The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant

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The influence of succinate treatment on Zn toxicity was investigated using plant growth and mineral uptake as stress indicators. Pea plants (*Pisum sativum* L., cv. Citrine) were treated with various Zn concentrations (0.67 to 700 μM Zn) in the presence and absence of 0.2 mM Na-succinate. Plants pre-treated with succinate and then exposed to Zn exhibited higher dry root, stem and leaf weight than the plants treated with Zn alone. An increase in Zn supply resulted in a decrease in the concentrations of Ca, Mg, P in the roots and an increase of Ca and N levels in the stems and leaves. The amount of Zn in the roots, stems and leaves increased with greater Zn rates. The succinate treatment increased P in the roots but did not affect the Ca, N and Mg contents in Zn-treated plants. Most of the Zn taken up was retained in the roots after succinate treatment. The ameliorative effect of succinate on plant growth could be due to a lower Zn translocation in the leaves and stems and increased Zn accumulation in the roots. Lower Zn translocation in aboveground parts seemed to result from Zn complexing by organic anion in the roots. This probably caused less Zn transport to the stems and leaves and suggested that succinate has potential for complexing with Zn and may play a role in tolerance to high Zn levels.
Key words: Succinate, zinc toxicity amelioration.

Efeito do fornecimento de zinco e tratamento com succinato no crescimento e absorção de minerais em plantas de ervilha: A influência do ácido succínico na toxicidade de Zn foi estudada, tendo-se o crescimento das plantas e a absorção mineral como parâmetros indicadores de estresse. Plantas de ervilha (*Pisum sativum* L., cv. Citrine) foram tratadas com várias concentrações de Zn (0,67 a 700 μM) na presença ou ausência de 0,2 mM de succinato de sódio. Plantas pré tratadas com succinato e então expostas ao Zn apresentaram maior massa seca de raízes, caules e folhas que plantas apenas tratadas com Zn. Aumento no fornecimento de Zn resultou em decréscimo nas concentrações de Ca, Mg, P nas raízes, mas aumento de Ca e N levels nos caules e folhas. A quantidade de Zn nas raízes, caules e folhas aumentou com o aumento do suprimento de Zn. O tratamento com succinato levou ao aumento P nas raízes e não afetou os conteúdos de Ca, N, Mg nas plantas tratadas com Zn. A maior parte do Zn absorvido ficou retido nas raízes depois do tratamento com o succinato. A melhora causada pelo succinato no crescimento das plantas talvez tenha sido devido ao acúmulo de Zn nas raízes, em função da maior complexação pelo íon orgânico, havendo menor translocação para as folhas e caules.
Palavras-chave: Melhora da toxicidade de zinco, succinato.

INTRODUCTION

Zn is an essential element for both plants and animals. It plays an important role in several plant metabolic processes; it activates enzymes and is involved in protein synthesis and carbohydrate, nucleic acid and lipid metabolism (Marshner, 1986; Pahlsson, 1989). However, like other heavy metals (Doncheva et al., 1996; Doncheva, 1997, 1998) when Zn is accumulated in excess in plant tissues, it causes alterations in vital growth processes such as photosynthesis and chlorophyll biosynthesis (Doncheva et al., 2001) and membrane integrity (De Vos et al., 1991). An excess of Zn has been reported to have a negative effect on mineral nutrition (Chaoui et al., 1997). Toxic levels of Zn for different varieties of crops have very wide limits - from 64 $\mu g.L^{-1}$ Zn for sorghum to 2000 $\mu g.L^{-1}$ Zn for cotton (Ohki, 1984).

To counteract this metabolic dysfunction caused by Zn toxicity stress, higher plants employ defense strategies. To protect themselves from heavy metal, plant cells must develop a mechanism by which the metal ion, entering the cytosol of the cell, is immediately complexed and inactivated. This protection process is mediated by phytochelatins (Grill et al., 1985; Neuman et al., 1994) and organic acids (Brokes, 1981; Thurman and Rankin, 1982).

