The Effect of Citrate and pH on Zinc Uptake by Wheat

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ABSTRACT

Zinc uptake by plants may be influenced by its reaction with organic ligands in the rhizosphere. Therefore, four experiments were conducted to examine the effects of an organic ligand (citrate) and pH on the uptake of Zn by wheat (*Triticum aestivum* L. emend. Thell). Plants were grown for 21 to 28 d in a nutrient solution (containing 0–0.05 μmol L^{-1} Zn) in a temperature controlled tank, either in the absence or presence of citrate and at constant or variable pH (3.7–7.1). Dry matter weights of plant parts and Zn content in the shoots were determined. The activities of Zn in the nutrient solution were estimated. Shoot dry matter of the wheat plants in each experiment correlated well with either $Zn_T/[H^+]$ or Σ(moles Zn_i/charge)/$[H^+]$ (Zn_T = total Zn, Zn_i = each zinc species). Both parameters could explain the relative shoot dry matter of the plants from all experiments in one equation ($y = A + BE^{-cx}$; $r^2 = 0.79$ and 0.77, respectively). In the absence of citrate, shoot dry matter as well as Zn content increased with increasing pH and increasing total Zn concentrations in solution. In the presence of citrate, the shoot dry matter of wheat plants that were grown in nutrient solution with constant pH increased with the total Zn concentrations. However, the effect of the total Zn concentrations in the solution containing citrate with variable pH were less important than the effect of pH.

ZINC ABSORPTION BY PLANTS usually increases with Zn concentration in solution (Carroll and Loneragan, 1968). However, other factors such as the presence of organic ligands and pH may also affect uptake (Chaudhry and Loneragan, 1972; Halvorson and Lindsay, 1977). Zinc absorption by plants usually decreases as the concentration of H^+ increases presumably because of the direct effect of H^+ toxicity and because of an indirect effect of competition between Zn^{2+} and H^+ ions for uptake sites on the root surface (Chaudhry and Loneragan, 1972). The effect of pH may be modified by the presence of organic ligands. In the presence of DTPA (diethylenetriamine pentaacetic acid) the uptake of Zn by maize was lower at pH 7.5 than at pH 5.2 (Halvorson and Lindsay, 1977). Ethylenediaminetetraacetic acid (EDTA) also depressed the uptake of Zn at pH 7.5 but to a lesser extent. Both synthetic ligands complex Zn more strongly at pH 7.5, and hence it was thought this was the cause of decreased uptake. However, the charge of the metal complex may have also influenced the extent of uptake. DeKock and Mitchell (1957) found that uptake of metal ions decreased as the charge of the complex with EDTA and DTPA increased.

Several different types of organic ligands exist in the rhizosphere (Stevenson and Ardakani, 1972) and some of these have been shown to increase the concentration of Zn in the soil solution (Chairidchai and Ritchie, 1990). There is only limited information on the effect of naturally occurring organic ligands on Zn absorption by plants. Hence, the objective of these experiments was to study the Zn absorption by wheat plants in the presence and absence of citrate at various pH values.

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MATERIALS AND METHODS

Four experiments were conducted to examine the effects of an organic ligand (citrate) and pH on the uptake of Zn by Gamenya wheat grown in a nutrient solution (Table 1) at 20 ± 1°C. Preliminary experiments indicated that the nutrient concentrations in Table 1 were adequate for plant growth.

Experiment 1

We investigated the hypothesis that increasing pH increases Zn uptake by wheat and the extent of the increase depends on the concentration of total soluble Zn (Zn_T). A complete factorial design was used to study the growth of wheat in nutrient solution containing three total soluble concentrations of Zn at 3 pH values (Table 2). All treatments were in triplicate.

The seeds were germinated at 20 ± 1°C on a wire mesh suspended above an aerated solution containing 250 μmol L^{-1} $Ca(NO_3)_2$, 2 μmol L^{-1} H_3BO_3 and 4 μmol L^{-1} FeEDTA. Calcium and B are essential for root growth because they maintain the integrity of the root membrane (Haynes and Robbins, 1948). When the first leaf was fully open (7–10 d), three wheat plants were transferred to each 2.5-L pot containing an aerated, complete nutrient solution (Table 1). Zinc was supplied as pure metal dissolved in 0.27 *M* HCl. Other reagents were analytical grade, and macronutrients were purified with dithizone and chloroform (Hewitt, 1952). Nutrient solutions were replaced every 24 h.

Solution pH values from 4.0–5.2 ± 0.05 were created by the addition of 0.1 mol L^{-1} KOH and adjusted to the desired values, once at the time the nutrient solution was replaced and then 10 h later. The amount of K added was always <4% of the amount supplied by the complete nutrient solution.

