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Zinc rhizotoxicity in wheat and radish is alleviated by micromolar levels of magnesium and potassium in solution culture

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Abstract

The effects of excess zinc (Zn) on solution-cultured wheat (*Triticum aestivum* L., cv. Yecora Rojo) and radish (*Raphanus sativus* L., cv. Cherry Belle) were studied, using both short-term root elongation studies and longer term split-root experiments. Alleviation of Zn rhizotoxicity by Mg and K was observed, with especially dramatic alleviation of root stunting by Mg. In the short-term studies using a simple medium (2 mM $CaCl_2$, pH 6.0), Mg concentrations of 1–5 μM were able to significantly alleviate rhizotoxicity caused by Zn concentrations as high as 60 μM. In the split-root studies, 100 μM Mg was sufficient to abolish Zn toxicity in both wheat and radish. Paradoxically, Mg enhanced uptake and translocation of Zn while simultaneously alleviating toxicity in these longer-term experiments. In short-term experiments, additions of K (0 to 200 μM) to the basal medium alleviated Zn rhizotoxicity to a more limited extent. In split-root experiments, however, the absence or presence of K in test solutions did not affect plant growth or Zn uptake. When increased from a physiological minimum (e.g., 200 μM), Ca also alleviates Zn toxicity, but the effect is very modest in comparison to that of Mg. The results are discussed in relation to the use of short-term assays of metal tolerance in simple salt solutions, and in relation to possible roles of Mg in the physiology of Zn toxicity.

Introduction

There are many sources of Zn contamination in soils, often in association with Pb, Cu, and Cd. Smelter and incinerator emissions, dispersal from mine wastes, excessive applications of Zn-containing fertilizers and pesticides, use of Zn-contaminated sewage sludges, manures or industrial wastes as fertilizers, and even release from galvanized surfaces can all significantly increase Zn in soils (Chaney, 1993). Zinc bioavailability in soil solution is increased by low pH, while the presence of organic ligands and hardness cations such as Ca decrease Zn availability (Chaney, 1993).

Zinc toxicity to plants has been studied for many years, although the majority of studies have focused on the comparative resistance of wild ecotypes collected from mine spoils or other heavily polluted sites. There is comparatively little information on the physiology of nontolerant crop plants challenged with higher-than-normal Zn levels, e.g., $>\sim$ 1 μM Zn in soil solution (Welch, 1995). Adequate tissue levels of Zn in plant shoots appear to be around 20–50 mg kg^{-1} DW, while the onset of toxicity for field-grown wheat is seen when shoot tissues reach 500 mg kg^{-1} (Welch, 1995; Reuter and Robinson, 1997). Plants do not seem to restrict translocation of Zn (as they do with Cu and Pb), although Zn will usually accumulate to higher concentrations in roots than in shoots (Longnecker and Robson, 1993).

There is a surprising dichotomy in the reports of plant-growth responses to excessive Zn in solution culture. In *Festuca rubra* seedlings exposed to just

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2 μM Zn in a simple $Ca(NO_3)_2$ solution, dramatic stunting of root cell growth, reduced RNA synthesis, and increased vacuole formation were observed (Davies et al., 1995). Similar experiments using $Ca(NO_3)_2$ solutions have also demonstrated Zn phytotoxicity at concentrations less than 20 μM (Ruano et al., 1988; Wong and Bradshaw, 1982), and Wu and Antonovics (1975) reported complete stunting of root growth in *Agrostis stolonifera* by 40 μM Zn. In contrast, using complete nutrient solutions, Hogan and Rauser (1979) observed only a 15% reduction in root growth of *Agrostis gigantea* growth at 150 μM Zn. Other studies employing complete nutrient solutions have shown similar insensitivity to Zn concentrations of 100 μM or more (e.g., Berry and Wallace, 1989; Harrington et al., 1996). In the only comparative study of which we are aware, Baker (1978) did not observe any effect of 80 μM Zn on root growth of *Silene maritima* With. reared in complete nutrient solution, but also reported that root elongation was reduced some 50% by just 15 μM Zn in a simple $Ca(NO_3)_2$ medium, a discrepancy not discussed by the author.

These differences in sensitivity to Zn of plants grown under different nutrient regimes seems to have largely escaped the notice of researchers until now. With Cu, such a disparity might be explained by unrecognized complications arising from metal complexation (Parker et al., 1995a; Parker and Norvell, 1999), but Zn cannot compete effectively with Fe(III) for the chelating agents such as EDTA that are typically used in complete nutrient solutions. Moreover, there is seldom enough EDTA or other strong ligand present to inactivate the very large concentrations of Zn employed in the complete-solution studies of Zn toxicity.

