

The relationship between salinity and cadmium stress in barley

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Abstract

Distribution of cadmium between roots and shoots of barley was manipulated by the cadmium concentration (0.01 and 0.005 mM Cd²⁺), pH (4.6 and 5.9) as well as treatment duration. The prolongation of treatment increased dry mass and content of cadmium in plants. The cadmium is accumulated mainly in roots. Presence of both, 0.005 mM Cd²⁺ and 100 mM NaCl in medium at pH 5.9 (Cd-NaCl plants) resulted in the most severe growth inhibition of plants, but about one half accumulation of cadmium in roots then in a case of only Cd-treated plants. In the Cd-NaCl plants, the net photosynthetic and transpiration rates were less reduced than in a case of only NaCl-treated plants. The treatments also influenced uptake of Ca, Cd, Cu, K, Mg, Na and Zn predominantly in roots.

Additional key words: growth, *Hordeum vulgare*, nutrient uptake, transpiration, photosynthesis.

Introduction

High salinity and cadmium pollution lead to significant fall-off in health and productivity of crops. Availability of Cd to plants is regulated by pH, redox potential and other physico-chemical parameters (Prasad 1995). Cadmium is preferably accumulated in the roots and decreases the water uptake in roots (Barceló and Poschendrieder 1990, Vozáry *et al.* 1997). The transfer of cadmium ions to upper parts of the plants is usually restricted (Leita *et al.* 1991, Brune and Dietz 1995). However, cadmium

accumulates also in the leaves (Di Cagno *et al.* 1999). Salinity stress (Kinraide 1999) also results in decreased water and nutrient uptake (Vögeli-Lange and Wagner 1990, Rivetta *et al.* 1997, Larsson *et al.* 1998). The relationships among cadmium, salinity, and mineral nutrition are relatively little known. The present study examined the effect of salinity and cadmium on the growth, gas exchange and nutrient uptake of hydroponically growing spring barley seedlings.

Materials and methods

The seeds of spring barley (*Hordeum vulgare* L. cv. Rubín) were sterilized by 15 % sodium hypochlorite (NaClO), and washed 3-times with distilled water. The seeds were germinated on the filter paper in Petri dishes at temperature of 19 °C and relative humidity of 90 % for 4 d in the dark. After germination they were grown hydroponically in 550 cm³ pots containing a half strength Knop's solution. The final concentrations of micro-elements were following: 0.925 mM H₃BO₃, 0.099 mM MnCl₂·H₂O, 0.247 mM ZnSO₄·7 H₂O, 0.053 mM CuSO₄·5 H₂O, 0.099 mM MoO₃. Cadmium was added

from stock solution of 1 mM CdCl₂ as volume aliquot to the medium to final concentrations of 0.005 and 0.01 mM Cd²⁺, and pH was adjusted to 5.9 and 4.6 (acidification with H₂SO₄). The plants were cultivated in growth chamber at temperature of 22 °C, 16-h photoperiod, irradiance of 300 μmol m⁻² s⁻¹, and relative humidity of 70 %. The germinated seedlings were exposed for 7 d to stress treatments or they were cultivated 7d without stress and then exposed to stress treatments for 7 d. In the later case the plants were treated with 100 mM NaCl, 0.01 mM Cd²⁺, or 0.01 mM Cd²⁺ + 100 mM NaCl at pH 5.9. All

Received 24 January 2002, accepted 18 April 2002.

Abbreviations: E - transpiration rate; g_s - stomatal conductance; P_N - net photosynthesis rate.

Acknowledgements: This work was supported by Grant No. 10/055/1995-1997 of the University of Agriculture, Prague. Authors thank Dr. J. Száková for elemental analysis, Dr. H. Vodičková and Dr. J. Černohorská for critical reading of the paper.

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control plants were grown in the basic nutrient medium (pH 5.9 or 4.6).

After cultivation, the roots were washed by 10^{-3} M solution of ethylenediaminetetraacetic acid (EDTA), length of leaves and root (of 2×10 plants) was measured, and the shoots, roots, and seeds were freeze-dried. The experiments were done in triplicate. Freeze-dried biomass of seeds, shoots and roots was decomposed by dry ashing in a mixture of oxidizing gases (*Dry Mode Mineralizer Apion, Tessek, Prague, Czech Republic*). The concentrations of Ca, Cd, Cu, K, Mg, Na, Zn in the digests and cultivation medium, before and after experiments were determined by atomic absorption spectrometry (*Varian SpectraAA-300, Victoria, Australia*).

