

Effects of lanthanum on growth and accumulation in roots of rice seedlings

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ABSTRACT

Hormetic effects on the growth were found in the roots of rice (*Oryza sativa* L. cv. Shengdao 16) exposed to increasing concentrations of La³⁺ (0.05, 0.1, 0.5, 1.0, and 1.5 mmol/L). The results indicated that La³⁺ promoted the growth of rice roots at 0.05 mmol/L, but inhibited the growth at 1.0 and 1.5 mmol/L La³⁺ after 13 days of exposure. Transmission electron microscope showed that La³⁺ was mainly deposited in the cell walls of the roots. In addition, the accumulation of K, Mg, Ca, Na, Fe, Mn, Zn, Cu, and Mo in the roots was also affected with the exposure of different La³⁺ treatments. It showed that La³⁺ affected the nutritional status of roots and further regulated the growth of rice.

Keywords: trivalent lanthanum; hormesis; nutrient element; rare earth element antagonism

The rare earth elements (REEs) comprise a group of 17 elements with similar chemical properties. REEs enriched fertilizers have been used in China since the 1970s (d'Aquino et al. 2009), and the effects of REEs on physiological responses were reported in different plant species (Fashui et al. 2000, Chen et al. 2001, Hu et al. 2002). Chlorophyll content, photosynthetic rate, and plant biomass could be enhanced by REEs (Wu et al. 1983, Chang 1991, He and Xue 2005). It was found that trivalent lanthanum (La³⁺) stimulates the growth of tobacco seedlings and accelerates the light reactions of photosynthesis at suitable concentration *in vivo* (Chen et al. 2001). Moreover, appropriate amount of REEs not only promotes seed germination, harvest quality, shoot growth, and root development, but also improves the plant resistance against stress and element uptake (He and Xue 2005, d'Aquino et al. 2009, Liu et al. 2012). REEs can enter into mesophyll cells via apoplast and symplast channels or plasmodesmata, and participate in the regulation of physiological and biochemical function (Gao et

al. 2003, Guo et al. 2007, Ye et al. 2008). However, the results of field trials and laboratory studies on REEs are inconsistent until now (Diatloff et al. 1995, 2008, von Tucher and Schmidhalter 2005).

Hormesis is defined as a phenomenon that low doses of an otherwise harmful agent can result in stimulatory or beneficial effects (Calabrese and Baldwin 2003). Accurate description of hormetic dose-response is an important step for the determination of the efficacy and hazards of La³⁺ with the hormetic phenomenon. However, how to evaluate the ecotoxicity of La³⁺ by combination of traditional threshold models and hormesis has not been thoroughly documented. Furthermore, there is little information available on how REEs increases the sensitivity of cell to plant growth regulators. The aims of the present study are to investigate the bioaccumulation of La and the uptake of mineral elements in seedlings of *Oryza sativa* L. (cv. Shengdao 16) exposed to increasing concentrations of La³⁺. In addition, the hormetic effects of La³⁺ on nutrient accumulation and plant growth was investigated.

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MATERIAL AND METHODS

Plant material and plant growth. Seeds of rice (*O. sativa* L. cv. Shengdao 16) were sterilized by soaking in 75% alcohol for 1 min, in 0.1% mercury chloride for 15 min, and in 1.0% sodium hypochlorite for 20 min before being rinsed five times with sterilized water. Seeds were germinated in half-strength Murashige and Skoog agar medium at pH 5.8 (0.75 mmol/L $MgSO_4$, 10 mmol/L NH_4NO_3 , 9.4 mmol/L KNO_3 , 0.625 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 2.5 μ mol/L KI, 50 μ mol/L H_3BO_3 , 50 μ mol/L $FeSO_4$, 50 μ mol/L $MnSO_4$, 15 μ mol/L $ZnSO_4$, 0.05 μ mol/L $CuSO_4$, 0.05 μ mol/L $CoCl_2$, 0.5 μ mol/L Na_2MoO_4 , 50 μ mol/L Na_2H_2EDTA , 0.15 μ mol/L thiamine, 1.2 μ mol/L pyridoxine, 2.0 μ mol/L nicotinic acid, 275 μ mol/L inositol, 0.56% agar, 3.0% sucrose, 0.05% Mes). Then the different concentrations of $La(NO_3)_3$ (0, 0.05, 0.1, 0.5, 1.0, and 1.5 mmol/L) were added to the basal medium. The plants were grown at $25.0 \pm 2^\circ C$ using a 14/10 h light/dark cycle in a growth chamber under a light intensity of 200 μ mol/m²/s and cultivated for 13 days.

