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Protective effects of phosphonomethyl-sarcosine against the copper and cadmium induced inhibition of leaf development in poplar

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ABSTRACT Heavy metals can disrupt plant metabolism, including photosynthesis, in a number of different ways. One common mechanism in acclimation of plants to abiotic stresses is the accumulation of compatible solutes. In these study we analysed the effect of exogenously added phosphonomethyl-sarcosine, a derivative of a synthesis intermedier of glycinebetaine, on leaf development in poplar under Cu and Cd stress. While growth was more strongly reduced by Cu treatment, Cd preferably inhibited photosynthesis. These effects were connected to the disturbed water (Cu stress) and ion balance (Cd stress) of plants. The symptoms of Cu and Cd stress were totally and partially abolished by exogenously added phosphonomethyl-sarcosine, respectively. Its protective effect might be based on the complexation of heavy metals (Cu stress), but glycinebetaine-like macromolecule/membrane protecting effects (Cd stress) are also possible.
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KEY WORDS

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Heavy metals can inhibit the activity of enzymes directly by binding to protein sulphydryl groups, by producing deficiency of essential ions and eventually by substituting essential ions (Van Assche and Clijsters 1990). Due to their interaction with water and ion balance heavy metals reduce growth both in roots and shoots (Titov et al. 1995; Fodor 2002), elicit oxidative damage of membranes (Alscher 1989), and consequently inhibit physiological processes and primarily decrease the bio-production by inhibiting photosynthesis (Krupa and Baszynski 1995; Mishliwa-Kurdziel et al. 2002).

Cell protection against toxic metals involves many processes, such as immobilization, exclusion, chelation, compartmentalization, and (6) synthesis of stress proteins (Hall 2002). One common mechanism to cope with various abiotic stresses is the accumulation of compatible solutes including betaines (Chen and Murata 2002). The supposed effects of glycinebetaine include protection of membranes, as well as stabilization of the quaternary structures of enzymes and complex proteins (Johvet et al. 1982; Sakamoto and Murata 2001). Moreover, exogenous application of GB improves the growth and survival of a wide variety of plants under various stresses (for example, see Hayashi et al.1998; Chen et al. 2000). The exact functions of these substances within the living organism, however, are still unclear, and their effect has not been investigated in connection with heavy metal toxicity.

In the present paper we investigated the possible protecting effects of phosphonomethyl-sarcosine (PmeS) on leaf development under Cd and Cu stress. PmeS is a derivative of N,N-dimethyl-glycine, which is an intermedier of the glycinebetaine biosynthetic pathway via N-methylation of glycine found in some halobacteria (Chen and Murata 2002).

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Materials and Methods

Poplar plants (*Populus glauca* L. var. Kopeczky) were used in the experiments. Micropropagated seedlings were transferred to a modified Hoagland solution with Fe-citrate as an iron source (Fodor et al. 1998). Treatments started four weeks after replanting, when the acclimated plants had developed 3-4 leaves of normal size. Plants were grown thereafter in the presence or absence of 1 or 10 μM $Cd(NO_3)_2$ (Cd1, Cd10) or 10 μM $CuSO_4$ (Cu10), and, in addition, 100 μM PmeS was added or not. Two weeks later, samples were taken from leaves developed before the treatment (lower leaves) or grown during the treatment (upper leaves).

Chlorophyll (Chl) concentration was determined photometrically in 80% acetone extracts (Porra et al. 1989).

The rate of light-induced CO_2 fixation was studied in detached leaves according to the method of Láng et al. (1985). The radioactivity of the samples was determined by means of the liquid scintillation technique (Beckman LS5000TD).

Metal concentrations were measured by means of total reflection X-ray fluorescence spectrometry (TXRF; Varga et al. 1997).

Results

The fresh weight of poplar plants moderately and strongly decreased under Cu10 and Cd10 treatments, respectively (Table 1). Growth inhibition was the strongest in the upper leaves emerging during the treatment. Dry weight percentage

