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Tolerance to Zn deficiency and P-Zn interaction in wheat seedlings cultured in chelator-buffered solutions

WenXuan MAI^{1,2,3}, XiaoHong TIAN^{1*}, Willam Jeffery GALE¹, XiWen YANG¹, XinChun LU¹

¹ College of Resources and Environment, Northwest A&F University, Yangling 712100, China;

² Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China;

³ Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: Zinc deficiency is a common constraint for wheat production in the regions with limited precipitation, particularly in the regions with high levels of available phosphate (P) in soil. Two experiments were conducted using chelator-buffered nutrient solutions to characterize differences in tolerance to Zn deficiency among three winter wheat (*Triticum aestivum* L.) genotypes and to investigate the relationship between P and Zn nutrition in wheat species. Four indices, Zn efficiency, relative shoot-to-root ratio, total Zn uptake in shoot, and shoot dry weight were used to compare the tolerance to Zn deficiency among three wheat genotypes. The results indicated that the four indices could be used in breeding selection for Zn uptake-efficient genotypes. The genotype H6712 was the most tolerant to Zn deficient, followed by M19, and then X13. Specifically, H6712 had the highest Zn uptake efficiency among the three genotypes. The addition of P to the growth medium increased Zn uptake and translocation from roots to shoots. Total Zn content of the wheat plant was 43% higher with 0.6 mmol/L P treatment than that of control with 0 mmol/L P treatment. The Zn translocation ratios from roots to shoots were increased by 16% and 26% with 0.6 mmol/L P treatment and 3 mmol/L P treatment, respectively, compared with 0 mmol/L P treatment. In contrast, high Zn concentrations in the growth medium inhibited P translocation from roots to shoots, but the inhibitive effects were not strong. Sixty-six percent of P taken up by wheat plants was translocated to the wheat shoots at 0 $\mu\text{mol/L}$ Zn treatment, while the percent was 60% at 3 $\mu\text{mol/L}$ Zn treatment. The result may be due to the fact that the wheat plants need more P than Zn.

Keywords: chelator-buffered solution; tolerance to Zn deficiency; P and Zn interaction

Soil Zn deficiency is a widespread constraint for wheat (*Triticum aestivum* L.) production, particularly in arid and semiarid regions where soil pH value and CaCO_3 content are high and organic matter content is low (Cakmak *et al.*, 2001). Soil Zn deficiency reduced both grain yield and quality (Lombnas and Singh, 2003) and may lead to human Zn deficiency, especially in developing countries where diets are abundant in cereal-based foods and deficient in animal protein (Cakmak *et al.*, 1999; Ackland and Michalczyk, 2006).

Wheat is the most important grain crop in northern China. It is mainly planted on calcareous soil which has low level of available Zn. Yang *et al.* (2007) reported that Diethylenetriaminepentaacetic Acid (DTPA)-

extractable Zn in the calcareous soil was average 0.37 mg/kg, whereas a critical value of 0.5 mg/kg is required for optimal wheat growth (Liu, 1994). The low level of Zn in soil has a negative effect on wheat yield and nutritional value.

Zinc efficiency means the ability of crop to grow and yield well in Zn deficient soil. Wheat genotypes differ greatly in the adaptation to Zn-deficient soil conditions (Cakmak *et al.*, 1997a, b). Therefore, selecting and breeding Zn-efficient genotypes has been recognized as the most logical approach for solving the problem of Zn deficiency in plants and humans

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* Corresponding author: XiaoHong TIAN (E-mail: txhong@hotmail.com)

(Cakmak *et al.*, 2001). The improvement of Zn efficiency in wheat has become a major task for plant breeders in many countries (Cakmak, 2002). The first step is to identify Zn efficient wheat genotypes from existing genetic resources (Cakmak *et al.*, 2001).

Zinc deficiency is common in crops that are grown in areas with high level of available soil P (Bogdanovic *et al.*, 1999). High level of available soil P can also reduce the bioavailability of Zn to humans by increasing the phytic acid content in cereal grain (Kuвано *et al.*, 2006). For these reasons, understanding the relationship between P and Zn is an important component of effective agronomy practices to apply P fertilizer. The relationship between P and Zn in plants is complex and there are many conflicting reports in literatures (Orabi *et al.*, 1985; Webb and Loneragan, 1988; Günes *et al.*, 1999; Huang *et al.*, 2000).

