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Growth and Nutrient Uptake by Barley (*Hordeum vulgare* L. cv Herta): Studies Using an *N*-(2-Hydroxyethyl)ethylenedinitrilotriacetic Acid-Buffered Nutrient Solution Technique

I. Zinc Ion Requirements

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The critical range of Zn^{2+} activity in nutrient solution required for optimum growth of barley (*Hordeum vulgare* L. cv Herta) was studied using the synthetic chelating agent *N*-(2-hydroxyethyl)ethylenedinitrilotriacetic acid to buffer micronutrient metal ions. The activity of Zn^{2+} was varied over a wide range from approximately 0.1×10^{-11} to 22×10^{-11} M Zn^{2+}. The dry weight of barley shoots reached a maximum at Zn^{2+} activities above approximately 3×10^{-11} M and was clearly depressed when Zn^{2+} activities were below about 1×10^{-11} M. The relationship in shoots between dry weight and Zn concentrations supports the view that there is a critical Zn concentration of about 25 $\mu g\ g^{-1}$ dry weight in whole shoots of barley seedlings. When Zn^{2+} activities in solution were near or below approximately 3×10^{-11} M, barley shoots accumulated higher concentrations of P, Mn, Ca, Mg, and Na, whereas Cu concentrations were reduced. P and Mn began to accumulate in the shoots before differences in dry weights were apparent and provided the earliest index of Zn deficiency. In Zn-deficient roots, concentrations of Ca and Mg increased by 25 to 30%, and those of Fe and Mn more than doubled. Zn appears to play a special role in regulating uptake of several mineral nutrients in barley.

Controlling micronutrient cation activities in nutrient media at the extremely low levels commonly found in soil solutions is an important but difficult objective. Until recently, this option was limited to a few laboratories worldwide because of the large expenditures involved in constructing and maintaining the equipment required (Norvell, 1991; Parker et al., 1993a). As a result, the critical activity of Zn^{2+} ions in nutrient media required for optimum plant performance has not been established for many crop species. It is now possible to perform such experiments in conventional low-volume nutrient solutions using chelating agents to regulate metal ion availability (Parker et al., 1993a). This approach has been facilitated by the development of convenient computer programs, such as GEOCHEM-PC (Parker et al., 1993b), that model metal speciation in aqueous solutions, and by the commercial availability of suitable and well-characterized chelating agents for micronutrient cations. Several applications of chelate buffering used as a means to regulate metal ion availability were discussed by Chaney (1988), Norvell (1991), and Parker et al. (1993a).

Here, we describe results of experiments to determine the critical range of solution activity of Zn^{2+} required by seedlings of barley (*Hordeum vulgare* L. cv Herta). This requirement was established by responses in dry matter production, visual symptoms, concentrations of Zn in plant shoots, and disturbances in the accumulation of several mineral nutrients. The activity of Zn^{2+} in nutrient solutions was buffered over a wide and physiologically important range by the chelating agent HEDTA.

MATERIALS AND METHODS

Preparation of Nutrient Solutions

The composition of the nutrient solutions (Table I) was adapted from that reported by Johnson et al. (1957). Five millimolar Mes, adjusted to pH 6.1 with KOH, was included as a pH buffer (Miyasaka et al., 1989). NaCl (0.5 mM) was included to allow the uptake and distribution of Na to be measured. Micronutrient metals were included as chelates of HEDTA. Zn treatments consisted of five levels of added Zn-HEDTA ranging from 0.1 to 20 μM. Each of the solutions contained 25 μM HEDTA in excess of that needed to chelate the five micronutrient metals. This excess served to buffer the activities of all of the micronutrient cations and to depress the activity of any contaminant Zn in those solutions intended to be low in Zn availability (i.e. the 0.1 and 1.0 μM Zn-HEDTA treatments).