Root growth measurements. The lengths of seminal and nodal roots, the dry weight (DW) and fresh weight (FW) of roots, as well as the number of nodal roots and lateral roots on seminal or nodal roots (20 plants) were determined in 13 day-old seedlings.

La and mineral nutrition elements analysis. Roots were washed with deionized water, and 0.1 g sample (dry matter basis) was weighed into teflon bombs and digested in 10 cm³ of HNO_3 using a microwave-assisted procedure. The content of La, K, Mg, Ca, Na, Fe, Mn, Zn, Cu, and Mo was determined by inductively coupled plasma sector field mass spectrometry (ICP-SF-MS) (Agilent, Tokyo, Japan).

Transmission electron microscope analysis. The root samples were prepared according to the method described by Xu et al. (2010). In brief, the roots were fixed for 2 h at 4°C in 2.5% (v/v) glutaraldehyde and 0.1 mol/L phosphate buffer solution (pH 7.3), and postfixed in 1% (w/v) aqueous osmium tetroxide for 2 h. Samples were dehydrated in a 50–100% ethanol series and embedded in Epon 812 resin. Ultra-thin sections of 70 nm thickness were cut with an Ultracut Ultramicrotome (Leica, Bensheim, Germany) and stained with uranyl acetate and lead citrate. Then the subcellular distribution of La was detected by a Hitachi

H-600 transmission electron microscope (TEM, Hitachi, Tokyo, Japan).

Statistical analyses. The values in the text are mean \pm standard deviation (SD) from two individual experiments. Statistical comparisons were done with one-way ANOVA using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA). Tukey's test was performed for post hoc comparisons when the difference was significant ($P < 0.05$).

RESULTS

Effect of La^{3+} on root length. The length of total nodal roots was induced by 0.05 mmol/L La^{3+} , but no effects were observed on the length of seminal roots at 0.05 and 0.1 mmol/L La^{3+} (Figures 1 and 2A). Moreover, the length of seminal roots and total nodal roots was significantly inhibited by increasing La concentrations from 0.5 to 1.5 mmol/L (Figures 1 and 2A).

Effect of La^{3+} on the root number of rice. Effects of La^{3+} on the root number were reported in Figure 2B. Although the lateral root number of seminal roots was unaffected by 0.05 mmol/L La^{3+} , it was significantly inhibited by increasing La concentrations from 0.1 to 1.5 mmol/L (Figure 2B). The effects of La^{3+} on nodal roots and total lateral roots were closely similar to those described for the lateral root number of seminal roots. The number of nodal roots and total lateral roots was significantly inhibited by increasing La concentrations from 0.5 to 1.5 mmol/L (Figure 2B). However, the number of nodal roots was significantly increased by

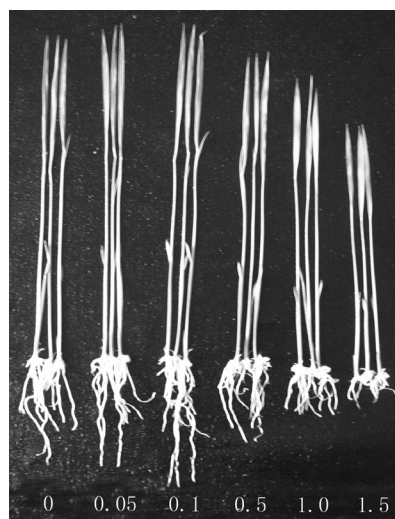


Figure 1. Effect of 0.05, 0.1, 0.5, 1.0, and 1.5 mmol/L La^{3+} on the length of rice root