Soil-free culture systems can provide important information about the interaction between nutrients and plant roots that can not be obtained from field studies completely. However, Zn contamination in chemical reagents and low plant requirements make it difficult to simulate Zn deficient soils using traditional nutrient solutions (Yang *et al.*, 1994). Chelator-buffered nutrient solution culture is an alternative method which uses a chelating agent such as N-(2-hydroxyethyl) ethylenediaminetriacetic acid (HEDTA) to buffer the free activity of micronutrients to appropriately low levels (Rengel, 1999). Chelator-buffered nutrient solutions offer two advantages. Firstly, chelator-buffered nutrient solutions can stabilize and maintain low micronutrient activity, thus mimicking the situation occurring in soil. Secondly, micronutrient stress of varying severity can be imposed predictably and reproducibly (Parker *et al.*, 1992).

In this paper, we reported the results of two experiments which used chelator-buffered solutions to investigate the effect of Zn and P nutrition on wheat growth. The objective of the first experiment was to compare the tolerance to Zn deficiency among three winter wheat genotypes through four plant indices. The objective of the second experiment was to investigate the interrelationship between P and Zn nutrition in wheat plants.

1 Materials and methods

1.1 Experiment 1

Three winter wheat genotypes were grown in chelator-buffered solutions containing 0.5 $\mu\text{mol/L}$ or 5 $\mu\text{mol/L}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Norvell and Welch, 1993). The composition of the chelator-buffered solution without Zn is shown in Table 1. The three winter wheat genotypes in this experiment were Mianyang19 (M19), Han6172 (H6172) and Xinmai13 (X13) which were widely planted in China.

Table 1 Composition of chelator-buffered nutrient solution

Reagent	Concentration ($\mu\text{mol/L}$)	Reagent	Concentration ($\mu\text{mol/L}$)
KNO_3	1,500	$\text{H}_6\text{Mo}_7\text{O}_{24}$	0.1
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1,000	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	2.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	250	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	3.0
H_3BO_3	12.5	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.1
$\text{C}_6\text{H}_5\text{O}_7\text{Fe} \cdot 5\text{H}_2\text{O}$	20.0	K_3HEDTA	50.0
MES	5,000		

Notes: ① 2-(N-morpholino) ethanesulfonic acid (MES) was adjusted to pH 6.1 with KOH; ② Equal molar amounts of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and K_3HEDTA were dissolved in water and allowed to stabilize for half an hour before use; ③ Macro- and micro-nutrient concentrations in the 1/2 strength chelator-buffered solution were 50% of the amount shown in the table, but MES and K_3HEDTA concentrations were unchanged.

Uniform wheat seeds were selected from each genotype, immersed in distilled water (55°C) for 15 min, and sterilized in 3% H_2O_2 solution (v/v) for 10 min. After sterilization, the seeds were rinsed with distilled water to remove residual H_2O_2 . The sterilized seeds were immersed in distilled water for 3 h and then cultured for 7 d on filter paper moistened with distilled water.

Seedlings of similar size were selected and transferred to 200 ml buckets containing 1/2 strength chelator-buffered solution (without Zn added). Each container was covered with plastic sheets; the sheets had four holes. Three seedlings were placed in each hole. The fourth hole was used for aeration.

Prior to grow in full strength chelator-buffered solution, the seedlings were pre-cultured in 1/2 strength solution for 3 d in order to overcome transplanting stress. The full strength chelator-buffered solution contained either 0.5 or 5 $\mu\text{mol/L}$ Zn treatment. Zinc was added to the chelator-buffered solution in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. Each Zn treatment was repli-

cated four times. Nutrient solutions were continuously aerated with air pumps. The solutions were completely replaced every three days.

The containers were arranged on the top of a laboratory table in a randomized complete block design. The plants were exposed to 10/14 h light/dark cycle each day and the light intensity was $300 \mu\text{mol}/(\text{m}^2 \cdot \text{s})$.