Several chelating agents were considered as candidates to control Zn^{2+} in nutrient solution (Norvell, 1991), but HEDTA was chosen because its stability constants are readily available, it forms Zn chelates of appropriate stability within a suitable pH range for barley (*Hordeum vulgare* L.) culture,

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Abbreviations: HEDTA, *N*-(2-hydroxyethyl)ethylenedinitrilotriacetic acid; ICPES, inductively coupled argon plasma emission spectrophotometry.

Table I. *Nutrients and other constituents in HEDTA-buffered nutrient solution for growth of barley at five levels of regulated Zn^{2+} activity*

Major Constituents		Minor Constituents		Zn-HEDTA[c] Treatments
	mM		μM	
KNO_3	1.50	H_3BO_4	12.5	
$Ca(NO_3)_2 \cdot 4H_2O$	1.00	H_2MoO_4	0.1	0.1, 1.0, 3.0, 8.0, 20.0 μM
$MgSO_4 \cdot 7H_2O$	0.25	$CuSO_4 \cdot H_2O$	2.0	
$NH_4H_2PO_4$	0.60	$MnSO_4 \cdot H_2O$	3.0	
NaCl	0.50	$NiSO_4 \cdot 6H_2O$	0.1	
MES (pH buffer)[a]	5.00	$Fe(NO_3)_3 \cdot 9H_2O$[b]	20.0	
		K_3-HEDTA	50.0	

[a] Mes, adjusted with KOH to pH 6.1. [b] $Fe(NO_3)_3 \cdot 9H_2O$ and HEDTA were dissolved in water, mixed, and equilibrated before addition to nutrient solutions to facilitate complete formation of Fe-HEDTA and to avoid precipitation of hydrous ferric oxides. [c] Equimolar amounts of $ZnSO_4 \cdot 7H_2O$ and K_3-HEDTA were dissolved in water, mixed, and equilibrated before addition to nutrient solutions.

and it does not bind Fe^{3+} so tightly that roots of barley seedlings (classified as having the strategy II Fe efficiency mechanism [Marschner, 1986]) would have difficulty in absorbing adequate Fe.

The chemical activities of Zn^{2+} and other ions in the five nutrient solutions were calculated using version 2.0 of GEOCHEM-PC (Parker et al., 1993b). All calculations were corrected for ionic strength using the Davies equation, which is incorporated within GEOCHEM-PC. Stability constants for HEDTA chelates were selected from the critical compilations by Smith and Martell (Martell and Smith, 1974; Smith and Martell, 1989). The principal stability constants (concentration constants at an ionic strength of 0.1) required for the calculation of Zn^{2+} activities are logK = 8.2 for the chelate species $CaHEDTA^-$ and logK = 14.6 for $ZnHEDTA^-$. Any error in these stability constants will contribute to errors in calculated activities of Zn^{2+}.

Plant Culture

Grains of barley cv Herta were soaked in aerated, deionized water overnight in the dark. Grains with radicals showing were transferred to seedling cups (No. 6, hollow polyethylene stoppers with bottoms severed and replaced with 4-mm mesh polyethylene screens heat fused to the stopper bases) and covered with black polyethylene beads (4-mm diameter). Five grains were positioned in seedling cups with their radicals protruding through the plastic screen. Four seedling cups per replicate were placed in holes drilled into lids of 1-L black polyethylene pots. Initially, these pots contained 850 mL of nutrient solution with macronutrients at one-quarter the concentrations shown in Table I but with micronutrients as shown, except that no Zn was included. The pots were positioned in a 20°C water bath under fluorescent lights (a combination of high intensity Grow-lux[1] and Sylvania fluorescent lights). Light intensity at the surface of plant shoots was 300 $\mu mol\ s^{-1}\ m^{-2}$. The nutrient solutions were aerated with filtered compressed air. Water used throughout the experiment was of high purity (18 MΩ resistance). All nutrient salts used to prepare the solutions were of analytical grade. All experimental apparatuses were washed in a detergent solution, rinsed with water, dipped in 3 M HCl, and finally rinsed free of acid with water before use.