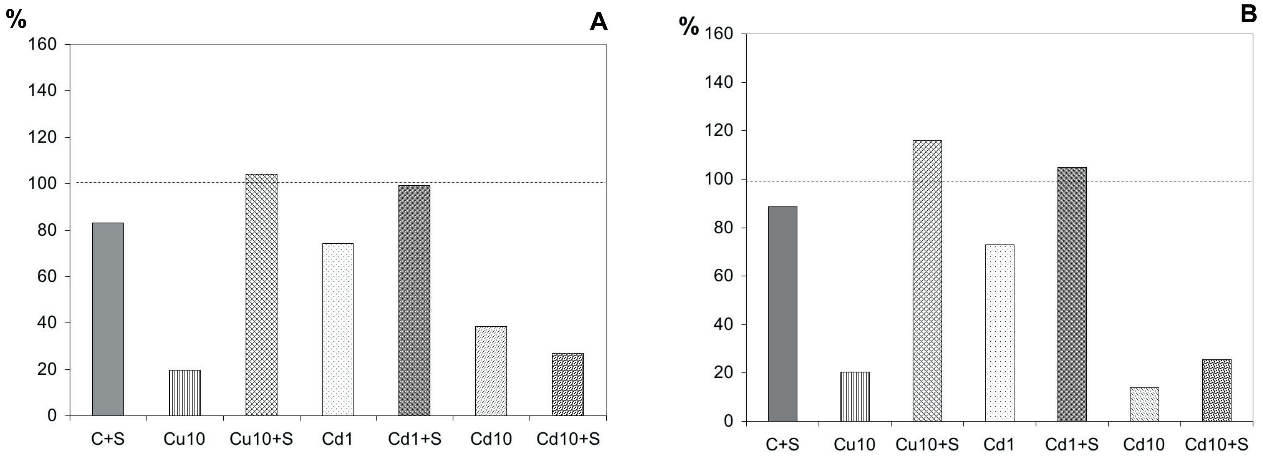

Figure 1. Surface area (A) and total Chl content (B) of Cd and Cu treated upper leaves in poplar. Values are given as the percentage of the corresponding control values. The average size of control leaves was 110.5 cm^2. and their Chl content was 2709.3 µg. S – PmeS treatment.

significantly increased only in Cu treated plants both in roots (from 8.5 to 13.5%) and in upper and lower leaves (from 16.5 to around 29.0%). PmeS eliminated the growth inhibition and elevation of dry weight percentage caused by Cu10, but it was not protective against the growth inhibition produced by Cd10 stress.

Chl accumulation followed the leaf surface changes in most cases except the Cd10 treatment where it was more strongly inhibited (Fig. 1). It means that Chl accumulation significantly decreased in heavy metal treated plants. On a leaf area basis, however, it was the same in the control, Cu10 and Cd1 treated leaves, and only decreased in Cd10 treated plants. PmeS slightly elevated the Chl accumulation in Cd10 treated leaves, and the inhibition was totally eliminated on a leaf area basis. Chl a/b ratio was only lowered in Cd10 treated plants referring to changes of the relative proportion of Chl-protein complexes.

Carbon dioxide assimilation, representing the overall photosynthetic capacity, was strongly diminished by both Cd10 and Cu10 treatment (Fig. 2). PmeS supply increased carbon dioxide fixation parallel with the increase in the Chl content in both cases.

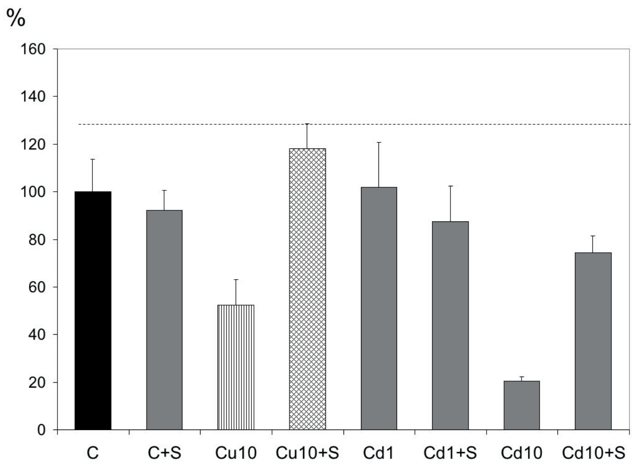

Figure 2. $^{14}CO_2$ fixation of Cd and Cu treated upper leaves in poplar. Values are given as the percentage of the corresponding control values. $^{14}CO_2$ fixation of control plants was around 13700 cpm cm^{-2}. S – PmeS treatment.

Table 1. Changes in fresh weight of poplar under treatments. Relative values are given as the percentage of the corresponding control values. C – control.

Treatments	root	lower leaves	upper leaves
		Absolute values (g)	
C	5.9±2.0	2.7±1.0	5.4±0.3
		Relative values (%)	
C + PmeS	79.7	43.2±16.2	89.5±5.9
Cu10	20.7	40.6±8.6	7.2±16.2
Cu10 + PmeS	80.3±6.6	72.1±12.9	97.6±5.9
Cd1	106.8±8.5	100.3±8.0	84.7±6.4
Cd1 + PmeS	102.0±9.1	79.0±11.7	92.9±5.3
Cd10	54.5±1.9	47.0±12.8	38.3±8.1
Cd10 + PmeS	63.6	79.3	35.2

Table 2. Ion contents of upper leaves. Values are given as the percentage of the corresponding control. Control (C) values are calculated in nmol cm^{-2} leaf material.

