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Original Research

Effect of Calcium and Cadmium on Growth and Accumulation of Cadmium, Calcium, Potassium and Sodium in Maize Seedlings

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Abstract

We examined cadmium (0.1 mmol/l) toxicity and effect of different calcium concentrations (0.1, 1.0, and 10.0 mmol/l) on the growth of organs (root, mesocotyl and coleoptile) of 4-day-old maize seedlings in hydroponic cultures. The influence of both metals on distribution of cadmium, calcium, potassium and sodium in the organs of maize seedlings was also studied. Calcium at a concentration of 1.0 mmol/l increased the growth of all organs in comparison with the growth in 0.1 and 10.0 mmol/l $CaCl_2$. Cadmium inhibited maize organ growth in the presence of 0.1 and 1.0 mmol/l $CaCl_2$, whereas cadmium toxicity was not observed at 10.0 mmol/l $CaCl_2$. It was found that the content of both metals in maize seedling organs rose with increasing concentrations of Ca or Cd in the hydroponic solutions. Cadmium administration together with 10.0 mmol/l $CaCl_2$ led to an increase in calcium concentrations in roots and coleoptiles. The highest calcium concentration (10.0 mmol/l) caused a decrease in the cadmium concentration in roots. The addition of cadmium with 0.1 or 1.0 mmol/l $CaCl_2$ diminished potassium accumulation in the roots. By contrast, potassium content in the roots was not altered by cadmium when calcium was applied at a concentration of 10.0 mmol/l $CaCl_2$. Sodium content in maize seedlings was not affected in the presence of both cadmium and calcium at a concentration of 0.1 or 1.0 mmol/l, whereas cadmium with 10.0 mmol/l $CaCl_2$ decreased sodium accumulation in the roots. Thus, we conclude that the positive effect of highest concentration of calcium on the growth in the presence of Cd relay on the maintenance of high concentration of potassium in the roots.

Keywords: concentration of metals (Ca, Cd, K, Na), *Zea mays* L., growth, roots, mesocotyls, coleoptiles

Introduction

Cadmium is a non-essential and highly toxic heavy metal, whose concentration in air, soil and waters is progressively increasing due to human activities. The most common symptom of Cd phytotoxicity is growth reduction, in part due to interference of Cd with mineral nutrition by hampering the uptake and translocation of essential elements, such as Ca and K [1, 2]. Since Ca and K are two plant nutrients that have a direct role in cell growth regulation [3, 4] the elucidation of Cd effect on uptake, accumulation and distribution of both elements in plants seems indispensable.

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It has been proposed that Cd is taken up by plants using the path of Ca transport [5], thus the presence of Cd in a medium may change the concentration of Ca in the plants [6]. On the other hand Cd content in plant tissue depends on the Ca level in the culture medium [7]. Cd also affects K concentration in plants [8, 9].

It was found that maize is the natrophobic plant species in which Na can disturb the growth processes. In the natrophobic plants an effective exclusion mechanism exists for restricting Na transport to the shoots [10]. It was found that in maize plants this Na exclusion mechanism is located in the mesocotyl [11]. However, there is a lack of data in literature concerning the effect of Cd on Na exclusion mechanism and Na accumulation in maize shoots.

Until now most experiments on the uptake of Cd and other ions were performed with maize plants older than 10 days [12-15], whereas our knowledge concerning the uptake of Cd and its effect on mineral nutrition at early stages of maize seedling development is still limited. On the other hand, 4-d-old maize seedlings were used by Małkowski et al. [16] in experiments on the effect of Pb on growth and mineral nutrition. Furthermore, organs excised from 4-d-old maize seedlings are the model system for studying cell expansion [17-19].

In the present study the effect of Cd at the concentration of 0.1 mmol/l and Ca at various concentrations (0.1, 1.0 and 10.0 mmol/l) on the growth and distribution of K, Ca and Cd in organs of 4-d-old maize seedlings were investigated. Since maize is a natrophobic plant species and Na was present in the medium, the concentration and distribution of Na in the seedlings were also examined.

Materials and Methods

Plant Material and Growth Conditions

Experiments were carried out with etiolated 96-h-old *Zea mays* L. (cv. K33xF2) seedlings. Seeds were soaked in distilled water for 2 h, spread on a paper towel that was also watered with distilled water, and placed for three days (72 h) in the incubator at 27°C in darkness. After 72 h 120 seedlings were transferred to containers filled with a hydroponic solution at the ratio of 1 seedling per 160 ml and incubated for the next 24 h. This solution was composed of: 1 mmol/l KCl and 0.1 mmol/l NaCl. Different calcium concentrations (0.1, 1.0, or 10.0 mmol/l $CaCl_2$) both without cadmium and with cadmium at the final concentration 0.1 mmol/l ($CdCl_2 \cdot 2.5H_2O$) were added to this solution. The initial pH of the solutions was adjusted to 5.8-6.0, with 0.1 N NaOH or 0.1 N HCl. It was found that pH of the solutions was not changed by seedlings after 24 h of incubation.

