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Abstract

This study employed rice as the test plant to investigate changes in rice biomass and the distribution and translocation of Cd within rice plants under hydroponic conditions with mild cadmium (Cd) pollution [Cd concentrations: 0 mg · L 1, 0.01 mg · L 1 (low dose), 0.03 mg · L 1 (medium dose), and 0.09 mg · L 1 (high dose)], through exogenous addition of different zinc (Zn) doses (concentrations: 0 mg · L 1, 0.025 mg · L 1, 0.05 mg · L 1, 0.1 mg · L 1, and 0.2 mg · L 1), explore the interaction between Zn and Cd, and identify the optimal exogenous Zn concentration for mitigating Cd contamination in rice. The results demonstrated that exogenous Zn application increased the biomass of rice roots, stems, and leaves, with the most pronounced effect at a Zn concentration of 0.05 mg · L 1. Under Zn-deficient conditions (0 mg · L 1), the ratio of Cd content in rice root cytoplasm to cell wall decreased with increasing exogenous Cd concentration; following exogenous Zn addition, this ratio exhibited an upward trend, with significant changes observed at the 0.03 mg · L 1 Cd level. At low to medium Cd doses (0.01-0.03 mg · L 1), Zn application reduced Cd uptake and translocation in rice roots. Specifically, at a Zn concentration of 0.05 mg · L 1, Cd content in rice roots, stems, and leaves decreased most significantly by 38%, 71%, and 65% (low Cd dose) and 44%, 79%, and 69% (medium Cd dose), respectively, while the translocation factors from root to stem and root to leaf decreased by 53% and 44% (low Cd dose) and 62% and 40% (medium Cd dose), respectively; however, further increases in Zn concentration did not produce significant changes in Cd content or translocation factors in various rice tissues. Under high-dose Cd conditions, exogenous Zn application showed no significant inhibitory effect on Cd content in rice roots, stems, or leaves. Therefore, under low to medium dose Cd pollution conditions, a clear antagonistic relationship exists between Zn and Cd, and exogenous addition of 0.05 mg · L 1 Zn represents the optimal concentration for reducing Cd uptake and translocation in rice while increasing rice yield.

Full Text

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Effects of Different Zinc Levels on the Translocation Capacity of Low-Dose Cadmium in Rice*

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Abstract

In this study, rice was used as the test plant. Under hydroponic conditions with slight cadmium (Cd) pollution at Cd concentrations of 0 mg·L—1, 0.01 mg·L—1 (low dose), 0.03 mg·L—1 (medium dose), and 0.09 mg·L—1 (high dose), different exogenous zinc (Zn) doses were added (Zn concentrations of 0 mg·L—1, 0.025 mg·L—1, 0.05 mg·L—1, 0.1 mg·L—1, and 0.2 mg·L—1) to investigate changes in rice biomass and the distribution and translocation of Cd within rice plants, explore the relationship between Zn and Cd, and screen for the optimal exogenous Zn concentration for controlling Cd pollution in rice. The results showed that application of exogenous Zn increased the biomass of rice roots, stems, and leaves, with the most significant effect at a Zn concentration of 0.05 mg·L—1. Under Zn-deficient conditions (0 mg·L—1), the ratio of Cd content in the cytoplasm to that in the cell wall of rice roots decreased as the exogenous Cd concentration increased; after the addition of exogenous Zn, the ratio of Cd content in the cytoplasm to that in the cell wall tended to increase, with a marked change at the 0.03 mg·L—1 Cd level. Under low- and medium-dose Cd levels (0.01-0.03 mg·L—1), Zn application reduced Cd uptake and translocation in rice roots. When the Zn concentration was 0.05 mg·L—1, the decreases in Cd content in rice roots, stems, and leaves were most pronounced, decreasing by 38%, 71%, and 65% (low-dose Cd) and by 44%, 79%, and 69% (medium-dose Cd), respectively; moreover, the translocation coefficients from rice roots to stems and from roots to leaves decreased by 53% and 44% (low-dose Cd), and by 62% and 40% (medium-dose Cd), respectively. Thereafter, as Zn concentration increased, the Cd contents and translocation coefficients in different rice plant parts showed no significant changes. Under high-dose Cd conditions, exogenous Zn application had no significant inhibitory effect on the Cd contents in rice roots, stems, and leaves. Therefore, under low- and medium-dose Cd pollution conditions, there is an evident antagonistic effect between Zn and Cd, and exogenous addition of 0.05 mg·L—1 Zn is the optimal concentration for reducing Cd uptake and translocation in rice and increasing rice yield.

