SALICYLIC ACID PROTECTS PHOTOSYNTHESIS AGAINST CADMIUM TOXICITY IN PEA PLANTS

L. Popova[1*], L. Maslenkova[1], R. Yordanova[1], A. Krantev[1], G. Szalai[2], T. Janda[2]

[1]Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
[2]Agricultural Research Institute at the Hungarian Academy of Sciences, H-2462, Martonvasar, POP 19, Hungary

Summary. In the present study we have investigated the possible mediatory role of salicylic acid (SA) in protecting photosynthesis against cadmium (Cd) toxicity. The exposure of pea plants to increasing Cd concentrations (0.5, 1.0, 2.0 and 5.0 μM) during early stages of their establishment caused a gradual decrease in shoot and root fresh weight accumulation, the rate of CO_2 fixation and the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC, E.C. 4.1.1.39), the effect being most expressed at higher Cd concentrations. *In vivo* the excess of Cd induced alterations in the redox cycling of oxygen evolving centers and the assimilatory capacity of the pea leaves as revealed by the changes in the thermoluminescense emission after flash illumination. Seed pretreatment with SA alleviated the negative effect of Cd on growth, photosynthesis, carboxylation reactions, thermoluminescence characteristics and chlorophyll content and led to a decrease in the oxidative injuries caused by Cd. The data suggest that the beneficial effect of SA during an earlier growth period could be related to avoidance of cumulative damage upon exposure to Cd, thus reducing the negative consequences of oxidative stress caused by heavy metal toxicity. In addition, the observed high endogenous levels of SA after treatment with

*Corresponding author, e-mail: lpopova@obzor.bio21.bas.bg

Cd suggest that SA may act directly as an antioxidant to scavenge the reactive oxygen species and/or it may modulate indirectly the redox balance through activation of antioxidant responses. Taken together these results could explain to some extent the protective role of SA on the photochemical activity of chloroplast membranes and photosynthetic carboxylation reactions in Cd-stressed pea plants.

Key words: antioxidants, cadmium, photosynthesis, *Pisum sativum* L., salicylic acid, thermoluminescence.

Abbreviations: A - net CO_2 assimilation rate; DTT - dithiothreitol; Gs - stomatal conductance; *O*-ANI - *O*-anisic acid; *O*-HCA - *O*-hydroxy-cinnamic acid (*O*-coumaric acid), pHBA - para-hydroxy-benzoic acid; PS II - photosystem II; RuBPC – ribulose-1,5-bisphosphate carboxylase; SA - salicylic acid; TL - thermoluminescence; WUE -water use efficiency.

INTRODUCTION

Cadmium (Cd) is a highly toxic trace element which enters the environment mainly from industrial processes and phosphate fertilizers. Cadmium is easily taken up by plant roots and can be loaded into the xylem for its transport into leaves. A large number of studies have demonstrated the toxic effect of Cd on plant metabolism, such as decrease uptake of nutrient elements (Sandalio et al., 2001), changes in nitrogen metabolism (Boussama et al., 1999), inhibition of photosynthesis through effects on the chlorophyll metabolism and chloroplasts structure (Gadallah, 1995) the activity of both photosystem II and the enzymes of photosynthetic carbon metabolism (Atal et al., 1993; Siedlecka et al., 1998). The toxicity of Cd has been related with the increase of lipid peroxidation and alterations in the antioxidant system (Sandalio et al., 2001; Romero-Puertas, 2002). Cadmium also produces alterations in the functionality of membranes by inducing changes in their lipid composition (Hernandez and Cook, 1977) and this can affect some enzymatic activities associated with membranes such as H^+- ATPase (Fodor et al., 1995).

Different plant species and varieties show a wide range of plasticity

in Cd tolerance, reaching from the high degree of sensitivity to the hyper accumulating phenotype of some tolerant higher plants. Legume plants are less tolerant to Cd toxicity than cereals and grasses (Metwally et al., 2005). A first barrier against Cd stress is retention of Cd in the cell wall (Nishizono et al., 1989). Once Cd enters the cytosol other detoxification mechanisms are induced, primarily the formation of complexes between Cd and phytochelatins (PCs) and their subsequent compartmentalization (Cobbett and Goldsbrough, 2002; Vazquez, 2006). Other mechanisms that plants have developed to cope with damages caused by cadmium are related with some stress signaling molecules, such as salicylic acid, jasmonic acid and ethylene. All these compounds were induced by Cd treatment, which suggest that they are involved in cell response to Cd toxicity (Rodriguez-Serrano, 2006).

