BIOLOGIA PLANTARUM 55 (1): 112-118, 2011

Effects of jasmonate and some other signalling factors on bean and onion growth during the initial phase of cadmium action

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Abstract

Short-time direct and indirect effects of 25 μM Cd on the growth of dicotyledon (*Phaseolus coccineus*) and monocotyledon (*Allium cepa*) plants were investigated in the presence of inhibitors of ethylene synthesis, NADPH oxidase, and the octadecanoid pathway. Only 5 min-long action of Cd was enough for inhibition of growth in bean roots, but its recovery time was extended to several days. After 7 h treatment, Cd was significantly accumulated in bean roots, but maximum H_2O_2 accumulation was seen after 1 h. Cd-induced H_2O_2 accumulation decreased especially after addition of ethylene inhibitor silver thiosulphate (STS). Low Cd accumulation and high growth inhibition were observed also in bean leaves and in *A. cepa* roots. The inhibitors of the octadecanoid pathway greatly weakened the inhibitory effect of Cd in *P. coccineus* roots, while no significant effect was observed in *A. cepa*. NADPH oxidase and ethylene blockade reversed (in the case of bean plants and indirectly treated *A. cepa* plants) or significantly diminished Cd action. Cd-induced growth inhibition of *P. coccineus* leaves was also alleviated by most inhibitors of the jasmonate pathway and by STS. These results indicate that Cd may have indirect and direct effects on growth processes.

Additional key words: *Allium cepa*, ethylene, heavy metals, hydrogen peroxide, NADPH oxidase, *Phaseolus coccineus*, stress.

Introduction

Contamination of soils and water with heavy metals is one of the major problems faced by the industrialized world today. Cadmium is a toxic, nonredox and nonessential metal without a known biological function. It is highly reactive and inactivates various enzymatic processes. Many studies indicated inhibition of plant growth (Maksymiec and Baszyński 1996, Maksymiec 1997, Sobkowiak and Dekert 2003, Guo *et al*. 2007, Sabreen and Sugiyama 2008). The exact mechanism of cadmium-induced growth inhibition is still unclear. In some cases the inhibitory effect of Cd on plant cell division was the main reason for leaf growth diminution, which was shown in pea and *Elodea canadensis* plants (Sandalio *et al.* 2001, Vecchia *et al.* 2005), and in roots of *Pisum sativum* (Fusconi *et al*. 2007). Apart from growth rate inhibition correlated with cell division, cadmium also inhibit cell elongation (Poschenrieder *et al.* 1989, Ranieri *et al*. 2005). Cd-induced root growth inhibition can be a consequence of Cd-stimulated premature xylogenesis and shortening of the root elongation zone (Ďurčeková *et al*. 2007). Indirect effects of Cd on plant growth include microtubule alteration (Xu *et al.* 2009), depressed K, Fe, Mg or Ca accumulation, inhibition of photosynthesis (Greger and Bertel 1992, Trivedi and Erdei 1992, Moya *et al.* 1993, Skórzyńska and Baszyński 1998, Sandalio *et al.* 2001) or reduction in the cyclin B1 and cyclin D content (Cockcroft *et al.* 2000, Sobkowiak and Dekert 2003). However, these effects were usually observed after a few days of treatment with the heavy metal and did not indicate any preliminary changes induced within cells.

More recently Pasternak *et al.* (2005) proposed that changes in ROS metabolism integrated with changes in auxin distribution were the cause of stress-induced reorientation of growth followed by cessation of roots and leaf growth. Lin *et al.* (2005) showed that Cu could act through changes in H_2O_2-dependent peroxidase activity followed by cell wall stiffening caused by cross-linking formation among its polymers. Because Cd is