Recently, Silva et al., (2001a–c) reported that micromolar concentrations of Mg can significantly alleviate the rhizotoxicity of Al, while millimolar levels of Ca are required to elicit the same effect. In exploring the causes underlying the disparate results from complete nutrient solutions versus simple salt solutions described above, we obtained similar evidence for a unique effect of Mg on Zn toxicity, and the experiments reported here explore this finding in some detail. We also present evidence that K concentrations of 10–100 μM also afford some protection against Zn toxicity in chemically simple media. We used both short-term root elongation assays and longer term split-root experiments to study the alleviation of Zn rhizotoxicity by Mg, K, and Ca. Such short-term assays have long been used to quantify the rhizotoxicity of metals (Wilkins, 1957), and their continued use lies in their simplicity; the effect of single or few elements on plant growth is clearly seen, and the endpoint is rapidly and readily determined. Split-root systems provide plants with all mineral nutrients to half of the root system, while the treatment half can be exposed to the intoxicating metal in a minimal medium (e.g., a Ca salt plus B) to which potentially interacting ions can be added individually or in combination. Longer-term effects of both root and shoot growth can be observed, and any toxicity due to impaired mineral nutrition can usually be eliminated or minimized.

Materials and methods

In all experiments, the salts used were analytical grade and double-deionized water was used throughout for stock and treatment solutions. All glassware, tanks and pots were acid-washed, and thoroughly rinsed with double-deionized water. Metal-ion activities were calculated using GEOCHEM-PC (Parker et al., 1995b.)

Short term root-elongation experiments

Seeds of wheat (*Triticum aestivum* L., cv. Yecora Rojo) and radish (*Raphanus sativus* L., cv. Cherry Belle) were surface-sterilized for 15 min in 0.04 M NaOCl, rinsed, and germinated for 44 h in the dark on filter papers soaked in 0.2 mM $CaCl_2$. Five (wheat) or 10 (radish) seedlings with uniform root length (longest of three seminal wheat roots ca 20 mm; taproot of radish ca 30 mm) were supported in polyethylene foam floats on the aerated, 3-L test solutions. Seedlings were grown in darkness for 48 h in a growth cabinet at 25 °C, 65% relative humidity. At termination, the two longest seminal roots per wheat seedling, and the single taproot of all radish seedlings, were measured and relative net elongation (RNE) calculated using the equation

$$\mathrm{RNE}, \% = (L_{\mathrm{final,\ treatment}} - L_{\mathrm{initial}})/(L_{\mathrm{final,\ control}} - L_{\mathrm{initial}}) \times 100, \quad (1)$$

where L refers to the measured root lengths. Treatments were replicated at least thrice. When treatments were sometimes repeated in separate experiments, the RNE results for each replicate have been combined across experiments into the means presented (thus, n was 3, 6, or occasionally 9). The basal solution used throughout was 2.0 mM $CaCl_2$, buffered at pH 6.0 with 0.25 mM MES and ~0.13 mM NaOH. Solutions

were continuously aerated, and pH checks indicated that drift over 24 h was not significant.

Concentration-response curves of Zn toxicity to wheat and radish roots were established by adding $ZnCl_2$ to the basal solution. We measured the alleviation of Zn rhizotoxicity by Mg and K at concentrations from 0 to 200 μM, and by Ca over the range of 200 to 5000 μM; alleviating cations were always added as the chloride salt. Other details of the test solutions are presented in the Results.

Split root experiments

Split root experiments were carried out using custom-made culture vessels constructed from 1.3-cm-thick, black acrylic. These consisted of two 3-L chambers separated by a dividing wall upon which seedling roots could straddle the two solutions. Four plants were secured by foam plugs fitted into the light-tight lid of each vessel, with roots evenly distributed between a complete nutrient solution and an incomplete test solution which contained the imposed concentrations of Zn, Mg and K. The complete solution (COM), contained (in mM) $Ca(NO_3)_2$ 2.0, KNO_3 1.0, $MgSO_4$ 0.5, KCl 0.1, NaH_2PO_4 0.1, and (in μM) H_3BO_3 10, Na_2MoO_4 0.1, Mn 1.0, Cu 2.0, Fe 100, Zn 8.0, and Ni 0.1. The trace metals were added as chloride salts in stock solution with equimolar HEDTA, plus a 50 μM excess of HEDTA for chelator-buffering these metals (Parker et al., 1995a). The free-ion activities of the trace metals were calculated as $(Mn^{2+}) = 10^{-7.8}$, $(Cu^{2+}) = 10^{-13.5}$, (Fe3+) $= 10^{-16.7}$, (Zn2+) $= 10^{-10.1}$, and $(Ni^{2+}) = 10^{-14.5}$. The basal, incomplete solution (INC) contained 2.0 mM $Ca(NO_3)_2$, and 10 μM H_3BO_3. Additions of Zn, Mg and K were made using chloride salts, with Mg and K at 100 μM when present. Both complete and incomplete solutions were buffered at pH 6.0, with 2.0 mM MES and $\sim$1.0 mM NaOH.

