

Lanthanum Resulted in Unbalance of Nutrient Elements and Disturbance of Cell Proliferation Cycles in *V. faba* L. Seedlings

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Abstract Effects of lanthanum (La) on mineral nutrients, cell cycles, and root lengthening have been little reported. The present work investigated these physiological responses in roots of *Vicia faba* seedlings cultivated in La³⁺-contained solutions for 15 days. The results showed that the increasing contents of La in the roots and leaves contributed to disbalances of contents of Ca, Fe, Cu, Zn, Mg, Mn, P, and K elements, and potential redistributions of some elements in the roots and leaves. These disbalances might be involved in the subsequent alteration of cell cycle phases in the root tips. Low-dose promotion and high-dose inhibition (Hormetic effects) were demonstrated as the dose responses of G0/G1-, S- or G2/M-phase ratios. The cell cycles were most probably arrested at G1/S interphase by La³⁺ in the root tips. The fact that the root lengths were not consistent with the changes of cell cycle phases suggested that the cell proliferation activities might be masked by other factors (e.g., cell expansion) under long-time exposure to La³⁺.

Keywords Rare earth · Mineral nutrients · Cell cycle phase · Growth regulation · Hormesis

Introduction

Over the past 30 years, rare earth element (REE)-based microfertilizers have been widely applied to enhance yield and improve quality of crops and vegetables (e.g., seed germination, root lengthening, chlorophyll content, nutrient absorption, and photosynthesis)

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owing to the specific properties of REEs [1–3]. However, effects of REEs on plant development vary a lot due to growth medium (pH value, cation exchange capacity, organic carbon, etc.), bioavailability of REEs, and growth stages of plants [4–6].

Concerns about the ecotoxicological effects and physiological mechanisms of REEs on organisms have been rising in recent years. REEs were found to regulate plant growth by affecting contents and distribution of mineral elements (Ca, Fe, Cu, K, P, Mg, etc.) [1, 7]. REE has stronger reactivity to non-metallic elements than calcium in biomolecules (oxygen, hydrogen, and nitrogen). REEs can also displace biometals in metalloproteins of membrane, metallic proteins and enzymes, leading to disturbance of mineral elements and cell membrane permeability [6, 8]. The binding of REE can thus modify active conformations of biomolecules and disorder biological functions [8]. REEs also have the capability to interfere with some metallic and non-metallic enzymes in cell mitotic activity, leading to disturbance of cell cycles. However, little reports are available about the correlation among the disturbance of nutrient elements, cell proliferation cycles, mitotic activity, and plant growth.

La, as a crucial component of REE-based microfertilizers, was used as a representative of light REEs in this experiment. The objectives are aimed to investigate (1) alterations of mineral nutrient contents and cell cycles, and (2) their possible relationship to root lengthening of *V. faba* seedlings cultured in La³⁺-polluted nutrient solutions.

Materials and Methods

Plant Material and La³⁺ Treatments

Seeds of *V. faba* were surface-sterilized with 0.1% (*m/v*) sodium hypochlorite solution for 10 min and rinsed thoroughly in distilled water. After germination at 22–24°C, six uniform seeds were selected and transplanted into 1.2-L containers filled with equal Hoagland solution [9]. Ammonium dihydrogen phosphate (0.5 mM) was directly sprayed over the seedlings everyday instead of dissolution in the solutions. Extraneous La³⁺ were 0, 1, 2, 4, and 8 mg/L in the solutions by appropriate dissolution of La (NO₃)₃, respectively. The solutions were replaced every 2 days, and pH values were maintained between 5.5 and 5.8. The containers were placed in a growth chamber under controlled conditions (15-h photoperiod with active radiation of 200 μmol m⁻² s⁻¹, 75% relative humidity, and 23/19°C day/night regime), and aerated for 24 h everyday. Three containers were prepared in each treatment in two independent experiments. Roots and leaves were harvested for chemical analysis and biological measurements after the treatment of 15 days.

Measurement of Root Lengths and Shoot Heights

Lengths between apical buds and stem base were measured denoting as heights of seedlings. Root lengths were measured from stem base to primary root tips.

Measurement of Element Contents by ICP-OES

Fresh roots were rinsed first with 1 M HCl and then with distilled water. Digestion of samples was performed according to the previous protocol [10, 11]. Elemental contents were detected by inductively coupled plasma optical emission (ICP-OES) and expressed as micrograms per gram dry weight (DW). Certified standard samples (GBW07429) and triplicates of all samples were used to ensure accuracy and precision.