Received 8 May 2009, *accepted* 30 October 2009.
Abbreviations: IB - ibuprofen; IM - imidazole; MJ - methyl jasmonate; PG - propyl gallate; ROS - reactive oxygen species; SHAM - salicylhydroxamic acid; STS - silver thiosulphate.
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accumulated in cell walls (Vecchia *et al.* 2005) and enhances H_2O_2 accumulation (Schützendübel *et al*. 2001, Cho and Seo 2005, Maksymiec and Krupa 2006, Guo *et al*. 2007), it is possible that the increased H_2O_2, usually formed by NAD(P)H oxidase in the walls, can decrease cell wall extensibility. NADPH oxidase was shown to be involved in plant growth and development (Papadakis and Roubelakis-Angelakis 1999, Liszkay *et al*. 2003) and plant response to several biotic and abiotic stresses (Lamb and Dixon 1997, Orozco-Cárdenas *et al.* 2001, Quartacci *et al.* 2001). Vassiliev *et al.* (2004) showed that Cd induces production of ethylene, which in stress conditions can also increase rigidity of cell walls through their lignification (Enyedi *et al.* 1992).

Recent investigations indicated that Cd induces accumulation of jasmonic acid in *A. thaliana* and runner bean plants (Maksymiec *et al.* 2005). This signalling factor can inhibit growth of various plant tissues (Maciejewska and Kopcewicz 2003) through depression of elongation or cell cycle processes (Irving *et al.* 1999, Saniewski *et al.* 1987, 2002, Świątek *et al.* 2002, Merkouropoulos and Shirsat 2003). This indicates that Cd can influence plant growth indirectly through induction of the jasmonate signalling pathway. This assumption was supported by accumulation of jasmonate-like inducible proteins after metal exposure during initial plantlet elongation (Gianazza *et al*. 2007) and by the alleviating effect of salicylic acid (an antagonist of jasmonates) pre-treatment on Cd-induced inhibition of rice roots growth and oxidative damage (Guo *et al*. 2007). However, investigations on the SA-deficient *A. thaliana* ecotype suggest that endogenous SA may function as a signalling molecule which increases Cd-induced oxidative stress (Zawoznik *et al*. 2007). Yeh *et al*. (2007) showed that in rice roots Cd also induces a signalling pathway connected with MAP kinase activation. Recently Groppa *et al*. (2008), in two day-long experiments, observed that nitric oxide is a signalling molecule involved in metal-induced wheat root growth inhibition. Since transcriptional regulation in response to Cd treatment is relatively rapid, the participation of signalling pathways in heavy metal-induced growth inhibition still remains unclear (Herbette *et al*. 2006, Maksymiec 2007).

The aim of the present work was to obtain information on the influence of some signalling pathways in Cd-induced growth inhibition during the first phase of heavy metal action.

Materials and methods

Runner bean (*Phaseolus coccineus* L. cv. Piękny Jaś) and onion (*Allium cepa* L. cv. Wolska) plants were cultivated in Knop nutrient solution after 5-d germination at 25 °C. The plants were grown at 16-h photoperiod, photon flux density (PFD) of 140 µmol(photon) m^{-2} s^{-1} and day/night temperature of 25/19 °C. After 3 d, the seedlings were treated by 25 µM Cd^{2+} ($CdSO_4 \cdot 7H_2O$) for different time periods. Additionally, 2 h before heavy metal treatment, the following substances were added to one part of plants: 0.3 mM ibuprofen (IB) – an inhibitor of lipoxygenase, 0.1 mM salicylhydroxamic acid (SHAM) or 0.1 mM propyl gallate (PG) – inhibitors of the jasmonic acid synthesis, 20 mM imidazole (IM) – an inhibitor of NADPH oxidase (the main source of H_2O_2 in the stress condition), 0.1 mM silver thiosulphate (STS) – a blocker of ethylene receptors, and 0.05 mM methyl jasmonate (MJ). 50 mM sucrose was added 4 h before treatment. STS was composed by mixing equal volumes of 10 mM $AgNO_3$ and 40 mM $Na_2S_2O_4$. SHAM was dissolved in minimal amounts of dimethylsulfoxid (DMSO), and MJ and PG in ethanol. To investigate the inductive effects of Cd, the plants were treated with 50 µM Cd^{2+} for 5 min and then transferred to the control nutrient solution and measured during the next 96 h. Another group of *Allium cepa* plants included seedlings, the roots of which were divided into two parts: one - growing in the control Knop solution and regarded as roots treated indirectly (RTND), and the other - in the solution with addition of the above mentioned substances (RTD). The control plants were those having all the roots untreated. Because the data obtained indicated a presumably main role of NADPH oxidase in the mechanism of Cd-induced *P. coccineus* root growth inhibition, measurements of the H_2O_2 content in these plants have been performed.