Experiment 2

We studied the effect of increasing citrate concentration on the uptake of Zn by wheat at a constant pH of 5.5 ± 0.1 (Table 2). The experiment was an incomplete factorial of three total soluble concentrations of Zn and three concentrations of citrate. The experimental preparation and method were the same as in experiment 1, except a plant density of five plants per 5 L pot was used and pH was controlled at pH 5.5 ± 0.1 by the presence of 1 mmol L^{-1} MES (2-[*N*-morpholino] ethane sulphonic acid) as described by Ewing and Robson (1990). Citrate was added as the potassium salt. The nutrient solution

Table 1. Concentrations (μmol L^{-1}) of basal nutrients in wheat growth experiments.

Nutrients	Exp. 1, 3, and 4	Exp. 2
Macro	—— Concentration μmol L^{-1} ——	
NH_4NO_3	100	–
$Ca(NO_3)_2.4H_2O$	500	400
KH_2PO_4	20	20
K_2SO_4	200	150
$MgCl_2.6H_2O$	100	100
$CaCl_2$	–	100
$(NH_4)_2SO_4$	–	50
Micro		
H_3BO_3	2	2
$CuSO_4.5H_2O$	0.1	0.1
$MnSO_4.H_2O$	0.25	0.25
$FeSO_4$	4	4

Table 2. Treatments imposed in Exp. 1–4.

Experiment no.	Treatments: Total Zn	Total citrate	pH	Zn Speciation†: Zn^{2+} (×10³)	$ZnOH^+$ (×10⁶)	$Zncit^-$ (×10³)	Σ(μmoles‡ Zn_i/charge) (×10³)
	μmol L⁻¹			μmol L⁻¹			
1	0.008	0	4.0	7.67	1.91	—	3.84
		0	4.6	7.67	7.77	—	3.85
		0	5.2	7.64	29.54	—	3.85
	0.015	0	4.0	14.4	3.66	—	7.20
		0	4.6	14.4	14.64	—	7.21
		0	5.2	14.4	47.39	—	7.23
	0.050	0	4.0	48.0	12.21	—	24.0
		0	4.6	48.0	43.21	—	24.0
		0	5.2	47.9	139.9	—	24.1
2	0.004	0	5.5	3.81	24.63	—	1.93
	0.008	0	5.5	7.62	49.26	—	3.86
		19	5.5	3.72	23.98	4.00	5.88
	0.015	0	5.5	14.3	92.36	—	7.24
		16	5.5	7.60	49.04	6.85	10.7
		50	5.5	3.68	23.76	10.9	12.7
3	0.004	0	4.7	3.84	3.82	—	1.92
	0.008	0	4.7	7.67	7.12	—	3.84
		25	4.5	6.17	3.66	1.21	4.30
	0.015	0	4.6	14.1	12.13	—	7.21
		20	4.5	11.9	7.45	2.01	7.97
		65	4.0	12.4	2.53	1.08	7.30
4	0.015	0	4.7	14.4	13.26	—	7.21
		0	4.5	14.4	8.89	—	7.20
		0	3.7	14.4	1.45	—	7.20
		65	6.3	2.29	102.7	12.5	13.71
		65	6.9	2.18	337.6	12.4	13.80
		65	7.1	2.13	587.5	12.2	13.30

† Actual Zn concentrations have been multiplied by either 10³ or 10⁶.
‡ Σ(the concentration of each Zn species divided by its charge)

was replaced every 72 and 48 h in the first and second weeks of the experiment, respectively, and every 24 h thereafter.

Experiment 3

An incomplete factorial design was used to study the effect of citrate on Zn uptake by wheat at acidic pH values (4.3–4.7 ± 0.05) and three total concentrations of Zn considered to be deficient, moderately deficient and adequate for plant growth (Table 2). The experimental procedures were the same as in Exp. 1 except the plant density which was five plants per 5 L pot. Citric acid was the source of the citrate and pH variation; Zn was supplied as $Zn(NO_3)_2$.

Experiment 4

The effect of a wide range of pH (4.3–7.1) on Zn uptake by wheat in the presence and absence of citrate was studied using an incomplete factorial design (Table 2). The Zn concentration used was considered adequate for plant growth in the absence of citrate. The pH values were adjusted to the desired values ± 0.05 (Table 2) with 5 mL of 0.05, 0.1, or 0.15 mol L⁻¹ KOH or 0, 0.01, or 0.15 mol L⁻¹ HCl. Sources of Zn and citrate were the same as in Exp. 3. The experimental preparation and methods were the same as in experiment 1 except the plant density which was five plants per pot.

Plant Analysis

The plants in each of the experiments were harvested at 21 d, except for Exp. 2, in which the plants were harvested at 28 d. The total fresh weight of the plants was measured, and shoot and root dry weights determined after drying at 70 ± 5°C for at least 72 h. The shoots were digested in HNO_3 and $HClO_4$ (Johnson and Ulrich, 1959), and the concentration of Zn in the digest was determined by atomic absorption spectrophotometry (Perkin Elmer 5000, Wilton, CT).