Salicylic acid (SA) as a potent signaling molecule in plants is involved in eliciting specific responses to biotic and abiotic stresses. It has been shown that SA provides protection in maize (Janda et al., 1999) and winter wheat plants (Tasgin et al., 2003) against low-temperature stress, induces termotolerence in mustard seedlings (Chen et al., 1997; Dat et al., 1998) or modulates plant responses to salt and osmotic stresses (Borsani et al., 2001) ozone or UV light (Sharma et al., 1996) drought (Senaratna et al., 2002) and herbicides (Ananieva et al., 2004). Furthermore, SA is also known to be involved in plant protection to heavy metals. SA pretreatment alleviates Pb- and Hg-induced membrane damage in rice (Mishra and Chudhuri, 1999) and Cd toxicity in barley (Metwally et al., 2003) and maize plants (Pal et al., 2002). Although there have been many reports on the photochemical and biochemical events occurring in photosynthesis during Cd toxicity, a lot of contradictory data can be found in the literature. Probably this is due to the very heterogeneous experimental approaches, including both laboratory grown conditions and field experiments. Only limited number of studies have been carried out during the germinating stage of plants.

In the present work, we studied the effect of exposure of pea plants to Cd during early stages of their establishment, on the physiological and biochemical properties of pea leaves. Our study was mainly focused on the mechanisms by which SA might influence the photosynthetic processes to overcome Cd toxicity in pea plants.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L., *cv. Ran*) were sterilized and divided into two groups. One half of the seeds was soaked in 500 μM SA solution for 6 h, the other half of the seeds was soaked in water (control), and then the both groups were allowed to germinate on moist filter paper in the dark. Three-day-old, dark grown seedlings, were placed in polyethylene pots filled with 0.6 L modified Hoagland solution. $CdCl_2$ was added at a concentration of 0.5, 1, 2 and 5 μM. Plants were grown in a growth chamber at a day/night cycle 16h/8h, at 22/18 ^oC, respectively, relative humidity between 50 % and 60 %, and 120 $\mu mol.m^{-2}s^{-1}$ PAR. After 12 days of growth the plants were harvested for analysis.

The growth of the shoot and root was analyzed in terms of their fresh weight. Chlorophyll was extracted by acetone and measured spectrophotometrically according to Arnon (1949).

The measurements were performed by a portable photosynthesis system Li-6400 (Li-Cor, Lincoln, USA). Leaves of 5-6 plants (second well-expanded leaf pairs) were placed in a 250 cm^3 camber. Quantum flux density was 870 $\mu mol\ m^{-2}\ s^{-1}$ PAR. Flow rate through the cuvette was 18-20 cm^3s^{-1}, boundary layer resistance (r_a) was 0.8 s cm^{-1}. Leaf temperature was 26±2 ^oC.

Leaf tissue was ground in a mortar on ice at a ratio of 1 g fresh mass to 5 ml cold extraction medium containing 0.33 M sorbitol, 0.05 M HEPES-NaOH, 2 mM KNO_3, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 0.5 mM K_2HPO_4, 0.02 M NaCl, and 0.2 M Na-isoascorbate, pH 7.6. The homogenate was quickly filtered through four layers of cheesecloth and centrifuged at 20, 000 × g for 15 min, and the supernatant used directly for enzyme assay.

RuBPC (E.C. 4.1.1. 39) activity was assayed from the activated crude preparation by following the incorporation of $NaH^{14}CO_3$ into acid stable products (Popova et all., 1988). The assay mixture for RuBPC contained in 50 mM HEPES-NaOH (pH 8.0): 20 μmol $MgCl_2$, 1 μmol ditiothreitol (DTT), 20 μmol $NaHCO_3$ (containing 1.48 MBq, specific radioactivity 0.38 $MBq\ \mu mol^{-1}$), and the enzyme extract equivalent to 0.3-0.4 mg protein. Reaction, at 25±1 ^oC, was initiated by addition of 2 μmol RuBP and stopped

after 1 min reaction time with 6 M HCl. Reaction volume was 1 ml. The amount of fixed $^{14}CO_2$ was measured in a liquid scintillation spectrometer.

TL glow curves of *Pisum sativum* leaf discs were measured using the apparatus and software described earlier (Miranda and Ducruet, 1995). Luminescence was detected by a Hamamatsu H-5701-50 photomultiplier linked to an amplifier. A 4 × 4 cm Peltier element was used for temperature control. Dark adapted leaf samples were cooled within a few seconds and irradiated via a fiber optic either by far-red radiation by a PAM 102-FR light source for 30 s or by a single turnover flash lamp (XST 103; Walz, Effertrich, Germany) at 1 °C. The leaf sample was gently pressed against the plate by a rubber ring and a Pyrex window, with the addition of 100 µl water for better thermal conduction. The rate of heating during measurements was 0.5 °C s^{-1}. The TL measurements were repeated several times and representative glow curves are presented in the figures.