Determination of Cell Cycles and Proliferation Index in Root Tips

Fifty root tips were cut from each container and immediately fixed in 4% (v/v) formaldehyde in Galbraith buffer [12], supplemented with 1% (w/v) polyvinylpyrrolidone and 10 mM sodium metabisulfite (pH 7.0) for 30 min at 4°C, and then washed thoroughly in cold Galbraith buffer for 10 min. Nuclei were isolated and purified as described by [13]. The pelleted nuclei were resuspended in 0.75 M hexanediol and incubated with PI/RNase Staining Buffer (BD Pharmingen™) in dark for 2 h at room temperature. Cell cycles were detected by flow cytometry (FACSCalibur, Becton Dickinson, USA) at 488-nm excitation and 525-nm emission. A total of 20,000 nuclei were measured for each sample. Peak at channel 200 denotes G0/G1 phase, peak at 400 denotes G2/M phase, and region between channel 200 and 400 represents S phase. G0/G1-, S-, and G2/M-phase ratios were calculated with the equipped software in the apparatus according to DNA distribution. Proliferation index was calculated according to formula $(S\text{-phase ratio} + G2/M\text{-phase ratio}) / (G0/G1\text{-phase ratio} + S\text{-phase ratio} + G2/M\text{-phase ratio}) \times 100\%$.

Statistical Analyses

All the statistical analyses were performed using SPSS 13.0. The data were all presented as mean \pm standard deviations of three replicates. Difference was considered to be significant at $p < 0.05$ and highly significant at $p < 0.01$ using one-way ANOVA by Dunnett's *t* test. Representative photographs from each treatment were presented.

Results

Changes of Element Contents in Roots and Leaves

The contents of La, K, and Cu in the roots changed in parallel with those in the leaves (Table 1). La contents in both of the roots and leaves increased with the increase of extraneous La. K contents tended to decrease in the roots and leaves in all the treatments. Cu contents in roots and leaves were first reduced, and then enhanced with the increasing La in the culture solutions.

The contents of Mg, Mn, Ca, Fe, Zn, Na, and P in the roots changed inversely to those in the leaves especially under low concentrations of extraneous La (Tables 2 and 3). In the roots, Ca

Table 1 Contents of mineral nutrients in roots changed synchronously with those in leaves

Extraneous La (mg/L)	La content in roots ($\mu\text{g/g DW}$)	La content in leaves ($\mu\text{g/g DW}$)	Cu content in roots ($\mu\text{g/g DW}$)	Cu content in leaves ($\mu\text{g/g DW}$)	K content in roots ($\mu\text{g/g DW}$)	K content in leaves ($\mu\text{g/g DW}$)
0	319 \pm 48	6.8 \pm 0.9	32.6 \pm 2.2	13.7 \pm 2.0	32,785 \pm 2,958	23,972 \pm 2,610
1	933 \pm 111*	11.5 \pm 2.0	26.8 \pm 2.8	12.5 \pm 2.0	31,778 \pm 3,640	21,869 \pm 2,832
2	1,048 \pm 185*	13.2 \pm 2.3*	24.8 \pm 2.2	13.8 \pm 1.7	29,635 \pm 2,584	22,537 \pm 3,329
4	1,699 \pm 245**	15.5 \pm 2.5**	28.8 \pm 5.0	14.7 \pm 2.5	27,399 \pm 3,560	21,064 \pm 3,340
8	2,594 \pm 467**	17.7 \pm 2.9**	41.3 \pm 6.8	17.4 \pm 3.1	23,964 \pm 4,119*	18,499 \pm 2,345

Values are denoted as mean \pm SD, $n=3$

* $p < 0.05$; ** $p < 0.01$

Table 2 Contents of mineral nutrients in leaves higher than those in roots

Extraneous La (mg/L)	Mg content in roots (μg/g DW)	Mg content in leaves (μg/g DW)	Mn content in roots (μg/g DW)	Mn content in leaves (μg/g DW)	P content in roots (μg/g DW)	P content in leaves (μg/g DW)
0	738±67	2,522±226	5.3±0.6	14.7±2.2	3,565±490	7,210±770
1	768±111	2,340±356	4.7±0.6	15.5±2.7	3,634±669	6,205±928
2	860±93	2,261±364	4.4±0.6	16.0±2.6	4,306±822	6,742±902
4	927±122	1,987±348	3.7±0.4*	17.5±2.5	3,938±730	7,463±859
8	905±104	1,683±314*	2.4±0.47**	16.9±3.1	3,642±653	7,632±1,152