Table 3. Experiment 1. The effects of total Zn concentration in solution [Zn_T], and pH on dry weight, Zn concentration and Zn content of wheat shoots, root fresh weight and the root/total fresh weight ratio.

Treatments: [Zn_T]	pH	Shoots: dry wt.	Zn conc.	Zn content	Root fresh wt.	Root/ Total fresh wt.
μmol L⁻¹		g plant⁻¹	μg g⁻¹	μg plant⁻¹	g plant⁻¹	
0.008	4.0	0.036	14.10	0.50	0.41	0.65
	4.6	0.070	15.32	1.07	0.65	0.52
	5.2	0.106	14.91	1.60	0.68	0.42
0.015	4.0	0.046	17.40	0.80	0.45	0.58
	4.6	0.097	15.47	1.49	0.67	0.46
	5.2	0.175	13.67	2.40	0.88	0.39
0.05	4.0	0.070	21.37	1.47	0.37	0.39
	4.6	0.106	17.95	1.90	0.69	0.45
	5.2	0.177	17.75	3.14	0.92	0.40
		Analysis of Variance				
[Zn_T]		***	***	***	NS	***
pH		***	NS	***	***	***
[Zn_T] × pH		*	NS	NS	NS	***

*,*** Significant at the 0.05 and 0.001 probability levels, respectively, NS, Not significant.

Speciation Calculations

The activities of the free Zn ion (Zn^{2+}), hydrolysed Zn ($ZnOH^+$) and Zn citrate complex ($Zncit^-$) (Table 1 and 3) in the nutrient solutions were calculated with a chemical equilibrium program TITRATOR (Cabaniss, 1987) and the thermodynamic stability constants at 20°C given by Lindsay (1979)

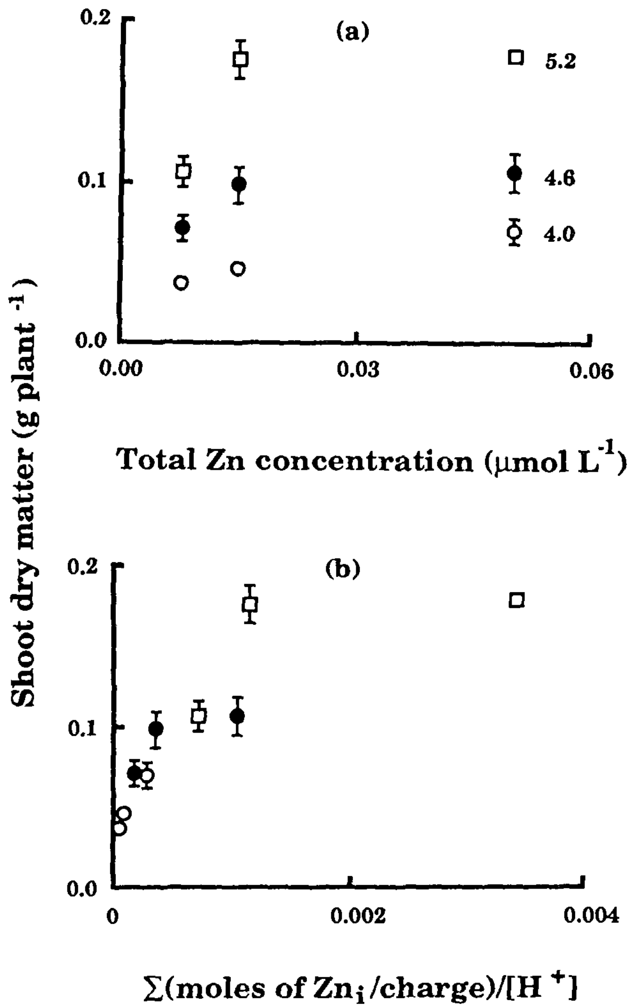

Fig. 1. The relationship between (a) total Zn concentration or (b) Σ(moles Zn_i/charge)/[H^+] and shoot dry matter of wheat plants at pH 4.0 (○), 4.6 (●) and 5.2 (□).