Approximately 1 g of dry root material was wet digested in H_2SO_4/HNO_3 mixture (1/5, v/v) for 24h, and then it was treated with HNO_3/$HClO_4$ mixture (5:1, v/v). Cadmium concentration in the digest was measured by atomic absorption spectrophotometer Perkin- Elmer (Germany).

Salicylic acid and its precursors were measured according to Meuwly and Métraux, (1995). Two g of the 3rd leaves were ground in liquid nitrogen in a mortal and pestle, in the presence of 1 g quartz sand. The tissue powder was transferred to a centrifugation tube and mixed with 2 ml of 70 % methanol containing 250 ng *ortho*-anisic acid (oANI) (used as internal standard) and 25 µg *para*-hydroxy-benzoic acid (pHBA) (used as extraction carrier). The extract was centrifuged at 10,000 × *g* for 20 min. The pellet was resuspended in 2 ml 90 % methanol and centrifuged as above.

The methanol content was evaporated from 2 ml of the mixed supernatants at room temperature under a vacuum. One ml of 5 % (w/v) TCA was added to the residual aqueous phase and the mixture was centrifuged at 15,000 × *g* for 10 min. The supernatant was gently partitioned twice against 3 ml of a 1/1 (v/v) mixture of ethyl acetate/cyclohexane. The upper organic layers contained the free phenolic portion. The aqueous phases containing the methanol-soluble bound phenolics were acid hydrolysed. 250 ng oANI together with 25 µg pHBA and 1.3 ml 8 N HCl were added to the aqueous phase and incubated for 60 min at 80 °C before partitioning

twice as above. Just prior to the HPLC analysis, the organic phases were evaporated to dryness under a vacuum and resuspended in 1 ml of the HPLC starting mobile phase. SA were quantified fluorimetrically (W474 scanning fluorescence detector, Waters, USA), with excitation at 305 nm and emission at 407 nm for SA.

Statistical analysis

The experiments were repeated several times and the average of the values was used. The data were statistically evaluated using the standard deviation and *T*-test methods.

RESULTS

Exposure to high Cd concentrations resulted in dramatic decreases in both shoot and root fresh weights (34.9 and 22.8 % at 2 μM Cd and 21.4 and 13.4 % at 5 μM Cd, respectively) (Table 1).

Chlorophyll content showed approximately 35 and 63 % reduction in 2 and 5 μM Cd-treated plants, respectively. Pretreatment with SA before exposure to Cd, however, showed 22.7 % and 33 % restoration of the chlorophyll levels. Taken together these measurements indicate that Cd concentrations above 0.5 μM impose a significant level of stress on pea plants in accordance with the increased Cd content in root tissue (Table 1). SA pretreatment reduced root accumulation of Cd in all Cd treated variants.

In the present work, it was found that control leaves initially contained little free SA compared to the bound forms. Treatment with Cd did not affect their content. One and 2 μM Cd increased the level of bound o-HCA and SA, the effect was higher expressed in 2 μM Cd-teated plants (approximately 3-fold increase, compared with the controls). The total content of the free and bound o- HCA and SA was significantly high in all Cd treated variants (Table 2).

The growth inhibition of pea plants by Cd treatment was accompanied by a decrease in the rate of photosynthesis (A). The most marked reduction (almost 2-fold) as compared with the control was observed after treatment