H_2O_2 content was determined according to the method of Pick (1986), with modification of two-fold concentration extract of *P. coccineus* roots used for analysis as described in detail previously (Maksymiec and Krupa 2006).

Cd toxicity was determined by measuring the plant leaf area and root elongation. Measurements of the length of the roots usually commenced when they were about 4 cm long. The leaf area was measured using a *GeniScan GS-4500* scanner (*Genius*, Taipei, Taiwan) and calculated by dedicated computer software manufactured by *Witra* (Warsaw, Poland).

For estimation of the elements, the roots were washed in 0.1 M HCl (4 °C) for 10 min and in distilled water for 30 min, and both roots and leaves were dried at 105 °C. Cd content was determined by atomic absorption spectrophotometer (*Unicam 939 A*, Cambridge, UK) after wetting the dried material in $HNO_3/HClO_4$ mixture (4:1, v/v).

The estimated values are means of the samples of three independent experiments, each with at least 5 - 6 replicates. Student's *t*-test was used for statistical evaluation of the differences between the control and treated plants.

Results

Cadmium ions substantially inhibited the growth of *P. coccineus* roots as early as after 2-h treatment (Fig. 1). The inhibitors of the octadecanoid pathway markedly weakened the inhibitory effect of Cd. Imidazole, i.e., the NADPH oxidase inhibitor reversed the Cd action, whilst STS, the ethylene inhibitor, significantly diminished it. Sucrose slightly increased the inhibitory effect of Cd. To reveal the recovery and sensitivity of bean roots to Cd action, the effect of 5 min treatment with 50 µM Cd was investigated. Four days after this treatment, the rate of roots growth was still maintained on about 58 % of the control level (data not shown).

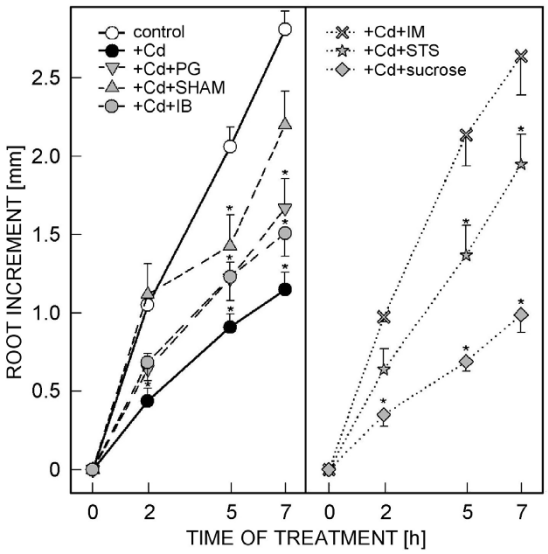

Fig. 1. Growth of *P. coccineus* roots during exposure to 25 µM Cd^{2+} alone or with propyl gallate (PG), salicylhydroxamic acid (SHAM), ibuprofen (IB), imidazole (IM), silver thiosulphate (STS) and sucrose. Means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

The growth of *A. cepa* roots was sharply diminished by Cd (60 and 64 % inhibition after 2 and 7 h of treatment, respectively) when the roots were growing in soil with Cd (Fig. 2). These roots were regarded as treated directly (RTD). When a part of the roots of the treated plants were in the control soil (RTND, roots treated non-directly), their growth was also diminished by Cd; this effect, however, was gradually decreased with time from about 60 % inhibition after 2 h to about 30 % after 7 h. Of the additionally used substances only STS and IM significantly diminished the inhibitory effect of Cd applied directly to *A. cepa* plants (RTD roots) or reversed this effect in indirectly treated (RTND roots). Sucrose slightly increased the inhibitory effect of Cd on RTD and RTND roots.

The growth of bean leaves was inhibited by Cd to 65 % of the control already after 7 h (Fig. 3). This effect was partially diminished by IB, PG and STS. The influence of STS was observed during the whole investigated period, but that of PG only after a longer time. IM insignificantly diminished Cd action.

