DOI: 10.1007/s10535-012-0135-x BIOLOGIA PLANTARUM 57 (1): 121-127, 2013

Abscisic acid is required in transduction of cadmium signal to potato roots

A. STROIŃSKI*, K. GIŻEWSKA, and M. ZIELEZIŃSKA

Department of Plant Physiology, University of Life Sciences, Wołyńska 35, PL-60637 Poznań, Poland

Abstract

Treatment of potato (*Solanum tuberosum*) plants with cadmium or abscisic acid (ABA) enhanced the content of *StPCS1* transcript and activity of phytochelatin synthase (PCS) in roots. These treatments enhanced the contents of ABA and expression of genes coding 9-cis-epoxycarotenoid dioxygenase 1 (*NCED1*) and basic leucine zipper (*b-ZIP*). Simultaneous treatment of potato plants with Cd and fluridone (Flu), an inhibitor of ABA biosynthesis, completely halted the Cd-induced transcription of *StPCS1*, *NCED1*, and *StbZIP* genes and limited the increases in PCS activity and ABA content. The data suggest that ABA participates in transduction of the Cd signal to the cells of potato roots.

Additional key words: basic leucine zipper; 9-cis-epoxycarotenoid dioxygenase, phytochelatin synthase.

Introduction

Heavy metals, such as cadmium, can induce oxidative stress and damage of macromolecules and cellular structures (Stroiński 1999, Clemens 2001). The resistance of plant cells to these effects partly depends on the content of phytochelatins (PCs) and other chelators which bind Cd ions. In the presence of heavy metals, PCs are synthesized from reduced glutathione (GSH) and related thiols by phytochelatin synthase (PCS; γ-glutamylcysteine dipeptidyl transpeptidase; EC 2.3.2.15) (Grill *et al*. 1989, Maitani *et al*. 1996, Pal and Rai 2010).

PCS genes have been characterized in *Arabidopsis thaliana* (*AtPCS1, AtPCS2*), wheat (*TaPCS1*), *Brassica juncea* (*BjPCS1*), rice (*OsPCS1*) (Cobbett and Goldsbrout 2002, Clemens 2006, Ogawa *et al*. 2011), potato (*StPCS1*) (Nuc *et al*. 2003), and *Sesbania rostrata* (Li *et al*. 2009). Some reports indicated that AtPCS1 did not exhibit transcriptional regulation by Cd although its activity increased in the presence of Cd (Ha *et al*. 1999, Vatamaniuk *et al*. 2000). In other studies, wheat and potato PCS (TaPCS1, StPCS) showed transcriptional and post-transcriptional regulation by Cd (Clemens *et al*. 1999, Lee and Korban 2002, Wang *et al*. 2009, Stroiński *et al*. 2010).

So far, there have been few reports characterizing participation of hormones in plant response to Cd. Short-term treatment of potato tuber discs with $CdCl_2$ solution enhanced the endogenous concentration of abscisic acid (ABA) and treatment with ABA elevated the *StPCS1* transcript level and PCS activity (Stroiński *et al*. 2010). Cd treatment increased the concentration of endogenous ABA in leaves of rice (Hsu and Kao 2003, 2005), in roots of *Typha latifolia* and *Phragmites australis* (Fediuc *et al*. 2005), and in leaves of citrus (Lopez-Climent *et al*. 2011).

ABA is synthesized from neoxanthin or violaxanthin by their cleavage by 9-cis-epoxycarotenoid dioxygenase (NCED). It is the key regulatory step of ABA biosynthesis (Schwartz *et al*. 1997a,b, 2003). Activities of ABA synthesizing enzymes are positively regulated by ABA. Initial induction of ABA biosynthesis by different factors, including Cd, stimulates this biosynthesis by means of this positive feedback loop (Audran *et al*. 2001, Xiong and Zhu, 2003). The ABA signal induces the expression of genes that have G-boxes in the promoter. Basic leucine zipper (b-ZIP) proteins are transcription factors that specifically bind to G-boxes; thus, they are important elements of the ABA signal transduction pathway (Jakoby *et al*. 2002). On the other hand, ABA can regulate biosynthesis of cysteine which is an essential substrate for synthesis of heavy metal chelators such as GSH and PCs. ABA and Cd induce accumulation of mRNA of cytosolic O-acetylserine (thiol)lyase (OASTL; EC 2.5.1.47) synthesizing cysteine (Barroso *et al*. 2006).

Received 17 February 2011, *accepted* 2 January 2012.

Abbreviations: ABA - abscisic acid; b-ZIP - basic leucine zipper; cADPR - cyclic ADP-ribose; Flu - fluridone; GSH - reduced glutathione; IP_3 - inositol-1,4,5-trisphosphate; NCED - 9-cis-epoxycarotenoid dioxygenase; PCs - phytochelatins; PCS - phytochelatin synthase; ROS - reactive oxygen species.