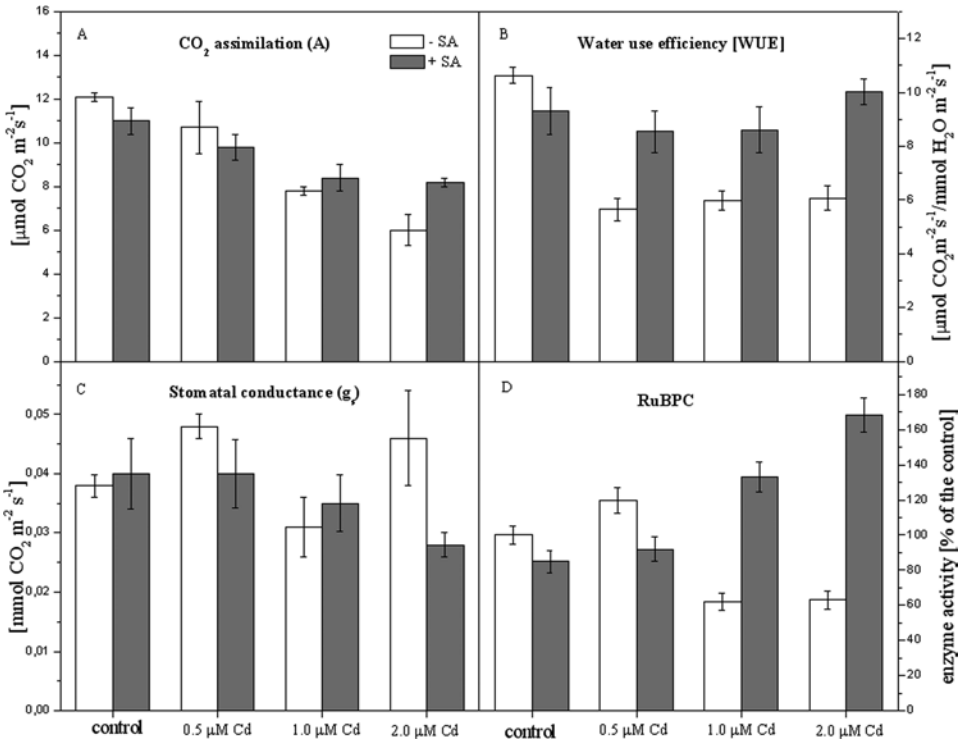

Fig. 1. Changes in gas-exchange parameters and RuBPC activity in pea plants treated with Cd and SA, A, net CO_2 assimilation rate; B, water use efficiency, WUE; C, stomatal conductance, gs; D, ribulose-1,5, bisphosphate carboxylase, RuBPC. Variants and treatment as described in Table 1. The values are means ± s.e. (n=3).

of pea plants with 2 µM Cd. Pretreatment with SA for 6 h before exposure to Cd recovered considerably the rate of A (Fig. 1 A). The water use efficiency was also affected by Cd treatment, undergoing a significant reduction in Cd-treated variants. Pretreatment with SA before exposure to Cd alleviated considerably the inhibitory effect of Cd on the values of WUE (Fig. 1B). The effects of Cd and SA on stomatal conductance showed the same trend (Fig. 1C). Treatment of pea plants with 1 and 2 µM Cd caused a decline in the activity of RuBPC approximately by 40 %. A very strong protective effect of SA was observed on RuBPC activity (Fig. 1D).

The illumination of unfrozen dark adapted leaves from non-treated

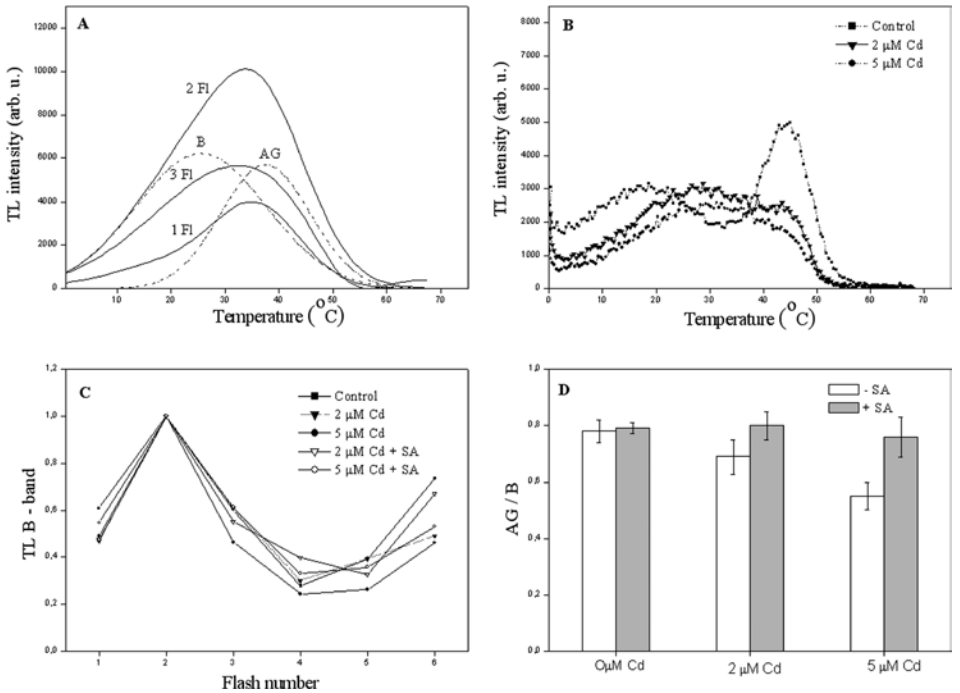

Fig. 2. Thermoluminescense glow curves in control pea leaves induced by 1 to 3 flashes (A). The components representing B- and AG-bend after TL curve decomposition are shown in dots; changes in TL B-band oscillation pattern (C) and in the ratio of AG to B band integrated intensities (D) in leaves of Pisum sativum plants treated with 2 and 5 µM Cd and after pre-treatment of the seeds with 500 µM SA; effect of Cd on TL curves recorded after 30 s far-red radiation (B). TL was recorded immediately after corresponding excitation, with a 0.5 ^{o}C s^{-1} heating rate.

