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Effects of zinc on cadmium uptake by spring wheat (*Triticum aestivum*, *L.*): long-time hydroponic study and short-time ^{109}Cd tracing study*

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Abstract: To investigate effects of Zn on Cd uptake by spring wheat (*Triticum aestivum*, *L.*) in solution culture, long-time hydroponic experiment (1 month) (Experiment 1) and short-time Cd isotope (^{109}Cd) tracing experiment (24 h) (Experiment 2) were conducted. In Experiment 1, spring wheat (cv. Brookton) was grown in nutrient solution at uniform cadmium concentration of 20 μmol/L and 10 zinc concentrations (0, 1, 5, 10, 20, 100, 200, 500, 1000, 2000 μmol/L). In Experiment 2, spring wheat seedlings, pre-cultivated in complete nutrient solution, were treated with ^{109}Cd of uniform activity and the same series of Zn concentrations as those in Experiment 1 for 24 h. Cd concentrations in shoots and roots in Experiment 1 increased marginally but not consistently with Zn increasing at Zn rates of 1~200 μmol/L, and then decreased significantly at high rates (>200 μmol/L). In Experiment 2, the response of ^{109}Cd activities in shoots and roots to increasing Zn was greatly similar to the response of Cd concentrations to Zn increasing in Experiment 1. The results of the two experiments indicated that the short-time and long-time exposure of spring wheat to Zn had similar effects on Cd accumulation.

Key words: Spring wheat, Effects of Zn, Cd uptake, ^{109}Cd tracing
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INTRODUCTION

Cadmium is a probably nonessential element to human and animal, and is also a highly toxic heavy metal which can be absorbed readily by crops and accumulated in human body through the food chains and result in human body health problems (Schroeder, 1965; Basta *et al.*, 1998). With the addition of Cd-containing manures, fertilizers, sewage, biosolids and other soil supplements, Cd contamination exists in many agricultural soils, and is becoming serious and large in area. So how to minimize the concentration of Cd in plants has become a pressing problem for resolution. Many factors such as soil pH, soil redox potential, cation exchange capacity, plant species, and fertilizer (nitrogen, phosphorus, potassium, zinc, etc.) application affect Cd transport in soil-plant system (Chaney and Hornick, 1978). Zinc, because of its chemical similarity to Cd, has long been a concern due to its effect on Cd uptake by plants (Chaney *et al*., 1976; Abdel-Sabour *et al*., 1988; Oliver *et al*., 1994; Welch *et al*., 1999; Nan *et al*., 2002). But the results are unconclusive or contradictory, and the mechanisms are not known yet. In the present project, two experiments were conducted: long-time hydroponic culture with a large range of Zn levels for studying Zn's effects on Cd accumulation in spring wheat; and short-time Cd isotope (^{109}Cd) tracing for studying the short-time effects of Zn (in the same range) on Cd uptake by spring wheat.

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MATERIALS AND METHODS

Experiment 1: long-time hydroponic experiment

1. Plant culture

Seeds of spring wheat (cv. Brookton), were sterilized in the solution ($\rho(H_2O_2)$=100 g/L) for 10 min followed by thorough washing in deionized water, then allowed to germinate on moist filter paper for 2 d. Geminated seeds were planted in moist perlite, and grown for about 6 d. Then the seedlings were removed from the perlite, and washed thoroughly and carefully under tap water to get rid of any adhering particles, then transferred to PVC pots containing 500 ml nutrient solution (modified Hoagland nutrient solution containing: in mmol/L, KNO_3, 4; $Ca(NO_3)_2$, 4; $MgSO_4$, 1.5; KH_2PO_4, 1.3; in μmol/L, FeEDTA, 50; $CuSO_4$, 1; $MnSO_4$, 5; H_3BO_3, 10; Na_2MoO_4, 0.5; $CoSO_4$, 0.19; NaCl, 100). There were 10 treatments for a large range of Zn ($ZnSO_4$) levels (0, 1, 5, 10, 20, 100, 200, 500, 1000, 2000 μmol/L), with each treatment having four replicates. Cd ($CdCl_2$) was added to all the treatments at a uniform rate of 20 μmol/L. The nutrient solution pH was adjusted to 6.0 using 0.1 mol/L KOH. All chemicals used for the experiments were of AR grade. The seedlings were grown in a growth chamber with 14/10 h light/dark cycles. Light intensity was about 280 μmol protons/(m^2·s). The nutrient solution was renewed twice a week and aerated continuously. The pots were randomly arranged several times during the growth period. After four weeks of growth, the seedlings were harvested. Seedlings roots were washed in deionized water, and blotted using rough filter paper, then the seedlings were divided into shoots and roots whose fresh weights were determined immediately. Plant shoots and roots were then oven dried at 70 °C for 48 h, and their dry weights were determined.