Acknowledgments: We thank Dr. Jerzy Roszyk for help in Cd determination. This study was supported by KBN grant No. 2 P06A 014 27.

* Corresponding author; fax: (+48) 609 489 360, e-mail: astroins@o2.pl

Both factors evoked the oxidative stress in plant cells (Stroiński and Kozłowska 1997, Stroiński and Zielezińska 1997, Jiang and Zhang 2001) that modulates the calcium content the cytoplasm by various ways (Murata *et al*. 2001, Desikan *et al*. 2004).

This study was undertaken to answer the question whether ABA participates in transduction of the Cd signal to the potato root cells.

Materials and methods

Sterile plants of *Solanum tuberosum* cv. Bzura were placed in *Sigma* (St. Louis, USA) culture boxes containing 300 cm^3 of Murashige and Skoog (MS) liquid medium with Gamborg's vitamins (*Sigma*) without or with 0.02 - 0.1 mM $CdCl_2$, 0.01 - 0.1 mM ABA, and/or 0.04 and 0.1 mM fluridone [Flu; 1-methyl-3-phenyl-5-(α,α,α-trifluoro-*m*-tolyl)-4-pyridone]. Plants were grown under *Philips* fluorescent lamps (*TLD 58W/840*) giving irradiance of 130 μmol m^{-2} s^{-1} (PAR) at 20 °C for 24 h. Two-week-old plants were incubated for 24 and 48 h under stress conditions and for 5 - 48 h for experiments with $CdCl_2$. In experiments with double stress factors, the plant treatment with ABA preceded its treatment with $CdCl_2$, whereas in the experiment with Flu and Cd, these reagents were introduced to the medium simultaneously and plants grew 24 h. Subsequently, the whole plants were immediately frozen in liquid nitrogen.

The isolation of PCS from frozen materials and activity estimation were described previously by Stroiński and Zielezińska (2001). PCS activity was determined according to Grill *et al*. (1989). Total protein contents were measured according to Bradford (1976). Phytochelatins were estimated by using a reverse-phase high-performance liquid chromatography (HPLC) with post-column derivatization with Ellman's reagent and measurement of the A_{405} (for detail see Stroiński and Zielezińska 1997). The amount of PCs, as a sum of PC_2, PC_3, and PC_4, was calculated from a comparison with the GSH standards.

The isolation and estimation of ABA by HPLC and competitive ELISA were performed according to Moore (1990). Plant material (3 - 5 g fresh mass) was freeze-dried and stored at -20 °C until analysis. Tissues were homogenized in 15 cm^3 of 80 % (v/v) methanol in 0.2 M acetic acid at 4 °C. During homogenization, ^{3}[H]-ABA was added as an internal standard for estimating extraction efficiency. Homogenized tissues were centrifuged at 5 000 *g* for 10 min. Supernatants were poured into 15 cm^3 tubes and dried in a speed vacuum concentrator. The dried sample were resuspended in 0.5 cm^3 of 80 % (v/v) methanol in 0.2 M acetic acid, mixed with 5 cm^3 of saturated NaCl solution and extracted three times with 5 cm^3 of diethyl ether. The ether phase was collected, evaporated to dryness and stored at -20 °C until estimation of ABA. ABA was determined after reconsti-tution of the samples in 80 % (v/v) methanol in 0.2 M acetic acid and centrifugation at 3 000 *g* for 10 min. Samples of 0.05 cm^3 were injected onto a reverse-phase column (*Discovery C18*, 24 × 4.6 mm, *Supelco*, St. Louis, USA). ABA was eluted with a gradient of acetonitrile (20 - 70 %) in 0.1 % (v/v) trifluoroacetic acid at a flow rate of 1 cm^3 min^{-1} and monitored at 254 nm. The fractions with ABA were collected and evaporated to dryness. ABA was quantified by the use of competitive ELISA kit (*Agdia*, Elkhart, USA).