Measurement of Growth and Metal Concentrations

After 24 h of incubation in the hydroponic containers the lengths of roots, mesocotyls, and coleoptiles of 120 seedlings were measured with a ruler to the nearest 1 mm. Roots were divided into segments 0-15 mm (apical root segments) and 15-30 mm, and shoots into mesocotyl and coleoptile (with the first leaf removed). The plant tissue was then dried at 80°C for 3 days to determine its dry weight.

For metal analyses, dry plant tissue was digested with ultra-pure concentrated nitric acid (Merck). The concentrations of calcium, cadmium, potassium and sodium were measured by emission spectrometry with excitation by an argon inductively coupled plasma technique (ICP-AES). Standard solutions of metals (Merck) were used as reference. All chemicals were of analytical grade. All experiments were replicated at least four times, with 120 seedlings in each replicate. The results are the mean values ± SE.

Statistical Analysis

Data were analyzed by using the computer software Statistical for Windows, version 5.1 ˙97, StatSoft Inc., Tulsa, Oklahoma, USA (Licence SP711660411-NET5). Differences between individual treatments and control were analyzed using one-way ANOVA and LSD test. Statistical significance was defined at $p<0.05$.

Results

The growth response of maize seedling organs to different Ca and Cd levels in the hydroponic solution is shown in Fig. 1. As indicate in Fig. 1, the growth of maize seedling organs depended on the Ca concentration and was highest in the presence of 1.0 mmol/l $CaCl_2$. When

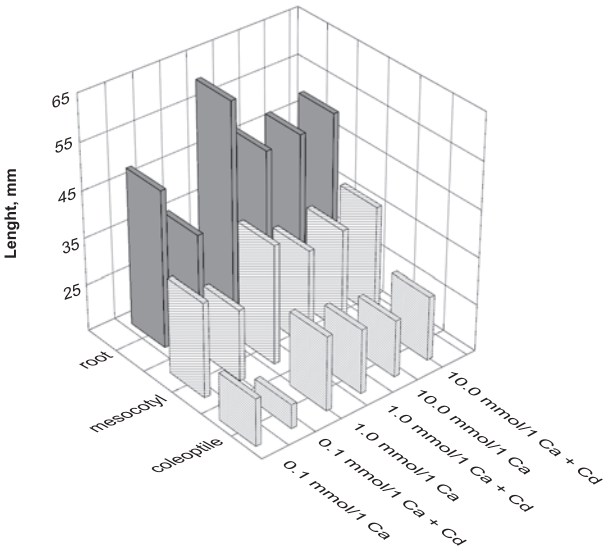

Fig. 1. The growth response of maize seedling organs to different Ca (0.1, 1.0 and 10.0 mmol/l) and Cd (0.0 and 0.1 mmol/l) levels in the hydroponic solution. S.E. did not exceed 10% (n=5).

0.1 mmol/l $CdCl_2$ was present with 0.1 mmol/l $CaCl_2$ in the medium the growth of roots, mesocotyls and coleoptiles was inhibited by 25%, 10% and 20%, respectively, as compared to the solution without Cd. In the presence of 1.0 mmol/l $CaCl_2$ the toxic effect of cadmium on the growth of roots and mesocotyls was the same as with 0.1 mmol/l $CaCl_2$ and only the coleoptile growth was less affected (inhibition by 7%). Cadmium did not affect the growth of maize seedling organs in the presence of 10.0 mmol/l $CaCl_2$ in the hydroponic solution (Fig. 1).

Calcium taken up from the growth medium was distributed over the plant organs. The increase of calcium concentration in the medium from 0.1 mmol/l to 10.0 mmol/l caused a significant increase of its accumulation in the roots and the mesocotyls, but this dependence was not observed in the coleoptile (Table 1). In plants treated with 0.1 mmol/l Cd together with 0.1 mmol/l $CaCl_2$ calcium concentration significantly increased in the roots and the coleoptiles. Cadmium administration together with 10.0 mmol/l $CaCl_2$ led to an increase in calcium concentrations in the roots and coleoptiles, although this increase for root segments excised between 0-15 mm was statistically significant at $p \leq 0.063$.