Keywords rice; cadmium; zinc; antagonism; cadmium translocation

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Effects of zinc level on low dose cadmium transport in rice plant*

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Abstract

The interaction of zinc (Zn) and cadmium (Cd) in soil is critical for the uptake and transport of Cd in rice plants. However, the effect of Zn levels on the interactions of Zn and Cd or on the rate of transport of Cd in rice plants is still not entirely clear. In this study, rice plant biomass, and Cd transport and distribution in rice plant were investigated in hydroponic experiment of mild Cd pollution with exogenous Zn addition. In the experiment, Cd concentrations were 0.01 mg·L—1 (low dose), 0.03 mg·L—1 (medium dose), 0.09 mg·L—1 (high dose), and 0 mg·L—1 (control); and exogenous Zn were 0.025 mg·L—1, 0.05 mg·L—1, 0.1 mg·L—1, 0.2 mg·L—1. The aim of the study was to determine the mechanism of interactions between Zn and Cd and to identify the optimal Zn concentration that effectively reduced Cd pollution in rice. The results showed that biomasses of different parts of rice plant increased significantly with increasing concentration of Zn. The increase of biomass was highest in 0.05 mg·L—1 exogenous Zn treatment. However, there was no significant increase when Zn concentration exceeded 0.05 mg·L—1. Due to the low dose of Cd in the experiment, there was no significant variation in rice plant biomass with increasing Cd concentration without exogenous Zn. Under Zn-free condition, the ratio of cytoplasm Cd to cell-wall Cd in rice root reduced from 2.88 to 1.04 with increasing Cd concentration. With the applying of exogenous Zn, the ratio of cytoplasm Cd to cell-wall

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The tissue homogenate was centrifuged at 20 000 × g for 45 min; the bottom pellet was the cell-wall fraction, and the supernatant was the cytoplasmic fraction[^{14}]. The supernatant cytoplasmic solution was kept at low temperature until analysis (the test was operated at 4 °C).

The remaining roots, stems, and leaves were placed in an oven for enzyme deactivation, dried, and weighed for biomass of each fraction. They were then transferred, together with the dried cell-wall fractions, into digestion vessels; HNO_3 and H_2O_2 (3 1, V/V) were added, and microwave digestion was carried out in a microwave digestion system (MLS1200). After complete digestion, the samples were transferred into volumetric tubes and brought to volume, then transferred to clean bottles for analysis. Zn and Cd contents in the treated samples were determined using inductively coupled plasma mass spectrometry (ICP-MS).

1.3 Data processing

The experimental data were statistically processed using Microsoft Excel, and significant differences among treatments were analyzed using SPSS 18.0. Figures were prepared with Excel.

2 Results and analysis

2.1 Changes in rice biomass under different Zn-Cd treatments

Cd is a nonessential element for plant growth and development. When the Cd dose in the environment exceeds the threshold that plants can tolerate, plant growth and development are affected. As shown in Table 1, for rice cultured at the same Zn concentration, biomass did not change significantly with increasing exogenous Cd dose ($P > 0.05$), indicating that the externally applied Cd dose was relatively low, within the tolerance range of rice, and insufficient to significantly affect rice growth. In addition, at the same Cd concentration, with increasing Zn concentration, the biomass of rice roots, stems, and leaves as a whole showed an increasing trend, indicating that Zn can promote rice growth. When the Zn concentration was 0.05 $mg \cdot L^{-1}$, the biomass of rice roots, stems, and leaves all increased significantly ($P < 0.05$). As Zn concentration continued to increase, the increasing trend in rice biomass was not significant ($P >$

0.05), indicating that under the conditions of this experiment, the optimal Zn concentration for promoting rice growth was 0.05 mg · L^{-1}.

Table 1 Biomass of the roots, stems and leaves of the rice under different Zn-Cd treatments
g · $plant^{-1}$

Zn treatment	Root biomass				Stem biomass				Leaf biomass			
	Cd0	Cd0.01	Cd0.03	Cd0.09	Cd0	Cd0.01	Cd0.03	Cd0.09	Cd0	Cd0.01	Cd0.03	Cd0.09
Zn0	0.09$±0.00*b*	0.10±0.00*c*	0.09±0.00*b*	0.09±0.00*c*	0.05±0.00*c*	0.04±0.01*c*	0.04±0.00*b*	0.03±0.01*c*	0.11±0.00			

Zn0, Zn0.025, Zn0.05, Zn0.1, and Zn0.2 represent added Zn amounts of 0 mg · L^{-1}, 0.025 mg · L^{-1}, 0.05 mg · L^{-1}, 0.1 mg · L^{-1}, and 0.2 mg · L^{-1}, respectively; Cd0, Cd0.01, Cd0.03, and Cd0.09 represent added Cd amounts of 0 mg · L^{-1}, 0.01 mg · L^{-1}, 0.03 mg · L^{-1}, and 0.09 mg · L^{-1}, respectively. Different letters in the same column indicate significant differences among different treatments within the same tissue ($P < 0.05$). Values in the table are expressed as mean ± standard deviation. The same below.