At day 7 after grain imbibition, seedlings were thinned to a population of three per cup for the harvests at day 10 and day 13, and two per cup for harvests on days 15 and 17. Treatments were imposed after thinning by replacing the initial nutrient solutions with those described in Table I. Nutrient solutions were replaced again on days 10, 13, and 15 to prevent depletion of nutrients.

Seedlings were harvested on days 7, 10, 13, 15, and 17. The small plants harvested during thinning on day 7 were combined for elemental analyses. Subsequently, three plants from each replicate pot were harvested on days 10 and 13, and two plants per replicate were harvested on days 15 and 17. At each harvest, seedling roots were rinsed briefly in deionized water. Seedlings were separated into roots and shoots. Roots were gently blotted between several layers of laboratory tissue paper to remove excess water. Fresh weights (not reported) were determined immediately after harvest, and dry weights were obtained after the plant parts were dried overnight at 60°C. One seedling from each replicate harvested on day 17 was dried separately, pulverized by hand in a plastic bag, and weighed, and soluble anions and Na were extracted as described below.

Analyses

Weighed samples of dried plant parts were heated in glass beakers overnight at 450°C in a muffle furnace. Ash residue was dissolved in concentrated HNO_3 and diluted to 3 M HNO_3 with deionized water (18 MΩ). Mineral element concentrations (Zn, Mn, Fe, Cu, K, Ca, Mg, and P) were determined in the 3 M HNO_3 digests by simultaneous ICPES. Nutrient solution aliquots, adjusted to 3 M HNO_3, were also assayed for mineral elements via ICPES.

Dried plant shoots and roots from harvest day 17 were extracted three times successively in 5 mL of boiling deionized water for 5 min. The combined extracts were filtered through filter paper (water rinsed, Whatman No. 42, ashless), and the

[1] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product by the U.S. Department of Agriculture.

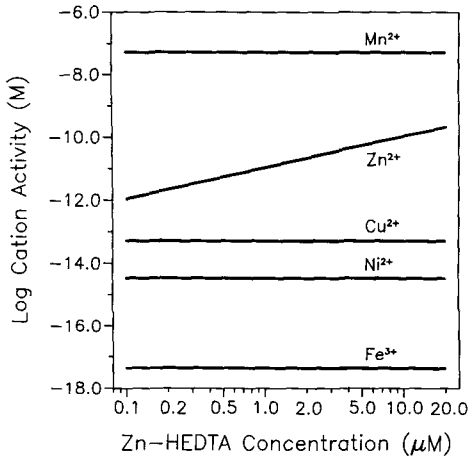

Figure 1. Plot of calculated cation activities as a function of varying Zn-HEDTA supply in nutrient solution (Table I, pH 6.0) in the presence of a 25 μM excess of HEDTA. These computations were carried out using GEOCHEM-PC (Parker et al., 1993b).

filtrate was brought to 25 mL with water. Anion concentrations (Cl^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-}) in 0.10-mL aliquots of diluted extract were determined by ion chromatography (Dionex series 2010i, Dionex Corp., Sunnyvale, CA) using an anion exchange column (Dionex IonPac AS4) and isocratically eluted with 3 mM $NaHCO_3$ and 2.4 mM Na_2CO_3. Anions were detected by suppressed conductivity (Dionex Micromembrane Suppressor, using 0.0125 M H_2SO_4 as the conductivity suppressor).

Concentrations of Na in dry-ashed plant samples are not reported because of Na contamination from the Pyrex beakers used to ash the samples. Instead, Na in plant material harvested on day 17 was determined by ICPES analyses of acidified filtrates of the hot water extracts.

Statistical analyses of data were performed using the Statistical Analysis System software (SAS Institute, Inc., Cary, NC). Throughout the text and tables, data are reported as means ± SE for each treatment listed.