Plant cells also develop a variety of biochemical reactions which leads to alteration in plasma membrane structures to prevent heavy metal effects on the membrane permeability and potassium, phosphate or organic compound leakage from plant cells (Nishizono et al., 1987; De Vos et al., 1989, 1991; Stranges and Macnair, 1991) to induction of the synthesis of heat shock proteins (Neuman et al., 1994).

The restricted Zn uptake in plants plays a key role in protection against the damaging effects of excess Zn. This protection may result from Zn binding to the wall of root cells or due to an intracellular tolerance mechanism. Mathys (1977) explained Zn tolerance as resulting from an increased ability to transport Zn into the vacuole. It has been proposed that Zn could be more efficiently transported by Zn-malate shuttle to the vacuole. Godbold et al. (1984), Wang et al. (1992) and Harmens et al. (1993, 1994) concluded that citrate has a high potential for complexing Zn and that cytosolic citrate is more likely to shuttle Zn into the vacuole than malate. However, it is not known whether succinate plays a role in Zn tolerance.

The aim of the present study was to examine the effect of increased Zn concentrations in the nutrient medium on plant growth, Zn, N, P, K, Ca and Mg uptake and their distribution in pea plants and whether the exogenous added succinate could reduce the toxicity effect of Zn.

MATERIAL AND METHODS

Plant material: Five-day-old pea seedlings (*Pisum sativum* L., cv. Citrine) were grown in a greenhouse under natural light, in aerated nutrient solution (Hellriegels, 1898) containing 1.0 mM KH_2PO_4, 0.5 mM $MgSO_4.7H_2O$, 3 mM $Ca(NO_3)_2.4H_2O$, 0.017 mM $FeSO_4.7H_2O$ and 0.14 mM EDTA, with the addition of microelements according to Hoagland and Arnon (1950). Zn treatment was performed by supplementing greater concentrations of Zn ions in the form of $ZnSO_4$; control - 0.7 (after Hoagland and Arnon, 1950), 35, 70, 350 and 700 µM. However, the plants were exposed or not to succinate before or after Zn treatment, defining three experimental groups:

a) Part of the plants were grown in nutrient solution but with different Zn concentrations: 0.7 (control), 35, 70, 350 and 700 µM Zn.

b) Plants were cultured for 24 h in control nutrient solution containing 0.2 mM Na-succinate. After 24h incubation, the succinate-treated plants were transferred to fresh nutrient solutions with different Zn concentrations: 35, 70, 350 and 700 µM

c) Plants was transferred to nutrient solutions supplemented with Zn concentrations 35, 70, 350, 700 µM and after Zn toxicity symptoms appeared (7days) they were sprayed with 1 mM Na-succinate.

After succinate treatment the plants were grown in nutrient solutions that were continuously aerated and replaced twice weekly so that the Zn concentrations of the nutrient solutions remained relatively constant during the entire period. Sixteen plants were used for each treatment and there were three independent experiments. Plants were harvested 23 d after treatment.

Plant growth parameters: Plants were separated into roots, shoots and leaves to obtain the fresh and dry weights.

Determination of elements: Plants were separated into roots, shoots and leaves and dried at 60 ^oC. Dry plant material (0.1 g) was separately ashed at 550 ^oC and the residue was brought to standard volume with 20 % HCl. The Zn, Mg and Ca contents were determined directly by atomic absorption spectrometry (Karl Zeiss, Jena, Germany) using air-acetylene flame. The total N content was measured according to Kjeldahl. P was determined colorimetricaly and K was determined on a flame photometer.

RESULTS AND DISCUSSION

Zn toxicity symptoms were observed seven days after the treatment began. Excess Zn caused leaf chlorosis and root browning. Greater Zn concentration in the nutrient solution along with the appearance of toxicity symptoms

significantly depressed the fresh mass of leaves, stems and roots compared to the control plants (figure 1). Reduction in the fresh weight due to high Zn supply was accompanied by desiccation of the aboveground plant parts. The accumulated dry mass was dependent on the Zn concentration in the nutrient solution (figure 1).