Total RNA was extracted from leaf and root tissues by using the phenol-chloroform method. Tissue was ground in liquid N_2 to a fine powder by using a mortar and a pestle. The ground tissue was transferred to a tube containing extraction buffer with phenol (Branch *et al*. 1989). Cellular debris was removed by centrifugation (10 000 *g* for 15 min) and the supernatant was extracted with chloroform. Then 8 M LiCl was added to the aqueous phase. RNA was pelleted by centrifugation (10 000 *g* for 20 min). The pellet was resuspended in deionized, distilled water and RNA was quantified by measuring absorbance at 260 and 280 nm. Poly(A)RNA was isolated from the total RNA with a *Dynabeads* mRNA isolation kit (*Dynal*, Grand Island, USA) and was used to preparation of the cDNA. The first-strand cDNAs were synthesized by reverse transcription from 0.1 - 0.5 μg of mRNA isolated from *Solanum tuberosum* by using AMV reverse transcriptase (*Promega*, Madison, USA) and an oligo dT primer according to the manufacturer's instructions. Amounts of synthesized cDNA for the PCR reaction were measured spectrofluorometrically by using the *OligoGreen* dye with oligonucleotide standard M13 (*Molecular Probes*). The PCR reaction was performed by the use of 2 × PCR *Master Mix* (*Fermentas*, Glen Burnie, USA) which contained a concentrated solution of 0.05 U cm^{-3} *Taq* DNA Polymerase (recombinant). The following specific primers were used for the PCS gene (*StPCS1*): 5'-AAATGGAAAGGGCCTTGGAG-3' and 5'-GAC CCAGTGAGGGGGATA-3' (Nuc *et al*. 2003), for carotenoid dioxygenase (*NCED1*): 5'-CACCGGAAA AATACCCAAATG-3' and 5'-GCAAATCATCTT CAGACATAGC-3' (Burbidge *et al*. 2000), for b-ZIP gen (*Stb-ZIP*): 5'-CCATTGGGTGGAAGAGCAAG-3' and 5'-TTGCTAACCTCCTGGATTTG-3' (Prat 1994), and for actin gen (*PoAc58*): 5'-GGTTTGCGGGAGATG ATGCT3' and 5'-TCCATATCATCCCAATTGCTG-3' (X55749). The amplification of the sample cDNA (20 ng) in the PCR reaction was performed in a thermocycler at the following conditions: initial denaturation step at 94.5 °C for 2 min; primer annealing step at 59 °C for 25 s; extending step at 69 °C for 1 min; 35 cycles at 95 °C for 15 s, 59 °C for 30 s, and 69 °C for 1 min; and final extending step at 69 °C for 7 min. The PCR products were separated electrophoretically on a agarose gel (11 g dm^{-3})

with ethidium bromide. The relative intensity of the cDNA signal was measured using *ScionImage Beta3b* (*Scion Corporation*, Frederick, USA). The results related to the respective actin were quantified and averaged from three independent experiments.

To determine the soluble (Cd_s) and bound (Cd_b) cadmium, the potato tissue was homogenized in 200 mM phosphate buffer at pH 7.8 and the resultant homogenate was centrifuged at 20 000 *g* for 20 min. Supernatants was collected to estimate Cd_S concentrations. To determine Cd_B content, the precipitate was wet-mineralized to ash by using an acid mixture (360 g dm^{-3} HCl + 200 g dm^{-3} $HClO_4$ + H_2O; 2:1:1; v/v). Cd was determined by means of flame atomic absorption spectrometry.

The data presented in the figures are means of three or more independent replications. The significance of differences between means was calculated by using unpaired *t*-test (*SigmaPlot*).

Results

Control plants (Cd-untreated) contained some detectable amounts of Cd: 0.3 ± 0.1 μg g^{-1}(f.m.) of Cd_s and 0.9 ± 1.4 μg g^{-1}(f.m.) of Cd_b. The treatment of 2-week-old plants with 0.1 mM $CdCl_2$ solution increased the Cd_s to 76 ± 7.7 μg g^{-1}(f.m.) and Cd_b to 10.9 ± 1.1 μg g^{-1}(f.m.).

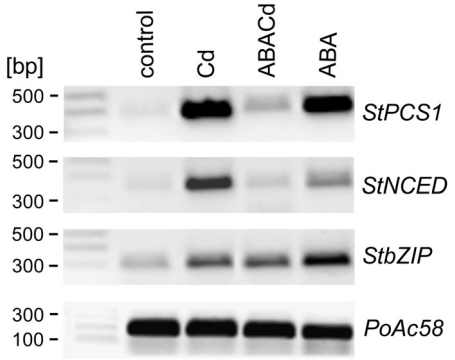

Fig. 1. Effects of exogenous Cd (0.1 mM) and ABA (0.1 mM) and their combination on expression of *NCED*. *StPCS1* and *StbZip* genes in potato roots. Specific primers to *NCED* for the 378 bp product, to *StPCS1* for the 352 bp product, to *StbZIP* for the 300 bp product, and to *PoAc58* for the 190 bp product were used.

