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Effects of Lanthanum and Cerium on the Vegetable Growth of Wheat (*Triticum aestivum* L.) Seedlings

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Lanthanum and cerium are two important elements of the rare earth elements (REEs) and their effects on biological systems have attracted much attention since the 1970s. They are the main components of commercial REEs micro-fertilizer, which are widely used in agriculture in China. For example, cultivated area used REEs micro-fertilizer in China has added up to 4.33×10^8 ha and 1.10×10^7 kg REO (rare earth oxide) has been consumed in the last twenty years (Wang and Zheng, 2001). Numerous applications suggest that REEs micro-fertilizer can import many visual benefits to plants such as darker green foliage, an enhanced rate of development, a greater production of roots and better fruit color. Above all, REEs micro-fertilizer can increase the yield by about 10%(Zheng et al., 2000). But no proof shows that rare earth elements are the essential elements for plant growth and the physiological mechanism to increase yields is still unclear. Results from limited studies on the effect of REEs on plant growth are conflicting. For example, Diatleff et al (1998) reported that La or Ce in culture medium from 0.1 to 2 ppm (1-16muM) were found to be toxic to the root elongation of corn and mungbean and Ce at 0.03ppm(0.2muM) reduced the total dry weight of mungbean by 44%. La inhibits root elongation of wheat (Parker et al., 1988). Colloidal lanthanum causes an almost complete inhibition of cell division and root elongation in the root tips of barley plants (Van Steveninck et al., 1976). But He and Loh (2000) reported that cerium nitrate (0.5-10uM) or lanthanum nitrate (0.5-50uM) in the culture medium significantly increased the length of the primary roots of *Arabidopsis thaliana*. Meehan et al (1993) reported that the applications of lanthanum and cerium increased spike production in wheat. Many questions remain unresolved, such as the toxic and physiological effects of REE fertilizer on plant and animals. So there are many arguments on the agricultural application of REEs. Up to now, the reports regarding REEs on plant growth had been only conducted on one or two stages of plant growth and development. And, tens of trace elements such as Fe, Mn, Mo was also found in commercial REEs micro-fertilizer of China. Maybe the beneficial effects resulted from those trace elements. Further, no studies have been done to compare the effects of La and Ce on the vegetable growth of crops. In view of those, lanthanum nitrate and cerous chloride were applied to wheat, an important agricultural crop in China. Through exposing experiment, we determined the root length, dry weight of roots and shoots, mineral elements content and bioaccumulation of lanthanum and cerium. From four stages, we investigated the physiological and toxic effects of La and Ce on the vegetable growth of wheat. It will help for assessing the effects of REEs on crop growth.

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MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) was chosen as the test plant. Its seeds were surface sterilized with 2% NaClO for 30 minutes and washed thoroughly with sterilized distilled water. They were placed in an illuminating incubator at 25 ± 1℃ for germination. After 2d, they began to germinate and were transplanted to nylon net to be cultivated with complete nutrient solution. The experiments were conducted in a green house with a photoperiod of 14h/10h (light / dark). The composition of complete nutrient solution (pH5.5$\pm$0.2) was as follows:(in mM) 1 $Ca(NO_3)_2$, 1KNO_3, 1 KH_2PO_4, 0.5$MgSO_4$, 0.004Fe-EDTA, 0.0013$MnSO_4$, 0.153$ZnSO_4$, 0.111H_2MnO_4, 0.081H_3BO_3, 0.064$CuSO_4$.

Two-day-old seedlings were transferred to plastic pots (8 plants/pots) filled with 200mL nutrient solution as mentioned above (0.25 mM KCl in place of 1mM KH_2PO_4 avoiding the precipitation of $LaPO_4$(Ksp = 10^{-23}) and $CePO_4$(Ksp = 10^{-23})). Metals were directly added in separate treatments as follows (in mg/L): control (no additional La or Ce), five concentrations of La (0.5, 1.0, 2.0, 5.0, 10.0, 25.0 mg/L), five of Ce (0.5, 1.0, 2.0, 5.0, 10.0, 25.0 mg/L) and four concentrations of La+Ce (0.5+0.5, 0.5+10.0, 10.0+0.5, 10.0+10.0 mg/L). The test medium was changed every two days and 1mM KH_2PO_4 was applied through foliage at the end of each photoperiod. The seedlings were collected after 7-day culture. All the treatments were triplicate.

Plant samples were harvested and washed with distilled water, then cut into shoots and roots, and the root length were measured. Plant samples were dried at 85℃ in an oven, the dry weights of roots and shoots measured. Samples of shoots and roots were placed in 25 ml beakers, soaked with concentrated HNO_3 (4.0 ml) and $HClO_4$ (1.0 ml) for 24 hours, then were heated. When the solution evaporated to near dryness, the beakers were removed from the heat, after cooling, the residues were dissolved in 7% HCl. The resultant solutions were subsequently used for analysis by ICP-AES to determine the elements (K, Ca, Mg, Mn, Zn) and REEs (La, Ce) (detection limits of La and Ce in ICP-AES: Ce, 0.008mg/L and La, 0.0015mg/L).