2.2 Distribution of Cd in rice-root subcellular fractions under different Zn-Cd treatments

Cd in solution was absorbed by rice roots and diffused into the root cytoplasm (Cyt) and cell wall (CW). It can be seen from Fig. 1 that, under Zn-deficient conditions, as Cd concentration increased, the ratio of Cd content in Cyt to that in CW gradually decreased, reaching a significant level when Cd concentrations were 0.03 mg · L^{-1} and 0.09 mg · L^{-1} ($P < 0.05$). Under a low-Cd environment, Cd content in Cyt was higher than that in CW, indicating that under low-dose Cd stress, Cd was preferentially stored in Cyt, with a small amount present in CW[^{15}]. As the Cd dose gradually increased, the proportion of Cd in Cyt decreased from 72% to 51%, approaching a ratio of 1 relative to Cd content in CW. This indicates that under high-dose Cd pollution, the capacity of CW to immobilize Cd increased, causing the difference in Cd content between Cyt and CW to gradually decrease. With increasing added Zn concentration, the ratio of Cd content in Cyt to that in CW showed an increasing trend at Cd concentrations of 0.03 mg · L^{-1} and 0.09 mg · L^{-1}, and the change was significant at a Cd concentration of 0.03 mg · L^{-1}, indicating that Zn application may promote the transfer of Cd into Cyt.

Fig. 1 Changes in the ratios of Cd content (Cyt-Cd CW-Cd) between cytoplasm (Cyt) and cell wall (CW) in rice roots under treatments with different Zn and Cd concentrations

Different lowercase letters indicate significant differences among different Zn treatments under the same Cd treatment. The same below. Different letters

Fig. 2 Contents of Cd in cytoplasm (A) and cell wall (B) of rice roots under different Zn and Cd treatments

Figure 1: Fig. 2 Contents of Cd in cytoplasm (A) and cell wall (B) of rice roots under different Zn and Cd treatments

denote significant difference under the same Cd concentrations among different Zn treatments at $P < 0.05$. The same below.

As can be seen from Fig. 2, under medium-low Cd concentrations, by comparing the changes in Cd contents in the cytoplasm (Cyt) and cell wall (CW) of rice roots under conditions without Zn application and with different Zn application rates, it was found that Zn application could significantly inhibit Cd uptake by Cyt and CW in rice roots (P<0.05). With increasing Zn content, the Cd contents in Cyt and CW decreased. When the Zn concentration was 0.05 $\text{mg}\cdot\text{L}^{-1}$, the inhibitory effect reached a significant level (P<0.05). Under low Cd, when the Zn concentration was 0.05 $\text{mg}\cdot\text{L}^{-1}$, Cd uptake by Cyt decreased by 82%, and that by CW decreased by 77%; under the medium Cd dose, Cd uptake by Cyt and CW decreased by 80% and 84%, respectively. Under the high Cd concentration (0.09 $\text{mg}\cdot\text{L}^{-1}$), when the Zn concentration reached 0.05 $\text{mg}\cdot\text{L}^{-1}$, Cd uptake by Cyt decreased by 12%, and that in CW decreased by 35%. This indicates that, under medium-low Cd concentrations, Zn has a significant effect on reducing plant Cd uptake, whereas under high Cd concentrations, Zn application has no significant effect on controlling Cd pollution.

Fig. 2 Contents of Cd in cytoplasm (A) and cell wall (B) of rice roots under different Zn and Cd treatments