Treatment of plants with Cd increased the content of endogenous ABA in roots about 4-fold (Table 1). This effect was dependent on concentrations of Cd^{2+} and time of metal treatment (results not presented). When plants were treated simultaneously with Cd and Flu, an inhibitor of ABA biosynthesis, the increase in ABA content was markedly limited (Table 2). In another experiment, when plants were treated with ABA, the endogenous ABA content in roots was five times higher than in control (Table 1). The increase in endogenous ABA content depended on concentrations of the applied ABA (results not presented) and partly disappeared after 72 h (Table 1). The treatment of plants with ABA and 24 h later with Cd brought about smaller increase in endogenous ABA content than Cd or ABA alone (Table 1).

ABA or Cd treatment induced expression of the *NCED* gene in roots (Fig. 1, Table 1). These effects were concentration-, and time-dependent (results not shown). When the plants were treated simultaneously with Cd and Flu, the *NCED* transcription was halted (Table 2). When ABA treatment preceded Cd treatment, the antagonistic effect was observed (Table 1).

In controls containing a small amount of Cd, both low PCS activity and a low level of *StPCS1* transcript were found. Cd or ABA treatment resulted in a great elevation of the transcript level of *StPCS1* and in an increase of PCS activity. These effects depended on the ABA and Cd concentrations as well as on the duration of Cd treatment (results not presented). When ABA treatment preceded Cd treatment, a Cd-induced increase of *StPCS1* transcript content did not occur (Fig. 1, Table 1). Similarly, antagonistic effect was noted in PCS activity (Table 1). When plants were treated simultaneously with Cd and Flu, the Cd-induced effect on the transcription of *StPCS1* was abolished and increase of PCS activity was limited (Table 2).

Table 1. Effects of exogenous ABA [0.1 mM] and Cd [0.1 mM] on endogenous ABA [pmol g^{-1}(f.m.)] and PC [nmol g^{-1}(f.m.)] content, PCS activities [pmol(PC) mg^{-1}(protein) min^{-1}] and on *NCED, StPCS1* and *StbZip* genes relative expression (to the level of *PoAc58*) in potato roots. Means of three replicates ± SE. The values marked with different letters are significantly different at $P \leq 0.05$.

	NCED	ABA content	*StPCS1*	PCS activity	PC content	*St bZip*
Control	0.05 ± 0.01 ^{a}	129 ± 24 ^{a}	0.05 ± 0.03 ^{a}	17 ± 5 ^{a}	63 ± 10 ^{a}	0.19 ± 0.03 ^{a}
Cd	0.74 ± 0.10 ^{b}	717 ± 34 ^{b}	0.70 ± 0.05 ^{b}	200 ± 12 ^{b}	508 ± 49 ^{b}	0.57 ± 0.04 ^{b}
Cd + ABA	0.18 ± 0.04 ^{c}	319 ± 143 ^{c}	0.17 ± 0.02 ^{c}	177 ± 17 ^{b}	53 ± 11 ^{a}	0.36 ± 0.03 ^{c}
ABA 24 h	0.73 ± 0.10 ^{b}	800 ± 98 ^{b}	0.50 ± 0.03 ^{b}	255 ± 12 ^{c}	62 ± 14 ^{a}	0.66 ± 0.08 ^{b}
ABA 48 h	-	262 ± 26 ^{c}	-	70 ± 10 ^{d}	77 ± 9 ^{a}	0.19 ± 0.04 ^{a}

Table 2. Influence of 0.1 mM fluridone (Flu) on 0.1 mM Cd-induced *StPCS1*. *NCED* and *StbZIP* relative expression (to the level of *PoAc58*), PCS activity [pmol(PC) mg^{-1}(protein) min^{-1}], and in ABA content [pmol g^{-1}(f.m.)]. Means of three replicates ± SE. The values marked with different letters are significantly different at $P \leq 0.05$.

	NCED	ABA content	*StPCS1*	PCS activity	*St bZip*
Control	0.03 ± 0.01 [a]	149 ± 12 [a]	0.04 ± 0.02 [a]	15 ± 3 [a]	0.18 ± 0.03 [a]
Cd	0.30 ± 0.03 [b]	707 ± 34 [b]	0.40 ± 0.04 [b]	123 ± 25 [b]	0.45 ± 0.08 [b]
Flu + Cd	0.08 ± 0.03 [a]	317 ± 45 [c]	0.12 ± 0.04 [c]	52 ± 8 [c]	0.07 ± 0.03 [c]

Addition of ABA did not significantly change PC contents in potato roots (Table 1) although the induction of PCS enzyme was observed (Fig. 1, Table 1). The treatment with Cd caused a significant elevation (almost 10 times) of PC contents (Table 1). When ABA treatment preceded Cd treatment, antagonistic effects were again observed (Fig. 1, Table 1).

ABA or Cd treatment substantially increased the *StbZIP* transcript content in roots (Fig. 1, Table 1). These effects depended on the concentration of both substances as well as on the duration of Cd treatment (results not presented). Also in this experiment, when ABA treatment preceded the Cd treatment, a negative interaction was noted between these compounds (Fig. 1 and Table 1). Simultaneously treatment of plants with Cd and Flu halted the transcription of *StbZIP* (Table 2).

