5.2	166 ± 14	0.994 ± 0.051
100 μM Cd + SA	3.68 ± 0.14	19466 ± 1946	25 ± 1.5	79 ± 4.7	196 ± 8**	1.035 ± 0.046**
400 μM Cd + SA	3.50 ± 0.31*	16611 ± 1328*	19 ± 0.5*	67 ± 5.4*	216 ± 18*	0.876 ± 0.062**
1000 μM Cd + SA	2.56 ± 0.28**	10984 ± 1318**	14 ± 0.7**	46 ± 1.8**	274 ± 25*	0.549 ± 0.084**

containing numerous organelles and a large nucleus. Numerous ribosomes were distributed in cytoplasm or located on the surface of endoplasmic reticulum (Fig. 1*A*). Some small vacuoles were found in meristematic cells, whereas large vacuoles or several vacuoles in mature parenchyma cell of the root tip of the plants treated with SA before exposure to Cd (Fig. 1*B*). The root cells exposed to different concentrations of Cd exhibited various ultrastructural changes. At 100 μM Cd, advanced vacuolation appeared in the meristem and cortical cells of root tips and also in cortical cells of elongation zone (Fig. 1*C*). At Cd concentrations higher than 400 μM, the cortex and stellar parenchyma tissues cells were damaged in varying degree. A reduction in cristae number was observed in mitochondria (Fig. 1*D*). The multivesiculate bodies were exhibited in parenchyma cells. Nucleus disintegration was accompanied by the nucleoli containing electron-dense granules (Fig. 1*E*). Damaged membrane systems and serious plasmolysis with separations of the plasma membrane from the cell wall was noted in most root cells exposed to 1000 μM Cd. The structural damaged of the cells resulted in death of the cells, which gradually decreased from cortical cells to stelar cells. A few electron-dense granules precipitated in large vacuoles or in small vesicles in cytoplasm of the cortical parenchyma cells which were treated with 100 to 400 μM Cd (Fig. 1*F*). The precipitation increased with increasing concentrations of Cd. At 1000 μM Cd, the abundant electron dense granules containing Cd formed into bigger precipitates which were encircled by membrane in the vacuoles (Fig. 1*E*). Small amounts were scattered in the nucleus (Fig. 1*E*) and in the cytoplasm (Fig. 1*F*) of the cortical parenchyma cells.

The chloroplast of the control plants possessed well-developed granal and stromal thylakoids (Fig. 2*A*). Treatment with SA (500 μM) alone did not induce any ultrastructural changes (Fig. 2*B*). Cd treatment at 400 μM concentration caused thylakoid swelling (Fig. 2*C*). But Cd treatment at 1000 μM caused severe damage (Fig. 2*E*). On the other hand, in the chloroplast pretreated with SA, the distortion of thylakoid membrane was effectively suppressed under treatment with 400 μM Cd (Fig. 2*D*). Though the arrangement of thylakoids was disturbed under treatment with 1000 μM Cd, severe damage was suppressed by the pretreatment with SA (Fig. 2*F*).

Discussion

SA priming for 20 h resulted in increase in growth and root dry mass of control plants. Usually, Cd is retained in roots and very small amount of it is transported in shoots (Caltado *et al.* 1983). Cd content in SA non-treated roots was higher as compared to that in SA primed roots similarly as observed Choudhury and Panda (2004). This different accumulation of Cd can be considered as a basis for physiological effects of SA. Cd is known to inhibit plant growth (Moussa 2004). SA ameliorated negative effects of Cd on growth in barley (Metwalley *et al.* 2003) and rice (Choudhury and Panda 2004) and also in salt stressed tomato (He and Zhu 2008). Cd affected water balance manifested by the RWC decrease which was in agreement with results of Costa and Morel (1994). The presoaking of wheat seeds for 20 h with 500 μM SA before exposure to Cd had a protective effect on RWC. These results were in corroborations with the findings of Krantev *et al.* (2006).