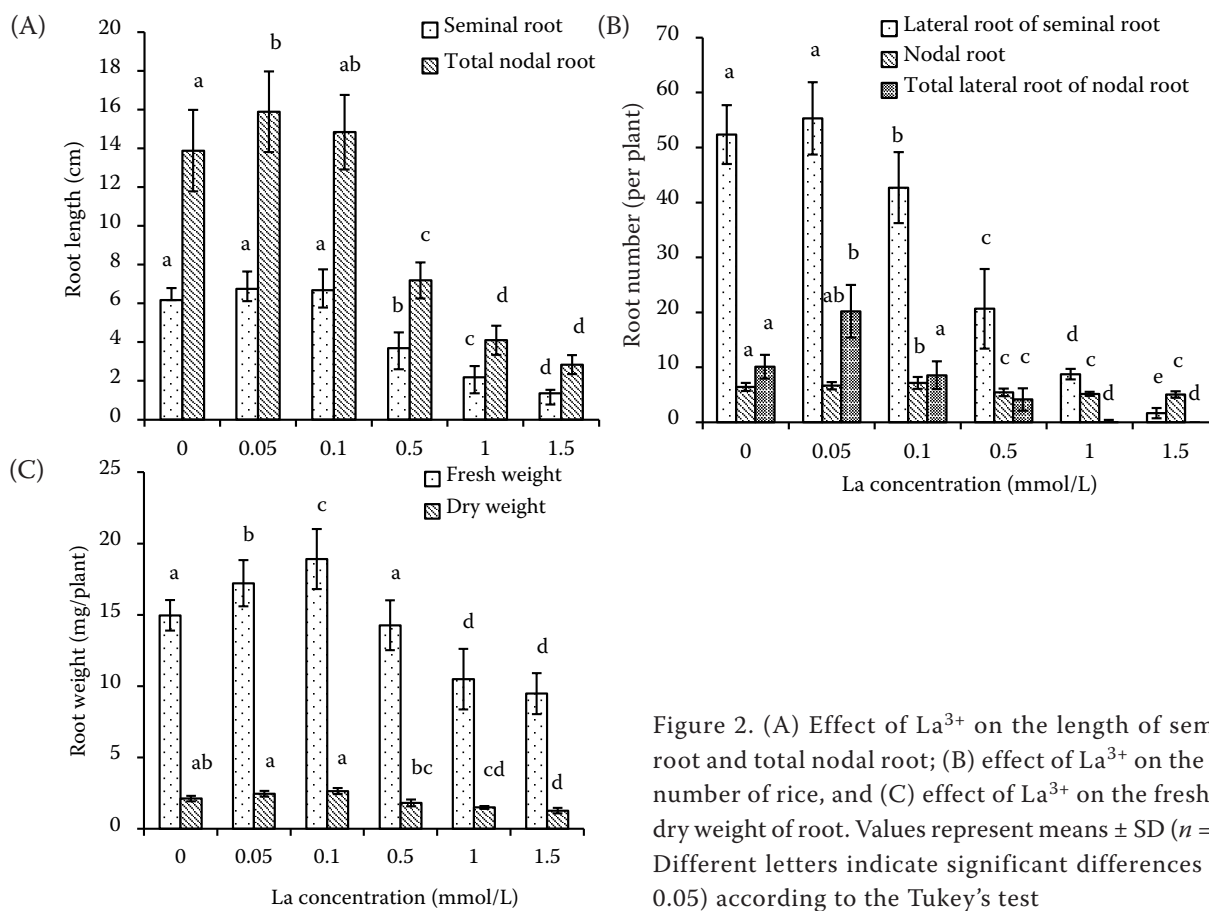


Figure 2. (A) Effect of La³⁺ on the length of seminal root and total nodal root; (B) effect of La³⁺ on the root number of rice, and (C) effect of La³⁺ on the fresh and dry weight of root. Values represent means \pm SD ($n = 20$). Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test

0.1 mmol/L La³⁺ and total lateral roots was increased by 0.05 mmol/L La³⁺, respectively (Figure 2B). The number of nodal roots was maximum at 0.1 mmol/L La³⁺, but the number of lateral roots of seminal roots and total lateral roots of nodal roots was maximum at 0.05 mmol/L La³⁺ (Figure 2B).

