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Effects of methyl jasmonate and excess copper on root and leaf growth

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Abstract

A short time effects of 25 and 150 μM Cu^{2+} or 50 μM methyl jasmonate (MJ) on growth of roots and leaves of *Phaseolus coccineus*, *Allium cepa* and *Zea mays* were investigated. Both Cu^{2+} and MJ inhibited root growth. Jasmonate synthesis inhibitors (ibuprofen, IB, salicylhydroxamic acid, SHAM, and propylgallate, PG) partially reversed the inhibitory effect of Cu^{2+} in *P. coccineus*, but in *A. cepa* this effect was not clear. Pretreatment with NADPH oxidase inhibitor (20 mM imidazole, IM), and especially ethylene inhibitor (silver thiosulphate, STS) mostly weakened Cu^{2+} effect on root growth in *P. coccineus* and *A. cepa*. The growth of *P. coccineus* leaves also slowed down by Cu^{2+} and this effect was partially ameliorated by IB, PG and IM, and completely by SHAM and STS. In *Z. mays* the effect of STS was considerably lower than that of PG and SHAM which reversed the effect of Cu^{2+}. These results indicate that jasmonate, ethylene and NADPH oxidase activity may be involved in Cu^{2+} inhibitory action on the roots of dicotyledon plants, but in *A. cepa* only ethylene and NADPH oxidase are involved. However, leaf growth inhibition induced by excess Cu^{2+} is connected in *Z. mays* especially with jasmonate, and in *P. coccineus* with ethylene, NADPH oxidase and, to a minor degree, with jasmonate.

Additional key words: ethylene, heavy metal, hydrogen peroxide, NADPH oxidase, signalling.

Introduction

Many studies have indicated that the main effect of heavy metals excess is growth inhibition (Maksymiec 1997, Alaoui-Sossé *et al.* 2004, Atanasova *et al.* 2004), but the exact mechanism remains still unclear. Although some authors consider that inhibition of the cell cycle is the main basis for growth inhibition (Eleftheriou and Karataglis 1989, Punz and Sieghardt 1993) cadmium (Poschenrieder *et al.* 1989) and especially copper commonly inhibit cell elongation (Wainwright and Woolhouse 1977, Maksymiec *et al.* 1995, Ouzounidou *et al.* 1995, Alaoui-Sossé *et al.* 2004). Growth of cell wall, its extensibility and increased osmotic potential are required for cell elongation. Alaoui-Sossé *et al.* (2004) indicated that in cucumber plants decreased potassium leaf uptake and inhibition of photosynthesis *via* sugar accumulation could be a reason of copper inhibition of cell expansion. Similarly, rice seedlings exposed to cadmium or nickel (Moya *et al.* 1993) as well as runner bean plants to cadmium (Skórzyńska and Baszyński 1998) and copper stress (Maksymiec and Baszyński 1998) showed an increase in sugar content and simultaneous decrease in photosynthesis. However, at present, it cannot be resolved what comes first - growth inhibition or photosynthetic activity decrease. These effects were observed usually after a few days of heavy metal treatment without preliminary changes induced within cells.

More recently, Lin *et al.* (2005) have shown that copper can act through changes in H_2O_2-dependent peroxidase activity followed by cell wall stiffening due to the formation of cross-linking among its polymers. It is possible that increased H_2O_2 formation in other sources, especially through increased activity of NADPH oxidase can decrease cell wall extensibility (Foreman *et al.* 2003). NADPH oxidase is involved in plant growth (Liszkay *et al.* 2003) and plant response to several biotic stresses as well as to copper (Lamb and Dixon 1997, Orozco-Cárdenas *et al.* 2001, Quartacci *et al.* 2001). Because growth inhibition occurs already after 1 d of metal treatment (Weckx and Clijsters 1996) the influence of signal molecules, including H_2O_2, is not excluded. Sandmann and Böger (1980) found that Cu induced production of ethylene, which can increase the rigidity of cell walls (Enyedi *et al.* 1992). The recent investigations indicated that Cu induced accumulation of jasmonic acid in *A. thaliana* and runner bean plants (Maksymiec *et al.*

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Abbreviations: IB - ibuprofen; IM - imidazole; JA - jasmonic acid; MJ - methyl jasmonate; PG - propylgallate, SHAM - salicylhydroxamic acid; STS - silver thiosulphate.