The growth inhibition produced by Cd could be at least partially due to the effect of this heavy metal on the photosynthesis rate (Metwally *et al.* 2003). Cd stress decreased the rate of CO_2 assimilation, photosynthetic efficiency and a degradation of chlorophyll as well as an inhibition of their biosynthesis, which could result in disturbances in the electron transport rates of PS 1 and PS 2, leading to the generation of oxygen free radicals (Moussa 2004). Treatment with SA, increased pigment contents (Moussa and Khodary 2003, Krantev *et al.* 2008). Pretreatment of plants with SA induced a considerable increase in the $^{14}CO_2$-assimilation, photosynthetic enzymes activity and the chlorophyll content, which was in accordance with (Khan *et al.* 2003, Moussa and Khodary 2003, Krantev *et al.* 2006, Mahdavian *et al.* 2008). The activities of both carboxylating enzymes (RuBPC and PEPC) were strongly reduced at all Cd concentrations applied and pretreatment of wheat plants with SA before exposure to Cd alleviated the inhibitory effect of Cd on these enzymes activity in agreement with Moussa and Khodary (2003), Moussa (2004) and Krantev *et al.* (2006). The malate formed as end product of CO_2 fixation by PEPC may function in cell osmoregulation (Harpster and Taylor 1986). SA application resulted in activation of PEPC in barley plants (Pancheva *et al.* 1996). RuBPC is the primary enzyme of photosynthetic carbon fixation. Cd^{2+}, as a divalent cation, may displace the Mg^{2+} resulting in a loss of RuBPC activity (Wildner and Henkel 1979). The highest Cd concentration decreased the RuBPC regeneration capacity (Moussa 2004).

An increase in H_2O_2 production is reported in plants under Cd treatments (Choudhury and Panda 2004,

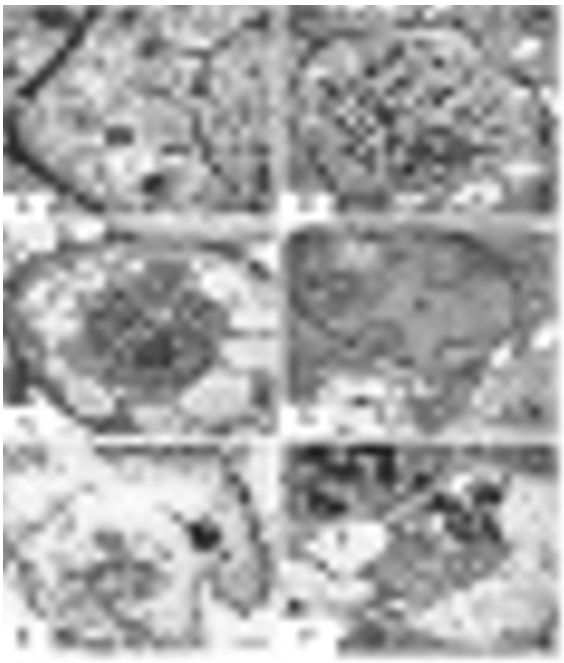

Fig. 1. TEM micrographs showing toxic effects of Cd on ultrastructure in the root tip cells of wheat. *A* - Control cells showing well developed root tip cells; *B* - SA primed cells showing no change compared to the control. *C* to *F* - The ultrastructural changes of root tip cells treated with Cd for 9 d; *C* - Increase in vacuolation (100 μM Cd); *D* - Reduction of mitochondria (400 μM Cd); *E* - Large precipitates encircled by membranes in the vacuoles and nucleoli containing electron-dense granules (1000 μM Cd); *F* - A few electron-dense granules distributed in vesicles in the cytoplasm (400 μM Cd). *Bar* = 25 mm (*D*), 5 mm (*A*, *F*) and 1 mm (*B*, *C*, *E*). *Arrows* point to electron-dense granules. CW - Cell wall, C - cytoplasm, D - dictyosome, ER - endoplasmic reticulum, IS - intercellular space, M - mitochondria, N - nucleus, NM - nuclear membrane, NU - nucleoli, V - vacuoles.