Values are denoted as mean ± SD, $n=3$

* $p<0.05$; ** $p<0.01$

and Fe contents increased along with the increasing La. The contents of Mg, P, Zn, and Na first increased, and then declined with the increase of extraneous La. In the leaves, the contents of P, Zn, and Na were initially reduced, and then enhanced along with the increase of La. The contents of Mg, Ca, and Fe tended to decline along with the increase of La. The contents of Mn in the roots declined linearly, but in the leaves, the Mn contents first increased and then tended to decrease along the treatments. In addition, the contents of Ca, Fe, Zn, or Na in the roots were higher than those in the leaves, while the contents of Mg, Mn, and P in the leaves were higher than those in the roots under the same treatments.

Changes of Root Lengths and Shoot Heights

Shoot heights and root lengths tended to decrease with the increasing La in the solutions, indicative of inhibition effects of La^{3+} on growth of the seedlings (Fig. 1).

Changes of Cell Cycle Phases and Proliferation Indexes in Meristem

Histograms of cell cycle phases and relative nuclear DNA contents were revealed by flow cytometric analysis of nuclei released from the root tips (Fig. 2). Ratios of G0/G1, S, and G2/M phase were also calculated according to the histograms of DNA distribution in the cell cycle phases, respectively (Fig. 3a).

The G0/G1-phase ratios changed synchronously with the G2/M-phase ratios. They first increased, then tended to decline, and finally enhanced with the increase of

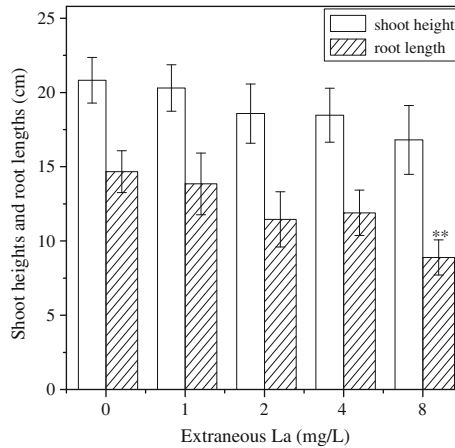
Table 3 Contents of mineral nutrients in roots higher than those in leaves

Extraneous La (mg/L)	Ca content in roots (μg/g DW)	Ca content in leaves (μg/g DW)	Fe content in roots (μg/g DW)	Fe content in leaves (μg/g DW)	Zn content in roots (μg/g DW)	Zn content in leaves (μg/g DW)	Na content in roots (μg/g DW)	Na content in leaves (μg/g DW)
0	1,923±231	1,237±137	463±74	133±16	156±17	101±11	2,464±310	136±18
1	2,202±309	1,104±106	566±79	120±21	187±26	95±17	2,480±397	126±23
2	2,191±177	1,097±150	544±99	111±20	204±35	96±17	2,514±397	110±13
4	2,306±293	1,092±110	710±100*	103±17	179±20	101±20	2,357±453	126±24
8	2,423±204	945±125	727±110*	96±18	120±21	109±18	2,180±385	149±23

Values are denoted as mean ± SD, $n=3$

* $p<0.05$; ** $p<0.01$

Fig. 1 Alteration of shoot heights and root lengths of *V. faba* seedlings cultivated in 0–8 mg/L of extraneous La in culture solution for 15 days. Values are denoted as mean \pm SD, $n=3$, $**p<0.01$



extraneous La^{3+} . However, the S-phase ratios altered inversely to both of the G0/G1 and G2/M phases in all the treatments. Thus, the cell cycles were most probably arrested by La^{3+} at G1/S interphase in the root tip cells.

The proliferation indexes (PI) of root tip cells changed in parallel with the S-phase ratios in the cell cycles of root tips. The S-phase ratios and PI values of the root tips were decreased to be the lowest at 1 mg/L of extraneous La^{3+} in all the treatments.