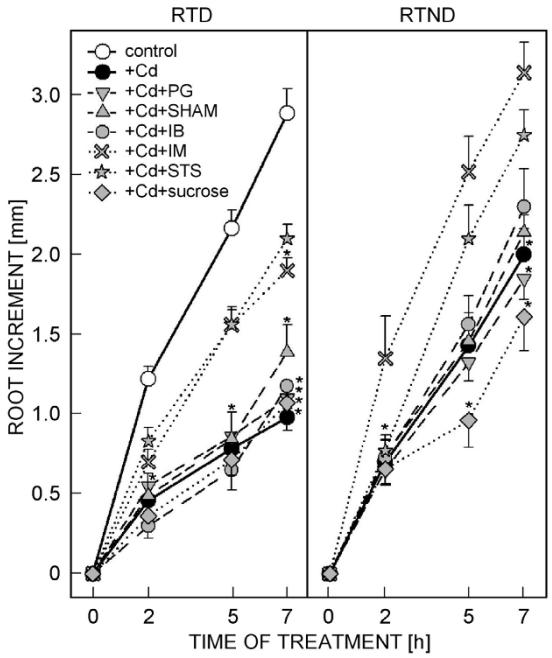

Fig. 2. Root growth of *A. cepa* treated plants divided into those immersed in the solution with 25 µM Cu^{2+} alone, or with PG, SHAM, IB IM, STS and sucrose (RTD, roots treated directly), and those immersed in the control solution (RTND - roots treated non-directly). The control plants were those with all untreated roots. Abbreviations used as in Fig. 1. Means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

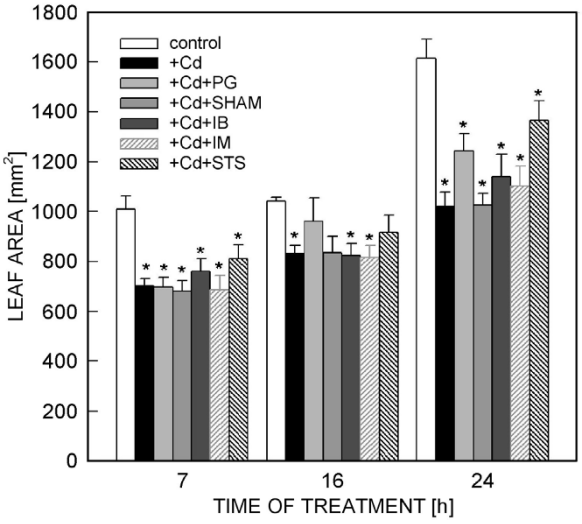

Fig. 3. Growth of *P. coccineus* leaves during exposure to 25 µM Cd^{2+} alone, or with PG, SHAM, IB, IM and STS. Abbreviations used as in Fig. 1. Data are means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

Blocking of ethylene perception by STS after preincubation time (2 h) or longer (5 h) increased significantly H_2O_2 concentration in *P. coccineus* roots. In the case of the other inhibitors used, no considerable effect was seen (data not presented). Cd ions induced strong and fast H_2O_2 accumulation in the roots, peaking after 1 h of exposure. The inhibitors, especially STS, diminished the inductive action of Cd on H_2O_2 content after 1 h, but after 5 h they (except for STS) increased it (Fig. 4).

After treatment with Cd for 2 and 7 h, its concentration in the roots of the bean plants increased from 0.27 to 1.90 and 17.26 μg g^{-1}(d.m.), respectively (Table 1). Cd was accumulated in *A. cepa* roots after 2 h of direct treatment to a higher degree than in the bean plants, but its accumulation did not increase with time. In RTND roots, its content was similar to that in the control. During the investigated time period, Cd did not accumulate considerably in the leaves of bean plants. All the inhibitors used generally did not change the Cd content or increase it significantly (except for the Cd level in the roots after adding IM).

Discussion

The inhibitory effect of Cd on the whole plant was usually analyzed after a few days of exposure. In such a case, additional processes, for example, transport of elements and assimilates can influence this phenomenon.