Wheat (cv. Yecora Rojo) and radish (cv. Cherry Belle) seeds were surface-sterilized as above, and germinated in papers soaked in complete nutrient solution. After 4 d, the taproots of radish seedlings were cut with a razor blade, 1.5 cm below the hypocotyl-root junction, to induce lateral root growth. After a further 4 d in germination papers, when the lateral roots were ca 15 cm long, the seedlings were transferred to the split root tanks. Wheat seedlings were grown undisturbed in papers for 5 days, then transferred to split root tanks when at least three (of five) seminal roots were ca 15 cm long.

Plants of both species were grown for an additional 16 days in an environmentally controlled growth cabinet with 65% relative humidity, and a daily 16-h photoperiod, with light intensity symmetrically ramped up to, and down from, an 8-h plateau at a photosynthetic photon flux of 500 μE m^{-2} s^{-1}. Zinc, Mg and K treatments were imposed on the third day after transfer of seedlings to the split-root vessels. Solutions were continuously aerated and replaced at regular intervals (5, 9, and 13 days), and the pH (both sides) checked daily and adjusted if necessary.

At harvest, plants were divided into shoots, 'complete' and 'incomplete' solution-grown roots (denoted COM-root and INC-root respectively), and in radish an additional top-root consisting of the bulbous growth between the hypocotyl and the lateral roots that were immersed in solution. After drying overnight at 65 °C, the plant material was weighed, ground, and microwave-digested in a mixture of concentrated HNO_3 and 9 *M* H_2O_2 prior to elemental analysis by inductively coupled plasma emission spectroscopy.

Two experiments were conducted with wheat; the first with seven concentrations of Zn (0, 2, 5, 10, 20, 40, 60 μM) in the INC solutions, and with both Mg and K present at 100 μM. Preliminary experiments had suggested that, of the salts usually present in complete solution culture, only Mg and K alleviated root-stunting due to Zn toxicity (data not shown). The second wheat experiment examined the effects of Mg and K in an incomplete factorial combination with 0 and 40 μM Zn. This experiment was repeated using radish, except that Zn was 0 or 20 μM, radish having shown a greater sensitivity to Zn than wheat in the short-term experiments (see Results). All treatments were replicated thrice with wheat and four times with radish. The split-root results were analyzed using standard ANOVA techniques; means were separated using Tukey's w procedure (Steel and Torrie, 1980).

Results

Short-term root elongation in wheat was quite sensitive to increasing Zn in the basal medium of 2.0 mM $CaCl_2$, pH 6.0 (Figure 1). Growth was reduced by 50% at a Zn^{2+} activity of about 19 μM, while the highest activity (42 μM) reduced RNE to 15%. Radish was even more sensitive than wheat, as a Zn^{2+} activity of 14 μM reduced elongation to about 10% of control (data not shown). Preliminary experiments (not shown) had suggested that, of the components in a

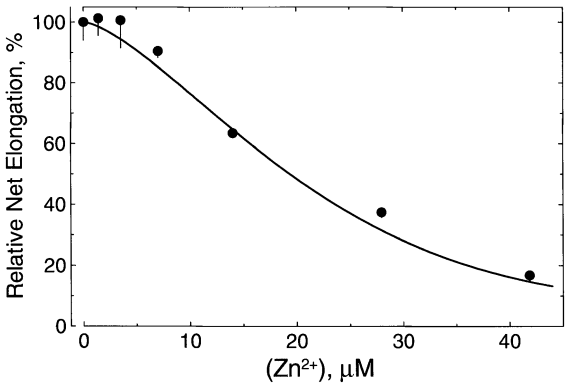

Figure 1. Effect of increasing Zn^{2+} activity on relative net root elongation of Yecora Rojo wheat seedlings, in a basal solution of 2.0 mM $CaCl_2$, pH 6.0 (total Zn ranged from 0 to 60 μM). The fitted line is a Weibull function, $y = c + (100\text{-}c) / \exp((ax)^b)$; $R^2 = 0.991$. Bars indicate 1 SE where it exceeds symbols size.