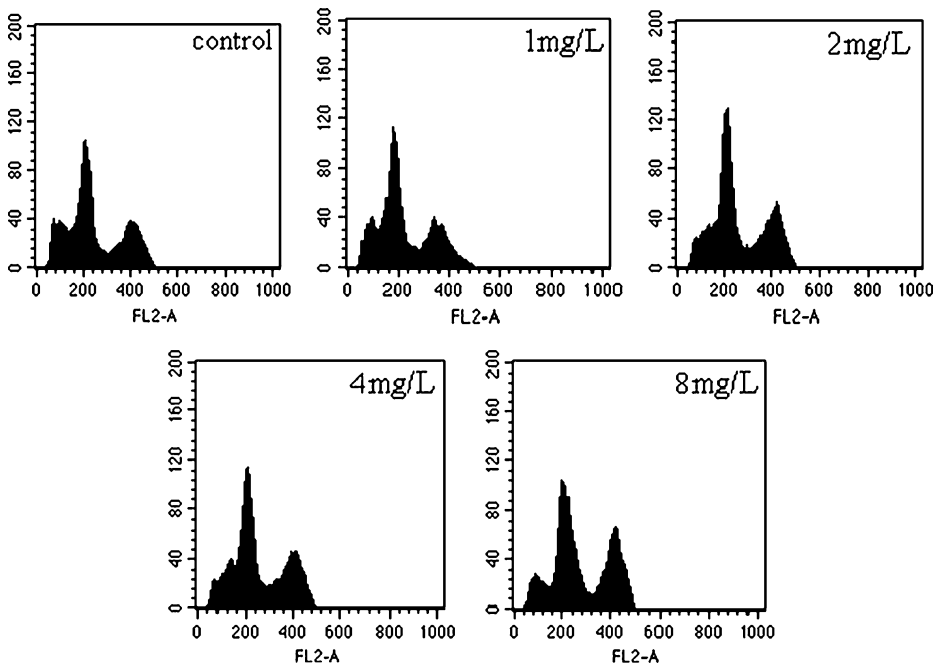
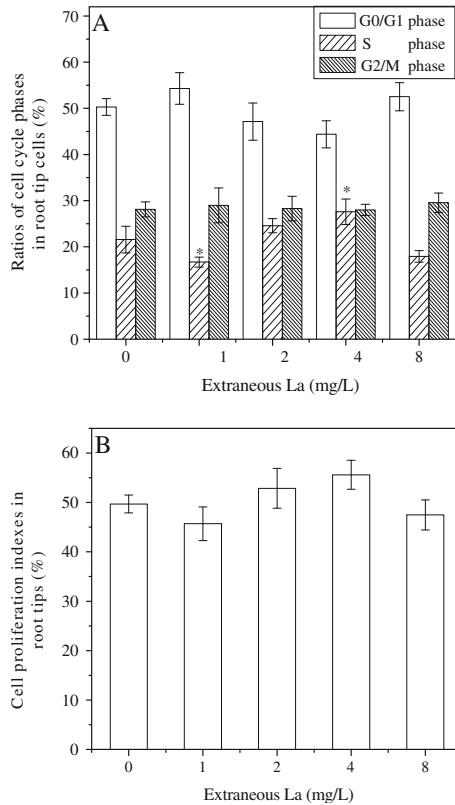


Fig. 2 Histograms of relative nuclear DNA contents and cell cycle phases obtained by flow cytometric analysis of nuclei released from root tips of *V. faba* seedlings cultivated in 0–8 mg/L of extraneous La for 15 days. Peak at channel 200 denotes G0/G1 phase, channel 400 denotes G2/M phase, and area between channel 200 and 400 denotes S phase

Fig. 3 G0/G1-, S-, and G2/M-phase ratios calculated according to histograms of DNA distribution in root tip cells (a). Proliferation index in cell cycle phases of root tips and apical buds of *V. faba* seedlings cultivated in 0–8 mg/L of extraneous La for 15 days (b). Values are denoted as mean \pm SD, $n=3$, * $p<0.05$



Discussion

REEs, as heavy metals, are mainly accumulated in roots and little in other organs of plants, which are reconfirmed by this experiment (Table 1). Low concentrations of REEs may accelerate nutrient element's uptake, improve photosynthesis, and stimulate plant growth [1, 14–16], while higher concentrations of REEs may reduce the uptake of nutrient elements and inhibit the development and growth of plants [17–19]. It is thus evident that REEs can lead to the unbalance of mineral nutrients and thereby interfere with plant growth.