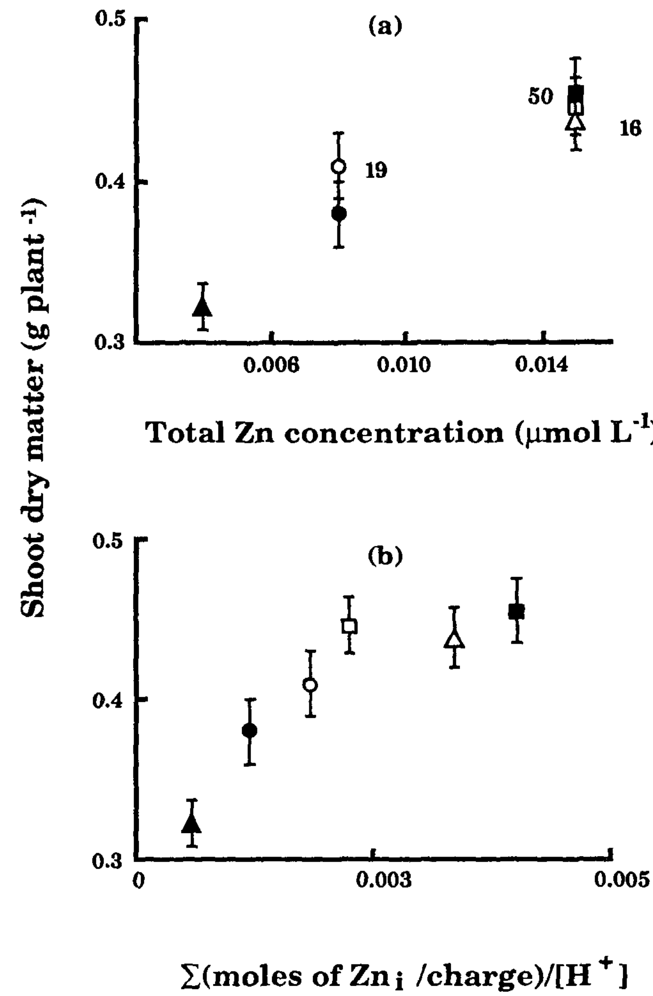

Fig. 2. The relationship between (a) total Zn concentration or (b) Σ(moles Zn_i/charge)/[H^+] and shoot dry matter of wheat plants in the absence (▲, ●, ■) or presence of 16 (△), 19 (○) or 50 (□) µmol L^{-1} of citrate at a constant solution pH of 5.5.

and Martell and Smith (1977). The calculations included reactions between all cations and anions in Table 2 as given in Lindsay (1979). Activity coefficients were calculated with the Guntelberg equation. Carbon dioxide concentrations were assumed to be equivalent to the atmospheric concentration because the solutions were continually aerated.

Statistical Analysis

The data were analyzed by estimating standard errors, Analysis of Variance, simple linear and non-linear regression ($y = A + Be^{-cx}$), and stepwise multiple linear regression.

RESULTS

Experiment 1

Zinc deficiency symptoms first occurred on Day 13 on the youngest fully expanded blade of the plants grown with 0.008 µmol L^{-1} Zn at pH 5.2. The symptom first appeared as a bruise patch in the middle of the leaves. One to 2 d later this area became necrotic and the necrotic area expanded to cover approximately 50% of the leaf area at harvest.

The plants grown in solutions at the other pHs at 0.008 µmol L^{-1} Zn started developing deficiency symptoms 2 d later. The symptoms in these plants were more severe than at pH 5.2 and the plants were stunted. Wheat grown in 0.015 µmol L^{-1} Zn at pH 4.6 developed symptoms on the same day, but the symptoms were not as severe. By harvest time only plants at 0.015 and 0.05 µmol L^{-1} Zn at pH 5.2 were without symptoms.

The shoot dry matter content of the plants increased asymptotically with Zn supply and pH (Fig. 1a). Maximum growth occurred at 0.015 µmol L^{-1} Zn at pH 5.2 and 4.6 and at 0.05 µmol L^{-1} Zn for pH 4.0. The increase in yield was 2.5 times greater at pH 5.2 than at pH 4.0. The difference in shoot dry matter could not be explained by a single effect of either the concentration in solution of Zn_T, Z, or H^+. However, there were good asymptotic correlations between shoot dry matter and $ZnOH^+$ (r^2 = 0.84), Zn_T/[H^+] (r^2 = 0.85) and the ratio of the sum of moles per charge of Zn species (Zn_i) and H^+, Σ(moles Zn_i/charge)/[H^+] (r^2 = 0.85, Fig. 1b).

The Zn concentration of the shoots increased with Zn supply (Table 3). At a constant supply of Zn, the Zn concentration of the shoots did not vary significantly with

Table 4. Experiment 2. Dry weight, Zn concentration, and Zn content of wheat shoots at various concentrations of Zn, $[Zn_T]$, and citrate, $[cit_T]$, in solution at pH 5.5.

Treatments		Shoots†		
$[Zn_T]$	$[cit_T]$	dry wt.	Zn conc.	Zn content
—— μmol L^{-1} ——		g $plant^{-1}$	$\mu g\ g^{-1}$	μg $plant^{-1}$
0.004	—	0.32 a	17.62	5.75
0.008	—	0.38 ab	18.62	8.98
0.008	19	0.41 bc	16.88	7.25
0.015	—	0.45 bc	20.97	9.65
0.015	16	0.44 c	17.17	7.65
0.015	50	0.46 c	19.48	9.35
		Analysis of Variance		
Treatments		**	NS	NS

** Significant at the 0.005 probability level, and NS, Not significant.
† a, b All values within a column with no common letter are significantly different ($p < 0.005$).

pH ($p < 0.05$). Dry matter yield was not well correlated with the Zn concentration of the shoots. Zinc content increased asymptotically with the ratio Σ(moles Zn_i/charge)/$[H^+]$ and with $Zn_T/[H^+]$ (data not shown).