Effect of La³⁺ on the fresh and dry weight of root. As shown in Figure 2, the fresh weight of roots was significantly increased at 0.05 and 0.1 mmol/L La³⁺, and the fresh and dry weight of rice roots was unaffected in the presence of 0.5 mmol/L La³⁺ (Figure 2C). However, increasing La concentration to 1.0 and 1.5 mmol/L significantly reduced the fresh and dry weight of roots (Figure 2C).

Bioaccumulation of La³⁺ and the effect of La³⁺ on nutrient uptake. While the La content in roots increased with La³⁺ supply, mineral nutrients responded differentially to different La³⁺ concentration (Table 1). The concentrations of K and Mo was significantly enhanced by 0.05 and 0.1 mmol/L La³⁺, but K, Ca, and Mo negatively affected by 1.0 mmol/L La³⁺ (Table 1). Though the content of Zn and Cu were decreased, the content of Mg and Mn were increased with the exposure

of different concentration of La³⁺ (Table 1). The concentration of Na was significantly increased by 0.05 and 0.1 mmol/L La³⁺, while the treatments of 0.5 and 1.0 mmol/L La³⁺ significantly increased the concentrations of Fe (Table 1). Furthermore, the data of TEM analysis showed that there was no La deposit in cell walls in the control (Figure 3A). However, La was mainly deposited in the cell walls of the roots exposed with 1.0 mmol/L La³⁺ (Figures 3B,C).

DISCUSSION

Our results showed that 0.05 and 0.1 mmol/L La³⁺ promoted the growth of rice roots, but 1.0 and 1.5 mmol/L La³⁺ inhibited the growth after 13 days of exposure. On the other hand, the fresh and dry weight of roots was also inhibited significantly by 1.0 and 1.5 mmol/L La³⁺. A similar result was obtained by Diatloff et al. (1995), who reported that corn root growth increased significantly with the applications of lower concentration of La³⁺. In contrast to the results of another study (d'Aquino et al. 2009), the results suggested that

Table 1. Bioaccumulation of La and some other elements in the roots of rice ($n = 3$)

Element	La concentration (mmol/L)				
	control	0.05	0.1	0.5	1.0
La ($\mu\text{g/g DW}$)	1.17 ± 0.10^a	566.52 ± 26.58^b	1228.60 ± 25.64^c	4111.57 ± 94.58^d	7216.96 ± 66.80^e
K (mg/g DW)	32.96 ± 0.48^a	35.36 ± 1.61^b	36.55 ± 0.38^b	31.08 ± 1.79^a	25.66 ± 1.38^c
Mg (mg/g DW)	0.82 ± 0.10^a	0.88 ± 0.07^a	1.23 ± 0.05^b	0.86 ± 0.06^a	0.92 ± 0.03^a
Ca (mg/g DW)	1.37 ± 0.07^a	1.59 ± 0.08^a	1.49 ± 0.16^a	0.92 ± 0.09^b	0.83 ± 0.18^b
Na (mg/g DW)	1.33 ± 0.04^a	2.07 ± 0.18^b	1.71 ± 0.14^c	1.35 ± 0.09^a	0.83 ± 0.09^d
Fe ($\mu\text{g/g DW}$)	331.99 ± 15.49^a	301.88 ± 9.65^b	315.54 ± 7.08^{ab}	374.27 ± 5.99^c	415.17 ± 7.61^d
Mn ($\mu\text{g/g DW}$)	13.11 ± 0.07^a	23.63 ± 1.26^b	30.58 ± 2.00^c	32.86 ± 2.34^c	44.14 ± 1.87^d
Zn ($\mu\text{g/g DW}$)	104.20 ± 5.06^a	92.88 ± 1.01^b	78.35 ± 5.82^c	70.83 ± 2.56^c	51.52 ± 1.56^d
Cu ($\mu\text{g/g DW}$)	12.13 ± 0.90^a	9.14 ± 0.79^b	7.62 ± 0.62^{bc}	7.00 ± 0.49^c	7.14 ± 0.92^{bc}
Mo ($\mu\text{g/g DW}$)	3.41 ± 0.21^a	4.08 ± 0.16^b	4.36 ± 0.14^b	1.85 ± 0.21^c	1.45 ± 0.09^c

Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test. DW – dry weight

REEs enhanced root growth at low concentrations and inhibited root growth at high concentrations.