Discussion

Our earlier study revealed that short-term treatment of potato tuber discs with $CdCl_2$ solution elevated the *StPCS1* transcript content, PCS activity, and content of PC and ABA (Stroiński *et al*. 2010). In the present study, we demonstrated that PCS in roots of potato was also regulated by Cd both at the level of transcription and enzyme activity. Cd treatment led to considerable accumulation of Cd in roots. Simultaneously, there was observed an increase in *StPCS1* transcript content, PCS activity, and PC content (Fig. 1, Table 1). However, some reports on *Arabidopsis* indicated that *AtPCS1* is not transcriptionaly regulated by Cd although its activity increased after treatment with Cd (Ha *et al*. 1999, Vatamaniuk *et al*. 2000). However, other studies showed transcriptional regulation of *AtPCS1* by Cd in wheat (*TaPCS1*) (Clemens *et al*. 1999), barley roots under N deficiency (Finkemeier *et al*. 2003), and during the early stages of plant development (Lee and Korban 2002). Recently, significant progress was made in understanding the role of heavy metals in PC synthesis. It turned out that the thiol groups of the PCS substrates, GSH or PC, are blocked by heavy metals (Cobbett 2002, Vatamaniuk *et al*. 2004). It seems that AtPCS1 possesses two Cd(II) binding sites, one activated and second inhibited the enzyme (Ogawa *et al*. 2011).

However, we do not know much about transduction of the Cd signal to the plant cell, in particular how Cd can induce transcription of the *PCS* gene. The results of our experiments allow us to postulate the participation of ABA in this process.

An increase in endogenous ABA concentration was observed in earlier studies (Hsu and Kao 2003, 2005, Fediuc *et al*. 2005, Stroiński *et al*. 2010) after treatment of these plants with Cd. These observations have been confirmed by the findings presented here. Cd treatment caused a marked increase in ABA *NCED* transcript contents in potato roots (Fig. 1, Table 1).

When during Cd stress the biosynthesis of ABA was limited by fluridone (Gamble and Mullet 1986, Yoshioka *et al*. 1998, Grappin *et al*. 2000), the transcription of *PCS1*, *NCED*, and *bZIP* was almost halted and the increases in PCS activity and ABA content were considerably limited (Table 2). Thus, we have proved, for the first time, the indispensability of a proper endogenous concentration of ABA in the Cd induction of the *PCS* gene.

Addition of ABA into the medium resulted in a great elevation of ABA content (Table 1). It was accompanied by a large rise of the *NCED* transcript level (Table 1), thus confirming the positive feedback between ABA and its biosynthesis. Surprisingly, the ABA treatment evidently increased the *StPCS1* transcript level and the amount of PCS (Table 1). However, the enzyme could not produce PC in the absence of Cd in the growth medium (Table 1). Also, these observations justify the claim that an elevated ABA content in tissue is prerequisite for the response of the plant to Cd, *i.e.* an increase of both the *StPCS1* transcript level and the amount of PCS in plant tissue.

These observations suggest that the Cd-signaling pathway is linked to the ABA-signaling pathway and their synchronization seems to be important for cell responses to Cd (Fig. 2). Unexpectedly this relation was disrupted when Cd stress was preceded by ABA treatment for 24 h (Fig. 1, Table 1). Since both stimuli may use the same signaling transducers, one stimulus might exhaust the pool of some transducers making them unavailable for the other stimulus (Knight and Knight 2001). Plausibly, when ABA treatment precedes Cd treatment, ABA exhausts the pool of one common transducer such as the calcium ion. Thus, lack of calcium may limit the Cd-signaling pathway (Fig. 2).

Our findings prompted us to propose the following scheme updating the knowledge concerning possible ABA participation in the transduction of the Cd signal to the plant cell (Fig. 2).

Previously, it was demonstrated that water deficit increased the ABA content in the plant and this activated a network of signaling pathways leading to stomatal closure (Bray 1997). A high ABA content in the cell can also activate NADPH oxidase and induce elevation of H_2O_2 content (Bray 1997, Murata *et al*. 2001). The results of other studies have shown a Cd-induced increase in H_2O_2 content (Stroiński and Zielezińska 1997, Rodriguez-Serrano 2006). H_2O_2 and ABA stimulate the permeability of Ca^{2+}-channels (Pei *et al*. 2000, Murata *et al*. 2001). Alternatively, ABA can modulate cytoplasmic Ca content by activation of synthesis of sphingosine phosphate, G proteins, lipase C, and IP_3 which regulate the mobilization of Ca^{2+} from internal stores. Calcium oscillation generates cascades of kinases and phosphatases, through which several transcription factors are activated (Himmelbach *et al*. 2003). In plants, basic region/leucine zipper transcription factors (b-ZIP) regulate several processes including drought, cold, and biotic stress signaling (Jakoby *et al*. 2002, Yamaguchi-Shinozaki and Shinozaki 2005).