2.3 Effects of different Zn-Cd treatments on Cd distribution in rice plants

As shown in Fig. 3, Cd was mainly distributed in rice roots, followed by stems, with only a small amount distributed in leaves. When the Zn concentration was 0.05 $\text{mg}\cdot\text{L}^{-1}$, under low Cd stress, Cd accumulation in roots, stems, and leaves of rice all decreased significantly (P<0.05). Similarly, when the Zn concentration was 0.05 $\text{mg}\cdot\text{L}^{-1}$, under the medium Cd concentration, except that the change in root Cd content did not reach a significant level compared with the treatment without Zn application, the Cd contents in stems and leaves both reached significant levels (P<0.05). Under low and medium Cd concentrations, affected by 0.05 $\text{mg}\cdot\text{L}^{-1}$ exogenous Zn, Cd accumulation in rice roots decreased by 38% and 44%, respectively; Cd accumulation in rice stems decreased by 71% and 79%, respectively; and Cd accumulation in rice leaves decreased by 65% and 69%, respectively. Meanwhile, it was found that under medium-low Cd pollution, with further increases in Zn concentration, 0.05 $\text{mg}\cdot\text{L}^{-1}$, 0.1 $\text{mg}\cdot\text{L}^{-1}$, and 0.2 $\text{mg}\cdot\text{L}^{-1}$ Zn had no significant differences in their effects on Cd accumulation in roots, stems, and leaves (P>0.05). This indicates that under medium-low Cd pollution conditions, Zn application can significantly reduce

Cd uptake and transport in rice, thereby reducing Cd enrichment in plants. At the same time, when the Zn concentration exceeded 0.05 mg $\cdot$ L^{-1}, the effective effect on reducing Cd in plants under low and medium Cd doses was not obvious, indicating that 0.05 mg $\cdot$ L^{-1} Zn was the optimal concentration for reducing Cd uptake by rice in this experiment. When the exogenous Cd concentration was 0.09 mg $\cdot$ L^{-1}, the Cd content in roots and stems was not significantly affected by Zn application ($P>0.05$); however, when the Zn concentration in leaves was greater than 0.05 mg $\cdot$ L^{-1}, the change was significant, showing a significant increasing trend compared with the Zn-deficient group.

2.4 Changes in Cd translocation coefficients in rice plants under different Zn-Cd treatments

The plant translocation coefficient (TF) (transfer factor) refers to the ratio of the heavy-metal content in aboveground stems and leaves to that in roots; it is a measure of the effective transfer of heavy metals in plants from roots to aboveground parts. In Fig. 4, the translocation coefficient of Cd from roots to stems under medium-low Cd concentrations decreased significantly with increasing Zn application ($P<0.05$). Under high Cd conditions, Zn application did not significantly reduce Cd translocation from roots to stems ($P>0.05$); instead, it showed an increasing trend. This indicates that under medium-low Cd conditions, Zn application reduces the ability of Cd to migrate from roots to stems, whereas under high Cd conditions, Zn application cannot reduce Cd migration to stems but instead promotes Cd migration. The translocation coefficient of Cd between roots and leaves under medium-low Cd concentrations also decreased with increasing Zn concentration, whereas the translocation coefficient between roots and leaves under high Cd increased with Zn application.

As can be seen from Fig. 4, without Zn application, under the three Cd doses—low, medium, and high—the order was $TF_{\text{low}} > TF_{\text{medium}} > TF_{\text{high}}$, indicating that the lower the Cd concentration, the more readily it was absorbed by the plant. Meanwhile, with increasing Zn application, the translocation coefficients under medium-low Cd doses decreased markedly and reached a significant level when the Zn concentration was 0.05 mg $\cdot$ L^{-1} ($P<0.05$), after which there was no significant change. Under the low and medium Cd treatments, the TF from roots to stems decreased by 53% and 62%, respectively, and the TF from roots to leaves decreased by 44% and 40%, respectively, indicating that under medium-low Cd pollution, 0.05 mg $\cdot$ L^{-1} Zn was the optimal concentration for reducing Cd translocation within the plant. Under high Cd conditions, the Cd translocation coefficient showed an increasing trend with increasing Zn concentration, indicating that under high Cd conditions there was no significant interaction between Zn and Cd ($P>0.05$).

3 Discussion

Zn is an essential trace element for plant growth and development. In this experiment,

Fig. 4 bar charts: transfer factors under different Zn and Cd treatments

Figure 2: Fig. 4 bar charts: transfer factors under different Zn and Cd treatments

As the applied exogenous Zn concentration increased, the biomass of rice roots, stems, and leaves all increased, possibly because Zn promotes photosynthesis in plants[^{16}]. When the Zn concentration exceeded 0.05 mg · L(^{-1}), rice biomass showed no significant change, possibly because the promotional effect of Zn concentration on rice growth had reached saturation; this also indicates that 0.05 mg · L(^{-1}) Zn was the optimal concentration for rice growth in this experiment. Under treatments with the same Zn concentration and different Cd concentrations, changes in rice biomass were not significant. This is because the amount of exogenous Cd applied in this experiment (<0.09 mg · L(^{-1})) was in the medium- to low-dose range and did not produce a significant effect on rice growth.