(control) pea plants with one short saturating flash induced a main TL peak at 35 ^{o}C, the so-called B-band ($S_2Q_B^-$) and a hardly distinguishable shoulder at temperature about 40 ^{o}C (Fig. 2A). Two consecutive flashes (2Fl) generate a maximal TL emission with B-band ($S_{2(3)}Q_B^-$) peaking at 33 ^{o}C. The amplitude of B-band decreased after three flashes (3Fl), but the shoulder at 40 ^{o}C designated as an afterglow (AG) band became better pronounced (Fig. 2A). The B-band results from the thermal activated recombination of the trapped electrons and positive charges on the

reduced quinone acceptor (Q_B^-) and the $S_2(S_3)$ oxidation state of the water-oxidizing complex of PSII, respectively (Rutherford et all, 1982). The AG TL emission corresponds to a back electron transfer towards PSII centers initially in the $S_{2(3)}Q_B$ state (Ducruet, 2003). A damping of period-four oscillation of B-band intensity, according to the exciting flash number was observed at the higher concentration of the heavy metal (Fig. 2C). The AG/B ratio, estimated from the TL glow curve after deconvolution showed a decrease from 0.8 in control to 0.55 in Cd-stressed plants (Fig. 2D). This trend was much better expressed in the case of TL induction by short (30 s) FR illumination of dark-adapted pea leaves (Fig. 2B). This kind of illumination excites mainly PSI, but a part of the energy can be absorbed in PSII antenna leading to charge separation and the induction of TL B-band. The TL glow curve of control plants showed an intense AG emission at 45 ^oC and a B-band peaking at a lower temperature as compared to the peak position after flash illumination, most probably as a consequence of lumen acidification and destabilization of S_2 and S_3 states of the oxygen-evolving complex (Miranda and Ducruet, 1995). Figure 2B demonstrates that high Cd concentrations led to a substantial decrease in AG emission without changes in the peak temperature maximum. In the same time the B-band looks broadened and shifted to higher temperature.

Short-term application of 500 μM SA to the pea seeds did not exert changes in the investigated TL parameters. However, pre-treatment with SA before the application of high heavy metal concentrations had a stabilizing effect on the photochemical reactions, as judged by some restoration of the AG/B ratio and the B-band oscillation pattern (Fig. 2C and 2D).

DISCUSSION

This study was undertaken to identify the mechanisms and the influence of SA on the photosynthetic processes in Cd exposed pea plants. Our results showed that Cd produced a concentration-dependant reduction in the growth of pea plants, measured as fresh weight of roots and shoots (Table 1). Under our experimental conditions, pea plants did not tolerate Cd concentrations higher than 5 μM without showing any visible toxicity symptoms. This confirms the data of other authors (Belimov et al., 2003)

Table 1. Effects of Cd and SA on some physiological parameters of pea plants and data on rood Cd accumulation. Dry seeds were soaked in 500 μM SA or water for 6h, and were germinated on moist filter paper for three days. Then they were transferred to hydroponic medium and grown for 14 days without or with Cd in the medium.
The data of shoot and root fresh weight and chlorophyll content are means ± s. e. (n=5) and for Cd content ± s. e. (n=3).

Conc. (μM Cd)	Shoot FW (g/plant)		Root FW (g/plant)		Chlorophyll (mg/g FW)		Cd content (mg/kg DW)	
	- SA	+ SA	- SA	+ SA	- SA	+ SA	- SA	+ SA
0	0.51 ± 0.04	0.54 ± 0.04	0.22 ± 0.05	0.21 ± 0.07	3.05 ± 0.3	2.76 ± 0.1	23.59	11.05
0.5	0.45 ± 0.04	0.55 ± 0.01	0.21 ± 0.03	0.21 ± 0.04	3.07 ± 0.3	3.04 ± 0.3	108.38	86.98
1.0	0.47 ± 0.02	0.48 ± 0.05	0.16 ± 0.04	0.19 ± 0.04	2.49 ± 0.2	2.80 ± 0.2	190.85	74.69
2.0	0.18 ± 0.03	0.22 ± 0.05	0.05 ± 0.01	0.08 ± 0.01	2.25 ± 0.2	2.76 ± 0.2	480.82	130.17
5.0	0.11 ± 0.04	0.17 ± 0.07	0.03 ± 0.00	0.03 ± 0.01	1.13 ± 0.4	1.49 ± 0.4	n.d.	n.d

Table 2. Effects of Cd and SA on the accumulation of free and bound oHCA and SA in pea leaves. The details of treatment and growth are as in Table 1. Each value corresponds to a typical experiment among at least tree replicates.