The addition of cadmium to the hydroponic medium led to an increase in Cd content of all maize organs (Table 2). Most of the Cd taken up by maize was accumulated in the roots. Cadmium contents in the mesocotyls were relatively high compared to the coleoptiles (Table 2). The highest calcium concentration (10.0 mmol/l) caused a decrease in the Cd concentration of the roots. By contrast to roots, there was no significant difference in cadmium content in the mesocotyls and the coleoptiles induced by different concentration of $CaCl_2$ in the hydroponic solutions (Table 2).

Table 1. Effect of different Ca and Cd concentrations in the solution on Ca concentration (mg/kg d.w.) in the organs of maize seedlings.

Concentration of $CaCl_2$ in the hydroponic medium	Plant organ			
	Root (0-15 mm)	Root (15-30 mm)	Mesocotyl	Coleoptile
0.0 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	961.6 ± 73.11 ef	843.4 ± 82.29 de	626.1 ± 46.26 bcd	336.6 ± 24.83 a
1.0 mmol/l $CaCl_2$	1919.4 ± 44.38 hi	2261.5 ± 92.67 ij	1505.9 ± 127.39 gh	606.2 ± 18.86 bc
10.0 mmol/l $CaCl_2$	3387.5 ± 197.97 kl	4312.7 ± 220.53 l	2275.2 ± 371.65 ij	504.6 ± 80.44 b
0.1 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	2757.1 ± 338.63 jk	2501.2 ± 316.9 ij	689.9 ± 10.83 cd	1185.4 ± 55.16 fg
1.0 mmol/l $CaCl_2$	2499.9 ± 404.00 ij	2332.9 ± 253.95 ij	1173.4 ± 122.05 fg	771.9 ± 63.39 cde
10.0 mmol/l $CaCl_2$	4555.6 ± 299.62 lm	6067.2 ± 780.71 m	2663.6 ± 393.72 ijk	2506.2 ± 667.28 ij

Values are the means ± SE (n=4). Means followed by the same letter are not significantly different from each other using the LSD test ($p \leq 0.05$).

Table 2. Effect of different Ca and Cd concentrations in the solution on Cd concentration (mg/kg d.w.) in the organs of maize seedlings.

Concentration of $CaCl_2$ in the hydroponic medium	Plant organ			
	Root (0-15 mm)	Root (15-30 mm)	Mesocotyl	Coleoptile
0.0 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	5.0 ± 3.85 b	16.2 ± 1.54 cde	0.0 ± 0.00 a	0.0 ± 0.00 a
1.0 mmol/l $CaCl_2$	2.9 ± 2.89 ab	3.9 ± 3.89 ab	0.0 ± 0.00 a	0.0 ± 0.00 a
10.0 mmol/l $CaCl_2$	14.9 ± 4.47 cd	24.8 ± 7.09 cde	0.1 ± 0.06 a	0.4 ± 0.42 a
0.1 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	3646.7 ± 80.23 j	1926.0 ± 78.27 ij	81.1 ± 9.15 ef	14.8 ± 0.57 cd
1.0 mmol/l $CaCl_2$	714.7 ± 53.00 hij	502.3 ± 20.04 ghi	168.8 ± 9.54 fgh	25.8 ± 4.95 cde
10.0 mmol/l $CaCl_2$	255.5 ± 55.54 fgh	302.1 ± 43.4 fgh	66.1 ± 11.57 def	9.3 ± 0.72 c

Values are the means ± SE (n=4). Means followed by the same letter are not significantly different from each other using the LSD test ($p \leq 0.05$).

Potassium concentration in maize seedling organs is much higher than other elements investigated in the present work (Table 3). The data presented in Table 3 show that potassium accumulation in the apical root segments was significantly higher in the presence of 0.1 and 10.0 mmol/l $CaCl_2$ compared to 1.0 mmol/l $CaCl_2$ and other treatments. The apical root segments accumulated significantly lower amounts of K^+ when both $CaCl_2$ (0.1 mmol/l) and $CdCl_2$ (0.1 mmol/l) was added. Potassium accumulation by the segments excised from roots between 15-30 mm was decreased when $CdCl_2$ was added together with 0.1 or 1.0 mmol/l $CaCl_2$. By contrast, K accumulation by the roots was not decreased by $CdCl_2$ when Ca was added at the concentration of 10.0 mmol/l both in the apical root segments and in the segments excised from roots between 15-30 mm (Table 3). Potassium concentration in the aboveground parts of maize was unaffected by the cadmium.