2. Plant analysis

Dried shoots and roots were finely ground in a muller. Sub-samples (0.25 g) of the dried ground plant materials were digested at 160 °C in 5 ml high-purity mixed acid (HNO_3:$HClO_4$=6:1). The digest was diluted to 50 ml using high-purity water, and the concentrations of Cd and Zn in the solution were determined by Atomic Absorption Spectrophotometry (Z-6100, Hitachi Co., Japan)

Experiment 2: short-time ^{109}Cd tracing experiment

1. Plant culture

Preculture of spring wheat seedlings was in complete nutrient solution like that in Experiment 1 except that there was no Cd addition and Zn treatment. After ten days of growth, the seedlings were treated by Zn at different levels (same as Experiment 1) for 7 d.

2. Cd isotope (^{109}Cd) tracing

After 7 d of Zn treatment, ^{109}Cd and Cd were added to each of the treatments, all the seedlings were equally exposed to 666 KBq/L ^{109}Cd and 20 μmol/L Cd for 24 h. At the end of the 24 h, the seedlings were removed from the ^{109}Cd-containing nutrient solution, and the seedling roots were immediately dipped thrice into 5 mmol/L $CaCl_2$ solution for 30 min so that all the ^{109}Cd and Cd ions adsorbed on the surface of the roots were exchanged.

3. Plant analysis

After washing by 5 mmol/L $CaCl_2$ solution, the seedlings were separated into shoots and roots and were blotted up. Then the dry weights of shoots and roots were determined immediately. The ^{109}Cd activities in shoots and roots were analyzed by γ spectrometry (Gi-2519+Accuspe, Canberra, USA) in the Institute for Application of Atomic Energy, Chinese Academy of Agricultural Sciences.

RESULTS

Plant shoots and roots dry weights

In Experiment 1, dry weights of shoots and roots were affected significantly by the Zn treatments (p<0.001) (Table 1). At Zn levels of 1~100 μmol/L dry weights of shoots and roots were higher than non-Zn addition (0 μmol/L), and then decreased at high rates (above 100 μmol/L), especially at the highest rate of 2000 μmol/L when the shoot and root dry weights were reduced by almost two fold and by more than three fold. High Zn had more severe inhibiting effect on roots than on shoots. In Experiment 2, the shoot dry weights were unaffected by Zn treatments probably because of the short-time exposure, but the root biomass at high Zn rates (>500 μmol/L) were reduced significantly.

Cd and Zn uptake

Data on plants uptake of Cd and Zn in Experi-

ment 1 are shown in Table 2. Zn concentrations in shoots and roots increased significantly with increasing of Zn levels. Cd concentrations in shoots and roots increased marginally but not consistently with Zn increasing at Zn rates of 1~200 μmol/L. While with the Zn rates increasing (>200 μmol/L), Cd concentrations in shoots and roots decreased significantly, from 142.0 mg/kg without Zn addition to 93.5 mg/kg of 2000 μmol/L in shoots and 2534 mg/kg to 548.1 mg/kg in roots.

^{109}Cd activities

Table 3 shows the ^{109}Cd activities in shoots and roots in Experiment 2. At Zn levels of 1~200 μmol/L, the ^{109}Cd activities in shoots were changed marginally but not consistently (decreased at 1~100 μmol/L and

Table 1 Shoot and root biomass of spring wheat grown in nutrient solution with different levels of Zn (g DW)

Zn concentrations (μmol/L)	Experiment 1		Experiment 2	
	Shoots	Roots	Shoots	Roots
0	0.23±0.01	0.14±0.02	0.17±0.02	0.08±0.01
1	0.40±0.03	0.21±0.01	0.15±0.02	0.11±0.01
5	0.35±0.03	0.16±0.01	0.14±0.01	0.08±0.01
10	0.36±0.05	0.20±0.01	0.15±0.01	0.09±0.01
20	0.34±0.05	0.17±0.02	0.16±0.02	0.12±0.01
100	0.29±0.06	0.16±0.01	0.17±0.01	0.11±0.01
200	0.22±0.01	0.14±0.01	0.14±0.01	0.09±0.01
500	0.22±0.01	0.14±0.02	0.16±0.01	0.11±0.01
1000	0.24±0.01	0.12±0.01	0.13±0.01	0.07±0.00
2000	0.15±0.01	0.04±0.00	0.12±0.02	0.05±0.00
Analysis of variance	p<0.001	p<0.001	Not significant	p<0.001