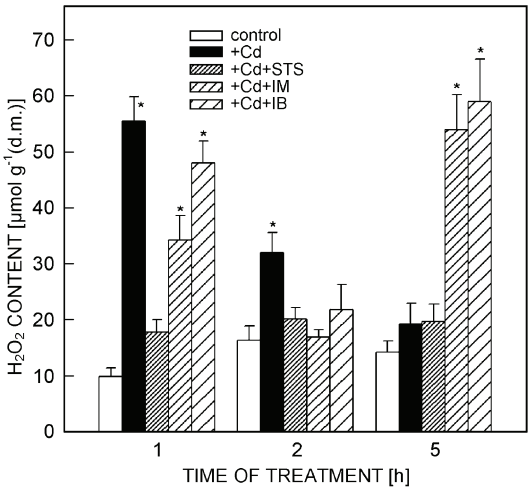

Fig. 4. Content of hydrogen peroxide (H_2O_2) in the roots of *P. coccineus* plants treated with 25 μM Cd alone, or with STS, IM and IB. Abbreviations used as in Fig. 1. Mans ± SE, $n = 18$, * - values significantly different at $P < 0.05$ between treatments and the control.

Our results indicated that the inhibitory effect was expressed in the roots immediately after heavy metal supply (within 2 h). Since the Cd content in the bean plants increased only slightly during this time and 5-min Cd action showed a long lasting inhibitory effect, it may be concluded that Cd can display (at least partially) an indirect effect on the growth processes. The experiment with *A. cepa* plants supported the above assumption because the inhibitory effect of Cd was also seen in the roots treated indirectly at Cd concentration similar to the control. Similar dynamics of Cd-induced root inhibition was also shown in bean and onion plants for MJ (Maksymiec and Krupa 2007).

Inhibitors of jasmonate synthesis diminished the inhibitory effect of Cd only in *P. coccineus* plants. This result is in contrast to Miyamoto *et al.* (1997), who showed that jasmonic acid (JA) was less effective in dicotyledons than in monocotyledons. This may be a result of the difference in the action of endogenously formed JA and of that introduced exogenously.

Ueda *et al.* (1995) showed that sucrose can reverse the inhibitory effect of JA in oat coleoptile segments. In our investigations sucrose slightly increased the inhibitory effect of Cd in the bean and onion plants. NADPH oxidase blockade (connected with H_2O_2 decrease) remarkably weakened Cd action on roots of *P. coccineus* and *A. cepa* plants.

It is worth noting that, in the roots treated indirectly, high inhibition was observed after 2 h of Cd action, but it was reversed only after depression of NADPH oxidase activity. This is correlated with the strong increase in H_2O_2 observed in the *P. coccineus* roots and coherent with the findings of Lin *et al.* (2005), who showed that H_2O_2 rapidly increased in soybean roots after Cu supply followed by inhibition of roots. The maximum H_2O_2 increase resulting from NADPH oxidase activity was obtained after 1 h Cd action also in *A. thaliana* leaves (Maksymiec and Krupa 2006) and after 45 min in alfalfa seedlings (Ortega-Villasante *et al.* 2007). The diminution of H_2O_2 formation can impede cell wall stiffening (Liszkay *et al.* 2003) observed after Cd treatment. After a longer time, together with NADPH oxidase also ethylene was involved in Cd-induced growth inhibition. In contrast to the roots treated indirectly, the effect of IM and STS after direct treatment was expressed only partially. Therefore, we suppose that Cd affects also directly the growth of monocotyledon plants, maybe through a negative effect on plasma membrane H^+-ATPase activity, which is an element of the "acid-growth" processes (Karcz and Kurtyka 2007).

In dicotyledons the addition of IM was most effective in abolishing the inhibitory Cd effect, but STS as well as the inhibitors of the octadecanoid pathway showed a lower effect. This may indicate that, in Cd stress, the main role of growth inhibition is expressed by NADPH oxidase with partial contribution of jasmonate and ethylene. This is quite possible because maximum JA accumulation was shown in the leaves between 6 - 14 h after Cd addition depending on the plant species (Maksymiec *et al.* 2005). Ethylene production

Table 1. Cadmium concentration [μg g^{-1} (d.m.)] in the roots and leaves of *P. coccineus* and *A. cepa* plants treated with 25 μM Cd^{2+}, and in the control. RTND (roots treated non-directly) indicated that the roots growing in the soil without contamination were collected from the treated plants, and RTD (roots treated directly) indicated the roots of the treated plants immersed in the soil with contamination. The samples were taken after 2, 7 or 24 h. Means ± SE of 5 replications. Abbreviations are used as in Fig. 1.

