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BRIEF COMMUNICATION

Alleviation of cadmium toxicity by naphthenate treatment

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Abstract

The work is concerned with the effect of low concentrations (10^{-7} mol dm^{-3}) of sodium naphthenate on total content of Cd and its particular forms in the intercellular space and inside cells, as well as on some physiological and biochemical parameters of young soybean plants grown in the presence of 1 mmol dm^{-3} solution of cadmium chloride. Presence of naphthenate reduced in average by 40 % content total and intracellular Cd in root, stem and leaves and alleviated the harmful effect of Cd on activity of nitrate reductase and content of photosynthetic pigments.

Additional key words: soybean, intracellular Cd, intercellular Cd, detoxification

In an attempt to increase plant tolerance to elevated concentrations of heavy metals various methods have been applied such as treatment with plant hormones (Ghorbanli *et al*. 1999), specific mineral nutrition, involving, *e.g*., sulfur (Popović *et al*. 1996), phosphorus (Sajwan *et al*. 2002) and nitrogen (Panković *et al*. 2000), cultivation conditions (Lunáčková *et al*. 2003/4), or treatment with some other substances such as biomin (Kamenova-Jouhimenko *et al*. 1997/8).

Petroleum acids of naphthenic type represent a complex mixture of cycloalkyl and carboxylic acids. As we previously found that mixture of naphthenic acids from Vojvodina crude oil "Velebit" exhibited physiological activity similar to that of plant hormones of auxin and gibberelin type (Ćirin-Novta *et al*. 2002), known to play a role in detoxification of heavy metals, it was interesting to examine potential effect of naphthenate in this respect. To this aim we studied the effect of treatment with sodium naphthenate on content of Cd, its particular forms in the intercellular space and inside cells, as well as on some physiological and biochemical parameters of young soybean plants grown in the presence of Cd.

Naphthenic acids were isolated from oil fraction (distillation interval 168 - 290 °C) of Vojvodina crude oil "Velebit" by alkaline extraction (Ćirin-Novta *et al*. 2003).

Young plants of soybean (*Glycine max* L.), genotype NS-L-320-251, were grown at Hoagland nutrient solution of the following composition [mmol dm^{-3}]: 2.5 $Ca(NO_3)_2$, 2.5 KNO_3, 1.0 KH_2PO_4, 1.0 $MgSO_4 . 7 H_2O$; and [μmol dm^{-3}] 23.1 B; 4.6 Mn; 0.38 Zn; 0.16 Cu; 0.052 Mo; 8.95 Fe in the form of Fe(III)-EDTA, until complete formation of the first physiologically active pair of leaves. After that they were divided into four groups: control, in which plants were continued to grow in the same solution and three test groups in which plants were grown in the same medium with the addition of Cd^{2+} at a concentration of 1 mmol dm^{-3} in the form of $CdCl_2$, sodium naphthenate at a concentration of 10^{-7} mol dm^{-3},

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Abbreviations: Car - carotenoids; Chl - chlorophyll.
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and both Cd (1 mmol dm^{-3} Cd^{2+}) and sodium naphthenate (10^{-7} mol dm^{-3}), respectively.

After growing plants under the given conditions for two weeks, *i.e.* to the appearance of third pair of leaves, contents of particular forms of Cd in the intercellular space and in cells of root, stem and leaves were determined as described in previous work (Kevrešan *et al*. 2003). Contents of total intercellular Cd was obtained by summing contents of its particular forms in the intercellular space while overall Cd content in root, stem and leaves was obtained by summing up total extracellular and intracellular Cd. Nitrate reductase activity was determined according to Witt (1975) and content of photosynthetic pigments according to Von Wettstein (1957).

Treatment with sodium naphthenate caused a significant decrease in content of total Cd in all plant parts by about 40 % (36 % in root, 37 % in stem, and 44 % in leaves). Content of intracellular Cd in root and leaves was similarly lowered as total Cd (44 % in root and 34 % in leaves) compared with Cd content treated only with Cd, whereas Cd content in stem remained practically unchanged. Also, content of intercellular Cd in root did not practically change, whereas its contents in stem and leaves was significantly lower (by 77 % in stem and by 59 % in leaves) in the treatment with naphthenate compared with the contents in plants treated with only Cd (Table 1).

The decrease in Cd content in leaves in the presence of sodium naphthenate influenced also the investigated biochemical parameters of young soybean leaves (Table 2).