RESULTS AND DISCUSSION

Mineral elements were analyzed (Table 1) to test whether La and Ce could have beneficial effects on the uptake and accumulation of nutrient mineral elements. The concentrations of main mineral elements in seedlings were lower than that in control (Table 1). Ca, Mg, Mn and Zn in shoots and Ca, Mg in roots decreased with the increase of La or Ce in medium. They were negative correlation with the concentrations of La or Ce in culture medium. Table 1 also showed that mineral element contents in shoots of (La+Ce)-treatments were lower than that of control. So addition of La and Ce in combination found no great protective effects on the uptake of mineral nutrient elements. It was consistent with the results of Diatleff et al (1998). La had similar effects as Ce on the uptake of mineral elements. Because of a similar ionic radius to Ca^{2+} and a valence higher than Ca^{2+}, La^{3+} and Ce^{3+} could bind to superficially located Ca^{2+} absorption sites in a less reversible manner than Ca^{2}. La^{3+} can block calcium ionic channels (Pineros and Tester, 1997). It also reported that La^{3+} was nonselective block of ion channel involved

Table 1. Contents of mineral elements in shoots and roots after 7 days exposure.

	Ca (‰)	K (%)	Mg (‰)	Cu (mg/Kg)	Fe (mg/Kg)	Mn (mg/Kg)	Zn (mg/Kg)
Shoots							
control	3.39	5.44	2.11	24.0	142	50.0	58.8
0.5La	2.83	4.73	1.77	12.1	128	49.5	60.0
1.0La	2.15	4.85	1.72	13.2	125	42.4	59.1
2.0La	1.89	4.96	1.68	13.2	120	38.7	58.2
5.0La	1.75	4.91	1.65	14.2	116	36.4	55.6
10La	1.66	4.88	1.61	14.1	123	35.1	53.1
25La	1.29	4.67	1.57	15.9	124	34.6	46.2
0.5Ce	2.18	4.79	1.81	11.3	111	48.9	56.0
1.0Ce	2.03	4.68	1.8	13.7	116	45.4	53.7
2.0Ce	1.98	4.32	1.79	12.5	107	40.8	52.2
5.0Ce	1.57	4.53	1.68	12.9	112	39.6	51.9
10Ce	1.27	4.67	1.62	14.0	123	38.8	51.1
25Ce	1.12	4.48	1.61	14.3	106	33.6	50.5
0.5Ce0.5La	2.17	4.89	2.08	13.4	117	47.2	58.3
0.5Ce10La	1.36	4.26	1.86	12.8	111	38.2	55.7
10Ce0.5La	1.40	4.53	1.48	12.9	96.4	35.1	54.9
10Ce10La	1.13	4.84	1.24	13.7	93.8	33.4	49.7
Roots							
control	2.87	8.31	3.98	24.5	257	98.7	96.5
0.5La	2.69	8.82	2.89	16.5	350	50.4	96.5
1.0La	2.43	8.19	2.79	16.8	327	51.7	97.4
2.0La	2.07	7.34	2.75	17.1	343	52.4	98.4
5.0La	2.04	7.05	2.43	16.9	335	48.7	98.6
10.0La	2.02	6.81	2.03	16.4	313	39.6	97.6
25La	1.90	6.61	1.64	17.8	325	45.6	96.6
0.5Ce	2.26	7.67	2.35	16.7	252	52.3	88.0
1.0Ce	2.19	7.59	2.22	14.9	294	49.9	86.5
2.0Ce	2.11	7.63	2.15	13.7	314	45.4	92.9
5.0Ce	1.87	7.35	1.98	15.7	305	43.3	89.9
10Ce	1.43	7.18	1.94	15.9	309	40.3	82.5
25Ce	1.08	6.49	1.86	17.1	335	44.6	85.1
0.5Ce0.5La	2.58	8.64	2.49	21.5	327	53.0	103
10Ce0.5La	1.78	6.71	158	18.3	263	43.0	89.3
0.5Ce10La	1.41	6.37	1.26	18.4	300	49.8	86.9
10Ce10La	1.09	6.15	1.06	17.7	290	45.2	92.0

in signal transduction (Lewis and Spalding, 1998). So they may disturb the uptake of nutrient ions through ions channels.

Table 2. Effects of La and Ce on the mean primary root length(±SE) after 7-day exposure to La and Ce applied alone and in combination.