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2005) within 6 - 7 h of exposure. Jasmonates can inhibit growth of various plant tissues through depression of the elongation (Irving *et al.* 1999, Saniewski *et al.* 2002, Maciejewska and Kopcewicz 2003) or cell cycle (Świątek *et al.* 2002). Recently Ouzounidou and Ilias (2005) showed that gibberellic acid and auxins lessened the inhibitory effect of Cu on root and shoot elongation, which suggested the influence of Cu on cytoskeleton organization.

The aim of the present work was to obtain information on the cause of growth inhibition by excess Cu at the first phase of heavy metal action. Therefore we investigated the influence of the inhibitors of ethylene, jasmonic cid and NADPH oxidase on Cu stress.

Materials and methods

Runner bean (*Phaseolus coccineus* L., cv. Piękny Jaś), *Allium cepa* L. (cv. Wolska) and *Zea mays* L. (cv. Hidosil) plants after 5 d of germination in a thermostated chamber (25 °C) were cultivated in Knop nutrient solution. The plants were grown at 16-h photoperiod, irradiance of 140 μmol(photon) m^{-2} s^{-1} and day/night temperature of 25/19 °C. After 3 d the seedlings (when the roots were 4 - 5 cm long) were treated for different time periods with 25 μM Cu^{2+} ($CuSO_4$. 5 H_2O), or by 50 μM Cu^{2+} if the seedlings were treated only for 5 min. The *Z. mays* plants were treated with 150 μM Cu^{2+}. Additionally, 2 h before Cu-treatment, the plants were pre-treated either with inhibitors of jasmonic acid synthesis pathway – 0.1 mM propylgallate (PG), 0.1 mM salicylhydroxamic acid (SHAM) or 0.3 mM ibuprofen (IB) (also an inhibitor of lipoxygenase), and inhibitor of NADPH oxidase (the main source of H_2O_2 in the stress condition) – 20 mM imidazole (IM) or a blocker of ethylene receptors – 0.1 mM silver thiosulphate (STS), and 50 μM methyl jasmonate (MJ). In some experiments 50 mM sucrose was added 4 h before treatment. STS was composed by mixing equal volume 10 mM $AgNO_3$ and 40 mM $Na_2S_2O_4$. SHAM was dissolved in minimal amounts of DMSO (0.02 cm^3 DMSO on 0.1 mM SHAM), whereas MJ and PG in ethanol.

Another group of *Allium cepa* plants were seedlings which roots were divided in to two parts, one growing in control Knop solution (estimated as RTND), and the other in solution with addition of the above mentioned substances (RTD).

Cu^{2+} toxicity was determined by measuring the plant leaf area and root elongation. Measurement of roots length was started than they were 4 - 5 cm long. The leaf area was measured using a *GeniScan GS-4500* scanner (*Genius*, Taiwan) and calculated by dedicated computer software manufactured by *Witra* (Warsaw, Poland).

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

The estimated values were the means of samples from three independent experiments, each with at least 6 - 7 replicates. For statistical evaluation of the differences established within control and treated plants the Student's *t*-test was used.

Results

Copper ions in excess as well as Cu^{2+} combined with 50 μM MJ substantially inhibited the growth of *P. coccineus* roots as early as after 2-h treatment (Fig. 1) and this inhibition lasted over the whole experiment (7 h). 50 % inhibition was obtained by 25 μM Cu^{2+} after 2-h treatment. MJ diminished root growth to 42 % of the control level after 7 h. The inhibitors of octadecanoid pathway differently influenced root growth. IB diminished Cu-induced growth inhibition after 2 h, however, after 5 h also PG and SHAM showed this effect which was greater than that of IB (nevertheless the growth inhibition in Cu+PG plants was still significant in comparison with control).

Imidazole and STS similarly ameliorate the inhibitory effect of 25 μM Cu^{2+}, but after 7 h IM acted slightly weaker than STS (Fig. 1).