Conc. (μM Cd)	Free oHCA (ng/g FW)		Bound oHCA (ng/g FW)		Free SA (ng/g FW)		Bound SA (ng/g FW)	
	- SA	+ SA	- SA	+ SA	- SA	+ SA	- SA	+ SA
0	31.5 ± 2.9	32.6 ± 2.1	131.1 ± 5.8	92.4 ± 8.9	73.2 ± 2.1	90.9 ± 3.1	478.8 ± 13	375.0 ± 9
0.5	15.7 ± 1.7	21.6 ± 1.9	64.7 ± 3.9	96.6 ± 7.9	71.9 ± 2.4	66.2 ± 2.9	437.8 ± 11	443.1 ± 7
1.0	33.7 ± 2.2	27.2 ± 2.5	149.2 ± 6.9	72.3 ± 3.0	68.1 ± 1.9	41.8 ± 1.9	1151.0 ± 25	464.1 ± 10
2.0	41.4 ± 2.1	61.7 ± 3.7	332.5 ± 9.4	59.4 ± 1.5	51.5 ± 1.3	46.7 ± 2.4	1235.2 ± 11	649.7 ± 10

that pea plants can be considered as Cd-sensitive species. In general, the sensitivity of a given plant species to heavy metal toxicity depends on its concentration, treatment duration, plant age, and the plant organ examined (Metwally et al., 2005). We explain our results with the fact that pea plants were exposed to Cd at the very early stage of their development. The growth inhibition of pea plants was accompanied by a significant decrease in net photosynthesis measured as CO_2 assimilation which at the highest Cd concentration (2 μM) was reduced about two times (Fig. 1A). The water use efficiency was also affected by Cd treatment, undergoing a progressive

reduction with increasing Cd concentrations (Fig. 1B). The activity of RuBPCase (Fig. 1D) and chlorophyll content (Table 1) also decreased with rising Cd concentrations.

In addition to the negative effects of Cd on the photosynthetic carboxylation reactions, PSII electron transport and especially oxygen-evolving complex were found to be very sensitive to the effect of Cd (Clijsters and van Assche, 1985). Different components of the electron transport chain were proposed as primary targets of Cd and different mechanisms of action were discussed. The donor side (Clijsters and van Assche, 1985; Krupa et al., 1993) or the acceptor side (Atal et al., 1993) have been implicated as the main sites of inhibition. In our attempt to contribute to the understanding the effects of Cd on PSII reactions, an analysis of the TL glow curve parameters was done in Cd-stressed pea. The traces in Fig. 2C revealed that higher Cd concentrations affected the B-band oscillation pattern and decreased the TL intensity. These results showed a reduction in the number of PSII reaction centers and an increase of misses in charge separation, thus suggesting that the centers can not reach their higher oxidation states, S_3 and S_4. Whereas the B-TL band is specific for PSII charge recombination (Rutherford et al.,1982), the AG band is thought to originate from a feedback of reducing equivalents from the stroma towards PSII centers initially in TL inactive $S_2(S_3)Qb$ state (Ducruet, 2003). It has been proposed that AG reflects the [NADPH + ATP] assimilatory potential in the chloroplasts when induced by flashes. The decrease of the assimilatory potential as evidenced by the lower AG/B ratio (Fig. 2D) can be due to the reduced electron transport. In the case of a high heavy metal concentration this can be the result of the harmful effect of reactive oxygen species on the thylakoid membrane composition and function. Geiken et al. (1998) reported that in the presence of high concentrations of Cd PSII oxygen-evolving complex was altered and disassembly of the stacked regions in chloroplasts of stressed pea plants could be seen.

Here we found that Cd treatment caused the accumulation of total SA levels, the effect being higher expressed on their conjugated forms (Table 2). A similar effect of Cd on SA accumulation in maize plants has been reported by Pal et al. (2005) and Krantev et al. (2007).

Cd content of dry seeds and root tissue was low in the absence of Cd in

the growth medium and strongly increased after treatment with Cd, being approximately 20 times higher in 2 μM Cd-treated plants (Table 1).