RESULTS AND DISCUSSION

Effects of Zn^{2+} on Dry Weights, Visual Symptoms, and Shoot and Root Concentrations of Mineral Nutrients

The Zn^{2+} activity supplied to barley seedlings was varied 200-fold by varying the concentration of Zn-HEDTA (Table I, Fig. 1). As Zn-HEDTA increased from 0.1 to 20 μM, the calculated activity of Zn^{2+} increased from 0.11×10^{-11} to 22×10^{-11} M, and the activities of other metals were unaffected. This capacity to vary the activity of one metal cation while holding the others constant is one of the greatest advantages of chelator buffering. In the presence of a constant excess of HEDTA in the form of Ca-HEDTA, the ratio of Zn^{2+} to Zn-HEDTA is constant, and Zn^{2+} can be varied simply by varying Zn-HEDTA. Varying Zn-HEDTA does not disturb the equilibria of other metals with HEDTA; nor do their equilibria disturb the levels of Zn^{2+} as long as the concentrations of Ca-HEDTA are not altered.

The dry weights of seedlings increased with increasing supply of Zn, reaching a maximum when Zn-HEDTA concentrations were 3 μM or greater (Table II, Figs. 2 and 3), and the −log activity of Zn^{2+}, i.e. $p(Zn^{2+})$, was 10.5 or less (Fig. 3). Dry weights of barley shoots in harvests on days 15 and 17 were clearly depressed when the $p(Zn^{2+})$ was 10.9 or above in the nutrient solution.

Relationships between the dry weight and the Zn concentration of barley shoots are shown in Figure 2 for all harvests. These results support the view that there is a critical Zn concentration in shoots of barley seedlings of about 25 μg g^{-1} dry weight, in agreement with the conclusions of Reuter (1986). At a $p(Zn^{2+})$ of 10.5, not only was growth of shoots greatest but the concentration of Zn in shoots was reasonably stable at 26 to 29 μg g^{-1} during the course of the experiment (Table II, Fig. 3).

Visual symptoms of Zn deficiency in crops may include

Table II. *Effects of Zn-HEDTA supply on shoot and root dry weight and Zn, Fe, Mn, Cu, K, Ca, Mg, and P concentrations in 15-d-old barley seedlings*

Zn-HEDTA	Plant Part	Dry Wt	Zn	Fe	Mn	Cu	K	Ca	Mg	P
μM		g			μg g^{-1a}				mg g^{-1a}	
0.1	Shoot	0.068	13.73	59.67	103.77	15.63	93.44	5.79	2.01	25.42
1.0	Shoot	0.075	18.30	54.00	89.57	20.43	97.49	5.52	2.06	20.29
3.0	Shoot	0.094	27.43	52.77	73.50	18.27	98.65	5.08	1.84	13.98
8.0	Shoot	0.085	45.87	61.13	71.90	21.63	96.01	5.00	1.79	12.91
20.0	Shoot	0.089	72.03	66.87	71.70	22.20	98.47	4.91	1.78	13.23
LSD[b]	Shoot	0.012	3.90	15.13	6.95	2.01	5.47	0.71	0.15	2.71
0.1	Root	0.026	12.93	120.73	284.93	1124	86.74	2.09	1.17	23.39
1.0	Root	0.021	18.80	50.30	243.23	1106	78.73	1.85	0.87	16.70
3.0	Root	0.024	25.33	29.93	149.57	768	82.14	1.71	0.75	13.02
8.0	Root	0.020	47.07	26.37	133.63	869	79.25	1.79	0.72	13.34
20.0	Root	0.022	80.77	37.78	116.23	981	76.82	1.72	0.73	13.05
LSD	Root	0.005	4.61	16.95	32.00	225	9.84	0.09	0.06	1.38

[a] Values are means ($n = 3$); concentrations are based on dry weights. [b] $df = 10$; $\alpha = 0.05$.