Results and discussion

Different application of Cd in the nutrient medium significantly affected plant growth and Cd content in plant tissue. The degree of the damage observed in Cd-treated plants, such as dry tip, chlorosis of leaves and browning of roots, increased with increasing of Cd concentration in medium. The Cd treatment reduced the dry mass at longer cultivation (7 + 7 d), but not significantly in the shorter cultivation (7 d) (Table 1). The more marked Cd toxicity symptoms were observed at pH 5.9 than at pH 4.6, although the similar final pH 6.67 ± 0.08 (at pH 5.9) and 6.60 ± 0.04 (at pH 4.6) were

The net photosynthetic rate (P_N), stomatal conductance (g_s) and the transpiration rate (E) were measured on 1st, 2nd and 3rd day of treatment by *LCA 4* infrared gas analyzer (*ADC BioScientific Ltd., Eijkelkamp, Netherlands*) on the second fully expanded leaf. Temperature in the chamber was in the range from 25 to 27 °C, the irradiance $260 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$, and relative humidity 25 to 30 %. The rate of gas exchange between leaves and atmosphere inside the chamber was estimated from the flow rate of air and changes of CO_2 concentrations between input and output from the leaf chamber. The data are presented as means of 10 measurements with standard deviation.

determined. In plants, at low pH the mobility of Cd ions from nutrient medium to roots is limited as was reported by Pendias and Pendias (1992). This corresponded with our results. Also Cd concentration in the seedlings increased with increasing concentration of Cd in the medium (Table 1). The higher Cd content was found at pH 5.9 and in older plants. However, the distribution of Cd between roots and shoots was not influenced. The distribution of Cd in plant tissues showed more than 90 % of total Cd content in roots. This is about one to two orders of magnitude higher than total Cd of shoots.

Table 1. The length, dry mass, and Cd content in shoots and roots of control plants and plants exposed for 7 d to 0.005 or 0.01 mM Cd^{2+} at medium pH 4.6 or 5.9. The germinated seedlings were directly exposed to Cd (7 d) or after 7 d of cultivation without stress (7 + 7 d). Means \pm SD, $n = 15$, * - differences significant at $P = 0.05$ determined by paired *t*-test in relation to control.

| | Cd [mM] | pH | 7 d shoots | roots | 7 + 7 d shoots | roots |
|--|---------|-----|---------------------|---------------------|----------------------|---------------------|
| Length [cm] | control | 5.9 | 15.55 ± 1.43 | 11.07 ± 2.35 | 16.56 ± 1.59 | 12.73 ± 2.20 |
| | | 4.6 | 16.29 ± 0.79 | 10.64 ± 0.09 | 16.85 ± 3.20 | 10.58 ± 1.72 |
| | 0.005 | 5.9 | 15.58 ± 0.83 | 10.55 ± 1.86 | 14.99 ± 1.69 | 11.87 ± 2.50 |
| | | 4.6 | 16.43 ± 1.17 | 10.75 ± 0.54 | 17.26 ± 2.51 | 11.13 ± 3.18 |
| | 0.01 | 5.9 | 14.20 ± 1.28 | 10.39 ± 0.02 | 13.29 ± 1.45 | 10.37 ± 2.55 |
| | | 4.6 | 15.50 ± 1.23 | 11.64 ± 0.09 | 16.37 ± 1.86 | 12.27 ± 2.85 |
| Dry mass [g] | control | 5.9 | 0.367 ± 0.024 | 0.133 ± 0.008 | $1.000 \pm 0.102^*$ | $0.360 \pm 0.012^*$ |
| | | 4.6 | 0.383 ± 0.054 | 0.120 ± 0.004 | $1.018 \pm 0.199^*$ | $0.447 \pm 0.033^*$ |
| | 0.005 | 5.9 | 0.382 ± 0.067 | 0.125 ± 0.004 | $0.798 \pm 0.210^*$ | $0.277 \pm 0.085^*$ |
| | | 4.6 | 0.380 ± 0.006 | 0.122 ± 0.003 | $0.990 \pm 0.250^*$ | $0.410 \pm 0.051^*$ |
| | 0.01 | 5.9 | 0.367 ± 0.016 | 0.144 ± 0.027 | $0.872 \pm 0.193^*$ | $0.269 \pm 0.090^*$ |
| | | 4.6 | 0.340 ± 0.040 | 0.135 ± 0.023 | $0.990 \pm 0.254^*$ | $0.314 \pm 0.084^*$ |
| Cd content [mg kg ⁻¹ (d.m.)] | control | 5.9 | 1.93 ± 1.54 | 1.09 ± 1.39 | 2.03 ± 0.98 | 0.62 ± 0.22 |
| | | 4.6 | 1.28 ± 0.32 | 1.76 ± 2.14 | 1.05 ± 0.53 | 0.18 ± 0.05 |
| | 0.005 | 5.9 | $482.3 \pm 82.9^*$ | $48.61 \pm 4.06^*$ | $549.3 \pm 56.2^*$ | $44.20 \pm 17.60^*$ |
| | | 4.6 | $363.1 \pm 28.9^*$ | $46.69 \pm 14.25^*$ | $330.0 \pm 35.6^*$ | $30.60 \pm 2.85^*$ |
| | 0.01 | 5.9 | $946.6 \pm 259.3^*$ | $72.49 \pm 6.03^*$ | $1114.8 \pm 118.3^*$ | $65.20 \pm 8.01^*$ |
| | | 4.6 | $735.2 \pm 140.7^*$ | $68.45 \pm 9.36^*$ | $892.4 \pm 199.8^*$ | $65.80 \pm 1.19^*$ |