Presoaking of pea seeds for 6 h with 500μM SA before exposure to Cd had a beneficial effect on growth, photosynthesis, carboxylation reactions, thermoluminescence characteristics and chlorophyll content, and led to a decreased oxidative injury caused by Cd. Although SA participates in the development of stress symptoms, it is also needed for the adaptation process and the induction of stress tolerance. Most abiotic stresses increase the plant concentration of SA, which points to its involvement in stress signaling. SA is a direct scavenger of hydroxyl radical and an iron-chelating compound, thereby inhibiting the direct impact of hydroxyl radicals as well as their generation via the Fenton-reaction (Dinis et al., 1994). The observed high levels of SA after treatment with Cd may act directly as an antioxidant to scavenge the reactive oxygen species and/or indirectly modulate redox balance through activation of antioxidant responses.

A hypothetical explanation may account for the positive effect of SA on photosynthesis in pea plants exposed to Cd stress: SA prevented cumulative damage development in response to Cd. The suggestion is supported by the data of the lowered root level of Cd in SA- pretreated pea plants (Table 2). Similar data have been reported by Szalai et al. (2005) in maize. Cd usually accumulates in the roots, because this is the first organ exposed to heavy metals in the soil, but it is also translocated into the shoots. Obviously, the lowered root level of Cd in SA-pretreated pea plants reduced the harmful effect of Cd and exerted a beneficial effect on the growth and photosynthesis. These results could explain to some extent the protective role of SA on the photochemical activity of chloroplast membranes and photosynthetic carboxylation reactions in Cd-stressed pea plants.

Acknowledgements: This study was supported by the Bulgarian-Hungarian Academy of Sciences Project (2004-2007), Bulgarian-Spanish project (P2005BG01) and by a grant from French Ministry of Foreign Affairs, ECO-NET project № 10149TB (2005-2006).

References

Ananieva, E., K. Christov, L. Popova, 2004. Exogenous treatment with Salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to Paraquat. J. Plant Physiol., 161, 319–328.

Arnon, D.I., 1949. Cooper enzymes in isolated chloroplasts. Polyphenol -oxidase in *Beta vulgaris.* Plant Physiol., 24**,** 1-15.

Atal, N., P.P. Sardini, P. Mohanty, 1993. Inhibition of the chloroplast photochemical reactions by treatment of wheat seedlings with low concentrations of cadmium. Analisys of electron transport activities and changes in fluorescence yield. Plant Cell Physiol., 32, 943-951.

Belimov, A. A., V.I. Safronova, V.E. Tsyganov, A.I. Borisov, A.P. Kozhemyakov, V.V. Stepanok, A. M. Martenson, V. Gianinazzi-Pearson, I.A. Tikhonovich, 2003. Genetic variability in tolerance to cadmium and accumulation of heavy metals in pea (*Pisum sativum* L.). Euphytica, 131, 25-35.

Borsani, O., V. Valpuesta, M. A. Botella, 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. Plant Physiol., 126, 1024-1030.

Boussama, N., O. Quariti, M.H. Ghorbal, 1999. Changes in growth and nitrogen assimilation in barley seedlings under cadmium stress. J. Plant Nutr., 22, 731-752.

Chen, Z., S. Iyer, A. Caplan, D.F. Klessig and B. Fan, 1997. Differential accumulation of salicylic acid and salicylic acid-sensitive catalase in different rice tissues. Plant Physiol., 114, 193-201.

Clijsters, H., F. van Assche, 1985. Inhibition of photosynthesis by heavy metals. Photosynth. Res., 7, 31-40.

Cobbett, C., P. Goldsbrough, 2002. Phytochelatins and metallothioneins: role of heavy metal detoxification and homeostasis. Annu Rev Plant Physiol Plant Mol Biol., 53, 159-182.

Dat, J.F., C.H. Foyer, I.M. Scott, 1998. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. Plant Physiol., 118, 1455-1461.

Dinis, T.C., V.M. Maderia, L.M. Almeida, 1994. Action of phenolic derivates (acetaminophen, salicylate, and 5- aminosalicylate) as inhibitors

of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315, 161-169.

Ducruet, J-M., 2003. Chlorophyll thermoluminescence of leaf disc: simple instruments and progress in signal interpretation open the way to new ecophysiological indicators. J. Exp. Bot., 54, 2419-2430.

Fodor, A., A. Szabo-Nagy, L. Erdei, 1995. 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.

Gadallah, M.A., 1995. Effects of cadmium and kinetin on chlorophyll content, saccahrides and dry matter accumulation in sunflower plants. Biol. Plant., 37, 233-240.

Geiken, B., J. Masojidek, M. Rizzuto, M.L. Pompili and M.T. Giardi, 1998. Incorporation of [^{35}S]methionine in higher plants reveals that stimulation of the D1 reaction center II protein turnover accompanies tolerance to heavy metal stress. Plant, Cell and Environ., 21, 1265-1273.