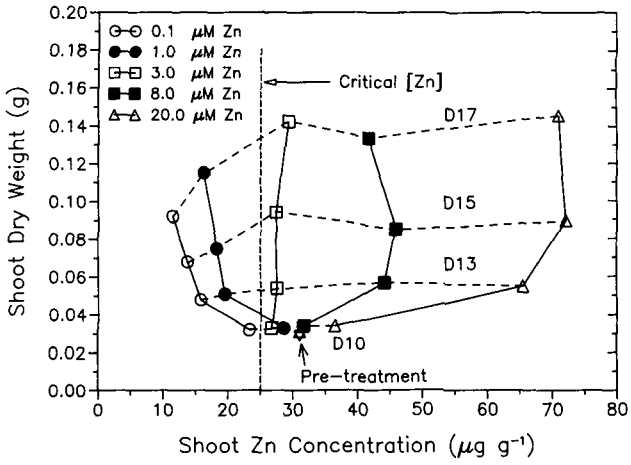

Figure 2. Relationship between dry weight and Zn concentration in shoots of barley seedlings harvested on days 10, 13, 15, and 17. The vertical dashed bar represents the approximate critical Zn concentration for young barley shoots (Reuter, 1986). The starred data point represents barley shoot characteristics at the onset of Zn treatments (day 7).

reduced shoot growth rate, shortened internodes, decreased leaf size, and leaf chlorosis with pale green stripes (Marschner, 1986). In barley, Zn deficiency is noted particularly by growth inhibition, especially of younger leaf blades, and pale green chlorotic stripes on leaf blades. In the studies reported here, these deficiency symptoms first occurred 13 d after grain imbibition in seedlings receiving the 0.1 and 1 μM Zn-HEDTA treatments. The older leaves of these low-concentration Zn treatments developed increasingly more severe chlorosis and necrotic spots during the course of the experiment. Symptoms were most severe in seedlings grown in 0.1 μM Zn-HEDTA, the lowest Zn concentration treatment. The symptoms were typical of those reported for Zn deficiency or P toxicity in barley (Loneragan et al., 1979), as discussed below. None of these symptoms were observed in seedlings grown in solutions containing 3 μM or more Zn-HEDTA.

Zn appears to play a special role in regulating the accumulation of mineral nutrients in barley seedlings. When Zn^{2+} activities were near or below the p(Zn^{2+}) critical value of 10.5 (i.e. about 3×10^{-11} M), the accumulation of a number of mineral elements in barley organs was affected. Increasing Zn^{2+} activity significantly increased shoot concentrations of Cu and significantly reduced the shoot concentrations of Mn, Ca, Mg, and P but had no significant effect on K or Fe shoot concentrations. These effects of Zn treatment are shown in Table II for seedlings harvested on day 15. Zn supply had the greatest effect on Zn, P, and Mn concentrations in the shoots. The effects of Zn supply on day 17 were similar, but considerably more P and Mn had accumulated in shoots (Fig. 3). In the low-concentration Zn treatments, the accumulation of P and Mn and the suppression of Cu in shoots were apparent on day 13, before effects on dry weight were observed. These changes provided the earliest index of Zn deficiency in these solutions. (Note that P accumulation was undoubtedly promoted by the moderately high P concentration in these nutrient solutions, 600 μM.)

Figure 4 depicts the effects of increasing Zn^{2+} activity on the shoot concentrations of water extractable Cl^-, $H_2PO_4^-$, NO_3^-, and SO_4^{2-} in 17-d-old barley seedlings. With the exception of NO_3^-, all of these anions decreased in concentration with increasing Zn^{2+} activity, especially $H_2PO_4^-$. The NO_3^- concentration in shoots appeared to increase with increasing Zn^{2+} activity, but this conclusion may not be reliable because of the possibility of NO_3^- reduction to NO_2^- during the anion extraction procedure.