Treatments	Root length(cm)	Treatments	Root length(cm)
control	13.9±1.68		13.9±1.68
0.5Ce	13.7±1.51	0.5La	14.2±1.12
1.0Ce	10.5±1.23	1.0La	11.2±0.967
2.0Ce	7.23±0.754	2.0La	7.60±1.21
5.0Ce	6.52±0.589	5.0La	6.45±0.893
10.0Ce	5.54±0.480	10.0La	5.53±0.822
25.0Ce	4.65±0.603	25.0La	4.96±0.406
0.5Ce0.5La	11.4±1.75	10.0Ce0.5La	5.08±0.868
0.5Ce10.0La	4.9±0.823	10.0Ce10.0La	4.64±0.736

Table 2 showed the mean root length of seedlings after exposure to La and Ce alone and in combination. La and Ce at low concentrations (0.5 mg/L) had no obvious effects on the elongation of roots. La and Ce at high concentrations (≥1. 0 mg/L) obviously inhibited the elongation of roots. For example, the primary mean root length was shorter than that of control in our study. The inhibition enhanced with the increase of La and Ce in culture medium. La and Ce had similar inhibiting effects on root elongation when La

Table 3. Effects of La and Ce on the relative dry weight of 9-day-old seedlings after seed germination (7days exposure).

Treatments	Relative yields and interaction					
	Shoots			Roots		
	Observe	Calculation	Interaction	Observe	Calculation	Interaction
Control	1.00			1.00		
0.5Ce	1.08			0.929		
10.0Ce	0.915			0.627		
0.5La	1.04			0.949		
10.0La	0.945			0.581		
0.5Ce0.5La	0.976	1.12	Synergistic	0.809	0.882	Synergistic
0.5Ce10La	0.945	1.02	Synergistic	0.652	0.54	Protective
10Ce0.5La	0.933	0.952	Synergistic	0.749	0.595	Protective
10Ce10La	0.903	0.865	Protective	0.601	0.364	Protective

The mean dry weight (mg/plant) ±SE. of control are 8.74±0.268(roots) and 16.36±1.50(shoots)

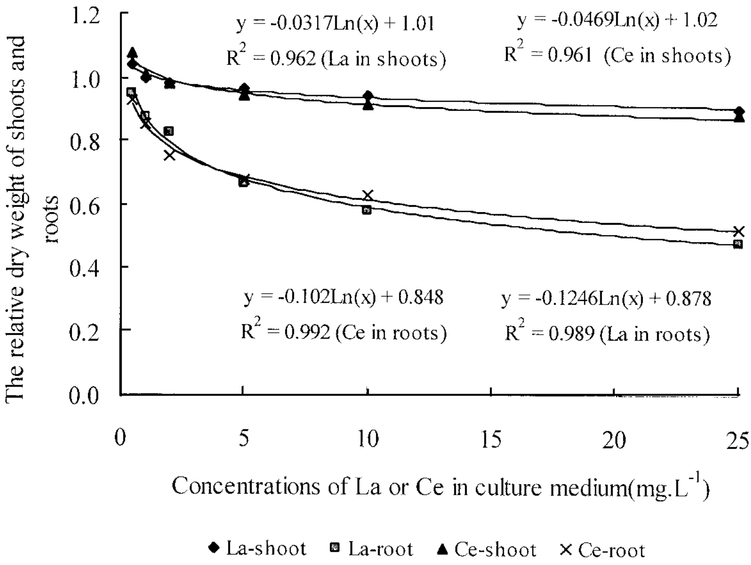

Figure 1. Changes of the relative yields with additional concentrations of La and Ce in medium.

and Ce applied alone. When La and Ce applied in combination, the primary root length of seedlings was almost equal to that of La or Ce-treatments alone. So combination of La and Ce didn't show protective effects on root elongation.

As compared to observed relative yield of control (1.00), the relative dry weight of shoots at 0.5mg/L La or Ce in culture medium were beyond 1.00 and they at 1.0mg/L La or Ce were almost equal to 1.00 (Figure 1). So La or Ce at low concentrations (below 1.0 mg/L) had some beneficial effects on the growth of shoots. But La or Ce at all additional concentrations significantly reduced the dry weight of roots (Figure 1). Roots were easier to be damaged by rare earth elements than shoots. The growth of roots could be considered as a sensitive biomarker of damage of rare earth elements. According to figure 1, La and Ce had similar effects on the growth of the seedlings. Regression equations between the relative yields and the additional concentrations of La and Ce were given (Figure 1). The growth of plant was inhibited when their relative yields were less than 1($W_{treatment}/W_{control}<1$). So the safe additional concentration threshold values were calculated at $W_{treatment}/W_{control}=1$ according to the regression equations. La or Ce applied alone at about 1.5mg/L and 0.3mg/L would inhibit the growth of shoots and roots respectively. When two metals were applied in combination, calculated relative yields, using the Colby procedure (cited by Chaoui et al., 1997) (A $\times$B = calculated relative yield), for the two plant parts are also given in Table 3. This technique of multiplication of observed relative yields was used to determine the nature yields of the La-Ce interactions (synergistic, additive or protective). For all treatments except 0.5Ce0.5La-treatment, observed relative yields of roots were higher than calculated relative yields predicted by multiplication of components, and for all

