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Effect of heavy metals on nitrate and protein metabolism in sugar beet

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Abstract

Nitrate content, activities of nitrate reductase and glutamine synthetase, soluble protein content, and proportion of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) protein in total proteins were measured in leaves of *Beta vulgaris* L. plants affected by nickel, cadmium, and molybdenum in concentrations of 10^{-4}, 10^{-2}, and 1 mM. The most harmful effect on the above mentioned parameters had Cd, less harmful Ni, whereas Mo stimulated the investigated parameters. The proportion of RuBPCO protein showed a high tolerance to heavy metals.

Additional key words: *Beta vulgaris* L., cadmium, glutamine synthetase, molybdenum, nickel, nitrate reductase, ribulose-1,5-bisphosphate carboxylase/oxygenase, soluble proteins.

Introduction

Heavy metals (HM) belong to the major pollutants that are accumulated in environment. Their availability in soil depends on natural processes, especially on the lithogenic and pedogenic ones, but also on anthropogenic factors. Heavy metal-induced stress causes various direct and indirect effects on practically all physiological processes in plants (for review see Woolhouse 1983). The HMs interfere with photosynthetic activities (Sheoran *et al.* 1990), mineral nutrition (Yang *et al.* 1996), protein metabolism (Tamás *et al.* 1997) enzymatic activity (Van Asshe and Clijsters 1990), membrane functions (Ouariti *et al.* 1997), water relations (Kastori *et al.* 1992) and some other biochemical processes.

Toxic effects of HM on plant physiology and metabolism are very complex, and they depend on plant species, nature of heavy metal and its concentration. The distinction between the direct and indirect effects of the HM action is very difficult.

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In spite of a large number of studies about the mechanisms of different HM action, further studies are needed, especially at the biochemical level. The aim of this study was to investigate changes in nitrate and protein metabolisms in sugar beet plants caused by Ni, Cd and Mo present in three different concentrations.

Materials and methods

Young sugar beet plants (*Beta vulgaris* L., hybrid NS Hy-11) were grown under semi-controlled greenhouse conditions, in a soil culture using Mitscherlich pots. Agrochemical characteristics of the soil were: pH in H_2O 8.1 and in KCl 6.7, $CaCO_3$ 2.09 %, organic C 2.5 %, total N 0.2 %, P_2O_5 0.01 % and K_2O 0.036 %; presence of the studied HM was not found. Conditions in the greenhouse were: day/night temperature of 25/15 °C, and 16-h photoperiod. After three weeks, the plants were treated with water solutions of Ni ($NiSO_4$), Cd ($CdCl_2$) and Mo (Na_2MoO_4) in concentrations of 10^{-4}, 10^{-2} and 1 mM, the control plants receiving simultaneously the same volume of distilled water. During the experiment the soil moisture was maintained at 60 % of maximal capillary capacity, and was constantly monitored gravimetrically. After 12 - 16 d, *i.e.*, when the symptoms of heavy metal excess appeared under the highest HM concentration, samples of sugar beet leaves for biochemical analyses were taken.

Nitrate content in leaves was determined spectrophotometrically using phenoldisulphonic acid. Nitrate reductase (NR) activity was assayed according to Hageman and Reed (1980), and glutamine synthetase (GS) activity according to Coombs and Hall (1982). The RuBPCO protein proportion was estimated as follows: total proteins from sugar beet leaves were extracted according to Damerval *et al.* (1986), and electrophoresis of the proteins was made on 10 % polyacrylamide gel under denaturing conditions after Laemmli (1970). Proteins on gel were quantified densitometrically by following the RuBPCO large subunit (known amount of RuBPCO standard protein was included in gels), and the results were expressed as percent of RuBPCO protein in total proteins. Soluble proteins from sugar beet leaves were extracted with 100 mol m^{-3} phosphate buffer, pH 7, and the protein content was determined after Lowry *et al.* (1951), using bovine serum albumin as standard.

All determinations were made in triplicates, and values were analyzed by Duncan's multiple range test and *t*-test.