Plant	Treatment	RTD		RTND		Leaves	
		2 h	7 h	2 h	7 h	2 h	24 h
P. coccineus	control	0.27 ± 0.03	0.27 ± 0.05	-	-	0.07 ± 0.01	0.06 ± 0.01
	Cd	1.90 ± 0.16	17.26 ± 4.44	-	-	1.01 ± 0.31	1.06 ± 0.15
	Cd+IB	2.80 ± 0.44	24.40 ± 5.08	-	-	1.26 ± 0.15	0.85 ± 0.10
	Cd+IM	2.45 ± 0.35	11.47 ± 1.50	-	-	1.25 ± 0.10	0.75 ± 0.09
	Cd+SHAM	3.55 ± 0.45	21.98 ± 1.12	-	-	0.76 ± 0.05	0.81 ± 0.13
	Cd+STS	3.20 ± 0.20	19.12 ± 0.19	-	-	0.47 ± 0.05	0.80 ± 0.05
	Cd+PG	1.82 ± 0.15	17.88 ± 1.25	-	-	0.98 ± 0.08	1.08 ± 0.09
A. cepa	control	0.85 ± 0.05	1.36 ± 0.16	0.86 ± 0.04	1.25 ± 0.12	-	-
	Cd	7.08 ± 0.07	8.34 ± 0.09	1.09 ± 0.15	1.36 ± 0.22	-	-
	Cd+IB	5.30 ± 0.37	9.34 ± 0.85	1.56 ± 0.21	2.05 ± 0.35	-	-
	Cd+IM	8.95 ± 0.65	8.64 ± 0.76	2.29 ± 0.35	1.35 ± 0.12	-	-
	Cd+SHAM	11.23 ± 0.85	15.40 ± 1.65	1.98 ± 0.25	1.97 ± 0.36	-	-
	Cd+STS	8.50 ± 0.95	7.08 ± 0.65	1.12 ± 0.11	0.96 ± 0.18	-	-
	Cd+PG	7.05 ± 0.55	9.66 ± 0.78	1.76 ± 0.55	1.16 ± 0.12	-	-

significantly increased after 5 h in JA-treated tomato suspension cells (Iakimova *et al*. 2008), and JA showed cross-talk action with ethylene (Saniewski *et al.* 1987). However, after 5 h of Cd action, inhibitors of ethylene, JA and NADPH oxidase increased H_2O_2 accumulation in *P. coccineus* roots. This phenomenon is difficult to explain at the present time, however, it may have resulted from starting additional mechanisms at a prolonged blockade of a particular signalling pathway, which induced H_2O_2 production or its translocation to the roots.

Analysis of Cd concentration showed that all the inhibitors used did not decrease Cd content in roots and leaves, which indicated that the effect of the inhibitors on Cd-induced growth inhibition did not result from the decreased content of this element.

In the leaves of the treated bean plants, Cd content was similar to the control level during the investigated periods, but the growth inhibition was significant. This suggests that rather an indirect effect of the heavy metal can also occur in the leaves. The participation of JA in the mechanism of Cd-induced growth inhibition was not as evident as in the roots. The growth inhibition induced by Cd was partially diminished by IB and PG but not by SHAM. This may be the result of unspecific reaction of SHAM with Cd. The effect of STS was more distinct because of the Cd action lowered by about 50 %.

I strongly believe that the data presented here indicate that Cd can rapidly affect root and leaf growth (at least partially) through signalling pathways. NADPH oxidase activity, and in a minor degree ethylene (through H_2O_2 increase) and jasmonate may be involved in the inhibitory action of Cd on the roots of dicotyledons, while in monocotyledons, only ethylene and NADPH oxidase play role. In leaves, the mechanism of growth inhibition induced by Cd may be connected with ethylene and, to a minor degree, with jasmonate. Further investigation is needed in future to resolve mechanisms connecting signalling pathways with the rapid inhibitory action of Cd on plant growth processes.

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