At a constant concentration of Zn supply, root fresh weight increased with pH in a similar manner to shoot growth (Table 3).

When root fresh weight was expressed as a proportion of total growth on a fresh weight basis, the proportion significantly decreased with Zn_T when pH was held constant (Table 3). The percentage of total fresh weight attributable to roots decreased as the Zn content of the plants increased and reached a minimum value when Zn content was ≥ 1.5 μg per plant (Table 3). A similar trend was also observed when the ratio of root to total fresh weight was plotted as a function of the ratio between the total moles per charge of Zn and H^+, the minimum occurred at the ratio $\geq 5 \times 10^{-3}$.

Experiment 2

At harvest, the plants at 0.004 μmol L^{-1} Zn were stunted. These plants had Zn deficiency symptoms as explained in Exp. 1 However, the symptoms which occurred by Day 20 did not become severe.

The shoot dry matter content of the plants increased with Zn supply (Fig. 2a). However, there was no significant difference ($p < 0.05$) in shoot growth in the presence and absence of citrate at each Zn supply of 0.008 and 0.015 μmol L^{-1} (Table 4). Dry matter yield increased with the ratio of the sum of moles per charge of Zn species and H^+ and reached the maximum value at ratio ≥ 0.003 (Fig. 2b; asymptotic regression, $r^2 = 0.94$). Dry matter yield was also asymptotically correlated with total $Zn_T/[H^+]$ ($r^2 = 0.92$).

Shoot dry matter accounted for 75% of the variation in the Zn content of the shoots. The Zn concentrations of the shoots were similar in all treatments (Table 4).

Experiment 3

The first Zn deficiency symptom appeared on the youngest fully expanded blades of the plants at 0.004 μmol L^{-1} Zn on Day 12, and it was very severe by harvest time. Two to 3 d later the symptoms also appeared on all the plants except those at 0.015 μmol L^{-1} with no citrate. Among these plants, the ones at 0.015

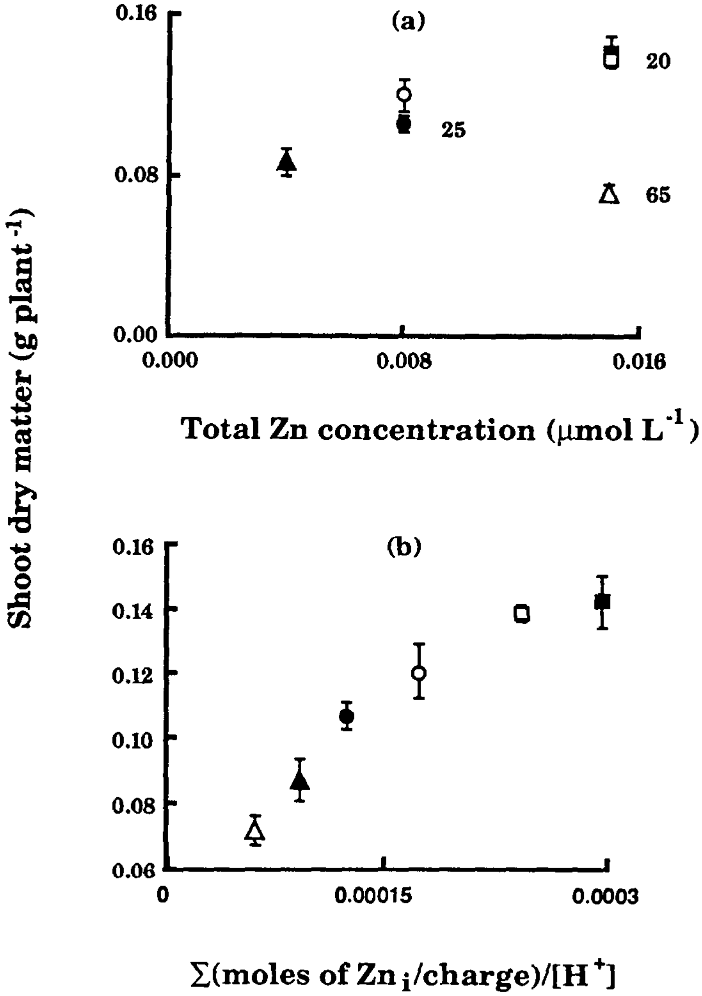

Fig. 3. The relationship between (a) total Zn concentration or (b) Σ(moles Zn_i/charge)/$[H^+]$ and shoot dry matter of wheat plants in the absence (▲, ○, ■) or presence of 20 (□), 25 (●) or 65 (△) μmol L^{-1} of citrate at various pHs.