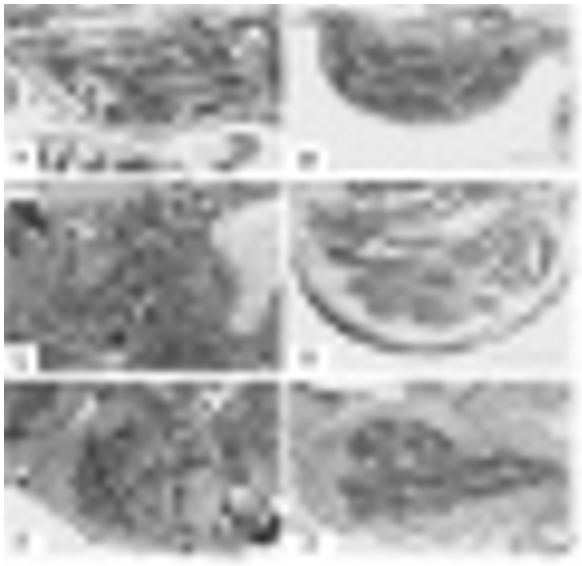

Fig. 2. Ultrastructure of a chloroplast in the cells of control plants (*A*), plants treated with 500 μM SA alone (*B*), plants treated with Cd (*C* - 400 μM, *D* - 1000 μM), plants treated with Cd (*E* - 400 μM, *F* - for 1000 μM) after pretreatment with SA (500 μM).

Krantev *et al.* 2006). In addition, it was demonstrated that SA pretreatment decreased MDA accumulation caused by Cd, which confirm the role of this compound against oxidation damage (Choudhury and Panda 2004, Krantev *et al.* 2006). The obtained results indicated that increased ABA content induced by the application of SA before Cd stress might be responsible for the alleviation of membrane injury under Cd stress (Bandurska and Stroinski 2005). Free proline accumulation appeared to be a suitable indicator for heavy metal stress. The observed decrease in proline content in plants grown from SA-pretreated seeds indicated partial recovery from Cd stress (Krantev *et al.* 2006). It has been suggested that free proline acts as an osmoprotectant (Delauney and Verma 1993) and as a metal chelator (Farago and Mullen 1979). Proline also acts directly as an antioxidant to protect the cell from free radical damage and maintain a more reducing environment that is favorable for phytochelation synthesis and Cd sequestration (Surasak *et al.* 2002)**.** At ultrastructure level, 100 μM Cd did not exhibit significant cellular damage to root cells. However, this concentration of Cd causes a greater degree of cell vacuolation, which was important for the compartmentation of Cd in meristems and cortical parenchyma cells. Sanità di Toppi and Gabbrielli (1999) indicated that a significant role in Cd detoxification and Cd tolerance was its vacuolar compartmentation. The toxicity symptoms seen in the presence of excessive amounts of Cd may be due to destroyed defense systems in cells. This paper provided evidence that at higher concentrations of Cd (400 - 1000 μM), the toxic symptoms of root cells were mainly continued disintegration of cell organelles, disruption of membranes, withdrawal of plasma membrane from cell walls, and formation of multi-vesiculate bodies in the cytoplasm. Former researches reported that the occurrence of electron-dense deposits in vacuoles and appearance of small vesicles in cytoplasm seemed to be common features of metal-stressed plants. The presence of metal-bearing granules in the vacuoles and vesicles was related to metal detoxification (Rauser and Ackerley 1987, Nassiri *et al.* 1997a,b). The content of Cd increased with increase of electron-dense granules. The high content of Cd was detected in vacuoles of cortical cells in differentiating and mature root cells. These results support the findings observed by X-ray microanalysis (Rauser and Ackerley 1987). Cd stress decreased the chlorophyll content and the thylakoids of the chloroplasts were swollen and showed a wavy shape. (Pietrini *et al.* 2003). It is reported that the structural change and swelling of thylakoid might be due to a change in the ionic composition of the stroma (Salama *et al*. 1994). In addition, the change of the thylakoids has been reported as a typical symptom of oxidative stress (Hernandez *et al.* 1995).

Other important role of SA in inducing resistance to various environmental stresses is stimulation of expression of genes coding pathogenesis-related proteins or defense-related enzymes (Merkouropoulos *et al.* 1999). SA could also form a complex with Cd that may provide Cd tolerance (Choudhury and Panda 2004).

References

Bandurska, H.: The effect of proline on nitrate reductase activity in water-stressed barley leaves. - Acta Physiol. Plant. **1**: 3-11, 1991.

Bandurska, H., Stroinski, A.: ABA and proline accumulation in leaves and roots of wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare* 'Maresi') barley genotypes under water deficit conditions. - Acta Physiol. Plant. **25**: 55-61, 2003.