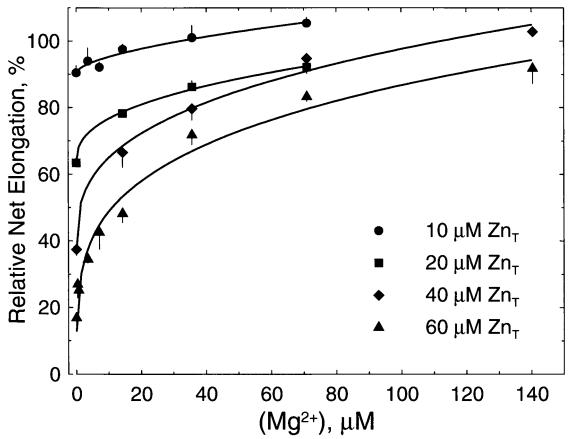

Figure 2. Alleviation of Zn rhizotoxicity in Yecora Rojo wheat seedlings by increasing Mg^{2+} activity in a basal solution of 2.0 mM $CaCl_2$, pH 6.0, containing the total Zn concentrations indicated (total Mg ranged from 0 to 200 μM). Bars indicate 1 SE where it exceeds symbols size.

complete nutrient solution, Mg and perhaps K could account for the reduced sensitivity to Zn as compared to that in a simple Ca salt. When we conducted an experiment identical to that in Figure 1, but with both Mg and K present at concentrations of 100 μM, a Zn^{2+} activity of $\sim$150 μM was required to inhibit root elongation by 50% (data not shown); this represents an 8-fold increase over that required in the Ca-only solution (Figure 1).

Similarly, wheat roots reared in split-root culture at total Zn concentrations up to 60 μM Zn in the presence of 100 μM each of Mg and K were not stunted, although tissue concentrations in the INC-side roots reached 228 μmol g^{-1} (14 900 μg g^{-1}) (Table 1). Some differences in root morphology were observed (with Zn, roots were thinner, longer, and less branched), but there was no significant difference in root dry weight between Zn treatments, nor between root weights grown in the complete (COM) *versus* incomplete (INC) solutions (Table 1). Shoot dry weights were similarly unaffected, even though shoot Zn concentrations were increased by some 10- to 20-fold by all Zn levels above the control (Table 1). Typically, shoot-yield reductions in crop plants are only observed when shoot Zn concentrations exceed $\sim$8 μmol g^{-1} DW (Chaney, 1993; Welch, 1995), so the absence of any statistically significant reduction in shoot weight in Table 1 is not unexpected. Moreover, despite the elevated Zn levels, shoot-tissue Cu, Fe, and Mn concentrations were not affected by Zn treatments (data not shown).

In short-term elongation experiments, the alleviation of Zn rhizotoxicity by Mg alone was dramatic. Increasing Mg^{2+} activities up to 140 μM consistently alleviated rhizotoxicity in wheat (Figure 2). In Zn-free controls, there was no evidence of any growth stimulation by comparable increases in Mg^{2+} activities (data not shown). At the highest Zn level (60 μM), we observed measurable alleviation at Mg^{2+} activities below 1 μM. Alleviation by Mg in short-term experiments with radish was similar to that in wheat, although not quite as clear-cut (data not shown). Variability within experiments was greater for radish than wheat, and the radish seedlings appeared to be more sensitive than wheat to inadequate aeration, which caused some stunting of root elongation. Radish was overall more sensitive to Zn toxicity, and alleviation by Mg was somewhat less dramatic. At a highly toxic Zn level of 20 μM, %RNE increased from 10 to 75 as the Mg^{2+} activity was increased from 0 to 140 μM (data not shown).

In split-root culture, the inclusion of Mg completely abolished the rhizotoxic effects of Zn on the INC-Side roots (Table 2). With 40 μM Zn in the INC solution, omission of Mg alone (treatment 4), or omission of both Mg and K (treatment 2), reduced root growth of wheat by some 70 to 80%. Without added Zn, the inclusion or omission of Mg did not significantly affect root growth in the INC side (compare treatments 1 and 6); dry weights of both shoots and the COM-side roots were unaffected by any treatment (Table 2). Thus the presence of Mg clearly and specifically affects the rhizotoxic effects of excess Zn in solution.