To reveal the inhibitory effect induced by 50 μM Cu^{2+} only 5-min treatment was sufficient Four days after this treatment the growth of roots was still diminished to 55 % of the control level (data not presented).

The growth of *A. cepa* roots was sharply diminished by Cu^{2+} (53 % inhibition), and especially MJ (78 % inhibition) if the roots were growing in the soil with the above mentioned substances (Fig. 2). These roots were regarded as treated directly (RTD). If a part of roots of the treated plants was in control soil (RNTD, roots not treated directly) their growth dynamics was also diminished by Cu^{2+} and MJ, however, this effect was weakly expressed after 2 h in the case of Cu^{2+} and MJ, but after 5 h only in the case of MJ.

Of the additionally used substances only STS and, partially after 7 h, SHAM diminished the inhibitory effect of Cu^{2+} directly applied to *A. cepa* plants (Fig. 2). If roots were treated with Cu^{2+} indirectly their growth was improved, especially by STS, and lesser by IM, SHAM and PG. IB did not show any positive effect.

The growth of bean leaves was significantly inhibited by Cu^{2+} (and also by MJ, data not shown) already after

7 h (Fig. 3). At prolonged exposure this effect was substantially weakened by IM and STS.

The inhibitors of octadecanoid pathway diminished the effect of Cu^{2+} after 16 h; SHAM showed the best effect.

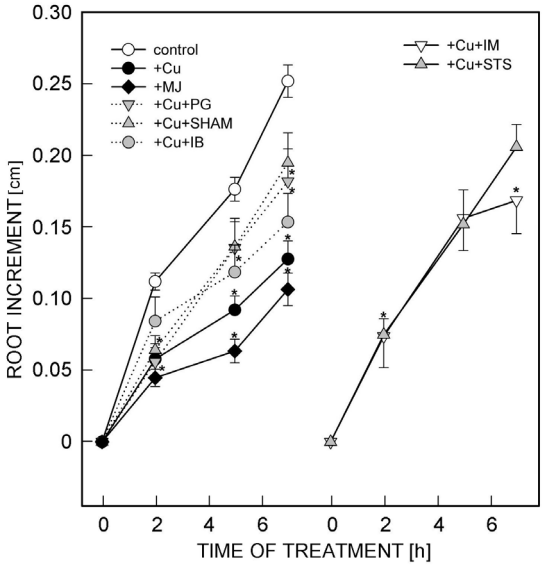

Fig. 1. Growth of *P. coccineus* roots during exposure to MJ or 25 μM Cu^{2+} alone, or with PG, SHAM, IB, IM and STS. Means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

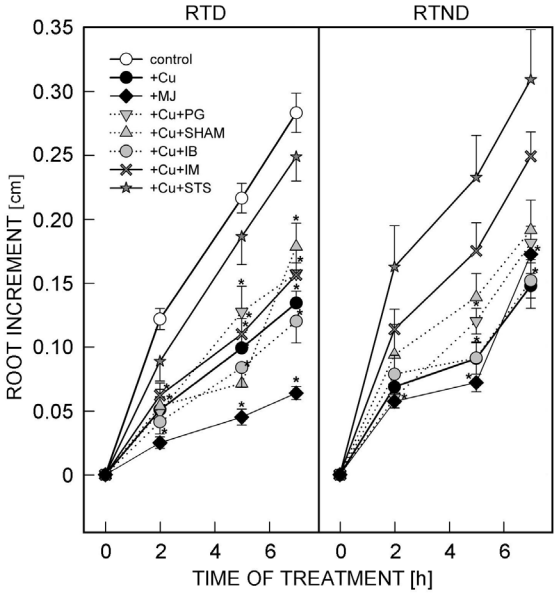

Fig. 2. Root growth of *A. cepa* treated plants divided into those immersed in nutrient solution with MJ or 25 μM Cu^{2+} alone, or with PG, SHAM, IB, IM and STS (RTD, roots treated directly), and those immersed in control solution (RTND - roots treated non-directly). Control were plants which roots were not treated. Means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