Distinct distributions of mineral elements in roots and leaves as well as their related mechanisms under the same treatments of REEs have been little reported, although many studies focused on the effects of REEs on nutrient's uptake [15]. The present study showed that the increasing extraneous La caused a distinct relocation of mineral nutrients in the roots and leaves. For instance, the contents of La, Cu, and K in the roots changed synchronously with those in the leaves, while the contents of Ca, Fe, Zn, Mg, Mn, Na, and P in the roots changed inversely to those in the leaves (Table 1). In addition, the contents of Ca and Fe in the roots increased with the increasing La^{3+} , while those in the leaves decreased along the treatments, which suggested that partial Ca and Fe in the leaves probably transferred downwards to the roots. The concentrations of La^{3+} were apparently responsible for the distinct redistribution of these elements between the roots and leaves. The difference may be attributed to mineral nutrients' capability of interactions with La^{3+} and penetration into cell membranes of roots and leaves. This is the first report concerning

the redistribution of mineral nutrients between the leaves and roots of plant exposed to concentrations of La^{3+} .

La^{3+} can bind to Ca^{2+} -located sites in cell membranes, block Ca^{2+} channels, and disturb the uptake of mineral ions because it has similar ionic radius as Ca^{2+} [20, 21]. In the leaves, for Mg, Ca, Fe, or K ions, La^{3+} might act as antagonists at all the tested concentrations; for Cu, P, Zn, and Na, as antagonists at low concentrations and as stimulator at high ones. In the roots, contents of Mn and K decreased and those of Fe and Ca increased at all tested doses. La^{3+} -dependent low-dose promotion and high-dose inhibition were observed in the contents of Mg, P, Zn, or Na in the roots and contents of Mn in the leaves. These findings suggested that La might play different roles for different elements in the roots/leaves. Transport of K^+ through Ca^{2+} channels was disturbed by REEs [20]. Transport of other elements is still unclear. The reason why La at higher concentrations turns to be the stimulator of uptake of some mineral ions also needs further investigation [22].

Disbalances of nutrient elements in the seedlings could be responsible for the growth delay of the roots and shoots. The distribution of mineral elements in the seedlings led to significant decrease of Mn and K contents in the roots and Mg contents in the leaves. Absence or deficiency of mineral elements could result in the poor growth or death of plants [23].

The disbalance of nutrient elements was also possibly involved in the alteration of cell proliferation cycles in the root tips. The changes of S-phase ratios and PI values describing cell proliferation cycles were supposed to be involved in the alteration of root lengths and shoot heights. However, the dose responses of the S-phase ratios and PI values were not consistent with the changes of the root lengths. The S-phase ratios and PI values increased to the highest at 4 mg/L, and then decreased to the lowest at 1 mg/L (Fig. 3). Inconsistently, the root lengths and shoot heights decreased to the lowest at 8 mg/L. This result demonstrated that the root lengths and shoot heights might be less controlled by the cell proliferation activity in root meristem for long-time cultivation. Similar conclusion was also made for roots of *Pisum sativum* L. cv. Frisson seedlings exposed to cadmium [24].

Root lengthening is generally related to apical meristem activity [24]. In spite of the lack of correlation between the root lengths and cell proliferation cycles in the roots, it may occur at early period of seedling growth and can be masked in the later development of plants. REEs exert low-dose promotion and high-dose inhibition (i.e., Hormetic effects) on plant growth [25], which was reconfirmed by the biphasic dose response curves of G0/G1-, S- and G2/M-phase ratios of the root tip cells in this study. The cell cycle phases were most probably arrested at G1/S interphase by La^{3+} in the root tips, which may be one of the mechanisms for REEs to interfere with plant growth.

Conclusion

Extraneous La in culture solution caused a disbalance of mineral elements, including the possible redistribution of some elements between the roots and leaves, which might be responsible for the alteration of cell cycle phases of root tip cells. Hormetic effects were shown in the changes of G0/G1-, S-, and G2/M-phase ratios, and the cell proliferation cycles were most probably arrested at G1/S interphase by La^{3+} , which may be one of the mechanisms for REEs to control and regulate plant growth. However, the root lengths were not consistent with the changes of cell cycle phases in the root tips. The mitotic activities in the roots might be overwhelmed by other factors (e.g., cell expansion) under long-time exposure to La^{3+} .

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