Previous study demonstrated that REEs can regulate plant growth by affecting the uptake of mineral elements (Hu et al. 2004). Such effects also occur in the case of La^{3+} in rice roots with stimulatory effects on nutrient uptake at low and inhibiting effects at high La concentrations. In this study, the bioaccumulation of K, Ca, and Mo was promoted by 0.05 and 0.1 mmol/L La^{3+} , but inhibited by 0.5 and 1.0 mmol/L La^{3+} . Though the levels of Zn and Cu were decreased, Mg and Mn level was increased by La^{3+} treatments. Intracellular Ca^{2+} is linked to physiological processes in plants, which may act as a second messenger in responses to most external stimuli in plants (Gehring et al. 1990, Irving et al. 1992, Webb et al. 1996). Tyler (2004) reported that REE ions could replace Ca^{2+} or interact with it in the variety of physiological

functions. Our study also showed that La^{3+} affected the accumulation of Ca^{2+} , but the regulatory mechanism needs to be further studied. In addition, since the bioaccumulation of K, Ca, and Mo was inhibited by higher concentrations of La^{3+} , higher concentrations of Fe, Mg and Mn would be required for the root growth and make up the deficiency of these elements.

In this study, it was found that the effect of La^{3+} on the growth of roots was related to the concentration of La^{3+} and non-uniform accumulation of nutrients. The study showed that, with increasing supply, the La content in the biomass of rice seedlings increased and deposited in cell walls. Once the accumulation exceeds the detoxification capacity of plant tissues, metals will become toxic to plants (Sinha et al. 1996). In our study, this threshold was reached at a concentration of 1.0 mmol/L La^{3+} in the nutrient solution. Hormetic

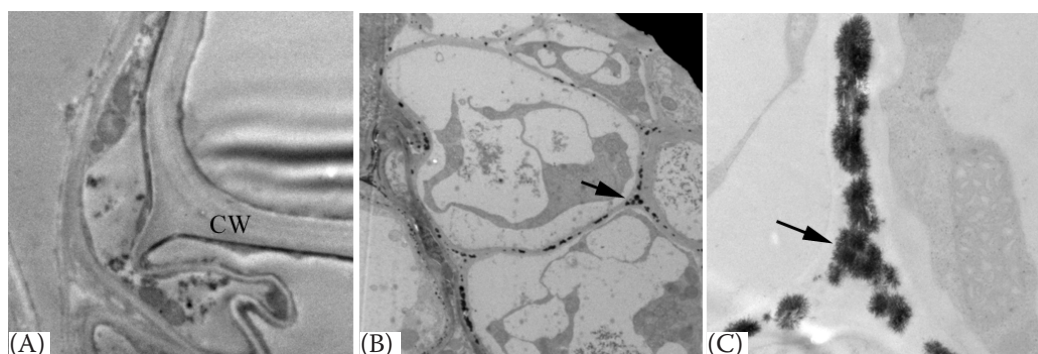


Figure 3. The subcellular distribution of La in the root. (A) Control, showing no La particle deposited in the cell wall (CW); (B) and (C) root cell treated with 1.0 mmol/L La^{3+} , showing La particles located in the CW (arrow indicates La particles)

effects generally show two kinds of trends, including the low-dose-stimulation and the high-dose-inhibition effects (Calabrese and Baldwin 2003, Rodricks 2003). In this study, the hormetic effects of La^{3+} on the growth were also observed. The growth of rice roots was promoted by 0.05 and 0.1 mmol/L La^{3+} , while 1.0 and 1.5 mmol/L La^{3+} inhibited the root growth. The hormetic effects of La^{3+} may be related with the uptake of some nutrients, such as K, Ca, and Mo.

In summary, the present study showed hormetic effects for La^{3+} supply to the growth of rice seedlings. La^{3+} could be accumulated in cell walls of rice seedling roots. In addition, the accumulation of K, Mg, Ca, Na, Fe, Mn, Zn, Cu, and Mo in the roots was also affected by La^{3+} treatments, which indicated that La^{3+} affected the nutritional status of roots and further regulated the growth of rice roots.

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