Presence of Cd in the nutrient medium had a negative effect on the activity of nitrate reductase as it led to its decrease by 36 % compared to control. However, the presence of naphthenate in the medium alleviated this adverse effect, the nitrate reductase activity in the presence of naphthenate and Cd being 93.5 % of the activity measured in control plants.

It is also evident that the presence of Cd in the nutrient solution had a negative effect on contents of chlorophyll (Chl) *a*, Chl *b* and carotenoids (Car) – their contents decreased by 32, 36, and 24.6 %, respectively, compared to the values measured for control. However, the presence of naphthenate alleviated the adverse effect of Cd, so that contents of Chl *a* and Car in plants subjected to combined treatment with Cd and naphthenate amounted to 91.5 % and 96.9 % of the respective contents in control plants. The presence of naphthenate in the nutrient medium also lowered the effect of Cd on content of Chl *b* but to a much smaller extent than in the case of Chl *a* – content of Chl *b* was 74.5 % of that measured for control plants.

Table 1. Effect of sodium naphthenate on content and distribution of Cd in young soybean plants [μg g^{-1}(d.m.)]. Plants were grown in the presence of Cd or at combined treatment with Cd and sodium naphthenate (Cd+NK). Each value represents the mean ± SD (n = 3).

Cd form	Roots		Stems		Leaves	
	Cd	Cd+NK	Cd	Cd+NK	Cd	Cd+NK
Intercellular water soluble Cd	3.89 ± 0.16	0.71 ± 0.03	2.41 ± 0.12	1.42 ± 0.05	1.01 ± 0.03	0.47 ± 0.03
Intercellular exchangeable Cd	2.41 ± 0.11	1.31 ± 0.06	0.62 ± 0.04	1.08 ± 0.04	0.45 ± 0.02	0.36 ± 0.04
Intercellular complexed Cd	38.77 ± 1.71	40.20 ± 1.80	45.55 ± 1.73	8.74 ± 0.39	12.23 ± 0.47	4.77 ± 0.21
Total intercellular Cd	45.07 ± 1.83	42.22 ± 1.89	48.58 ± 2.17	11.24 ± 0.43	13.69 ± 0.51	5.60 ± 0.29
Intracellular Cd	155.00 ± 6.92	86.53 ± 3.93	51.25 ± 2.11	51.73 ± 2.24	21.82 ± 1.14	14.41 ± 0.81
Total Cd	200.07 ± 9.91	128.75 ± 5.82	99.83 ± 4.22	62.97 ± 3.13	35.51 ± 1.63	20.01 ± 0.93

Table 2. Effect of sodium naphthenate and Cd on activity of nitrate reductase [μmol(NO_2^-) g^{-1}(f.m.) s^{-1}] and content of chloroplast pigments [mg g^{-1} (d.m.)] in young soybean plants. Plants were grown in the presence of Cd (Cd) or sodium naphthenate (NK) and at combined treatment with Cd and sodium naphthenate (Cd+NK). Each value represents the mean ± SD (n = 3). Values in the same column followed by different letters are significantly different ($P < 0.05$, Duncan's multiple range test).

Treatment	Activity of nitrate reductase	Chl *a*	Chl *b*	Car
Control	$2.53 \cdot 10^{-3} \pm 1.9 \cdot 10^{-4a}$	11.08 ± 0.28^{a}	4.35 ± 0.56^{a}	2.88 ± 0.02^{a}
Cd	$1.61 \cdot 10^{-3} \pm 8.6 \cdot 10^{-5b}$	7.57 ± 0.11^{b}	2.80 ± 0.11^{b}	2.17 ± 0.02^{b}
NK	$2.04 \cdot 10^{-3} \pm 2.3 \cdot 10^{-4c}$	11.70 ± 1.39^{a}	4.05 ± 1.10^{a}	3.17 ± 0.20^{a}
Cd+NK	$2.35 \cdot 10^{-3} \pm 3.0 \cdot 10^{-4a}$	10.14 ± 0.35^{a}	3.24 ± 0.10^{a}	2.79 ± 0.15^{a}

On the basis of the results obtained in this study it can be supposed that naphthenic acids applied in smaller concentrations can lower content of Cd in plants grown in the presence of its elevated concentrations and thus reduce its toxicity. The alleviating effect is probably due to subtle modifications of plasma membranes of root cells by altering their permeability to Cd.

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