Some of the effects of Zn supply on concentrations of Cl^-, $H_2PO_4^-$, and SO_4^{2-} in shoots of 17-d-old barley seedlings (Fig. 4) can be attributed to "growth dilution," i.e. a reduction in the amount of nutrients accumulated per unit mass of plant material when dry matter production is stimulated by overcoming a growth limitation. This accounts for much of the decrease in Cl^- and SO_4^{2-} but not for the much larger

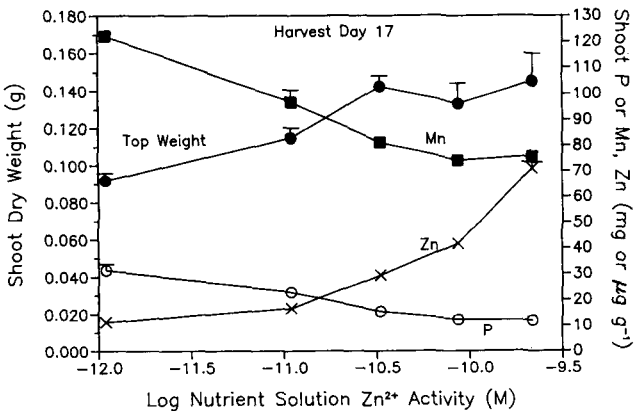

Figure 3. Effects of increasing nutrient solution Zn^{2+} activity on the dry weight and Zn, Mn, and P concentrations in shoots of 17-d-old barley seedlings grown in one-quarter concentration nutrient solutions. Zn was supplied as Zn-HEDTA. Error bars depict +SE ($n = 3$).

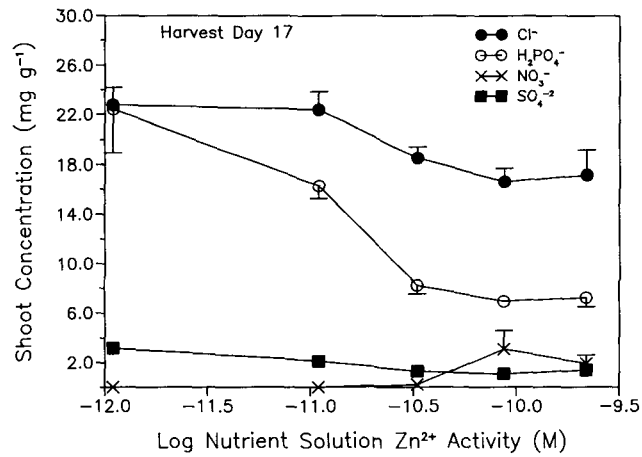

Figure 4. Effects of increasing nutrient solution Zn^{2+} activity on the concentration (dry weight basis) of Cl^-, $H_2PO_4^-$, NO_3^-, and SO_4^{2-} in shoots of 17-d-old barley seedlings. Zn was supplied as Zn-HEDTA. Error bars depict +SE ($n = 3$).

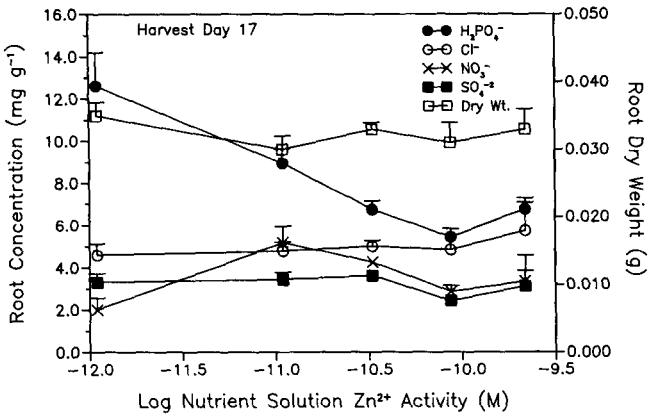

Figure 5. Effects of increasing nutrient solution Zn^{2+} activity on the concentration (dry weight basis) of Cl^-, $H_2PO_4^-$, NO_3^-, and SO_4^{2-} in roots of 17-d-old barley seedlings. Zn was supplied as Zn-HEDTA. Error bars depict +SE ($n = 3$).