Table 1. Dry weights and Zn tissue concentrations of wheat plant parts grown in a split-root system containing a complete solution (COM) on one side, and incomplete solution (INC) on the other. Zn treatments were added to the incomplete solutions, which contained both Mg and K at 100 μM. Values within a column are not significantly different ($P > 0.05$) when followed by the same letter

Zn (μ)M	Dry Matter			Zn Concentration		
	Shoot	Root-COM	Root-INC	Shoot	Root-COM	Root -INC
	(g)			(μmol g^{-1})		
0	1.16 a	0.278 a	0.249 a	0.39 a	0.98 a	0.66 a
2	0.97 a	0.228 a	0.221 a	4.00 b	1.90 a	33.5 ab
5	0.89 a	0.230 a	0.218 a	6.29 b	1.28 a	87.2 b
10	0.93 a	0.241 a	0.251 a	6.65 b	1.18 a	153 c
20	0.77 a	0.254 a	0.299 a	8.84 bc	1.54 a	185 cd
40	0.90 a	0.203 a	0.293 a	7.69 bc	1.83 a	212 cd
60	0.93 a	0.268 a	0.226 a	6.35 b	1.76 a	228 d

Table 2. Dry weights (g) of wheat and radish plant parts grown in a split-root system containing a complete solution (COM) on one side, and incomplete solution (INC) on the other. The + signs indicate the presence of Zn (40 μM for wheat, 20 μM Zn for radish), 100 μM Mg, and/or 100 μM K in the INC solutions. Values within a column are not significantly different ($P > 0.05$) when followed by the same letter

Treatment				Wheat			Radish			
	Zn	Mg	K	Shoot	Root-COM	Root-INC	Shoot	Root-top	Root-COM	Root-INC
1	−	−	−	0.98 a	0.281 a	0.249 a	2.06 a	1.45 a	0.148 a	0.098 a
2	+	−	−	0.89 a	0.229 a	0.044 b	2.43 a	1.46 a	0.249 b	0.009 b
3	+	+	−	1.10 a	0.331 a	0.336 a	2.25 a	1.59 a	0.189 ab	0.067 a
4	+	−	+	1.21 a	0.492 a	0.074 b	2.27 a	1.63 a	0.226 ab	0.009 b
5	+	+	+	1.02 a	0.307 a	0.326 a	2.42 a	2.27 a	0.186 ab	0.075 a
6	−	+	+	1.16 a	0.278 a	0.249 a	2.77 a	1.77 a	0.199 ab	0.100 a

Radish responded similarly to wheat in split-root culture, with root weights on the INC side of ~10% of control when 20 μM Zn was added and Mg was omitted from the INC solution (Table 2). Occasional poor growth of single plants contributed to larger variance in shoot and root dry-weights as compared with wheat. Shoot and root-top dry weights were not significantly affected by Zn treatments. In contrast to wheat, root growth of radish in the incomplete solutions was consistently less vigorous than in the complete solution (Table 2).

Although inclusion of Mg in the INC solutions dramatically reduced the toxic effects of Zn (treatment 2 *versus* 3, Table 2), it simultaneously caused 2.2- and 6-fold *increases* in Zn concentration of the INC-side roots and shoots of wheat, respectively (Table 3). The pattern in radish was slightly different: inclusion of Mg caused a 2.5-fold increase in shoot and root-top Zn, no significant change in the concentration of Zn in the INC-side roots, but a significant reduction in Zn in the COM-side roots (Table 3). In both species the INC-side roots had lower Mg contents when Mg was omitted (data not shown), indicating some limitations on phloem retranslocation to the INC-side roots. For example, the Mg content of the INC-side roots of wheat grown without Mg was one-third to one-quarter that of INC roots grown with Mg. Retranslocation of K was not similarly limited.

In short-term elongation experiments, K also alleviated Zn toxicity in wheat, although its efficacy was not as great as that of Mg at equivalent activities (Figures 2 and 3). At the higher two Zn concentrations (40 and 60 μM), low K^+ activities caused partial alleviation, but then seemed to reach a plateau wherein higher activities had little effect (Figure 3). With radish, K exhibited a similarly limited ability to

Table 3. Zn tissue concentrations (μmol g^{-1} DW) of the wheat and radish plant parts depicted in Table 2. Values within a column are not significantly different ($P > 0.05$) when followed by the same letter