μmol L^{-1} Zn with 65 μmol L^{-1} citrate became stunted and their roots were very short and produced a great amount of mucilage. The symptoms on the latter plants became as severe as those at lowest Zn concentration.

In the absence of citrate, shoot dry matter increased with total Zn supply (Fig. 3a). At 0.008 and 0.015 μmol L^{-1} Zn, however, dry matter decreased when citrate was added (Table 5). In the presence of 25 μmol L^{-1} citrate at 0.008 μmol L^{-1} Zn, the dry matter decreased slightly, whereas there was a marked decrease ($p < 0.05$) when 65 μmol L^{-1} citrate was added to 0.015 μmol L^{-1} Zn. Dry matter yield was well correlated with either $Zn_T/[H^+]$ ($r^2 = 0.95$; $y = A + Be^{-cx}$) or the ratio between the sum of moles per charge of Zn species and H^+ (r^2 ($r^2 = 0.98$; $y = A + Be^{-cx}$; Fig. 3b). None of the Zn species in solution could adequately predict dry matter yield by fitting a single equation to a plot of yield versus the concentration of an individual Zn species. The combined effect of Zn^{2+}, $Zncit^-$, and H^+ however could account for 88% of the variation in the dry matter content by multiple regression.

In the absence of citrate, Zn content and concentration of the plants both increased with Zn supply (Table 5).

Table 5. Experiment 3. Dry weight, Zn concentration and Zn content of wheat shoots at various concentrations of Zn, $[Zn_T]$, and citrate, $[cit_T]$, in solution.

Treatments			Shoots†		
$[Zn_T]$	$[cit_T]$	pH	dry wt.	Zn conc.	Zn content
— (μmol L^{-1}) —			g $plant^{-1}$	$\mu g\ g^{-1}$	μg $plant^{-1}$
0.004	—	4.7	0.09 a	7.01 a	0.63 a
0.008	—	4.7	0.12 bc	8.22 ab	0.99 b
0.008	25	4.5	0.11 b	10.17 bc	1.12 b
0.015	0	4.6	0.142 d	10.32 bc	1.47 c
0.015	20	4.5	0.138 cd	12.10 c	1.67 c
0.015	65	4.0	0.071 a	7.58 a	0.54 a
			Analysis of Variance		
Treatments			***	**	***

,* Significant at the 0.005 and 0.001 probability levels, respectively.
† a,b All values within a column with no common letter are significantly different ($p < 0.005$).

Table 6. Experiment 4. Shoot dry weight, Zn concentration and Zn content of wheat plants grown in the presence (+) or absence (−) of 65 μmol L^{-1} citrate, $[cit_T]$, and 0.015 μmol L^{-1} Zn in solution at various pH.

Treatments		Shoots†		
$[cit_T]$	pH	dry wt.	Zn conc.	Zn content
(μmol L^{-1})		g $plant^{-1}$	$\mu g\ g^{-1}$	μg $plant^{-1}$
0	4.7	0.14 a	12.56 a	1.79 ab
0	4.5	0.20 ab	13.78 a	2.04 b
0	3.7	0.15 a	9.28 bc	0.88 a
65	6.3	0.21 ab	17.48 b	3.58
65	6.9	0.267 b	18.28 b	4.69 c
65	7.1	0.280 b	18.58 b	5.02 c
		Analysis of Variance		
Treatments		NS	***	***

*** Significant at the 0.001 probability level, and NS = not significant.
† a,b All values within a column with no common letter are significantly different ($p < 0.005$).

At a constant concentration of Zn supply the Zn content of the plants decreased when 25 and 65 μmol L^{-1} of the ligand were added to 0.008 and 0.015 μmol L^{-1} Zn treatments, respectively. The presence of the citrate had no effect on Zn concentration in the plants except at the highest addition to the 0.015 μmol L^{-1} Zn treatment, when Zn concentration decreased.

Experiment 4

All plants grown in the absence of citrate produced Zn deficiency symptoms whereas those in the presence of citrate were free of symptoms. The severity of the symptom increased with decreasing pH.

Shoot dry matter of plants grown in the presence of citrate tended to be higher than for those grown in the absence and the difference in growth increased with pH (Table 6). The yield of plants from treatments with pH > 5.5 were greater than anticipated from Exp. 1 (by comparing the ratio of yields at pH 5.2 and 4.6 in Exp. 1 with the ratio of yields at pH 6.3 and 4.6 in Exp. 4). The moles per charge of Zn^{2+} and $Zncit^-$ separately accounted for 72 and 71% of the variation in shoot dry matter. The combined effect of the two Zn species, however, accounted for 87% of the variation. The shoot dry matter also correlated well with the ratio of moles per charge of Zn to H^+ (Fig. 4; $r^2 = 0.79$; $y = A + Be^{-cx}$) and $Zn_T/[H^+]$ ($r^2 = 0.79$; $y = A + Be^{-cx}$).