Four indices were used to compare the tolerance to Zn deficiency among wheat genotypes. They are: (1) Zn efficiency; (2) Relative shoot-to-root ratio at deficient conditions compared with normal Zn concentrations in the growth medium; (3) Total uptake of Zn in shoot under Zn deficient treatment; and (4) shoot dry weight under Zn deficient treatments.

1.2 Experiment 2

Two winter wheat genotypes were grown in chelator-buffered solutions containing two Zn concentrations and three P concentrations in a 2×3 factorial design. The $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ concentrations were $0 \mu\text{mol}/\text{L}$ and $3 \mu\text{mol}/\text{L}$, and the $\text{NH}_4\text{H}_2\text{PO}_4$ concentrations were $0 \text{ mmol}/\text{L}$, $0.6 \text{ mmol}/\text{L}$ and $3 \text{ mmol}/\text{L}$, respectively. Each of the six treatment combinations was replicated three times. Two winter wheat genotypes, Yuanfeng 998 (Y998) and Zhengmai 9023 (Z9023) were used in this experiment. The selection and germination of seeds and the conditions for the culture of seedlings in the chelator-buffered solutions were the same as described in experiment 1.

1.3 Chemical analyses

Plants from both experiments were harvested after 30 days of culture in the chelate buffered solutions. Excess water was removed by blotting, and the shoots and roots were dried in an oven at 75°C . Shoot and root dry weights were recorded, and the plant samples were ground and dry-ashed at 550°C for 8 h in a muffle furnace. The ash was dissolved in 1 : 1 (v/v) concentrated HNO_3 solution. Shoot and root Zn concentrations in the solutions were determined by atomic absorption spectroscopy (AA320CRT, CANY Co., Ltd., China). Shoot and root P concentrations in the solutions were determined colorimetrically at 660 nm (UV-7502, CANY Co., Ltd., China) (Bao, 1999).

1.4 Data analyses

Zinc efficiency (%) was calculated according to the method of Torun *et al.* (2000):

$$\text{Zn efficiency} = \frac{\text{Shoot dry weight at the } \text{Zn}_{0.5} \text{ treatment}}{\text{Shoot dry weight at the } \text{Zn}_{5.0} \text{ treatment}} \times 100\%$$

Zinc translocation ratio (%) was calculated according to the method of Rengel *et al.* (1995a, b):

$$\text{Zn translocation ratio} = \frac{\text{Shoot Zn content}}{\text{whole plant Zn content}} \times 100\%$$

Data from the two experiments were statistically analyzed with SAS 8.1 (SAS Institute Inc., Cary, NC, USA). Multiple comparisons of the means were conducted using Duncan's new multiple range test (SSR).

2 Results

2.1 Zn deficiency and P toxicity symptoms among wheat genotypes

In experiment 1, wheat plants at $0.5 \mu\text{mol}/\text{L}$ Zn treatment showed obvious Zn deficiency symptoms after one week of culture. Visual symptoms included intervening chlorosis between the mid-vein and leaf margin of young leaves, while the leaf tip, base and margin remained green. Wheat shoot dry weights was decreased ranging from 51% to 68% at $0.5 \mu\text{mol}/\text{L}$ Zn treatment compared with $5 \mu\text{mol}/\text{L}$ Zn treatment, while root dry weights was decreased ranging from 43% to 57% (Table 2). The decrease of shoot and root dry weight due to Zn deficiency was the smallest for H6172 and the largest for X13. The shoot-to-root ratios at $0.5 \mu\text{mol}/\text{L}$ Zn treatment were decreased ranging from 15% to 28% compared with $5 \mu\text{mol}/\text{L}$ Zn treatment which indicated that the inhabitation of Zn deficiency to the growth of wheat shoots was higher than that of wheat roots.

In experiment 2, the plants at $0 \mu\text{mol}/\text{L}$ Zn and $3 \text{ mmol}/\text{L}$ P treatments developed chlorosis and necrosis at the tips and margins of old leaves, which indicated slight P toxicity. Other researchers reported that it was difficult to distinguish between Zn deficiency and P toxicity symptoms in a number of species (Loneragan *et al.*, 1982). However, the visual differences between the two conditions were observed in the experiment.