	Treatment			Wheat			Radish			
	Zn	Mg	K	Shoot	Root-COM	Root-INC	Shoot	Root-Top	Root-COM	Root-INC
1	−	−	−	0.46 a	1.01 a	0.36 a	0.50 a	0.30 a	0.51 a	0.81 a
2	+	−	−	1.08 b	1.41 a	85.1 b	0.78 a	0.45 a	1.15 b	36.1 b
3	+	+	−	6.64 c	1.57 a	188 c	2.40 b	1.12 b	0.67 a	29.0 b
4	+	−	+	0.75 a	0.96 a	67.1 b	0.67 a	0.43 a	1.41 b	38.6 b
5	+	+	+	7.78 c	1.66 a	197 c	2.44 b	1.03 b	0.79 a	27.0 b
6	−	+	+	0.45 a	0.98 a	0.29 a	0.45 a	0.30 a	0.76 a	0.72 a

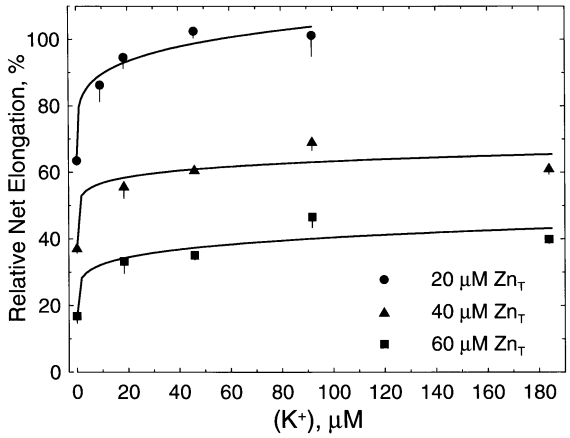

Figure 3. Alleviation of Zn rhizotoxicity in Yecora Rojo wheat seedlings by increasing K^+ activity in a basal solution of 2.0 mM $CaCl_2$, pH 6.0, containing the total Zn concentrations indicated (total K ranged from 0 to 200 μM). Bars indicate 1 SE where it exceeds symbols size.

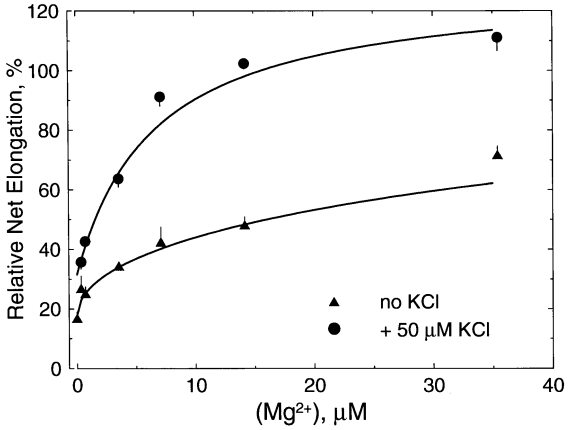

Figure 4. Alleviation of Zn rhizotoxicity in Yecora Rojo wheat seedlings by increasing Mg^{2+} activity with and without 50 μM KCl. For all points, total Zn was 60 μM in a basal solution of 2.0 mM $CaCl_2$, pH 6.0; total Mg ranged from 0 to 50 μM. The data for Mg alone are the same as in Figure 2. Bars indicate 1 SE where it exceeds symbols size.

alleviate toxicity in short-term experiments. At 20 μM Zn, K^+ activities of 92 and 184 μM elevated RNE from 10% (at zero K) to 37 and 43%, respectively (data not shown). With wheat (but not radish), these same K^+ activities caused small (<15%) increases in root elongation in the absence of Zn (not shown), but the effect was too inconsistent across experiments to yield any confidence in the result.

In contrast, the longer-term split-root experiments revealed no ability of K to alleviate Zn rhizotoxicity (Table 2). With both wheat and radish, addition of K but not Mg (treatment 4) caused no improvement in the growth of the INC-side roots relative to Zn alone (treatment 2). Moreover, the ability of Mg to alleviate toxicity in both species did not depend on the simultaneous presence of K (compare treatments 3 and 5, Table 2).

The possibility of a K × Mg interaction was probed using wheat in short-term experiments (Figure 4). At a highly toxic Zn level of 60 μM, Mg^{2+} activities up to 35 μM caused only a partial alleviation of toxicity. When K was also included at a concentration of 50 μM, however, Zn toxicity was fully abolished at $(Mg^{2+}) = 14$ μM (Figure 4). This result suggests that, despite its limited ability to alleviate Zn toxicity by itself (Figure 3), K has some ability to enhance the alleviating effect of Mg. Again, this interactive effect was not observable in the split-root experiments (Table 2).