Table 2 Cd and Zn concentrations in shoots and roots of spring wheat grown in nutrient solution with different levels of Zn (mg/kg)

Zn concentrations (μmol/L)	Cd		Zn	
	Shoots	Roots	Shoots	Roots
0	142.0±7.6	2534±64.7	42.5±7.10	360.0±59.2
1	159.5±6.2	2867±86.9	143.5±4.00	473.5±11.8
5	145.0±13.7	2678±257	212.5±14.7	1770±76.4
10	134.0±10.8	3015±169	288.7±14.5	3293±96.6
20	127.0±4.7	2471±185	482.7±37.0	5595±467
100	150.4±8.2	3028±164	1073±77.0	16109±1774
200	155.4±10.7	3717±174	1384±89.6	24253±2311
500	107.6±8.8	2287±571	2019±222	25308±1740
1000	96. 9±8.3	1058±70.8	3087±210	24671±3017
2000	93.5±2.2	548.1±41.3	7060±549	41509±1470
Analysis of variance	p<0.001	p<0.001	p<0.001	p<0.001

Table 3 ^{109}Cd activities in shoots and roots of spring wheat grown in nutrient solution with different levels of Zn (10^6 Bq/L)

Zn concentrations (μmol/L)	Shoot	Root
0	2.67±0.28	18.38±1.14
1	3.63±0.23	16.84±0.90
5	3.31±0.19	16.51±0.97
10	2.91±0.06	17.16±2.79
20	2.87±0.37	17.66±1.15
100	2.26±0.31	21.12±1.27
200	3.00±0.22	25.55±1.67
500	2.30±0.14	38.28±1.60
1000	2.09±0.32	24.19±1.40
2000	1.28±0.20	12.40±0.42
Analysis of variance	p<0.001	p<0.001

increased at 100~200 μmol/L) with Zn increasing, and then at high rates (>200 μmol/L) were reduced significantly, to less than half that of the control at highest rate of 2000 μmol/L (1.73×10^6 Bq/L vs 2.66×10^6 Bq/L). In roots, the ^{109}Cd activities did not decrease as rapidly as in shoots at high Zn rates. The data indicated that, in short time, the Cd uptake by spring wheat seedlings was similar to Cd uptake in long time in response to Zn supply (Table 2).

Correlation between Cd accumulation for long-time and ^{109}Cd activities for short-time

In shoots, there was significant (r=0.80) positive correlation between Cd accumulation for long time and ^{109}Cd activities for short time at different Zn rates (Fig.1), which indicated that the short time and long time exposure of spring wheat to Zn had similar response to Zn concentrations. Furthermore, The short-time ^{109}Cd tracing is a more direct and precise way for investigating the Cd accumulation in plants, moreover, it is relatively freer from effects of other factors which make the interpretation of plants response to Cd-Zn interaction difficult. So the good regression line suggested the short-time data were also valid for the long-time experiment.

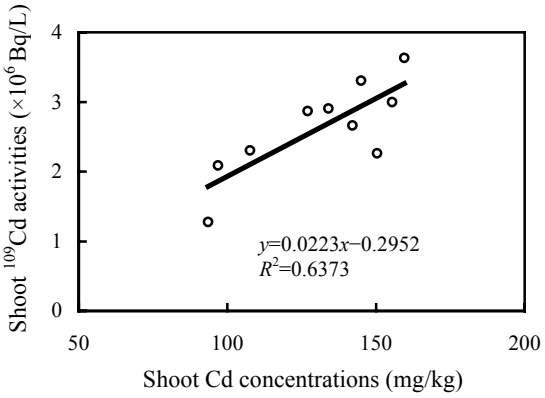

Fig.1 The correlation between Cd accumulation and ^{109}Cd activities in shoots for long-time and short-time exposure respectively