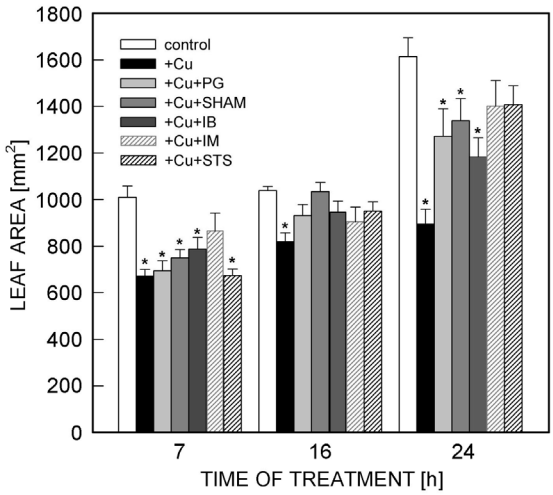

Fig. 3. The influence of PG, SHAM, IB, IM and STS on leaf growth of *P. coccineus* plants treated with 25 μM Cu^{2+}. Means ± SE, n = 18, * - values significantly different at $P < 0.05$ between treatments and the control.

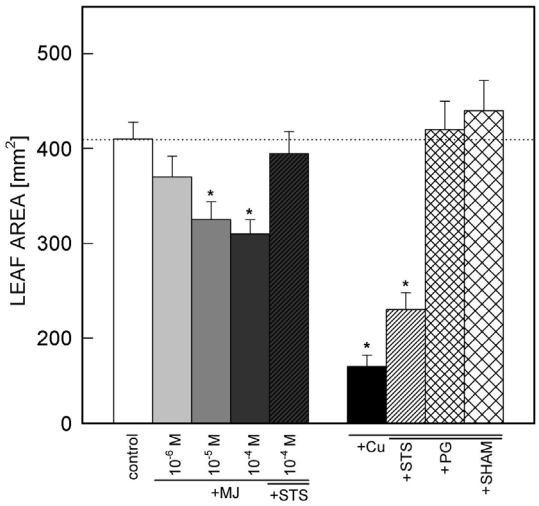

Fig. 4. Growth of *Z. mays* leaves during exposure to different MJ concentration or 150 μM Cu^{2+} alone, or with STS, PG, and SHAM. Means ± SE, n = 15, * - values significantly different at $P < 0.05$ between treatments and the control.

In monocotyledon plant (*Z. mays*) after 4-day treatment 150 μM Cu^{2+} decreased leaf growth to 40 % of control. MJ diminished the growth parallel to its concentration, but to a minor degree than Cu^{2+}. STS depressed Cu^{2+} and MJ action, and the inhibitors of JA synthesis (PG and SHAM) abolished the effect of Cu^{2+} (Fig. 4).

After Cu-treatment its concentration in the roots of bean plants increased 2- and 3-fold after 2 and 7 h, respectively (Table 1). In the leaves of *P. coccineus* and roots of *A. cepa* plants treated directly (RTD) and non-directly (RTND), Cu accumulation was smaller and did not exceeded 2-fold.

Table 1. Copper concentration [μg g^{-1} (d.m.)] in the roots and leaves of control and treated of *Phaseolus coccineus* and *Allium cepa* plants. RNTD (roots not treated directly) indicated the roots which grown in the soil without contaminations collected from treated plants, and RTD (roots treated directly) roots of treated plants growing in the soil with contaminations. The data are means ± SE of 6 experiments.

		RTD		RNTD		Leaves	
		2 h	7 h	2 h	7 h	7 h	24 h
Phaseolus	control	11.00 ± 2.04	11.50 ± 2.08	-	-	13.34 ± 1.55	11.99 ± 0.82
coccineus	+ Cu^{2+}	20.94 ± 0.45	34.82 ± 4.69	-	-	30.94 ± 1.95	14.25 ± 1.22
Allium	control	17.00 ± 1.90	31.20 ± 3.50	17.00 ± 1.90	31.20 ± 3.50	-	-
cepa	+ Cu^{2+}	22.16 ± 2.12	62.16 ± 5.20	26.51 ± 3.10	44.50 ± 3.28	-	-