Although Ca could alleviate Zn rhizotoxicity, its effect was much more modest than that of Mg (or perhaps even K). When increased from 200 μM (more than sufficient for optimal growth at pH 6 and in the absence of toxicants [Kinraide, 1998]) to 1000 μM,

Ca only improved the elongation of wheat roots exposed to 20 μM Zn from 13 to 40% of control (defined as 0 Zn, 2000 μM Ca); a further increase to 5000 μM total Ca only improved RNE to 72% (data not shown). At 60 μM Zn, the same range in Ca concentration only increased RNE from 10 to 34%, in marked contrast to the near-complete alleviation caused by 200 μM Mg (Figure 2)

Discussion

Silva et al. (2001a) recently reported that Mg concentrations as low as 10 μM significantly alleviated Al rhizotoxicity in soybean (*Glycine max* [L.] Merrill), but that millimolar concentrations were required to cause any alleviation in wheat. Here, we have shown that Mg activities in the μM range can alleviate or abolish the toxicity of Zn in both a representative monocot (wheat) and a dicot (radish). This ability was demonstrated in both 48-h root elongation assays, and in split-root experiments lasting for $>$2 weeks.

In the split-root studies, a perplexing and paradoxical result was obtained: inclusion of 100 μM Mg increased the dry weights of the Zn-exposed roots by some 10-fold, fully abolishing toxicity as measured by root weight (Table 2). At the same time, however, inclusion of Mg caused significant increases in the Zn concentrations of the INC-side roots of wheat, and in the shoots of both species (Table 3). From this, we conclude that the protective effect of Mg is not due to its ability to diminish uptake or translocation of Zn. Instead, it seems Mg must help to internally detoxify Zn by, for example, interfering with Zn's toxic mode of action, or by promoting its sequestration into some innocuous form (Clemens, 2001).

Potassium also exhibited some limited ability to alleviate Zn rhizotoxicity in short-term assays using a simple Ca salt medium (Figure 3). As with Mg, this alleviation occurred with μM levels of K, but the alleviation seemed to soon reach a plateau when Zn was present at highly toxic concentrations (Figure 3). In the split root experiments, where only a single, rather toxic level of Zn was used (40 μM for wheat, 20 μM for radish), the inclusion of K at 100 μM in the INC solutions caused no measurable alleviation, irrespective of Mg level (Table 2). It is possible that the levels of Zn chosen for the split-root studies may have masked K's alleviating ability, but a better explanation for this discrepancy may lie in the tissue K concentrations. In the INC-side roots, K levels were largely indifferent to treatment (data not shown), suggesting ample supply from the COM side via retranslocation of this phloem-mobile element; thus, the INC-side roots were indifferent to the presence of K in solution. In the short-term elongation experiments, however, root K would have been limited to the seed reserves except when included in the external medium.

Although we were not able to probe the interactive effects of Mg and K in detail, the data in Figure 4 suggest that the presence of K in a simple medium can significantly enhance the ability of Mg to alleviate Zn toxicity. No such synergism could be detected in the split root studies (Table 2), in large measure because Mg alone seemed to fully abolish toxicity in those experiments. Moreover, the K status of the INC-side roots was probably unaffected by treatment, as discussed above.

In contrast, the phloem mobility of Mg was sufficiently limited so that there were significant differences in Mg content (and presumably physiological status) of the INC-side roots in the split-root experiments. This leads us to speculate that inhibition of root growth by Zn in the absence of exogenous Mg might be, in part, an induced Mg deficiency in the apical tissues, where phloem development is incomplete. If limited retranslocation from seed sources (short-term experiments) or the COM-side roots (split-root studies) leads to a marginal Mg status of the root apices, then Zn might interfere with the utilization of this limited Mg supply. Silva et al. (2001c) described a similar scenario when discussing possible explanations for the alleviation of Al toxicity by such low external concentrations of Mg.

The mechanisms whereby Mg and K can alleviate the rhizotoxicity of Zn in chemically simple media are unknown, but they seem to be distinct from that of Ca, for which millimolar levels were required for even moderate alleviation. Kinraide (1998) has suggested three mechanisms of alleviation of rhizotoxicity, based on the Guoy-Chapman-Stern (GCS) model for metal binding on the plasma lemma exterior. Mechanism I describes the beneficial effect of cations which reduce the negativity of the plasma membrane, and thus the electrostatic attraction for toxic cations. This is not necessarily displacement in the sense of occupation of binding sites otherwise occupied by toxicant, but substitution of ameliorant for toxicant may be a component of Mechanism I. Mechanism II is Ca specific, sufficient extracellular Ca being essential for cell growth and thus root elongation, even in the absence of toxicant. The amelioration of rhizotoxicity by

Ca is at least partly described by the restoration and maintenance of Ca at the plasma membrane surface. Mechanism III encompasses any ameliorative effects of ions not explained by Mechanisms I and II, for example the blockage of ion channels by ameliorative ions, preventing uptake of the toxicant.