Results and discussion

The investigated HM caused a substantial (25 % in average) decrease of dry matter content only at the highest concentration applied (1 mM). Of the three metals, the excess of Mo caused the most significant decrease in dry matter content in shoots of sugar beet plants (Table 1). Similarly, Ni and Cd present in an excess in the substrate caused a significant decrease of dry matter content in maize plants (Woolhouse 1983). High HM concentrations in the majority of cases inhibit plant growth, especially the root growth (*e.g.*, Karataglis *et al.* 1991). The different effects of a particular HM on plant growth are usually observed at high concentrations.

The nitrate content in the leaves of young sugar beet plants was markedly dependent of both the HM and its concentration (Table 1). With the exception of Mo, an increased concentration of HM in the substrate caused a significant decrease of nitrate content in plant leaves, especially at the highest HM concentration. The presence of HM in the substrate inhibits uptake of nitrates by the root as well as their translocation by xylem to the site of their reduction. Thus, *e.g.*, the excess of Cd caused a decrease of nitrate content in shoots of pea plants by 90 % (Hernández *et al.* 1996). A significant nitrate increase observed in the presence of the highest Mo concentration was probably more a consequence of the pronounced reduction of dry matter content than a consequence of a favourable effect of the Mo excess on the uptake and transport of nitrate.

Table 1. Dry matter content in shoots, and nitrate and soluble protein contents, activities of enzymes of nitrate metabolism, and proportion of RuBPCO in total proteins in leaves of sugar beet plants as affected by nickel, cadmium and molybdenum at different concentrations (10^{-4}, 10^{-2}, and 1 mM). *,** - values significantly different from control at $P < 0.05$ and $P < 0.01$, respectively. Values in the same row labelled with different letters are significantly different at $P < 0.05$.

Parameter	HM	Control	10^{-4}	10^{-2}	1
Dry matter	Ni	0.601	0.631a	0.532b	0.455b**
[g plant^{-1}]	Cd	0.601	0.538a	0.520a	0.482a**
	Mo	0.601	0.566a	0.512ab	0.417b**
Nitrate content	Ni	3.351	3.338a	2.794ab*	2.501b**
[g kg^{-1}(d.m.)]	Cd	3.351	3.382a	2.842b *	2.455b**
	Mo	3.351	2.496b**	2.650b **	5.112a**
NR activity	Ni	0.81	0.77 a	0.65 a	0.53 b**
[μmol kg^{-1}(f.m.) s^{-1}]	Cd	0.81	0.51 a**	0.33 b **	0.26 c**
	Mo	0.81	1.32 a**	1.42 a **	1.51 b**
GS activity	Ni	14.0	10.1 a	9.3 a	6.1 a**
[μmol kg^{-1}(f.m.) s^{-1}]	Cd	14.0	-	-	-
	Mo	14.0	26.0 c**	31.0 b **	39.0 a**
Protein content	Ni	27.6	23.0 a**	18.3 b **	17.6 b**
[g kg^{-1}(f.m.)]	Cd	27.6	23.3 a*	21.3 ab**	19.3 b**
	Mo	27.6	30.6 a	32.6 a **	33.0 a**
RuBPCO protein	Ni	26.0	24.3 a	23.8 a	22.8 a
[%]	Cd	26.0	27.1 a	22.9 a	18.1 b*
	Mo	26.0	31.0 a	30.7 a	26.5 a

Sugar beet is a plant species in which nitrate is reduced primarily in shoots, whereas the nitrate reductase (NR) activity in the root is insignificant (Petrović and Kastori 1990). The measured NR activities were significantly dependent on the HM and their concentration (Table 1). Ni caused a significant inhibition of NR only at the highest concentration, whereas Cd reduced substantially this parameter at all concentrations used.