2.2 Tolerance of wheat genotypes to Zn deficiency

In experiment 1, the four indices were chosen to compare the response of the three wheat genotypes to Zn deficiency (Fig. 1). The four indices showed similar trends and indicated that H6712 was the most tolerant

Table 2 Effects of Zn concentrations in chelator-buffered solutions on shoot and root dry weights (g/plant) and shoot/root ratio of three wheat genotypes

Parameters	M19		H6172		X13	
	ZnSO ₄ ·7H ₂ O					
	0.5 µmol/L	5 µmol/L	0.5 µmol/L	5 µmol/L	0.5 µmol/L	5 µmol/L
Shoot dry weight (g/plant)	0.275bc	0.705ab	0.315c	0.643ab	0.283bc	0.910a
Root dry weigh (g/plant)	0.086c	0.175bc	0.112bc	0.195ab	0.118bc	0.273a
Shoot/root	3.19b	4.04a	2.81b	3.30ab	2.39b	3.33ab

Note: Data are from experiment 1; M19, H6172, and X13 represent Mianyang19, Han6172 and Xinmai13 wheat genotypes, respectively. The differences were first tested using one-way analysis of variance (ANOVA), and then multiple comparisons of the means within each Zn treatment were used. Within a column, the means followed by different letters are significantly different ($P < 0.05$).

to Zn deficient treatment followed by M19 and X13. Specifically, H6172 had the highest Zn efficiency the three genotypes. Due to Zn deficiency, the decrease of shoot dry weight for H6172 with 0.5 and 5 µmol/L Zn treatments was less than that of the other two genotypes. Meanwhile, the genotype H6172 had the highest relative shoot-to-root ratio, total plant Zn content and shoot dry weight. The consistent results of the four indices showed that characterizing the tolerance of wheat plants to Zn deficiency is useful. Moreover, three of the four indices showed large differences among the three genotypes. For example, Zn efficiency of H6172 was 58% higher than that of X13. The results indicated that the three genotypes differed in tolerance to Zn deficiency, suggesting that H6172 might have the potential in breeding and selection for Zn efficient wheat genotypes.

2.3 P and Zn nutritional status of wheat plants

2.3.1 Effect of P supply on the Zn concentration and content in wheat plants

The root Zn concentrations (µg/g) tended to decline as

the P concentrations increased in the chelator-buffered solutions (Fig. 2a). In contrast, the shoot Zn concentrations increased significantly as the P concentrations increased. The change of shoot and root Zn contents (µg/plant) have similar trend with shoot and root Zn concentrations (Fig. 2b). This indicated that P decreased Zn uptake by plant root. The total Zn content of the wheat plants was 43% higher at 0.6 mmol/L P treatment than that at 0 mmol/L P treatment (control). Total Zn content at 3 mmol/L P treatment was slightly smaller than that at 0.6 mmol/L P treatment. The root Zn content was decreased by 19% at 3 mmol/L P treatment than that of the control. This indicated that high P concentration in the nutrient solution resulted in the decrease of Zn uptake in wheat plants. The Zn translocation ratio from roots to shoots increased by 16% and 26% at 0.6 mmol/L and 3 mmol/L P treatments, respectively (Fig. 2c), compared with the control. The result indicated that P promoted the translocation of Zn from roots to shoots. In conclusion, the