Treatments	Ca	Fe	Mn	K
C	1329,5	19,7	12,3	8207,5
C + PmeS	96,1	89,4	117,9	104,2
Cu10	16,0	17,7	23,3	14,6
Cu10 + PmeS	90,6	97,7	106,7	106,7
Cd10	64,6	38,4	71,1	120,7
Cd10 + PmeS	82,4	67,1	104,3	89,4

Heavy metal treatment also caused changes in the ion composition of plants (Table 2). As a result of Cu10 treatment the quantity of all the examined ions decreased, but the ratios of the different ions were invariable. Cd10 treatment, however, resulted significant alteration in the ion ratios, particularly a marked reduction in the iron content was observed. In PmeS and heavy metal co-treated plants the ion balance recovered, and the concentration of ions approached the control level.

Discussion

The results verified the inhibitory effects of Cu and Cd on plant growth and photosynthesis (Titov et al. 1995; di Toppi et al. 1999; Fodor 2002). While growth was more strongly reduced by Cu treatment, Cd preferably inhibited photosynthesis.

The mechanism of growth inhibition was different in the case of these metals. Cu caused disturbance in water regime and inhibited the elongation of cells. Water deficiency resulted from the drop of K^+ ion concentration in plants under Cu stress (Bujtás and Cseh 1982). In contrast, Cd is associated with cell walls and middle lamellae in cells, and it was shown to increase the cross-linking of pectins in the middle lamellae (Prasad 1995). This cross-linking might be responsible for the inhibition of cell expansion.

Our results showed that changes in the ion balance and ion distribution had much more marked effect on photosynthesis, than a strong lowering of the ion content.

PmeS totally protected poplar plants against Cu stress. Its protective action might be based on the complexation of heavy metals in the nutrient solution. Glycinebetaine-like macromolecule/membrane protecting effects (Johvet et al. 1982; Chen and Murata 2002) inside and outside of cell could also contribute to its protective effects particularly in the case of Cd stress affecting photosynthesis (Sakamoto and Murata 2001).

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References

Alscher RG (1989) Biosynthesis and antioxidant function of glutathione in plants. Plant Physiol 77:457-464.

Bujtás K, Cseh E (1982) Effect of copper on the K^+ uptake system of wheat roots (in Hungarian). MTA Biol Oszt Közl 25:609-622.

Chen THH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol 5:250–257.

Chen WP, Li PH, Chen THH (2000) Glycinebetaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in Zea mays. L. Plant Cell Environ 23:609-618.

di Toppi S, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105-130.

Fodor F. (2002) Physiological responses of vascular plants to heavy metals. In Prasad MNV, and Strzalka K, eds., Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants, Kluwer Academic Publishers, Dordrecht, pp. 149-177.

Fodor F, Cseh E, Varga A, Záray Gy (1998) Lead uptake, distribution, and remobilization in cucumber. J Plant Nutr 21:1363-1373.

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1-11.

Hayashi H, Alia, Sakamoto A, Nonaka H, Chen THH, Murata N (1998) Enhanced germination under high-salt conditions of seeds of transgenic *Arabidopsis* with a bacterial gene (codA) for choline oxidase. J Plant Res 111:357-362.

Johvet Y, Larher F Hamelin J (1982) Osmoregulation in halophytic higher plants-the protective effect of glycinebetaine against the heat destabilization of membranes. Plant Sci Letts 25:193-201.

Krupa Z, Baszynski T (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus - direct and indirect effects on light and dark reactions. Acta Physiol Plant 17:177-190.

Láng F, Sárvári É Szigeti Z (1985) Apparatus and method for rapid determination of photosynthetic CO_2 fixation of leaves. Biochim Physiol Pflanz 180:333-336.

Mishliwa-Kurdziel B, Prasad MNV, Strzalka K (2002) Heavy metal influence on the light phase of photosynthesis. In In Prasad MNV, and Strzalka K, eds., Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants, Kluwer Academic Publishers, Dordrecht, pp. 229-255.

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384-394.

Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. Environm Exp Bot 35:525-545.

Sakamoto A, Murata N (2001) The use of bacterial choline oxidase, a glycinebetaine-synthesizing enzyme, to create stress-resistant transgenic plants. Plant Physiol 125:180-188.

Titov AF, Talanova VV, Boeva NP (1995) Growth responses of barley and wheat seedlings to lead and cadmium. Biol Plant 38:431-436.

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13:195-206.

Varga A, Záray Gy, Fodor F, Cseh E (1997) Study of interaction of iron and lead during their uptake process in wheat roots by total reflection X-ray fluorescence spectrometry. Spectrochim Acta 52B:1027-1032.