The nitrate present in plant cell cytoplasm participates directly in the expression of the NR gene, and thus influences the NR induction (Hoff et al. 1992, Tischner *et al.* 1993). Burzynski (1988) supposes that HM, and especially Cd, inhibit nitrate translocation from xylem to cytoplasm. The reduced NR activity in organs of plant photosynthesis is frequently related to the appearance of water deficit in the HM-contaminated plants (Rauser and Dumbroff 1981). Under these conditions, the rate of transpiration is lowered, and water and nitrate transport in xylem is hindered (Barcelo and Poschenrieder 1990).

A positive correlation was found between the NR activity and Mo concentrations in the substrate (Table 1). NR and xanthine dehydrogenase are plant enzymes requiring the presence of Mo for their activity. Of the total amount of Mo present in plants, about 10 % is built in as the co-factor Mo-pterin in the NR complex (Notton 1983). In relation to this, some authors have found that the presence of Mo up to a certain level in the substrate stimulates the NR activity. However, if Mo is present in an excess, then, like other HMs, it can, to a smaller or greater extent, reduce the NR activity (Srivastava 1980).

In the literature, there are different opinions on the change of nitrate content an on the activity of particular enzymes of nitrate metabolism in plants in the presence of HM. Yevdokimova (1994) found that nitrate is accumulated in plants grown in soils with high contents of HMs. A decrease in nitrate content, accompanied by an increase in the NR activity, was observed in potato plants (Mondy and Munshi 1993). The presence of Cd caused an increase in the NR and GS activities of maize leaves (Ferretti *et al.* 1993), whereas the presence of this element resulted in an insignificant change of GS activity and a reduction of NR activity in pea plants (Chugh *et al.* 1992).

The photosynthetic organs usually contain two GS isoenzymes: GS_1 and GS_2 (McNally *et al.* 1983). GS_2 may play a primary role in the metabolism of ammonia formed in the chloroplasts after nitrate reduction. With regard to this, the activity of the GS_2 isoenzyme is significantly influenced by NR, nitrite reductase, amount of the substrate, and nitrate content in the cytoplasm (Oaks *et al.* 1980). The GS activity in leaves of sugar beet plants was dependent of the HM and its concentration (Table 1). The used concentrations of Ni caused a significant decrease in GS activity, whereas the presence of Mo caused its increase. On the other hand, the presence of Cd completely suppressed the GS activity. The excess contents of Cu, Hg, Cd, Zn, Ni, and Fe in the substrate caused a specific inhibition of GS in rice plants (Miflin and Lea 1980). A significant reduction of GS activity by Cd in various plant species and its complete inhibition in sugar beet plants (Popović *et al.* 1996), is sometimes explained by Cd ability to form inactive complexes (the O-, N-, or S-ligands) of the natural proteins, especially of the enzyme proteins, inhibiting thus their activities (Van Assche and Clijsters 1990). Nevertheless, an excess of Cd inhibits nitrate translocation from the root to the shoot (Hernandez *et al.* 1996), and the NR activity is mainly influenced by the sum of nitrates present in cell cytoplasm (Burzynski 1988).

The investigated HMs exhibited different effects on the content of soluble proteins in sugar beet leaves. While Ni and Cd caused a decrease of soluble protein content, Mo produced an opposite effect. However, the changes were far less expressed than the changes in activity of the nitrogen assimilation enzymes (Table 1). A high resistance to the HM effect showed also the proportion of RuBPCO protein in total proteins, significant changes being observed only at the highest Cd concentration (Table 1). Stability of the RuBPCO protein proportion under stress conditions, such as drought, has been observed in sunflower (Panković 1996) and sugar beet (Kevrešan *et al.* 1997).

Of the investigated HMs the most harmful effect had Cd, then Ni, whereas Mo mostly stimulated the investigated parameters. Because Cd is a mobile element, it is accumulated in leaves, where GS is mostly located in photosynthetic tissues (Dubois *et al.* 1996). Hence, there is a positive correlation between Cd concentrations in the xylem and leaves (Guo and Marschner 1995) and its action on GS. The NR activity can be reduced in the presence of agents for modification of thiol groups of the cysteine moiety (Trimboli *et al.* 1996), and Cd is known for its specific affinity towards sulphur (Kaim and Schwederski 1994).

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