Cieslinski *et al.* (1996a) showed that Cd accumulation and distribution within the plants varied at different stages of plant development and were strongly affected by both soil type and plant. The retention of Cd in roots was found in many plant species, for example in seedlings of *Zea mays* 92 - 94 % at 0.003 mM Cd²⁺ (Leita *et al.* 1991) or *Hordeum vulgare* 95 % at 0.001 mM Cd²⁺

(Brune and Dietz 1995). In our study the reduced content of Cd in shoots of spring barley indicated that Cd was retained in the roots, perhaps by a mechanism involving regulation of water content and vacuolar sequestration of Cd in root cells (Vögeli-Lange and Wagner 1990, Hart *et al.* 1998).

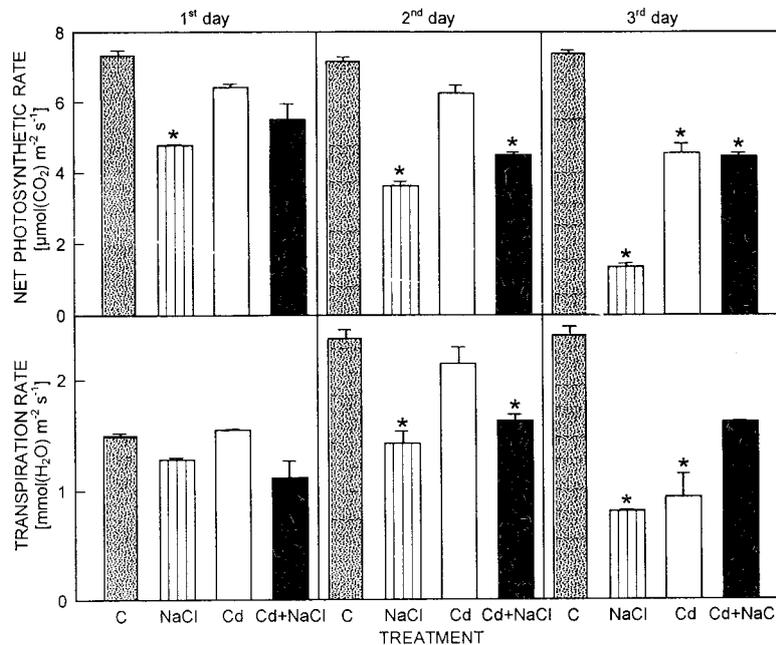


Fig. 1. Net photosynthetic rate (P_N) and transpiration rate (E) in the presence of 100 mM NaCl (NaCl), 0.01 mM Cd²⁺ (Cd), and combination of the both (Cd + NaCl) in comparison to control (C). The second fully expanded leaves were measured on the 1st, 2nd and 3rd d of treatments. Mean \pm SD, $n = 10$, * - differences significant at $P = 0.05$ determined by paired t -test in relation to control.