DISCUSSION

As an essential element, appropriate levels (1~200 μmol/L respectively in present hydroponic culture) can enhance plant growth compared to plant growth without addition of Zn, and can weaken inhibition of plant growth by Cd, probably by alleviating Cd induced damage to plant cell. Zn has been shown to serve in cell membrane as a structure–stabilizing component by participating in controlling the lateral mobility of membrane lipids and membrane water permeability (Rygol *et al*., 1992). Aßmann *et al*. (1996) reported that, Zn^{2+} could alleviate Cd^{2+}-induced damage (K^+ loss) to yeast cell plasma membrane. However, high rates of Zn showed severe phytotoxic effects on spring wheat, and significantly inhibited its growth. High level of Zn is likely to destroy the metabolic balance in plants, to result in disorder of other mineral nutrients states.

Long-time and short-time experiments showed that Zn application in a large range of levels (especially high rates of Zn) had significant effect on Cd accumulation in shoots and roots, and that Cd concentrations (in Experiment 1) and ^{109}Cd activities (in Experiment 2) in shoots affected by Zn levels were significantly correlated to the results of regression analysis (Fig.1). Therefore, it can be said that the short-time and long-time exposure of spring wheat to Zn have similar effects on Cd accumulation.

It is supposed that Zn at three levels may influence Cd uptake by plants: soil chemical processes, cell membrane transport and phloem transport. On this experiment condition, the latter two may be mainly involved. Broad-range transport systems for essential trace metals such as Zn allow the cell to effectively accumulate trace elements for future need, but this poses the risk of cell uptake of toxic metals such as Cd (Nies, 1992). Hart *et al*. (2002) suggested that Cd and Zn share a common transport system at the root cell membrane in wheat plants. So Cd and Zn when they exist simultaneously would compete for the same membrane binding sites and transport systems. Experiments on animal (Endo and Shaikh, 1993) and yeast cell (Aßmann *et al*., 1996) showed that inhibition of Cd uptake by Zn is ascribed to competition of increasing Zn levels (being dominating ion) with Cd for membrane binding sites and transport systems. In case Cd had been absorbed into plant, Zn may inhibit Cd transport through the phloem. Increasing Zn level in stem tissue can limit Cd transport from phloem to grains (Welch *et al*., 1999; Olliver *et al*., 1997; Herren and Feller, 1997). The percentage of re-translocation of ^{109}Cd in treated leaves appeared

negatively correlated to Zn concentrations. Zn inhibits the transfer of ^{109}Cd from treated leaf to other parts (Cakmak *et al.*, 2000). There are reports that Zn has synergistic and additive effect or has no effect on Cd uptake (Abdelilah *et al*., 1997; Grant and Bailey, 1998). Above two phenomena existed in the present experiments. Although the short-time and long-time exposure of spring wheat to Zn showed similar response to Zn concentrations, the effects of Zn were some what complicated. We suppose that there is probably another complicating factor-phosphorus (P) that should be taken into consideration. Many evidences have been reported on the effects of P on Cd and Zn uptake by plants. A 3-yr field study by Grant and Bailey (1998) indicated that Cd concentrations of durum wheat increased with applications of phosphorus and nitrogen fertilizers. Yang *et al*.(1999) found, in solution culture, Cd concentrations in wheat corn increased with P supply at pH 5.0. Maier *et al*. (2002) reported that P fertilization significantly increased tuber Cd concentrations of potato in glasshouse and field experiments. P-Zn interaction was more complex and had long been studied (Loneragan, 1951; Grant and Bailey, 1993; Bogdanovic *et al*., 1999; Zhu *et al*., 2001; Li and Zhu, 2002). So we suggest that the reason why the Cd-Zn interaction is so complicated is that, besides the known factors such as soil properties, plant species and Cd/Zn ratio, etc., phosphorus may be a important factor, especially in low P or high P medium. In our present experiments, the P level in the nutrient solution was quite high. So under lower rates of Zn, P is likely to be the dominant factor affecting Cd uptake. It may be the direct or indirect influence of Cd uptake that affect Zn uptake by plants. With the increasing of Zn application, Zn became the dominant factor instead and P has little effects on Cd uptake, and competed with Cd for membrane binding sites and transport system, so that Cd levels decreased in plants significantly.

Further studies are needed to investigate the relationship of P, influencing both Cd and Zn uptake by plants, to the Cd-Zn interaction, then to get further understanding of the mechanisms of Cd-Zn interaction.

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