Hernandez, L., D. Cook, 1977. Modification of root plasma membrane lipid composition of cadmium-treated *Pisum sativum.* J. Exp. Bot., 48, 1375-1381.

Janda, T., G. Szalai, I. Tari, E. Paldi, 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. Planta, 208, 175-180.

Krantev, A., R. Yordanova, T. Janda, G. Szalai, L. Popova, 2007. Treatment with salicilyc acid decreases the effect of cadmium on photosynthesis in maize plants. J. Plant Physiol., (in press).

Krupa, Z., G. Oquist, N. Nunner, 1993. The effect of cadmium on photosynthesis of *Phaseolus vulgaris* – a fluorescence analysis. Physiol. Plant., 88, 626-630.

Metwally, A., V.I. Safronova, A.A. Belimov, K.J. Dietz, 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J. Exp Bot., 56, 167-178.

Metwally, A., I. Finkermeier, M. Georgi, K.J. Dietz, 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol., 132, 272-281.

Meuwly, P., J.P. Métraux, 1993. *Ortho*-anisic acid as internal standard

for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. Anal. Biochem, 214, 500-505.

Miranda, T., J-M. Ducruet, 1995. Characterization of the chlorophyll thermoluminescence afterglow in dark-adapted or far-red illuminated plant leaves. Plant Physiol. Biochem., 33, 689-699.

Mishra, A., M.A. Chudhuri, 1999. Effect of salicylic acid on heavy metal-induced membrane deterioration in rice. Biol. Plant., 42, 409-415.

Nishizono, H., K. Kubota, S. Suzuki, F. Ishii, 1989. Accumulation of heavy metals in cell walls of *Polygonum cuspidatum* roots from metalliferous habitats. Plant Cell Physiol., 30, 595-598.

Pal, M., E. Horvath, T. Janda, E. Paldi, G. Szalai, 2005. Cadmium stimulates the accumulation of salicylic acid and its putative precursors in maize (*Zea mays)* plants. Physiol. Plant., 125, 356-364.

Pal, M., G. Szalai, E. Horvath, T. Janda, E. Paldi, 2002. Effect of salicylic acid during heavy metal stress. Proc. 7th Hungarian Cong. Plant Physiol., 46, 119-120.

Popova, L.P., T.D. Tsonev, S.G. Vaklinova, 1988. Changes in some photorespiratory and photosynthetic properties in barley leaves after treatment with jasmonic acid. J. Plant Physiol., 132, 257-261.

Rodriguez-Serrano, M., M. C. Romero-Puertas, A. Zabalza, F J. Corpas, M. Gomez, L. A. Del Rio, L. M. Sandalio, 2006. Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation *in vivo*. Plant Cell Environ., 29, 1532-1544.

Romero-Puertas, M.C., J.M. Palma, M. Gomes, L.A. del Rio, L.M.,Sandalio, 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ., 25, 677-686.

Rutherford, A.W., A.R. Crofts, Y. Inoue, 1982. Thermoluminescence as a probe of photosystem II photochemistry. The frigin of flash-induced glow curves. Biochim. Biophys. Acta, 682, 457-465.

Sandalio, L.M., H.C. Dalurzo, M. Gomes, M. Romero-Puertas, L.A. del Rio, 2001. Cadmium- induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot., 52, 2115-2126.

Senaratna, T., D. Touchell, E. Bunns, K. Dixon, 2000. Acetyl salicylic acid

(aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul., 30, 157-161.

Sharma, Y.K., J. Leon, I. Raskin, K.R. Davis, 1996. Ozone-induced responses in *Arabidopsis thaliana*- the role of salicylic acid in the accumulation of defence-related transcripts and induced resistance. Proc. Natl. Acad. Sci. USA, 93, 5099-5104.

Siedlecka, A., G. Samuelsson, P. Gardenstrom, L.A. Kleczkowski, Z. Krupa,1998. The „acvivatory model“ of plant response to moderate cadmium stress- relationship between carbonic anhydrase and Rubisco, In: Garab G. (ed.), Photosynthesis: Mechanisms and Effects, vol. IV, Kluwer Academic Publ., Dordrecht-Boston- London, pp. 2677-2680.

Szalai, G., M. Pal, E. Horvath, T. Janda, E. Paldi, 2005. Investigations on the adaptability of maize lines and hybrids to low temperature and cadmium. Acta Agronomica Hungarica, 53, 183-196

Tasgin, E., O. Attici, B. Nalbantogly, 2003. Effect of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regul., 41, 231-236.

Vazquez, S., P. Goldsbrough, R.O. Carpena, 2006. Assessing the relative contributions of phytochelatins and the cell wall to cadmium resistance in white lupin. Physiol. Plant., 128, 487-495.