When the plants were incubated in 0.2 mM Na-succinate for 24 h and then treated with increased Zn concentrations they accumulated greater dry mass than the plants treated with Zn alone. Plants exposed to 0.2 mM Na-succinate and then to 35 µM and 70 µM Zn increased the root, stem and leaf dry mass by 7, 94, and 29 % respectively, compared to 35 µM Zn-treated plants and by 45, 18, and 18 % respectively, compared to 70 µM Zn-treated plants. The same trend, although expressed to a lesser extent, could be detected in plants treated with higher Zn concentrations (figure 1).

The uptake of Zn in pea plants was dependent on the Zn concentration in the nutrient solution (table 1). Zn accumulation in the roots increased with higher Zn concentrations. The highest Zn levels were reached in the plants exposed to the highest Zn concentrations. The addition of succinate to the nutrient solution increased Zn accumulation in the roots compared with Zn treatment alone. At equal exogenous Zn concentration, the roots of succinate-Zn treated plants accumulated more Zn than the Zn-treated plants.

Similarly to the roots, the Zn concentrations measured in the leaves and stems treated with Zn alone increased with higher Zn supply (table 1). In contrast, the succinate-Zn treated plants accumulated significantly less Zn in their shoots. At equal levels of Zn supply, the Zn concentration in the leaves and stems depended on the type of succinate treatment. The Zn concentration in the stems and leaves of plants treated with succinate before Zn supply was lower compared to the plants treated with succinate in the presence of Zn. The higher root Zn uptake and decreased Zn translocation from roots to shoots by succinate treatment suggest that succinate facilitates the formation of metal-succinate complexes in the roots and may play a role in Zn accumulation. The formation of succinate complexes could provide an effective barrier to the movement of Zn, causing a decrease in its content in the aboveground parts.

The effects of Zn on the uptake and distribution of N, P, K, and Mg in the roots, stems and leaves are illustrated in figure 2. The N content increased in the leaves, stems and roots of the pea plants up to 70 µM Zn treatment but remained steady or decreased in root and leaf tissues from the 70 µM Zn treatment onwards (figure 2A). The N concentration in the stem increased significantly at the last two treatments. The N levels in the plants treated with succinate in the presence and absence of Zn did not change compared to the plants treated with Zn alone.

The uptake and distribution of P in plants was influenced by Zn treatment (figure 2B). It is known that at normal Zn concentrations in the nutrient medium the content of inorganic P decreases, whereas the organic (acid-soluble) form increases (Menser, 1985). The roots and leaves showed decreasing P contents up to 70 µM Zn and increasing P content from 70 µM Zn onwards. The accumulation of P was greatest in the stems of Zn-treated plants. The addition of succinate to the nutrient solution increased P accumulation by roots in plants treated with higher Zn concentrations compared with Zn treatment alone.

The K content of the stems and leaves (figure 2C) was not significantly affected by higher concentrations of Zn. A continuous increase in K accumulation was detected in the roots from the 350 µM Zn treatment onwards. The addition of succinate to the nutrient solution did not affect the K content of Zn-treated plants.

Table 1. Effect of Zn supply and succinate treatment on pea plant leaf, stem and root Zn contents during 23 days´ cultivation.

Treatments	Leaves[a]	Stems ($mg.kg^{-1}$ dry weight)	Roots
Control (0.7 µM Zn)	89.9 ± 0.4	190.1 ± 1.1	400.3 ± 2.1
35 µM Zn	149.2 ± 0.7	439.4 ± 2.2	825.2 ± 4.6
Suc. + 35 µM Zn	137.1 ± 0.7***	310.0 ± 1.6***	932.5 ± 4.7***
35 µM Zn + Suc.	126.9 ± 0.7***	303.4 ± 1.5***	835.0 ± 4.2***
70 µM Zn	293.6 ± 1.5	931.9 ± 4.7	3201.9 ± 16.3
Suc. + 70 µM Zn	294.7 ± 1.5*	419.4 ± 2.2***	3712.2 ± 18.3***
70 µM Zn + Suc.	269.7 ± 1.4***	512.3 ± 2.8***	2885.4 ± 14.6***
350 µM Zn	1698.2 ± 8.7	1494.0 ± 7.4	8896.4 ± 95.9
Suc. + 350 µM Zn	1675.6 ± 8.8*	1239.4 ± 6.4***	25104.8 ± 127.4***
350 µM Zn + Suc.	1543.4 ± 7.8**	1288.2 ± 6.3***	21312.1 ± 109.2***
700 µM Zn	2297.5 ± 11.9	3007.2 ± 13.3	24768.3 ± 125.6
Suc. + 700 µM Zn	2238.3 ± 11.4***	2975.8 ± 15.1***	24816.0 ± 126.0*
700 µM Zn + Suc.	2165.7 ± 11.1***	3311.4 ± 16.8***	26408.7 ± 70.4***