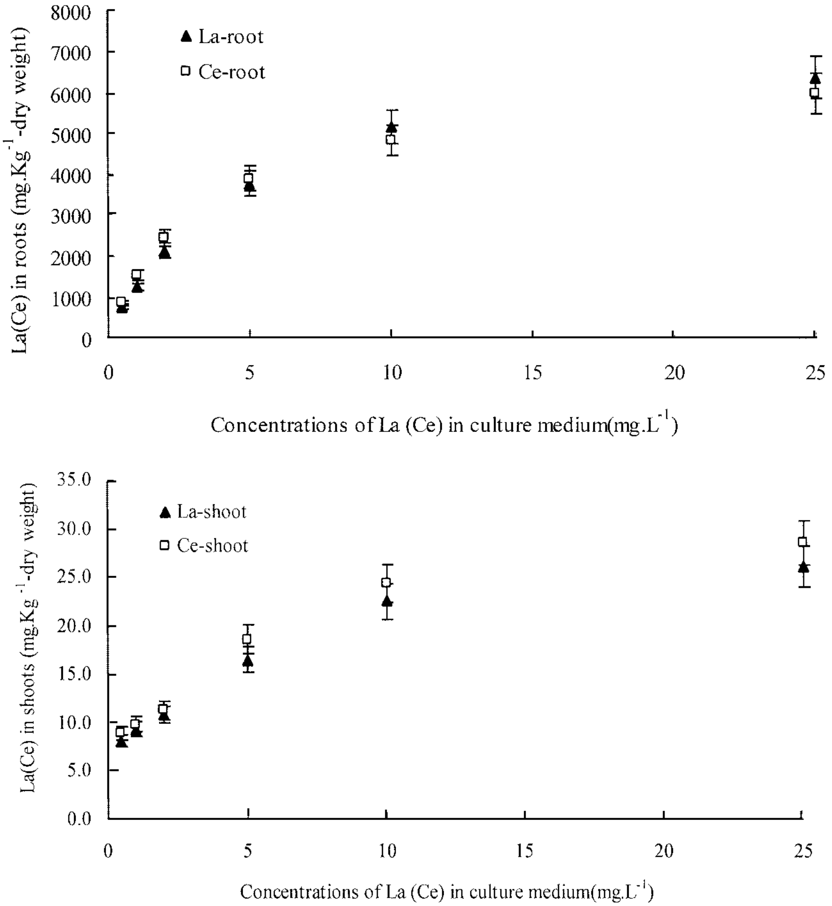

Figure 2. The concentrations of La and Ce accumulated in shoots and roots of seedlings when they were applied alone.

treatments except 10Ce10La-treatment, observed relative yields of shoots were lower than calculated relative yields. There were some protective effects on the growth of roots in the La+Ce-treatments. Compared to the damage of shoots by high concentrations of La and Ce, combination of La and Ce had protective effects on the growth of shoots.

Figure 2 showed the contents of La and Ce accumulated in shoots and roots. With the increase of concentrations of La and Ce added in culture medium, contents of La and Ce accumulated in shoots and roots increased obviously. La and Ce accumulated in shoots and roots were apparent positive correlation with their concentrations added to the culture medium. Their contents in roots were much higher than that in shoots (Figure 2). So La or Ce was mainly deposited in roots of plant and only part of them was

Table 4. Contents of lanthanum and cerium in shoots and roots when La and Ce applied in combination (mg/Kg-dry weight).

Treatments	Shoots		Roots	
	La	Ce	La	Ce
0.5Ce0.5La	4.53	6.61	720	774
0.5Ce10La	24.6	5.11	3945	179
10.0Ce0.5La	2.27	18.5	198	4084
10.0Ce10.0La	9.06	10.1	2490	2826

transported to shoots. Their high accumulation in roots may cause nutrient disturbance and growth inhibition of seedlings. In order to better understanding the interaction of La and Ce, their contents were listed in Table 4 when they were used in combination. The selective uptake was found between La and Ce by seedlings. For example, contents of Ce were higher than La in shoots and roots of seedlings at 0.5Ce+0.5La and 10.0Ce+10.0La-treatmments. Compared 0.5Ce0.5La-treatment with 0.5Ce10La-treatmment, high additional of La inhibited the uptake of Ce, so did Ce in 10.0Ce0.5La-treatment and 10.0Ce10.0La-treatment.

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