Our data suggest that the Cd signal firstly induces ABA biosynthesis and further ABA intensifies its synthesis through a positive feedback loop. The appropriate accumulation of ABA is required for the induction of *StPCS1, NCED*, and *StbZIP* transcription. These observations indicate that the increased expression of *StPCS1* under Cd stress is ABA-dependent (Fig. 2).

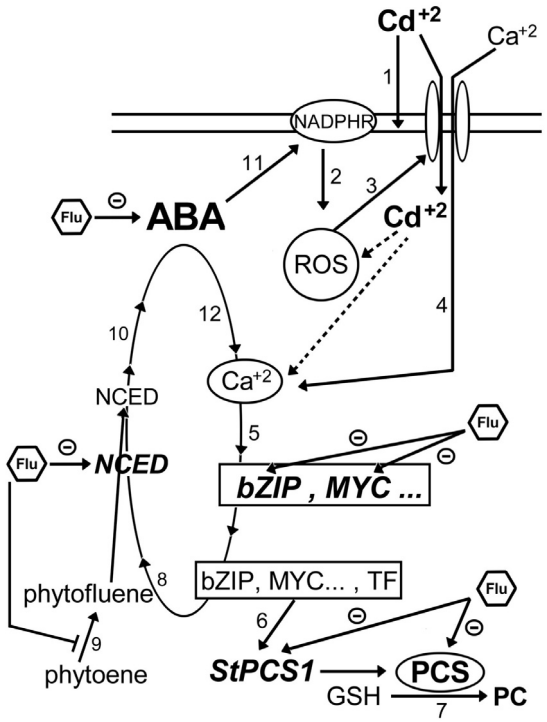

Fig. 2. Hypothetical model of interaction between Cd and ABA-signaling pathways. Cd ions can bind nonspecifically to several functional groups of membrane macromolecules (-SH, -COOH, -OH, $=HPO_2$) (1) and induce some structural and functional changes such as stimulation of NADPH oxidase activity, ROS production, and opening of Ca^{2+} channels (2, 3). These changes generate Ca^{2+} oscillation in the cytoplasm (4) which affects the activities of Ca-dependent protein kinases and phosphatases. This kinase-phosphatase cascade modulates the activities of several transcription factors (5) and consequently the activities of several enzymes. Cd in this study induced transcription of the *StbZIP, StMYC*, and *StPCS1* genes and elevated PCS activity (7). Simultaneously Cd induces the transcription of *NCED* (8). This is accompanied by stimulation of ABA biosynthesis (10) and an increase in ABA content. ABA can also stimulate ROS generation (11, 2) by the same pathway as Cd and activate *StbZIP, StPCS1* (6), and *NCED* (8) genes. The exogenous ABA treatment of plants, which resulted in higher levels of *STbZIP* and *NCED* transcripts as well as in a higher ABA content, confirmed earlier observations of a positive feedback between ABA and its biosynthesis. The high level of *StPCS1* transcript was correlated with high activity of PCS (7) which in the presence of Cd synthesized PC from GSH. When plants are treated simultaneously with Cd and Flu, the inhibitor of ABA biosynthesis (9), the Cd-induced transcription of *StbZIP* (5), *StPCS1* (6), and *NCED* (8) genes was completely blocked.

References

Audran, C., Liotenberg, S., Gonneau, M., North, H., Frey, A., Tap-Waksman, K.,Vartanian, N., Marion-Poll, A.: Localisation and expression of zeaxanthin epoxidase mRNA in *Arabidopsis* in response to drought stress and during seed development. - Aust. J. Plant. Physiol. **28**: 1161-1173, 2001.

Barroso, J.B., Francisco, J., Corpas, F.J., Carreras, A., Rodrıguez-Serrano, M., Francisco, J., Esteban, F.J., Fernandez-Ocanal, A., Chakil, M., Romero-Puertas, M.C., Valderrama, R., Luisa, M., Sandalio, L.M., Del Rio, L.A.: Localization of S-nitrosoglutathione and expression of S-nitrosoglutathione reductase in pea plants under cadmium stress. - J. exp. Bot. **57**: 1785-1793, 2006.

Bradford, M.M.: A rapid and sensitive method for the quantitation of mikrogram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Branch, A.D., Benenfeld, B.J., Robertson H.D.: RNA fingerprinting. - Methods Enzymol. **180**: 130-154, 1989.