Both Zn concentration and Zn content in plant shoots were higher in the presence of citrate than in the absence (Table 6). In the presence of citrate, Zn concentrations were similar at all pH values. In the absence of citrate, the concentration increased when pH changed from 3.7 to 4.5 ($p < 0.05$). In the presence of citrate, Zn content increased ($p < 0.05$) as pH rose from 6.3 to 6.9. In the absence of citrate, it increased ($p < 0.05$) when pH changed from 3.7 to 4.5.

All Experiments

The Zn content and relative yield of wheat from Exps. 1 through 4 for data at pH ≤ 5.5 could be explained by a single curve when plotted against the ratio of the sum of moles per charge of Zn species and H^+ (Fig. 5; $r^2 = 0.78$; $y = A + Be^{-cx}$) and when plotted versus $Zn_T/[H^+]$ ($r^2 = 0.70$; $y = A + Be^{-cx}$). Relative yield was estimated by assuming that maximum yield was obtained

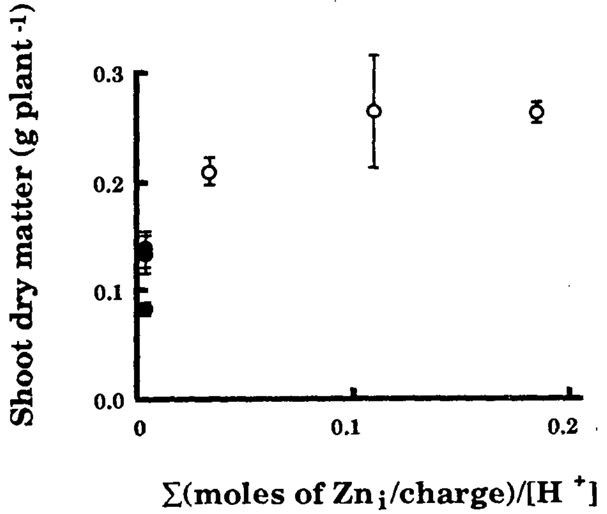

Fig. 4. The relationship between Σ(moles Zn_i/charge)/$[H^+]$ and shoot dry matter of wheat plants in the absence or presence of 65 μmol L^{-1} of citrate at pH 3.7 to 7.1.

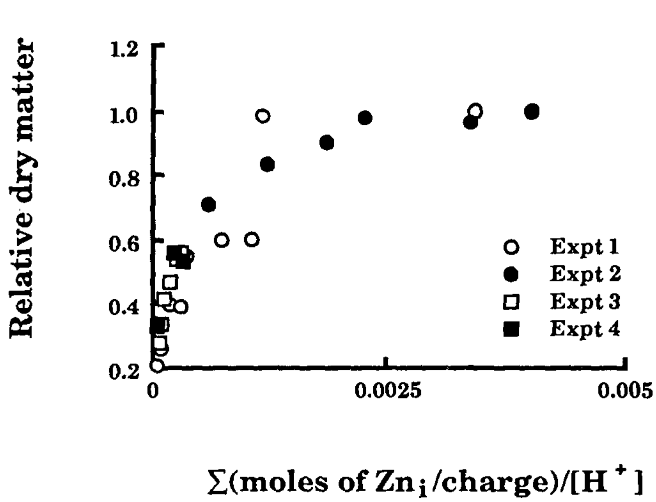

Fig. 5. The relationship between Σ(moles Zn_i/charge)/$[H^+]$ and relative dry matter of wheat plants from four experiments at pH ≤ 5.5.

at pH 5.2 or 5.5 at 0.015 μmol L^{-1} Zn_T and in the absence of citrate. For data from Exps. 3 and 4, maximum yield was estimated by assuming it equalled the yield at 0.015 μmol L^{-1} Zn_T and pH 4.6 or 4.7 divided by 0.557. The fraction 0.557 was estimated from the yield at pH 4.7 expressed as a fraction of the yield at pH 5.2 in Exp. 1 for a Zn supply of 0.015 μmol L^{-1}.

DISCUSSION

Plants were able to take up Zn that was initially complexed with citrate. Plant growth and Zn uptake at pH $\leq$ 5.5 was affected by pH, the Zn supply and possibly the form of Zn species in solution. At pH > 6.3 another unidentified factor affected Zn uptake.

Zinc Uptake in the Presence of Citrate

In the presence of citrate, the correlation between Σ(moles Zn_i/charge)/[H^+] and plant growth suggested that the number of charges on the species may affect the plants ability to take up Zn. In the presence of citrate there were more moles per charge of Zn because more Zn was in the form of single-charge species ($Zncit^-$). Further experiments (Similar to Exp. 2) would have to be conducted to confirm this hypothesis. A bigger variation in $Zncit^-$ would be necessary as well as a constant solution pH and possibly a lower Zn supply.