Table 2. The content of Cd and mineral elements [mg kg⁻¹(d.m.)] in the roots and shoots, dry mass [g] of the control plants and the plants treated for 7 d without and then for 7 d with 0.01 mM Cd²⁺, 100 mM NaCl, 0.01 mM Cd²⁺ + 100 mM NaCl. Means \pm SD, $n = 15$ plants per treatment and experiment was triplicate, * - differences significant at $P = 0.05$ determined by paired t -test in relation to control.

| Element | | Control | Cd | NaCl | Cd + NaCl |
|----------|-------|-------------------|-------------------|--------------------|---------------------|
| Shoots | Ca | 7617 \pm 1132 | 8003 \pm 680 | 3748 \pm 796* | 4127 \pm 284* |
| | Cd | 0.40 \pm 0.220 | 75.86 \pm 1.80* | 0.53 \pm 0.381 | 52.58 \pm 15.37* |
| | Cu | 9.63 \pm 3.37 | 7.70 \pm 2.10 | 11.94 \pm 6.98 | 8.92 \pm 3.18 |
| | K | 62431 \pm 4933 | 65852 \pm 5028 | 48854 \pm 6867* | 53997 \pm 3799 |
| | Mg | 2071 \pm 145 | 1956 \pm 172 | 1673 \pm 180 | 1857 \pm 416 |
| | Na | 256 \pm 160 | 418 \pm 195 | 22145 \pm 1577* | 14910 \pm 2348* |
| | Zn | 56.05 \pm 17.17 | 52.09 \pm 12.90 | 152.97 \pm 90.43 | 105.33 \pm 53.46* |
| | Roots | Ca | 4640 \pm 1281 | 5013 \pm 1111 | 1062 \pm 354* |
| Cd | | 1.59 \pm 0.50 | 1122 \pm 203* | 1.54 \pm 1.08 | 684.3 \pm 236* |
| Cu | | 5.97 \pm 2.84 | 8.95 \pm 3.74 | 14.62 \pm 12.42 | 30.5 \pm 6.51* |
| K | | 58295 \pm 8327 | 53088 \pm 5134 | 37245 \pm 5875 | 26347 \pm 2703* |
| Mg | | 1669 \pm 543 | 1501 \pm 343 | 1231 \pm 373 | 1440 \pm 255 |
| Na | | 1724 \pm 1632 | 1720 \pm 399 | 30154 \pm 6478* | 27586 \pm 1191* |
| Zn | | 65.12 \pm 36.36 | 116 \pm 28 | 84.87 \pm 41.55 | 150.92 \pm 11.65* |
| Dry mass | | 0.86 \pm 0.12 | 0.66 \pm 0.11 | 0.64 \pm 0.05 | 0.53 \pm 0.05* |

Although the roots accumulated the highest concentrations of Cd of all plant parts investigated, increased Cd application reduced the length of shoots, whereas the length of the roots was unchanged or slightly stimulated (Table 1). The stimulated growth of roots by Cd (Baker and Walker 1989, Verkleij *et al.* 1990) indicated quick response to Cd (Hart *et al.* 1998, Pineros *et al.* 1998) and the metal ion requirement for better root growth (Patra *et al.* 1995). From this view the barley plants via stimulation of root growth and the new adventitious stems formation from bases of stressed plants (the results not shown) could be also viewed as the increase of chelating capacity of these organs for Cd detoxification, as reported by Meuwly and Rauser (1992). The browning of roots observed at stressed plants may be a result of oxidative stress (Dietz *et al.* 1999).