Bandurska, H., Stroinski, A.: The effect of salicylic acid on barley response to water deficit. - Acta Physiol. Plant. **27**: 379-386, 2005.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Blanke, M., Notton, B., Hucklesby, D.: Physical and kinetic properties of photosynthetic PEP carboxylase in developing apple fruit. - Phytochemistry **25**: 601-606, 1986.

Bray, G.A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. - Anal. Biochem. **1**: 276-285, 1960.

Cataldo, D.A., Garland, R., Wildung, R.E.: Cadmium uptake kinetics in intact soybean plants. - Plant Physiol. **73**: 844-848, 1983.

Choudhury, S., Panda, S.K.: Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. - Bulg. J. Plant Physiol. **30**: 95-110, 2004.

Costa, G., Morel, J.: Water relations, gas exchange and amino acid content in Cd treated lettuce. - Plant Physiol. Biochem. **32**: 561-570, 1994.

Delauney, A.J., Verma, D.P.S.: Proline biosynthesis and osmoregulation in plants. - Plant J. **4**: 215-223, 1993.

Farago, M.E., Mullen, W.A.: Plants which accumulate metals. Part IV. A possible copper-proline complex from the roots of *Armeria maritima*. - Inorg. chim. Acta **32**: 93-94, 1979.

Fodor, A., Szabó-Nagy, A., Erdei, L.: The effects of cadmium on the fluidity and H^+-ATPase activity of plasma membrane from sunflower and wheat roots. - J. Plant Physiol. **14**: 787-792, 1995.

Gouia, H., Suzuki, A., Brulfert, J., Ghorbal, H.: Effect of cadmium on the coordination of nitrogen and carbon metabolism in bean seedlings. - Plant Physiol. **160**: 367-376, 2004.

Greger, M., Ögren, E.: Direct and indirect effects of Cd^{2+} on photosynthesis in sugar beet (*Beta vulgaris*). - Physiol. Plant. **83**: 129-135, 1991.

Harpster, M.H., Taylor, W.C.: Maize phosphoenolpyruvate carboxylase. - J. biol. Chem. **261**: 6132-6136, 1986.

He, Y., Zhu, Z.J.; Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicon esculentum*. - Biol. Plant. **52**: 792-795, 2008.

Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F., Del Rio, L.A.: Salt-induced oxidative stress in chloroplasts of pea