Bray, E.A.: Plant responses to water deficit. - Trends in Plant Sci. **2**: 48-54, 1997.

Burbidge, A., Taylor, I.B., Thompson, A.: 9-cis-epoxy-carotenoid dioxygenase cDNA. - EMBL/GenBank/ DDBJdatabases, 2000.

Clemens, C., Kim, E.J., Neumann, D., Schroeder, J.I.: Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. - EMBO J. **18**: 3325-3333,

1999.
Clemens, C.: Molecular mechanisms of plant metal tolerance and homeostasis. - Planta **212**: 475-486, 2001.
Clemens, C.: Evolution and function of phytochelatin synthase. - J. Plant Physiol. **163**: 319-332, 2006.
Cobbett, C., Goldsbrough, P.: Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. - Annu. Rev. Plant Biol. **53**: 159-182, 2002.
Desikan, R., Cheung, M.K., Bright, J., Henson, D., John, T., Hancock, J.T., Neill, S.J.: ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. - J. exp. Bot. **55**: 205-212, 2004.
Fediuc, E., Lips, H., Erdei, L.: *O*-acetylserine (thiol) lyase activity in *Pragmites* and *Typha* plants under cadmium and NaCl stress conditions and the involvement of ABA in the stress response. - J. Plant Physiol. **162**: 865-872, 2005.
Finkemeier, I., Kluge, C., Metwally, A.M., Georgi, M,N., Grotiohann, N., Dietz, K.-J.: Alterations in Cd-induced gene expression under nitrogen deficiency in *Hordeum vulgare.* - Plant Cell Environ. **26**: 821-833, 2003.
Gamble, P.E., Mullet, J.E.: Inhibition of carotenoid accumulation and abscisic acid biosynthesis in fluridone-treated dark-grown barley. - Eur. J. Biochem. **160**: 117-121, 1986.
Grappin, P., Bouinot, D., Sotta, B., Miginiac, E., Jullien, M.: Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. - Planta **210**: 279-285, 2000.
Grill, E., Löffler, S., Winnacker, E.-L., Zenk, M.H.: Phytochelatins, the heavy-metal binding peptides of plants, are synthesized from glutathione by a specific glutamyl-cysteine dipeptydyl transpeptidase (phytochelatin synthase). - Proc. nat. Acad. Sci. USA **86**: 6838-6842, 1989.
Ha, S.-B., Smith, A.P., Howden, R., Dietrich, W.M., Bugg, S., O'Connell, M.J., Goldsbrough, P.B., Cobbett, C.S.: Phytochelatin synthase genes from *Arabidopsis* and the yeast, *Schizosaccharomyces pombe*. - Plant Cell **1**: 153-1164, 1999.
Himmelbach, A., Yang, Y., Grill, E.: Relay and control of abscisic acid signaling. - Curr. Opin. Plant Biol. **6**: 470-479, 2003.
Hsu, Y.T., Kao, C.H.: Abscisic acid accumulation and cadmium tolerance in rice seedlings. - Plant Cell Environ. **6**: 867-874, 2003.
Hsu, Y.T., Kao, C.H.: Abscisic acid accumulation and cadmium tolerance in rice seedlings. - Physiol. Plant. **124**: 71-80, 2005.
Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., Parcy F.: bZIP transcription factors in *Arabidopsis*. - Trends Plant Sci. **7**: 106-107, 2002.
Jiang, M., Zhang J.: Effect of abscisic acid on active oxygen species, antioxidative system and oxidative damage in leaves of maize seedlings. - Plant Cell Physiol. **42**: 1265-1273, 2001.
Knight, H., Knight, M.R. Abiotic stress signalling pathways: specificity and cross-talk. - Trends Plant Sci. **6**: 262-267, 2001.
Lee, S., Korban, S.S. Transcriptional regulation of *Arabidopsis thaliana* phytochelatin synthase (AtPCS1) by cadmium during early stages of plant development. - Planta **215**: 689-693, 2002.
Li, A.M., Yu, B.Y., Chen, F.H., Gan, H.Y., Yuan, J.G., Qiu,R., Huang, J.C., Yang, Z.Y., Xu, Z.F.: Characterization of the *Sesbania rostrata* phytochelatin synthase gene: alternative splicing and function of four isoforms. - Int. J. mol. Sci. **10**: 3269-3282, 2009.
Lopez-Climent, M.F., Arbona, V., Perez-Clement, R.M., Gomez-Cadenas, A.: Effects of cadmium on gas exchange and phytohormone contents in citrus. - Biol. Plant. **55**: 187-190, 2011.
Maitani, T., Kubota, H., Sato, K., Yamada, T.: The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorum.* - Plant Physiol. **110**: 1145-1150, 1996.
Moore, R.: Abscisic acid is not necessary for gravitropism in primary roots of *Zea mays.* - Ann. Bot. **66**: 281-283, 1990.
Murata, Y., Pei, Z-M., Mori, I.C., Schroeder, J.: Abscisic acid activation of plasma membrane Ca^{+2} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. - Plant Cell **13**: 2513-2523, 2001.
Nuc, P.W., Chadzinikolau, T., Nuc, K., Stroiński, A.: *Solanum tuberosum* mRNA for phytochelatin synthase (*pcs1* gene). EMBL/GenBank/DDBJ database 2003.
Ogawa, S., Yoshidomi, T., Yoshimura, E.: Cadmium(II)-stimulated enzyme activation of *Arabidopsis thaliana* phytochelatin synthase 1. - J. inorg. Chem. **105**: 111-117, 2011.
Pal, R., Rai, J.P.N.: Phytochelatins: peptides involved in heavy metal detoxification. - Appl. Biochem.Biotechnol. **160**: 945-963, 2010.
Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G.J., Grill, E., Schroeder J.I.: Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. - Nature **406**: 731-734., 2000.
Prat, S.: *S. tuberosum* mRNA for leucine zipper transcription factor. - EMBL/GenBank/DDBJ databases 1994.
Rodriguez-Serrano, M., Romero-Puertas, M.C., Zabalza, A., Corpas, F.J., Gomez, M., Del Rio, L.A., Sandalio, L.M.: 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, 2006.
Sanders, D., Brownlee, C., Harper, J.F.: Communicating with calcium. - Plant Cell **11**: 691-706, 1999.
Schwartz, S.H., Léon-Kloosterziel, K.M., Koornneef, M., Zeevaart, J.A.I.: Biochemical characterization of the *aba2* and *aba3* mutants in *Arabidopsis thaliana*. - Plant Physiol. **114**: 161-166, 1997a.
Schwartz, S.H., Qin, X., Zeevaart, J.A.D.: Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. - Plant Physiol. **131**: 1591-1601, 2003.
Schwartz, S.H., Tan, B.C., Gage, D.A., Zeevaart, J.A.D., McCarty, D.R.: Specific oxidative cleavage of carotenoid by VP14 of maize. - Science **276**: 1872-1874, 1997b.
Stroiński, A.: Some physiological and biochemical aspects of plant resistance to cadmium effect. I. Antioxidative system. - Acta Physiol. Plant. **21**: 175-188, 1999.
Stroiński, A., Chadzinikolau, T., Giżewska, K., Zielezińska, M.: ABA or cadmium induced phytochelatin synthesis in potato tubers. - Biol. Plant. **54**: 117-120, 2010.
Stroiński, A., Kozłowska, M.: Cadmium-induced oxidative stress in potato tuber. - Acta Soc. Bot. Polon. **66**: 189-195, 1997.
Stroiński, A., Zielezińska, M.: Cadmium effect on hydrogen peroxide, glutathione and phytochelatins levels in potato tuber. - Acta Physiol. Plant. **19**: 127-136, 1997.