[a] Mean values ± s.e. (n = 5). Significant effects of Zn concentrations and treatments with succinate at the 0.05, 0.1 and 1 level respectively according to ANOVA are denoted as *** , ** and * .

The increase in Zn concentration above 70 µM led to an enhanced quantity of Mg in both the leaves and stems, accompanied by a drastic decrease in the Mg concentration in the roots (figure 2D). A change could not be detected for Mg concentration in Zn-treated plants after treatment with Na-succinate.

A different pattern for Ca accumulation was observed in Zn-treated plants in the aboveground parts and the roots (table 2). The Ca concentration increased in the leaves and stems (1.27 and 1.14 fold, respectively) whereas the Ca concentration in the roots decreased to 42 % by the highest Zn treatment. Zn:Ca interactions have been shown for bread and durum wheat cultivars (Hart et al., 1998). Zn uptake in these plants was inhibited dramatically by Ca, which suggested that Zn and Ca do not share a common transport mechanism. On the other hand, the decrease in the concentrations of Ca and Mg in the roots in response to higher Zn concentration was probably a result of osmotic adjustment.

The decrease in the Ca:Zn concentration ratio below 35 has been accepted as a quantitative criterion for Zn toxicity (Davis and Parker, 1993; Davis et al., 1995). The values for this ratio calculated for the leaves, stems and roots of the treated pea plants are presented in table 3. The ratio was found to decrease with increasing Zn concentration in the nutrient solution. Ca:Zn concentration ratios could not be improved by succinate treatment.

Table 2. Effect of Zn supply and succinate treatment on pea plant leaf, stem and root Ca contents during 23 days´ cultivation.

Treatments	Leaves[a]	Stems (Ca g.100g^{-1} dry weight)	Roots
Control (0.7 µM Zn)	1.52 ± 0.04	1.31 ± 0.03	1.82 ± 0.05
35 µM Zn	1.59 ± 0.03	1.50 ± 0.04	0.92 ± 0.03
Suc. + 35 µM Zn	1.77 ± 0.04***	1.35 ± 0.04***	1.01 ± 0.03***
35 µM Zn + Suc.	1.66 ± 0.04***	1.32 ± 0.03***	1.17 ± 0.03***
70 µM Zn	1.69 ± 0.04	1.40 ± 0.02	1.16 ± 0.03
Suc. + 70 µM Zn	1.86 ± 0.05***	1.51 ± 0.03**	1.08 ± 0.03*
70 µM Zn + Suc.	1.69 ± 0.04	1.40 ± 0.03	1.16 ± 0.03
350 µM Zn	2.07 ± 0.05	1.52 ± 0.04	1.24 ± 0.03
Suc. + 350 µM Zn	2.08 ± 0.05*	1.48 ± 0.04**	1.19 ± 0.03*
350 µM Zn + Suc.	2.02 ± 0.05*	1.38 ± 0.04**	1.30 ± 0.03*
700 µM Zn	1.94 ± 0.05	1.50 ± 0.04	0.79 ± 0.02
Suc. + 700 µM Zn	1.93 ± 0.05*	1.36 ± 0.04**	0.78 ± 0.02
700 µM Zn + Suc.	1.96 ± 0.05*	1.44 ± 0.04**	0.78 ± 0.02

[a] Mean values ± s.e. (n = 4). Significant effects of Zn concentrations and treatments with succinate at the 0.05, 0.1 and 1 levels respectively according to ANOVA are denoted as *** , ** and * .