Calculation of Zn^{2+} activities at the plasma membrane surface by a Gouy-Chapman-Stern model (Kinraide et al., 1998) indicates that Mechanism I is the principal means of Ca^{2+} alleviation of Zn^{2+} rhizotoxicity. For Mg^{2+} to alleviate toxicity by that mechanism, additions of 800 μM Mg^{2+} to 200 μM Ca^{2+} would have to produce the same modest effect as the elevation of 200 μM Ca^{2+} to 1000 μM (see Results). To produce the same Mechanism I effect with KCl would require that that salt to be added at 24,500 μM. Clearly, Mg^{2+} and K^{+} alleviate toxicity by some variation of Mechanisms III. Full descriptions of the calculations, and deeper discussion of these mechanisms are given in a companion paper (Kinraide et al., 2003).

Our understanding of the mechanisms underlying Zn toxicity and tolerance is still rudimentary (Chaney, 1993), although molecular approachs are beginning to yield important clues (Clemens, 2001). At present, it is difficult to do more than speculate about why Mg, at micromolar levels, so profoundly influences Zn toxicity and uptake. Sequestration in the vacuole has long been considered a likely component of Zn homeostasis and tolerance (e.g., Harmens et al., 1994; Verkleij et al., 1998), and an increasing number of Zn transporters are being identified in higher plants (Grotz et al., 1999; Pence et al., 2000), including some that have been implicated in Zn transport across the tonoplast (van der Zaal et al., 1999; Bloß et al., 2001). Shaul et al. (1999) have isolated a proton-driven antiporter from the vacuole of *Arabidopsis thaliana* that is highly selective for Mg and Zn (but not other divalent cations). Verkleij et al. (1998) found that tonoplast vesicles isolated from a tolerant genotype of *Silene vulgaris* had higher Zn transport rates than those from a sensitive genotype, and that transport required Mg-ATP. Despite these empirical connections between Mg and Zn, however, there is no obvious explanation for how Mg can alleviate Zn's toxicity while simultaneously increasing tissue Zn burdens in both roots and shoots (Tables 2 and 3). Moreover, the vacuolar sequestration model (Clemens, 2001) is not universally supported experimentally. Bruen et al. (1994) have shown that, in mildly Zn-intoxicated barley (*Hordeum vulgare* L.), the majority of the Zn in the primary leaves is apoplastic; their estimate of the vacuolar stores in the leaf mesophyll was $<10\%$ of the total leaf burden.

Our results are in clear contrast with those of Silva et al. (2001a–c). With soybean, Mg alleviates Al toxicity because apical tissue burdens of the toxicant are decreased (Silva et al., 2001c). This appears to be due to Mg's ability to stimulate efflux of citrate (but not malate) from the apical zones, and citrate is a potent chelator of Al. Such a mechanism seems unlikely here because (i) Zn is much more weakly bound by organic acids than is Al, and (ii) exogenous Mg simultaneously alleviates toxicity while enhancing accumulation of Zn in both roots and shoots (Tables 2 and 3). An alternative, which we think very unlikely, is that exogenous Mg somehow lowers Zn in sensitive apical tissues while at the same time increasing its accumulation in the remainder of the root system. Despite our present ignorance concerning the role of Mg (and K) in Zn toxicity, however, these findings may benefit future physiological studies, especially those aimed at understanding compartmentation of Zn and its importance in cellular homeostasis and tolerance (Clemens, 2001).

From a more practical standpoint, our results have implications for the use of simple Ca solutions for studying the physiology of metal toxicity, or even for screening species/genotypes for tolerance. Wilkins (1957) original root elongation assay was developed for determining metal toxicity indices, and the simple basal solution of $Ca(NO_3)_2$ was employed to minimize the possibility that the metals of interest would precipitate out of solution (e.g., with phosphate). Such chemical simplicity is still appealing today, because the extent of precipitation and complexation reactions in complete nutrient solutions cannot always be predicted with precision, even with the benefit of computerized chemical equilibrium models (Parker and Norvell, 1999). Surprisingly, differences in sensitivity to supraoptimal Zn among plants reared in simple versus complete solutions have not been commented upon, until now. Clearly, such differences could be crucial when attempting to make comparisons between experiments using the two systems, and certainly when extrapolating from results obtained in simple Ca-salt solutions to experimental or field situations involving soil-grown plants.

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