changes in $H_2PO_4^-$. The reasons for the apparent increase in NO_3^- concentrations in barley shoots with increasing Zn supply are not known. However, Cakmak and Marschner (1990) also reported that NO_3^- uptake was depressed in Zn-deficient plants, and they speculated that Zn could be required for the biosynthesis of membrane proteins involved in root-cell NO_3^- absorption.

Roots of 15-d-old barley grown in concentrations of Zn-HEDTA below 3 μM [i.e. $p(Zn^{2+}) > 10.5$] accumulated higher concentrations of Mn, Fe, Cu, Ca, Mg, K, and P than did roots of seedlings supplied higher concentrations of Zn-HEDTA (Table II). The largest increases were shown for Fe, Mn, and P. Root concentrations of Ca, Cu, Mg, and Fe were fairly stable when the Zn-HEDTA concentration exceeded 3 μM [i.e. $p(Zn^{2+}) < 10.5$]. However, concentrations of Mn in roots were decreased significantly by increases in Zn-HEDTA throughout the entire range of treatments.

The effect of increasing Zn supply on lowering the concentration of these elements in roots cannot be attributed to changes in root mass resulting from Zn treatment because Zn supply did not significantly affect root weight (Table II, Fig. 5). Nor can the depression in root cations be explained by competitive ion exchange with Zn^{2+}, because the amounts of accumulated Zn are inadequate. Specific effects of Zn on synthesis of ligand groups that bind Fe, Mn, and Cu in the apoplasm (i.e. within the root cell-wall matrix) cannot be excluded, but to our knowledge there is no evidence for this possibility. Also, the concentrations of free micronutrient cations available for binding to reactive sites in the root apoplasm were extremely low because of the presence of HEDTA (Fig. 1). Therefore, we conclude that the differences we observed in mineral nutrient cation concentrations in barley roots are the result of altered absorption of cations by root cells.

Zn-deficient roots also accumulated much higher concentrations of water-soluble $H_2PO_4^-$ and somewhat more SO_4^{2-} (Fig. 5) in comparison with plants receiving adequate Zn. In contrast, concentrations of Cl^- and possibly NO_3^- were lower in roots of Zn-deficient seedlings. These observations of anion accumulation by barley roots provide added support for the contention that Zn plays a role in root-cell ion absorption processes in general. Other investigators have also reported that Zn supply affects the accumulation of mineral nutrients by higher plant roots (Loughman et al., 1982; Kumar et al., 1986; Graham et al., 1987; Kennedy and Bonsalves, 1987; Schwartz et al., 1987; Cakmak and Marschner, 1990; Kochian, 1991).

Figure 6 shows the effects of increasing Zn supply on the accumulation of water-extractable Na by 17-d-old barley seedlings. Increasing the Zn^{2+} activity from about 0.1×10^{-11} M to 3×10^{-11} M depressed Na^+ concentrations in the shoots and increased Na^+ concentrations in the roots. Increasing Zn^{2+} activity above 3×10^{-11} M had no further significant effect on Na concentrations in either roots or shoots of barley seedlings. In shoots, growth dilution in response to Zn accounts for only part of the decrease in Na concentrations, and in roots, retention of Na was clearly increased when Zn supply was adequate. These results suggest the possibility of a protective role for Zn^{2+} in modifying the absorption of Na^+ by roots or the transport of Na^+ from roots to shoots. Our data support reports that increasing Zn supply may decrease the accumulation of Na by higher plants (Shukla and Prasad, 1974; Bilski, 1990; Saxena and Rewari, 1990). Correction of Zn deficiencies in crops growing in arid soils may have a dual benefit in that crops may also suffer from salinity injury in some of these soils.