The growth of seedlings cultivated for 7 + 7 d at pH 5.9 was also inhibited due to Cd and salinity treatments. In comparison to Cd-stress, the salinity treatment (100 mM NaCl) caused visually stronger symptoms of toxicity in barley plants probably via multiple stress conditions including water deficiency and disbalance in nutrient uptake (Kinraide 1999). The exposure of plants to NaCl led to chlorosis with small necrosis of upper parts of the leaves. The Cd + NaCl treated plants had shorter brown roots, large leaf chlorosis, the dry up and the advanced necrosis on the reduced leaves area. In Cd + NaCl treatment the growth and development of the plants were the most retarded (Table 2), although the other measured parameters unexpectedly did not correlate with this (Figs. 1, 2). It is interesting to note that these plants contained less Cd in both, roots and shoots than in Cd-treatments alone (Table 2.). Multiple bindings of Cd in cytosol (Klapheck *et al.* 1994), in vacuole (Vögeli-Lange and Wagner 1990) in cell wall and in apoplast (Hart *et al.* 1998) contribute to different mechanism of translocation of Cd from the roots to the shoots. The movement of Cd from roots to shoots via xylem is driven by transpiration from the leaves (Salt and Rauser 1995). In our study the transpiration rate (E) correlated with the stomatal conductance (g_s) (Fig. 2), but not with the net photosynthetic rate (P_N). The inhibition of E influenced a mobility and accumulation of Cd and Na in shoots. Vozáry *et al.* (1997) reported quick increase of water in apoplast of pea in the presence of 0.2 mM Cd^{2+} , which caused higher mobility of elements and induction of osmotic stress. Hagemayer and Weisel (1989) reported an increase in transpiration rate due to 0.09 mM Cd^{2+} in comparison to NaCl treatments. The different inhibition effect of Cd and NaCl and Cd + NaCl treatments on the E and P_N were observed (Fig. 2). If NaCl treatment caused promptly osmotic stress, the content of Cd in Cd + Na treated plants was limited by both, water deficiency and increase of Na in root. Similar to E the P_N inhibition of

NaCl-treated plants was surprisingly ameliorated by Cd addition to the nutrient medium (Fig. 1).

The barley plants at 100 mM NaCl accumulated large amount of Na by both, the shoots and roots. This is likely because they have abundant vacuolar Na^+/H^+ antiporter (Carborino and DuPont 1989). The increase in Ca observed at Cd-treatment in roots can have protective

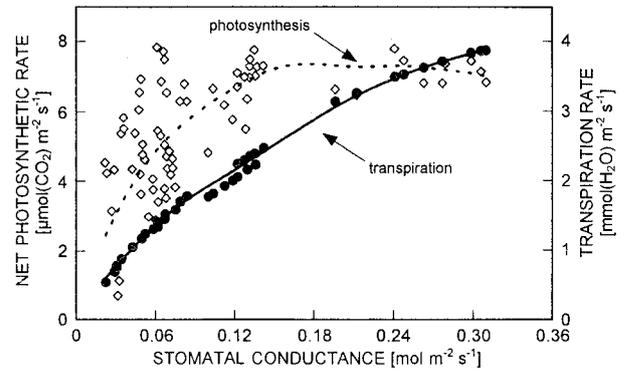


Fig. 2. Net photosynthetic rate (P_N) and transpiration rate (E) in relation to the stomatal conductance in the fully expanded leaf of barley.

effect against Cd excess in the roots. In a opposite to Cd treatment, the Ca-deficiency induced by NaCl-treatment was drastically increased at Cd + NaCl-treatment. Calcium is involved in the water flow regulation of root (Quintero *et al.* 1999). Sodium at high concentrations in the rooting medium can displaced Ca^{2+} from the cell surfaces (Kinraide 1999). The similar binding affinity for Na^+ , Cd^{2+} and Ca^{2+} at plasma membrane (Ouyang and Vogel 1998, Vögeli-Lange and Wagner 1990, Rivetta *et al.* 1997) may lead to the transient increase or decrease in Ca^{2+} inside root cells. This suggests that the sensitivity to ion imbalances observed in our experiments at NaCl and Cd + NaCl treatments is related to the symptoms of Ca-deficiency (Yang *et al.* 1996, Larsson *et al.* 1998). The content of other elements were also influenced concomitantly to Cd or NaCl-treatments (Table 2). In all the treatments (Cd, NaCl, Cd + NaCl) the higher content of Zn and lower content of Mg in comparison to control were found. Although kinetic constants for Zn and Cd uptake have been quite different (Hart *et al.* 1998), Zn competitively inhibited Cd uptake in plant roots (Costa and Morel 1993). The salinity stressed plants (NaCl, Cd + NaCl) showed decrease in content of K, which did not correspond with observation of Walker and Taiz (1988). They found the accumulation of large amount of potassium (up to 500 mM) in salinity stressed plants. This discrepancy was probably influenced by different type of plant. In our experiments the degree of Mg-deficiency correlated with the inhibition of P_N .

These results showed an important role of nutrients composition on modification of heavy metal toxicity.

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