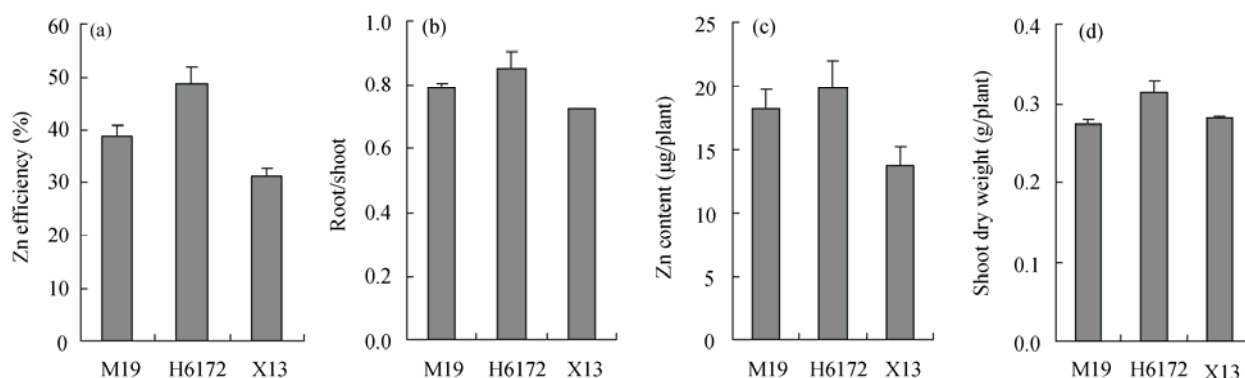


Fig. 1 Indices of experiment 1 characterized the tolerance to Zn deficiency of different wheat genotypes. (a) Zn efficiency; (b) shoot/root ratio at 0.5 µmol/L Zn treatment; (c) total plant Zn content at 0.5 µmol/L Zn treatment; and (d) shoot dry weight at 0.5 µmol/L Zn treatment. Data represent means ± SD ($n = 4$).

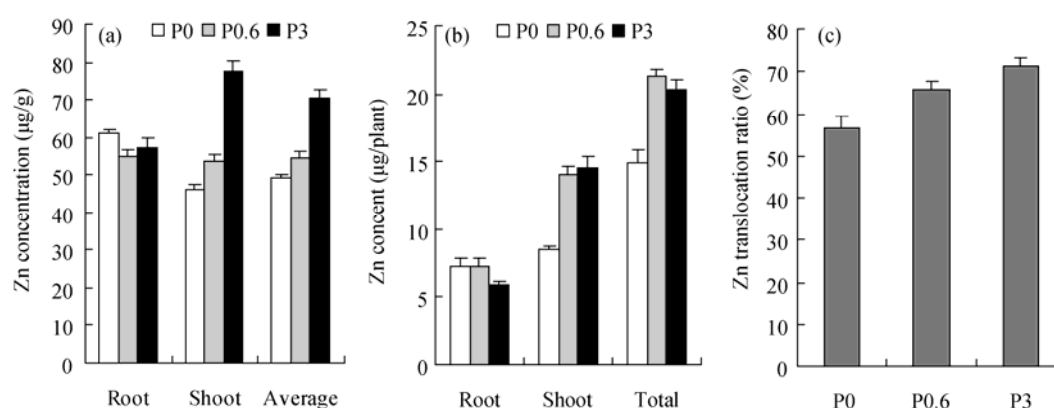


Fig. 2 Effects of P addition in chelator-buffered solutions on (a) the Zn concentration in wheat shoots and roots; (b) the Zn content in wheat shoots and roots; and (c) the Zn translocation ratio. Data are from experiment 2 and represent the means of three genotypes \pm SD ($n = 12$).

results indicated that high P concentrations in the growth medium reduced Zn uptake but increased Zn translocation from roots to shoots.

2.3.2 Effect of Zn supply on the P concentration and content in wheat plants

The addition of Zn in the chelator-buffered solution resulted in a significant increase in the P concentration (mg/g) and content (mg/plant) in wheat shoots and roots (Fig. 3). The total plant Zn concentration was increased by 43% and the shoot Zn concentration was increased by 67% as the P concentration in the chelator-buffered solutions was increased from 0 to 3 mmol/L (Fig. 2a). In contrast, the total plant P concentration was only increased by 14% and the shoot P concentration was only increased by 10% as the Zn concentration in the nutrient solution was increased from 0 to 3 μ mol/L (Fig. 3).

The addition of Zn in the chelator-buffered solutions resulted in a decrease in the proportion of plant P that was translocated from roots to shoots. At 0 μ mol/L Zn treatment, 66% of the P taken up by wheat plants was translocated to the shoots (Fig. 3c). In contrast, 60% of the P taken-up by wheat plants was translocated to the wheat shoots at 3 μ mol/L Zn treatment. The results indicated that the increase of Zn concentration in the growth medium resulted in an increase of P uptake in wheat plants, however, the proportion of P that was translocated to the shoots was declined.