Zn^{2+} Activity Required for Optimum Nutrient Accumulation and Growth

Zn deficiency results in several physiological changes in higher plants (Hewitt, 1979). Among these changes are disturbances in the absorption and translocation of nutrients (Welch et al., 1982; Römheld and Marschner, 1991). Our results provide clear evidence for such disturbances in the accumulation of P, Mn, Fe, Cu, and Na by shoots or roots of barley. P accumulated to especially high concentrations in shoots of Zn-deficient plants (Table II, Figs. 3 and 4), reaching

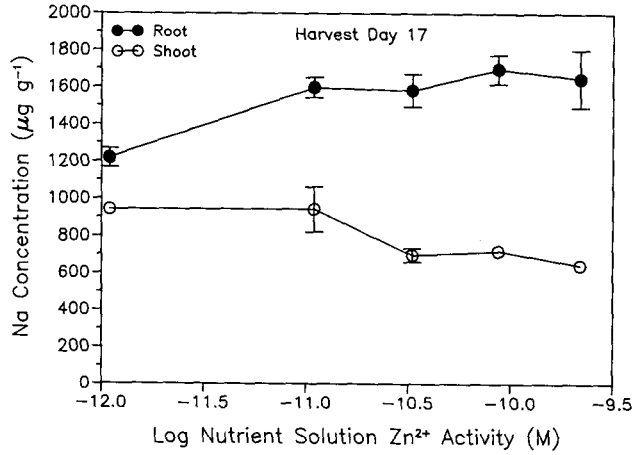

Figure 6. Effects of increasing nutrient solution Zn^{2+} activity on the concentration of Na in shoots and roots of 17-d-old barley seedlings. Error bars represent ±SE ($n = 3$).

levels >15 mg g^{-1}, which are usually considered to be toxic (Loneragan et al., 1979). The accumulation of excess P in shoots appeared to begin even at $p(Zn^{2+}) = 10.1$, suggesting that a higher level of Zn^{2+} may be required for regulation of ion accumulation than is needed for optimum shoot growth. However, Parker et al. (1992) reported that the older leaves of two tomato (*Lycopersicon esculentum* L.) cultivars accumulated excess P only at free Zn^{2+} activities that were lower than that needed for best growth.

Our results, shown in Table II and Figures 1 to 6, indicate that the activity of Zn^{2+} in nutrient solution required for optimum growth of barley was in the range of $p(Zn^{2+}) = 10.5$ to 10.1 and probably close to 10.5. This very low activity of Zn^{2+} ions, when maintained at the root surface, apparently supplies enough Zn to meet the needs of the plant. Others have reported similar requirements for several plant species grown in solution culture (Parker et al., 1993a). Laurie et al. (1991) estimated that the activity of Zn^{2+} required in solution for "healthy" growth of barley seedlings was between $p(Zn^{2+})$ of 10 and 11. Bell et al. (1991) found $p(Zn^{2+})$ of 9.8 adequate for normal growth of barley cv Klages in nutrient solutions containing several different chelating agents. Parker et al. (1992) reported that tomato required $p(Zn^{2+}) < 10.6$ for best growth. Halvorson and Lindsay (1977) estimated that a $p(Zn^{2+})$ of 10.6 was required to prevent Zn deficiency in corn (*Zea mays* L.) grown in solution culture. Apparently, many plant species require a continuing supply of Zn^{2+} at activities in the range of about $p(Zn^{2+}) = 9.8$ to 10.6 to maintain optimum growth and effective control of nutrient absorption and transport.

Our results, and those of others, support the suggestion by Loneragan et al. (1982) that Zn deficiency interferes with the control of ion uptake in a general way. In a companion paper (Welch and Norvell, 1993), we present evidence that Zn plays a role in the maintenance of root cell membranes, as first reported by Welch et al. (1982).

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