Table 3. The Ca/Zn concentration ratios in pea plant leaves, stems and roots during 23 days´ cultivation.

Treatments	Leaves	Stems	Roots
Control (0.7 µM Zn)	172.00	69.82	45.17
35 µM Zn	115.04	34.70	11.17
Suc. + 35 µM Zn	118.50	43.32	10.81
35 µM Zn + Suc.	130.61	43.55	13.96
70 µM Zn	57.59	14.94	5.65
Suc. + 70 µM Zn	62.91	35.80	2.92
70 µM Zn + Suc.	62.66	27.20	4.02
350 µM Zn	12.12	10.15	0.65
Suc. + 350 µM Zn	12.32	11.77	0.47
350 µM Zn + Suc.	13.04	10.74	0.60
700 µM Zn	8.45	4.99	0.32
Suc. + 700 µM Zn	8.62	4.21	0.31
700 µM Zn + Suc.	9.03	4.38	0.30

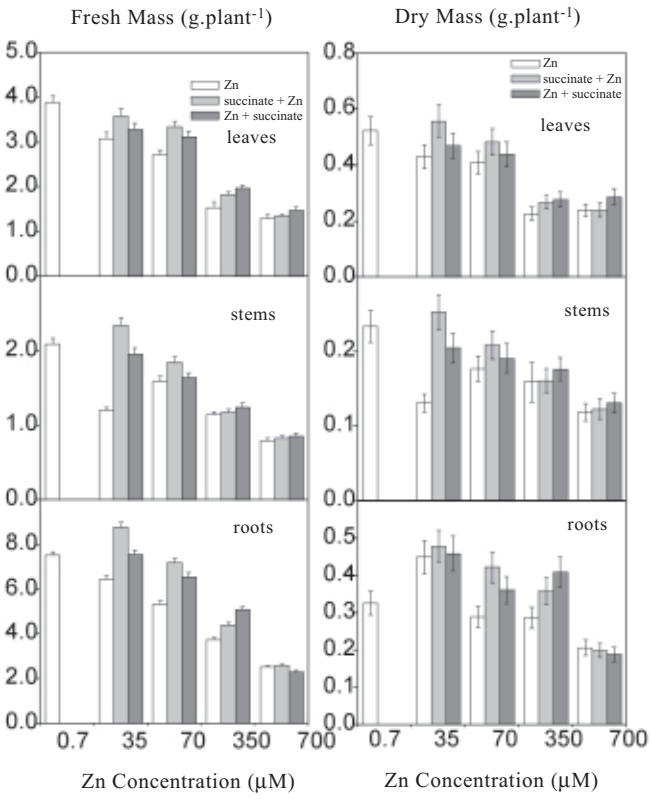

Figure 1. Fresh and dry mass of roots, stems and leaves expressed in gram per plant after 23 days´ treatments. Pea plants were treated with increasing Zn (35, 70, 350, 700 µM) alone; Succinate treated plants + Zn (35, 70, 350, 700 µM); Zn (35, 70, 350, 700 µM) treated plants + succinate.