Stroiński, A. Zielezińska, M. Cadmium and oxidative stress influence on phytochelatin synthase activity in potato tuber. - Acta Physiol. Plant. **23**: 157- 160, 2001.

Vatamaniuk, O.K., Mari, S., Lu, Y.P., Rea, P.A.: Mechanism of heavy metal ion activation of phytochelatin (PC) synthase: blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides. - J. biol. Chem. **275**: 31451-31459, 2000.

Vatamaniuk, O.K., Mari, S., Lang, A., Chalasani, S., Demkiv, L., Rea, P.A.: Phytochelatin synthase, a dipeptidyl-transferase that undergoes multisite acylation with γ-glutamylcysteine during catalysis. Stoichiometric and site-directed mutagenic analysis of *Arabidopsis thaliana* PCS1-catalyzed phytochelatin synthesis. - J. biol. Chem. **279**: 22449-22460, 2004.

Wang, H.C., Wu, J.S., Chia, J.C., Yang, C.C., Wu, Y.J., Juang, R.H.: Phytochelatin synthase is regulated by protein phosphorylation at a threonine residue near its catalytic site. - J. Agr. Food Chem. **57**: 7348-7355, 2009.

Xiong, L., Zhu, J.-K.: Regulation of abscisic acid biosynthesis. - Plant Physiol. **133**: 29-36, 2003.

Yamaguchi-Shinozaki, K., Shinozaki, K.: Ogranization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. - Trends Plant Sci. **10**: 88-94, 2005.

Yoshioka T, Endo T, Satoh S.: Restoration of seed germination supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis. - Plant Cell Physiol. **39**: 307-312, 1998.