2.3.3 P/Zn ratios in wheat plants

The supply of Zn in growth medium had different effects on the P/Zn ratio in shoots compared with roots. The shoot P/Zn ratio at 3 μ mol/L Zn treatment was lower than that at 0 μ mol/L Zn treatment (Fig. 4a). An

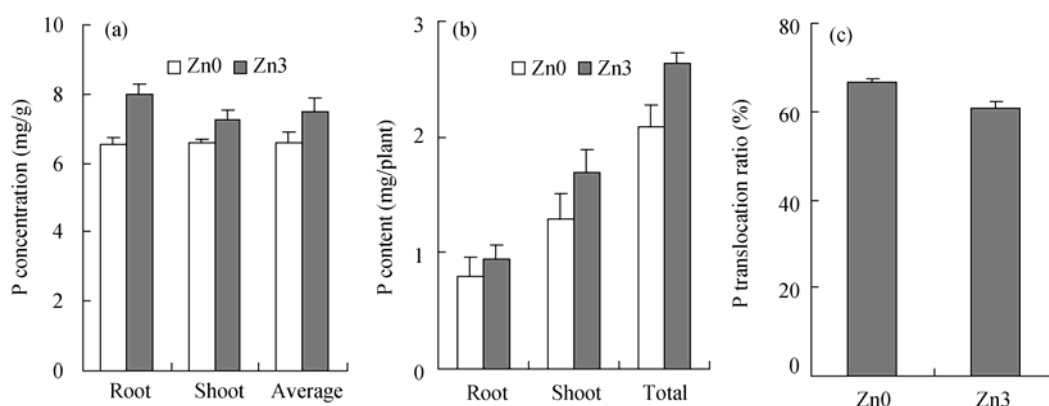


Fig. 3 Effects of Zn addition on (a) P concentration in shoots and roots, (b) P content in shoots and roots, and (c) P translocation ratio. Data are from experiment 2 and represent the means of two genotypes \pm SD ($n = 18$).

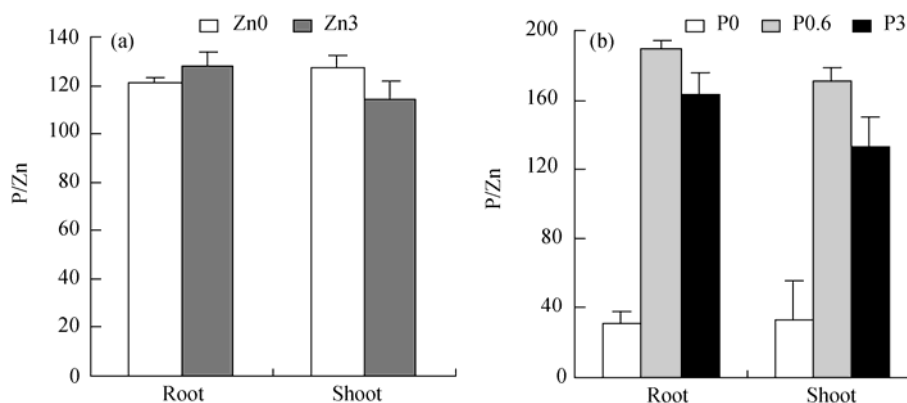


Fig. 4 Effects of Zn and P concentrations in chelator-buffered solutions on P/Zn ratios in shoots and roots of wheat. Data are from experiment 2 and represent the means of two genotypes \pm SD ($n = 18$ for Fig. 4a and $n = 12$ for Fig. 4b).

opposite trend was found for the root P/Zn ratio. The changes of P/Zn ratio suggested that an increase of Zn concentrations in the growth medium resulted in an increase in P uptake by wheat roots but a decrease in Zn translocation from roots to shoots. At 0.6 mmol/L P treatment, the P/Zn ratios in shoots and roots were 4.2 to 5.1 times than that in the control, however, at 3 mmol/L P treatment, the values were decreased (Fig. 4b). The results indicated that the effect of Zn on the P/Zn ratio of wheat shoots and roots in the growth medium was greater than that of P.