The data in Table 4 indicated that roots were particularly efficient in accumulating Na compared to shoots. Calcium did not affect the contents of Na in the apical root segments (0-15 mm). Increasing the Ca concentration from 0.1 mmol/l to 1.0 mmol/l or 10.0 mmol/l led to a decrease in Na content of the second root zone (15-30 mm) and mesocotyls. Coleoptiles from the 10 mmol/l Ca treatment had a significantly lower concentration of Na than coleoptiles from 0.1 or 1.0 mmol/l treatments. The presence of both Cd (0.1 mmol/l) and Ca at the concentration of 0.1 or 1.0 mmol/l did not affect the Na content in maize seedling organs, whereas Cd (0.1 mmol/l) and Ca (10.0 mmol/l) decreased Na concentration in the roots.

Discussion

Many experiments proved the necessity of Ca ions in auxin-induced plant cell elongation [20], but on the other hand the inhibition of auxin-induced growth caused by

Table 3. Effect of different Ca and Cd concentrations in the solution on K concentration (g/kg d.w.) in the organs of maize seedlings.

Concentration of $CaCl_2$ in the hydroponic medium	Plant organ			
	Root (0-15 mm)	Root (15-30 mm)	Mesocotyl	Coleoptile
0.0 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	41.7 ± 1.47 g	29.4 ± 0.27 cde	28.4 ± 1.16 bcd	29.1 ± 0.62 cde
1.0 mmol/l $CaCl_2$	34.4 ± 1.19 f	31.8 ± 1.05 ef	28.1 ± 0.67 bcd	24.4 ± 0.26 a
10.0 mmol/l $CaCl_2$	41.4 ± 2.59 g	33.1 ± 2.39 f	27.8 ± 0.87 bc	25.6 ± 0.40 ab
0.1 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	27.9 ± 0.12 bc	23.8 ± 0.31 a	27.5 ± 0.20 bc	29.4 ± 1.13 cde
1.0 mmol/l $CaCl_2$	31.4 ± 0.88 def	27.4 ± 0.75 bc	26.3 ± 0.48 abc	27.4 ± 0.08 bc
10.0 mmol/l $CaCl_2$	44.6 ± 2.78 g	34.5 ± 2.24 f	27.5 ± 1.58 bc	26.4 ± 1.72 abc

Values are the means ± SE (n=4). Means followed by the same letter are not significantly different from each other using the LSD test ($p \leq 0.05$).

Table 4. Effect of different Ca and Cd concentrations in the solution on Na concentration (mg/kg d.w.) in the organs of maize seedlings.

Concentration of $CaCl_2$ in the hydroponic medium	Plant organ			
	Root (0-15 mm)	Root (15-30 mm)	Mesocotyl	Coleoptile
0.0 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	1063.6 ± 157.68 ijkl	2499.7 ± 546.77 m	160.7 ± 26.96 cd	375.8 ± 41.26 fg
1.0 mmol/l $CaCl_2$	821.7 ± 132.96 ijk	1322.4 ± 307.39 jkl	88.4 ± 8.31 ab	643.0 ± 18.95 ghi
10.0 mmol/l $CaCl_2$	725.4 ± 50.9 hij	739.8 ± 98.64 hij	68.0 ± 12.62 a	162.6 ± 20.63 cd
0.1 mmol/l $CdCl_2$				
0.1 mmol/l $CaCl_2$	1748.2 ± 310.2 lm	2557.1 ± 869.18 m	205.9 ± 41.82 cde	350.8 ± 57.95 ef
1.0 mmol/l $CaCl_2$	991.1 ± 119.08 ijkl	1427.3 ± 372.53 klm	137.5 ± 33.06 bc	466.8 ± 92.57 fgh
10.0 mmol/l $CaCl_2$	259.1 ± 3.74 def	392.1 ± 78.60 fg	69.9 ± 15.18 a	84.5 ± 35.81 a

Values are the means ± SE (n=4). Means followed by the same letter are not significantly different from each other using the LSD test ($p \leq 0.05$).

calcium concentrations from 1 to 20 mmol/l has long been recognized [20-22]. The results presented in this paper (Fig. 1) showed that calcium in the hydroponic medium did not inhibit growth response of maize seedling organs. The 10.0 mmol/l $CaCl_2$ did not significantly affect the growth of maize seedling organs as compared to 0.1 mmol/l $CaCl_2$, whereas the growth of maize organs was even increased ca. 20% in the presence of 1.0 mmol/l $CaCl_2$ in the medium (Fig. 1). Similar results were recorded by Greger and Bertell [6], who observed that fresh weight of *Beta vulgaris* seedlings was higher in plants exposed to 0.72 mmol/l than in plants exposed to 0.18 mmol/l $CaCl_2$ in the culture medium.