The ratio Zn_T/[H^+] was well correlated with plant growth possibly because the effect of pH on uptake in Exps. 3 and 4 was greater than the effect of citrate and hence the effect of citrate on Zn uptake was overshadowed by the pH effect. In Exp. 2, the high correlation with Zn_T/[H^+] was partially a consequence of the regression being fitted through only three values of x (two of the six and three of the six x values were the same). In the case of the correlation between plant growth and Σ(moles Zn_i/charge)/[H^+], all six x values were evenly spread along the fitted curve and hence no individual point had a marked effect on the r^2 value (Fig. 2).

The importance of the charge of a metal chelate on its absorption by plants has been suggested by DeKock and Mitchell (1957). The effect of charge characteristics of Cu chelate on the uptake of the element from nutrient solutions has also been shown for Italian ryegrass (*Lolium multiflorum* Lam.) and red clover (*Trifolium pratense* L.) (Iwasaki and Takahashi, 1989). On the other hand, the total concentration in soil solution extracts of micronutrients such as Zn and Cu was shown to be less important as far as the uptake by plants was concerned, particularly in the presence of a ligand (Halvorson and Lindsay, 1977; Minnich et al., 1987).

Zinc Uptake in the Absence of Citrate

The growth of wheat was controlled by Zn supply, the direct effect of H^+ on yield and the effect of H^+ on Zn uptake and hence yield. The concentration of total Zn in solution was not a good indicator of dry matter yield unless the pH of the nutrient solution was also considered.

At a constant concentration of 0.008 μmol L^{-1} Zn, plant growth was affected by all three factors (i.e. Zn supply, H^+, and H^+ effects on Zn uptake). At 0.015 μmol L^{-1} Zn, H^+ decreased plant growth directly by decreasing Zn uptake whereas at 0.05 μmol L^{-1} Zn the effect of H^+ on Zn uptake was decreased by the luxury supply of Zn and hence variations in wheat growth were mainly related to the direct effects of H^+ on yield. Increasing the pH from 4.0 to 4.6 had a greater effect on yield at a Zn supply of 0.015 μmol L^{-1} than at 0.05 μmol L^{-1} because two factors were limiting yield in the former case whereas mainly one factor was limiting yield at 0.05 μmol L^{-1}. Nevertheless, the symptoms indicated that H^+ was still limiting Zn uptake at the two lower pH values even when there was a luxury supply of Zn. The increase in yield when the pH increased from 4.0 to 4.6 at a Zn supply of 0.015 μmol L^{-1} is greater than at 0.008 μmol L^{-1} because in the latter situation the plants are still restricted by an inadequate supply of Zn.

At pH 5.2 and Zn supplies greater than 0.008 μmol L^{-1} neither pH nor Zn supply were limiting growth. However, at 0.008 μmol L^{-1}, even though plants grown at this pH grew better than the plants at the other two lower pHs, they showed symptoms of Zn deficiency first. Dilution of Zn in the plants appeared to have occurred and been responsible for the earlier symptoms.

The free ion, Zn^{2+}, has been suggested as the available form of Zn (Halvorson and Lindsay, 1977). However, in this present study, we could not correlate Zn^{2+} with the shoot dry matter, Zn content, or Zn concentration in the plants. This phenomenon may be partially attributable to an overriding influence of pH on Zn uptake.

The success of the introduction of the effect of H^+ into the regression as a divisor factor to Zn_t or the total moles per charge of Zn_i is in agreement with the competition effect of H^+ as described earlier by Chaudhry and Loneragan (1972).

Root Growth

The response of the roots to Zn supply appeared to be pH dependent. At low pH (i.e. pH 4.0 and 4.6) when the toxicity effect of H^+ was greatest, the roots did not respond to an increasing concentration of Zn_T. On the other hand, when the H^+ toxicity was absent at pH 5.2, there was an increase in root growth with an increasing concentration of Zn_T. Shoot growth as influenced by Zn_T appeared to be independent of root growth because it responded to an increasing Zn_T despite the absence of a growth response by the roots.

Hydrogen ions did not affect the plants' ability to take up Zn as much as they affected the plants' ability to grow shoots and roots. The concentration of Zn in the plants grown at a deficient supply did not differ as pH increased even though shoot and root growth increased. On the other hand, the Zn concentration in plants decreased as pH increased at adequate and luxury supplies of Zn because Zn uptake was not hindered by H^+ at the lower pH values to the same extent as shoot and root growth.

Root/Total Growth Ratio

At each pH, the root/total growth ratio was highest at the lowest Zn supply. This may indicate that the plants acquired as much as possible when the supply was low in order to maintain their internal concentration. When Zn supply became more abundant, plants then developed more shoot and hence the ratio went down. Carroll and

Loneragan (1968) also found a greater root/shoot ratio at lower Zn supply in wheat and several other plants.

These results indicated the presence of organic ligands such as citrate in the rhizosphere may affect Zn uptake by wheat, but the effect will depend on pH and Zn supply.

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