plants. - Plant Sci. **105**: 151-167. 1995.
Janda, T., Szalai, G., Tari, I., Paldi, E.: Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. - Planta **208**: 175-180, 1999.
Kahle, H.: Response of roots of trees to heavy metals. - Environ. exp. Bot. **33**: 99-119, 1993.
Khan, W., Prithiviraj, B., Smith, D.L.: Photosynthetic responses of corn and soy bean to foliar application of salicylates. - J. Plant Physiol. **50**: 1-8, 2003.
Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L.: Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. - J. Plant Physiol. **165**: 920-931, 2008.
Krantev, A., Yordanova, R., Popova, L.: Salicylic acid decreases Cd toxicity in maize plants. - Gen. appl. Plant Physiol. **1**: 45-52, 2006.
Liu, Y., Wang, X., Zeng, G., Qu, D., Gu, J., Zhou, M., Chai, L.: Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea* (L.) Gaud. - Chemosphere **69**: 99-107, 2007.
Madhava Rao, K.V., Sresty, T.V.S.: Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. - Plant Sci. **157**: 113-128, 2000.
Mahdavian, K., Kalantari, K.M., Ghorbanli, M.; Torkzade, M.: The effects of salicylic acid on pigment contents in ultraviolet radiation stressed pepper plants. - Biol. Plant. **52**: 170-172, 2008.
Merkouropoulos, G., Barnett, D.C., Shirasat, A.H.: The *Arabidopsis* extension gene is developmentally regulated, is induced by wounding, methyl jasmonate, abscisic and salicylic acid, and codes for a protein with unusual motifs. - Planta **208**: 212-219, 1999.
Metwally, A., Finkermeier, I., Georgi, M., Dietz, K.J.: Salicylic acid alleviates the cadmium toxicity in barley seedlings. - Plant Physiol. **132**: 272-281, 2003.
Mishra, A., Choudhuri, M.A.: Effects of salicylic acid on heavy metal induced membrane degradation mediated by lipoxygenase in rice. - Biol. Plant **42**: 409-415, 1999.
Moussa, H.R.: Effect of cadmium on growth and oxidative metabolism of faba bean plants. - Acta agron. hung. **52**: 269-276, 2004.
Moussa, H.R., Abdel-Aziz, S.M.: Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. - Aust. J. Crop Sci. **1**: 31-36, 2008.
Moussa, H.R., Khodary S.E.A.: Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. - Isotope Radiat. Res. **35**: 179-187, 2003.
Nassiri, Y., Mansot, J.L., Wéry, J., Ginsburger-Vogel, T., Anuard, C.J.: Ultrastructureal and electron energy loss spectroscopy studies of sequestration mechanisms of Cd and Cu in the marine diaton *Skeletonema costatum*. - Arch. Environ. Contam. Toxicol. **33**: 147-155, 1997b.
Nassiri, Y., Wéry, J., Mansot, J.L., Ginsburger-Vogel, T.: Cadmium bioaccumulation in *Tetraselmis suecica*: an electron energy loss spectroscopy (EELS) study. - Arch. Environ. Contam. Toxicol. **33**: 156-161, 1997a.
Ouariti, O., Boussama, N., Zarrouk, M., Cherif, A., Ghorbal, M.H.: Cadmium- and copper-induced changes in tomato membrane lipids. - Phytochemistry **45**: 1343-1350, 1997.
Pancheva, T.V., Popova, L.P., Uzunova, A.N.: Effects of salicylic acid on growth and photosynthesis in barley plants. - J. Plant Physiol. **149**: 57-63, 1996.
Patterson, B.D., Elspeth, A., Ferguson, I.B.: Estimation of hydrogen peroxide in plant extracts using titanium (IV). - Anal. Biochem. **139**: 487-92, 1984.
Pietrini, F., Iannelli, M.A., Pasqualini, S., Massacci, A.: Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. *ex* Steudel. - Plant Physiol. **133**: 829-837, 2003.
Porra, R.J., Thompson, W.A., Kriedemann, P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. - Biochem. biophys. Acta **975**: 384-394, 1989.
Rafi, M.M., Epstein, E.: Silicon absorption by wheat (*Triticum aestivum* L.). - Plant Soil **211**: 223-230, 1999.
Raskin, I.: Role of salicylic acid in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 439-463, 1992.
Rauser, W.E., Ackerley, C.A.: Localization of cadmium in granules within differentiating and mature root cells. - Can. J. Bot. **65**: 643-646, 1987.
Salama, S., Trivedi, S., Busheva, M, Arafa, A.A., Garab, G., Erclei, L.: Effects of NaCl salinity on growth, cation accumulation, chloroplast structure and function in wheat cultivars differing in salt tolerance. - J. Plant Physiol. **144**: 241-247. 1994.
Sanità di Toppi, L., Gabrielli, R.: Response to cadmium in higher plants, - Environ. exp. Bot. **41**: 105-130, 1999.
Senaratana, T., Touchell, D., Bunn, E., Dixon, K.: Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. - Plant Growth Regul. **30**: 157-161, 2000.
Shakirova, F.M., Sakhabutdinovam A.R., Bezrukova, M.V., Fatkhutdinova, R.A., Fatkhutdinova, D.R.: Changes in hormonal status of wheat seed lings induced by salicylic acid and salinity. - Plant Sci. **164**: 317-322, 2003.
Somashekaraiah, B.V., Padmaja, K., Prasad, A.R.K.: Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxides in chlorophyll degradation. - Physiol. Plant. **85**: 85-89, 1992.
Surasak, S., Samuel, T., Desh-Pal, S.V., Richard, T.S.: Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. - Plant Cell **14**: 2837-2847, 2002.
Taylor, M.D.: Accumulation of cadmium derived from fertilizers in New Zealand soils. - Sci. total Environ. **208**: 123-126, 1997.
Warren, C.R., Adams, M.A., Chen, Z.: Is photosynthesis related to concentrations of nitrogen and Rubisco in leaves of Australian native plants? - Aust. J. Plant Physiol. **27**: 407-416, 2000.
Wildner, G.F., Henkel, J.: The effect of divalent metal ion on the activity of Mg^{2+}-depleted ribulose-1,5-bisphosphate oxygenase. - Planta **146**: 223-228, 1979.