3 Discussion and conclusion

Interactions between the elements in the soil-plant system affect many processes including absorption, translocation, distribution, accumulation, and physiological activity (Zhang *et al.*, 2005). The relationship between Zn and P in plant nutrition has been widely studied, however, many results were inconsistent (Orabi *et al.*, 1985). Shang and Bates (1987) found that P increased Zn deficiency in corn without Zn treatments, and Zn increased P deficiency in plants without P treatments. The deficiency in either case was readily corrected by applying the appropriate element. In contrast, Orabi *et al.* (1985) showed that the relationship between the two elements was positively correlative. The application of P increased the dry matter of the different parts of corn plant, meanwhile, Zn uptake and Zn content of corn plant were also increased. The large number of studies on the Zn and P relationship in the nutrition of plants and the great controversy about the obtained results showed

that the problem has still not completely be solved, and also indicated that the problem is complex. Our results showed that neither a positive relationship nor a passive relationship existed between Zn and P uptake by wheat plants. High P concentrations (3 mmol/L) in the chealator-buffered solutions resulted in the slight decrease in Zn uptake by plant roots compared with 0.6 mmol/L P treatment, however, the difference between the treatments was not significant. At the same time, the high P concentrations promoted the translocation of Zn from roots to shoots, which is different from the above mentioned results. One explanation is that high P concentrations in the chelator-buffered solutions inhibited wheat root growth. And, Zn was transported from the roots to shoots in order to meet the metabolic needs of the plants. Another explanation is that the proper P/Zn balance in metabolically active between root and shoot was different. P decreased Zn uptake in all plants, but the decrease was more significant in vegetative parts than in grain (Shukla and Hans, 1980). It is recognized that major interactions between Zn and P occur at the plant metabolic level, and a metabolic disorder within plant cells is related to an imbalance between P and Zn. Therefore, the maintenance of a proper P/Zn balance in metabolically active plant tissues for normal growth is necessary.

In contrast, the high Zn concentration in the nutrient solution increased P uptake by roots but reduced P translocation from roots to shoots. Our results are in agreement with Gianquinto *et al.* (2000) who found that the addition of small amount of Zn fertilizer increased P concentration in dwarf beans. And, it also

revealed that the Zn and P interactions originate in the wheat plant roots.

Wheat plants maintained a narrower P/Zn ratio which responded less to the application of Zn. However, the effect of P on plant Zn uptake in the growth medium was significantly greater than that of Zn. Thus, the P concentration in wheat plant plays a dominant role in maintaining the P and Zn nutritional balance in the plants. This opinion is indicated by the large difference in the P/Zn ratios between the 0 and 0.6 mmol/L P treatments. The Zn concentration in the growth medium affected the P/Zn ratio in plants by inhibiting P translocation from roots to shoots, however, this effect was relatively small. These results showed that Zn response pattern in wheat plants was partly associated with their capacity to accumulate and distribute P in different plant tissues as reported by

Ambler and Brown (1969) by using two cultivars of soybean and cowpea, respectively.

Chelator-buffered nutrient solutions provided important information about plant roots, which is difficult to be obtained in field studies. We found that P in the growth medium increased Zn uptake and translocation from roots to shoots, while excess Zn in the growth medium affected the distribution of P in wheat plants by inhibiting P translocation from roots to shoots. Further studies need to be done to understand the complex relationship between Zn and P in plant roots.

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State Key Laboratory of Xinjiang Institute of Ecology and Geography was successfully founded

Recently, State Key Laboratory of Desert and Oasis Ecology of Xinjiang Institute of Ecology and Geography (XIEG), Chinese Academy of Sciences, was approved by Ministry of Science and Technology of the People's Republic of China to be founded.

The laboratory will focus on the research fields including desert ecological processes and desertification prevention and control, oasis ecological processes and sustainable management, ecological and hydrological processes and water resources utilization, and mountain-oasis-desert ecosystem processes and regional integrated simulation. Meanwhile, the laboratory will intensify the researches of natural resources utilization and the relationship between ecological protection and economic development in arid land, in order to meet the need of social economic development and ecological construction in Western China.