Our data concerning the effect of cadmium on the growth of maize seedlings organs showed that root growth was more inhibited by cadmium in the presence of 0.1–1.0 mmol/l $CaCl_2$ than shoot growth. In the presence of 10.0 mmol/l $CaCl_2$ cadmium did not affect the growth of maize seedling organs, which suggests that calcium at this concentration counteracted the toxic effect of cadmium on the growth of maize organs (Fig. 1). It had already been shown that higher calcium concentrations decrease the toxic effect of cadmium on different physiological processes [7, 22, 23]. Greger and Bertell [6] suggested that the degree of inhibition of plant growth caused by Cd depends on the Ca level in the culture medium and plant tissue.

The data presented in this paper (Table 1) show that calcium concentration in the roots and the mesocotyls, but not in the coleoptiles, was significantly increased with the increase of calcium concentration in the medium. In plants treated with 0.1 mmol/l Cd together with 0.1 mmol/l $CaCl_2$ calcium concentration significantly increased in the roots and the coleoptiles. Similar results have recently been demonstrated by Małkowski et al. [24], who show that in maize seedlings exposed to Cd (at 0.1 mmol/l) the accumulation of Ca in root segments was enhanced 3-fold as compared to the control solution (without Cd), whereas the increase of Ca accumulation in the coleoptiles was not statistically significant. Compared to the seedlings treated with 10.0 mmol/l $CaCl_2$ without cadmium administration of cadmium together with 10.0 mmol/l $CaCl_2$ led to statistically significant increase in the calcium concentrations in the segments excised from roots between 15-30 mm and the coleoptiles (Table1).

According to literature data, effects of Ca on uptake and distribution of Cd were ambiguous. On the one hand, Ca considerably inhibited Cd uptake by roots of diverse plant species [25, 26], but on the other hand, a higher Ca concentration in the medium promoted Cd uptake by *Phaseolus coccineus* roots [7]. The data on accumulation of Cd have clearly shown the higher accumulation of this metal in roots compared to shoots (Table 2). These results are in agreement with the data obtained by other authors for different plant species [9, 24]. It had already been shown that cadmium accumulation in seedlings was affected by several nutrients [8, 26]. In our experiment with maize roots, 10.0 mmol/l $CaCl_2$ blocked both Cd^{2+} accumulation in the roots and Cd toxicity effects on their growth (Table 2). Results presented by Kim et al. [26] documented that Ca^{2+} blocked both Cd^{2+} transport into rice roots and Cd^{2+} toxicity on root growth [26] by competition of Cd^{2+} with Ca^{2+} for transport into root cells via transporters that pass Ca^{2+} [5].

Cadmium-induced K content decrease in the root was observed when both $CaCl_2$ and $CdCl_2$ were added at the concentration of 0.1 mmol/l (Table 3). By contrast, K accumulation by the roots was not decreased by $CdCl_2$ when Ca was added at the concentration of 10.0 mmol/l (Table 3). It was proposed by Drazic and Mihailovic [9] that decrease of K content in roots may be due to decreased K uptake, K-leakage into a nutrient medium or increased K transport into shoots induced by Cd. Results presented here (Table 3) showed that the concentration of K in maize shoots (mesocotyl + coleoptile) was not increased by Cd, suggesting that a lower concentration of K in the maize roots cannot be related to higher K transport to shoots induced by Cd, as it was proposed by Drazic and Mihailovic [9].

Sodium was strongly accumulated in the maize roots as compared to shoots (Table 4). In the second root zone (15-30 mm) the concentration of Na was several times higher compared to the concentration in the mesocotyl and coleoptile in all treatments. It is in agreement with results obtained by different authors, who demonstrated that the addition of extracellular Ca^{2+} reduced Na^+ uptake by plant cells [27, 28]. Results presented in the current study showed that in the treatments with cadmium the accumulation of Na in roots decreased with increasing Ca concentration in the medium, although statistically significant lower concentration of Na was found only with 10.0 mmol/l $CaCl_2$ (Table 4).

Maize is the natrophobic plant species in which function an effective exclusion mechanism of Na transport to the shoots [10]. It was found that this Na exclusion mechanism is located in the mesocotyl of maize plants [11]. Our data showed that cadmium did not break down the sodium exclusion mechanism, because sodium concentration in maize shoots was not enhanced by cadmium treatment (Table 4).

The amelioration of toxic effect of Cd on the growth of maize seedlings by 10.0 mmol/l $CaCl_2$ was connected with the enhancement of K concentration and with a decrease of Cd concentration in the roots. Thus, we conclude that positive effect of the high concentration of Ca on the growth relay on the maintenance of high concentration of K in the roots irrespective of presence of Cd in the medium.

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