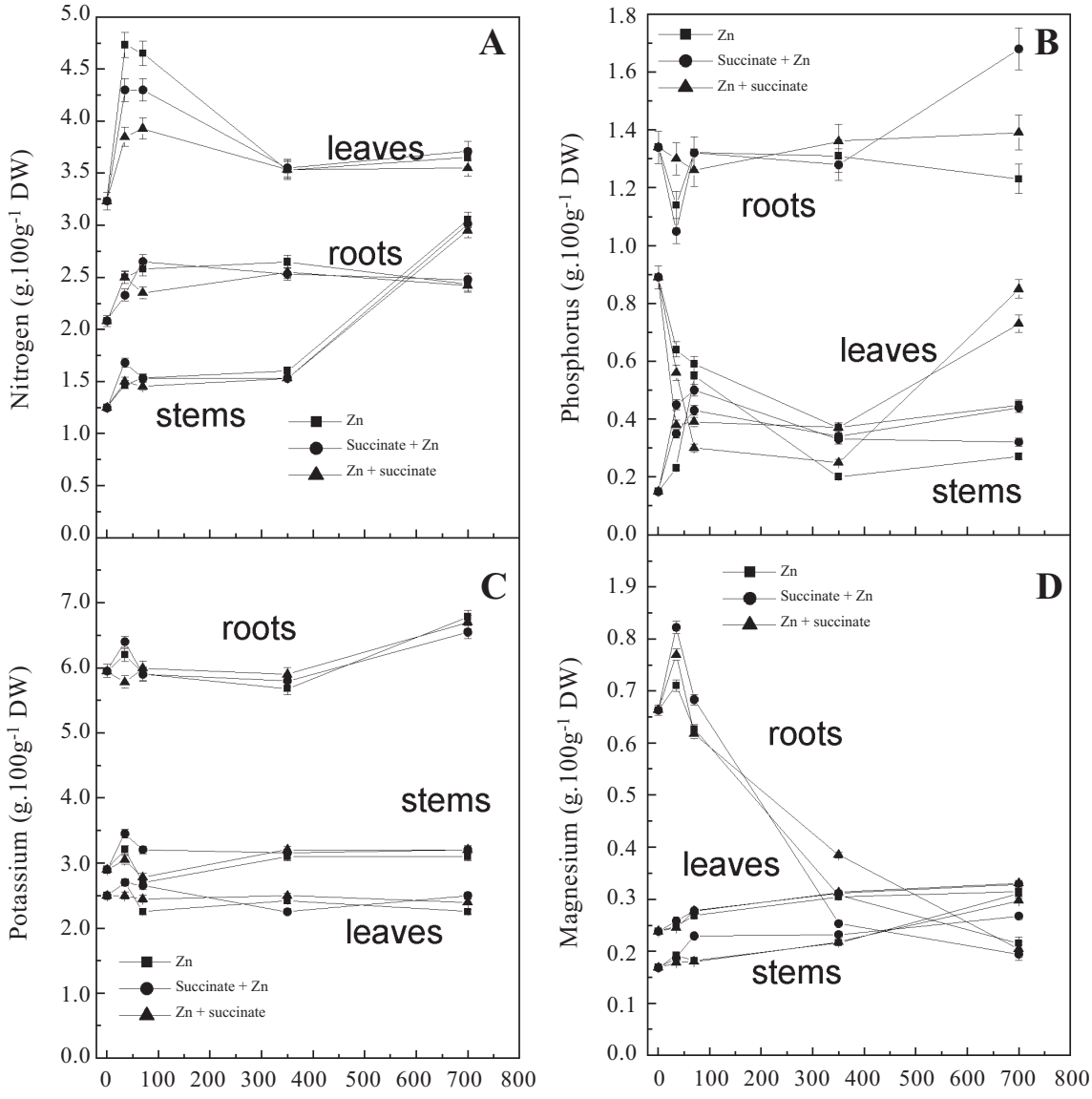

Figure 2. N, P, K and Mg concentrations in the roots, stems and leaves of pea plants after 23 days´ treatment. Pea plants were treated with increasing Zn (35, 70, 350, 700 µM) alone; Succinate treated plants + Zn (35, 70, 350, 700 µM); Zn (35, 70, 350, 700 µM) treated plants + succinate.

The results obtained for the content of the five major elements (N, P, K, Ca and Mg) in pea plants treated with increasing Zn concentrations demonstrated a similarity of the type of curves describing the degree of their accumulation. This indicated a nonspecific effect of Zn on ion absorption and translocation. The addition of Na-succinate was not able to change the cation-anion balance significantly in Zn-treated pea plants.

Our results indicate that Zn supplied at concentrations above 70 µM produced toxic effects typical of metal stress in pea plants. Treatment with Zn led to a reduction in the root, stem and leaf growth. The leaves showed signs of induced chlorosis. The addition of succinate before and after zinc treatment to the plants reduced the Zn toxicity symptoms to some extent. The protective effect of exogenous succinate might be due to the translocation of lower Zn concentrations to aboveground parts. This is indirect evidence supporting the idea that vacuolar accumulation of Zn-organic acid complexes may be a mechanism for Zn tolerance in naturally tolerant ecotypes.

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