Under Zn deficiency, Cd was distributed to a greater extent in the Cyt. In addition, Ma et al.[^{17}] also found that more than 90% of Cd was stored in protoplasts. This phenomenon is mainly related to the compartmentalized detoxification mechanism of vacuoles in plant cells. Once Cd enters plant cells, the cells fix the metal Cd in vacuoles through the tonoplast for effective internal detoxification[^{18}], thereby reducing the damage caused by Cd to intracellular organelles[^{19}]. As Cd concentration increased, the ratio of Cd in Cyt to that in CW continuously decreased. This may be because high Cd concentrations stimulated the CW of rice roots and promoted CW secretion of Cd-affinitive substances carrying negative charges, which adsorbed Cd ions and thereby enhanced the capacity of the rice-root CW to immobilize Cd[^{20}], preventing Cd from entering cells. With the addition of exogenous Zn, the ratio of Cd in Cyt to that in CW showed an increasing trend, possibly because Zn and Cd competed for adsorption sites on the CW surface, resulting in an increase in Cd content in Cyt.

The root is the first barrier by which plants resist heavy-metal toxicity. Cd(^{2+}) enters the xylem ducts through the symplastic and apoplastic pathways and is transported upward. Most Cd(^{2+}) precipitates in the intercellular spaces of epidermal cells[^{21}]; therefore most Cd is blocked in the roots, followed by the stems, and finally the leaves[^{22}-^{25}]. As the concentration of exogenous Zn increased, Cd accumulation in the Cyt and CW of roots of rice treated with medium to low doses decreased significantly. Hart et al.[^{26}] considered that Zn and Cd share the same transporter during uptake and transport, and that Zn is the main competitor for the binding sites of this transporter, resulting in reduced Cd uptake by roots. Hua Lu et al.[^{27}] considered that Zn and Cd have the same

Fig. 3 Changes of Cd contents in roots (A), stems (B) and leaves (C) of rice under different Zn and Cd treatments

Fig. 4 Effect of different Zn/Cd treatments on Cd transfer factors between rice root-stem (A) and root-leaf (B)

Fig. 4 Zn on Cd transfer factors from roots to stems (A) and from roots to leaves (B) of rice under different Zn and Cd treatments

transport channels, whereas Zn uptake is mainly active transport and Cd uptake is more often passive transport; therefore, Zn may have a certain advantage. Thus, the decrease in Cd uptake may be caused by competition between Zn and Cd for adsorption sites on root-cell membranes and for root transport channels, which is consistent with the conclusion of Mckenna et al.[1] At the same time, under medium- and low-dose Cd conditions, Cd accumulation in rice stems and leaves also decreased significantly with increasing Zn application. This may be because Zn–Cd competition for binding to proteins causes more Cd to remain in a free state; during transport in the xylem, CW has a strong exchange capacity for free ions, thereby reducing the transport rate of Cd[2]. Under medium- and low-dose Cd contamination, the results of this study are consistent with some other reports: application of exogenous Zn can significantly inhibit Cd uptake by plant roots[3] and the transport and translocation of Cd within plants[4]. Under high-Cd conditions, however, with increasing Zn application, Cd contents in roots and stems did not change significantly, whereas Cd content in leaves increased instead. This indicates that, under high-Cd conditions, Zn application cannot significantly reduce plant uptake and translocation of Cd. This may be because Cd and Zn have the same valence state and similar ionic radii and therefore compete for binding sites on plant cell surfaces, increasing Cd solubility and promoting Cd transfer from roots to shoots[5]. Therefore, at different levels of Cd contamination, the effect of controlling Cd pollution through Zn fertilization differs.

4 Conclusion

This study shows that applying Zn can increase rice biomass, reaching a significant level at 0.05 mg·L^—1 Zn. At the same time, under medium- and low-dose Cd contamination, application of 0.05 mg·L^—1 Zn can significantly reduce the phytoavailability of Cd and weaken Cd translocation within the plant, but the effect on reducing the availability of high-dose Cd is not obvious. Under medium- and low-dose Cd contamination, Zn and Cd show an antagonistic relationship; under high-dose Cd contamination, the antagonism between Zn and Cd is not obvious and may be accompanied by synergism. Therefore, for medium- and low-dose Cd contamination, maintaining the soil available Zn concentration at 0.05 mg·L^—1 through Zn fertilization can, to a relatively large extent, increase rice yield and reduce Cd uptake and translocation by rice. However, in environ-

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[2] 28

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ments contaminated with high-dose Cd, the effect of Zn fertilization on reducing the phytoavailability of Cd is not significant.

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Note: Figure translations are in progress. See original paper for figures.

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