Discussion

Data on rapid effect of excess Cu are limited. Information about Cu inhibitory effect on the whole plant mostly refers to a time longer than 1 day of exposition. However, recently Lin *et al.* (2005) showed that Cu at 5 - 10 μM concentration inhibited the growth of soybean roots after 12 h. Our results indicate that the inhibitory effect is expressed immediately after heavy metal supply (within 2 h), and a very short, 5-min Cu^{2+} action showed a long lasting inhibitory effect. It may indicate that Cu^{2+}, besides its direct effect (because the metal content increased 2- to 3-fold), can have also an inductive character. An experiment with *A. cepa* plants supported the above assumption because the inhibitory effect of Cu^{2+} was seen also in roots treated indirectly, in which Cu concentration was comparable to control. The dynamics similar to that of Cu-induced root inhibition showed MJ both in *P. coccineus* and *A. cepa* plants. However, the inhibitors of jasmonate synthesis mostly diminished the inhibitory effect of Cu^{2+} in *P. coccineus*, but in *A. cepa* this effect was unclear. Miyamoto *et al.* (1997) showed that in dicotyledons JA was less effective than in monocotyledons. In our paper a similar effect was noted after exogenous MJ application. However, inhibitors of JA synthesis used in our experiments did not support the above observation, indicating that the action mode of endogenously formed JA in the investigated plants may be different than that applied exogenously. Ueda *et al.* (1995) showed that sucrose can reverse the inhibitory effect of JA in oat coleoptile segments. In our experiments the effect of sucrose was only slightly accentuated in bean but not in *A. cepa* plants (data not shown). It may suggest that the plant species as well as the kind of tissue can determine the sensitivity to jasmonate.

Inhibitors of NADPH oxidase mostly weakened Cu action on roots of *P. coccineus* and *A. cepa* plants. It correlates with the findings of Lin *et al.* (2005) who showed that H_2O_2 content (connected with NADPH oxidase activity) rapidly increased in soybean roots 1 h after Cu supply, followed by root growth inhibition. We obtained similar results in *A. thaliana* leaves (Maksymiec and Krupa 2006). H_2O_2 rapid increase, observed after Cu or MJ-treatment was diminished in the former case by PG, IM and in the later by IM. The diminution of H_2O_2 formation can, according to Liszkay *et al.* (2003), impede cell wall stiffening observed after excess Cu supply.

Because blocking ethylene receptors by STS was most effective to abolish the inhibitory Cu^{2+} effect, we propose that the formation of ethylene and H_2O_2 may be the main cause of the Cu^{2+} inhibitory effect in roots. The partial improvement of growth in Cu-treated plants by inhibitors of the octadecanoid pathway indicated also JA affect on root growth of bean plants, but it needs additional analysis because Saniewski *et al.* (1987) and Tung *et al.* (1996) obtained different results on the influence of JA in ethylene production.

In the leaves of treated bean plants Cu concentration was similar to control level after 24 h and significantly exceeded it only after the first 7 h. It may suggest that rather indirect effect of Cu^{2+} is possible. It can be connected with the earliest root growth decrease or the influence of the investigated substances. The participation of JA in the mechanism of Cu-induced growth inhibition was greater in leaves than roots. Growth inhibition of *P. coccineus* leaves induced by Cu^{2+} was partially improved by IB, PG and IM, and completely by SHAM and STS. The influence increase of the inhibitors of the octadecanoid pathway was correlated with JA content increase observed after 14-h exposure (Maksymiec *et al.* 2005). In *Z. mays* leaves the effect of STS was substantially lower than that of PG and SHAM which reversed the effect of Cu, indicating that JA showed a greater role in the mechanism of Cu-induced leaf growth inhibition in monocotyledons than in dicotyledons.

Our data, for the first time, to our knowledge, indicate that the mechanism of growth inhibition induced in leaves by excess Cu may be connected especially with jasmonate in *Z. mays*, and *P. coccineus* plants with ethylene, NADPH oxidase activity and, to a minor degree with jasmonate. Ethylene and NADPH oxidase may be involved in the inhibitory action of Cu on roots of monocotyledon plants, but in dicotyledons especially ethylene, H_2O_2 and, to a minor degree, jasmonate.

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