Biol Trace Elem Res (2013) 152:82–90
DOI 10.1007/s12011-013-9600-4

Influence of Rare Earth Elements on Metabolism and Related Enzyme Activity and Isozyme Expression in *Tetrastigma hemsleyanum* Cell Suspension Cultures

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Received: 23 November 2012 / Accepted: 1 January 2013 / Published online: 10 January 2013
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Abstract The effects of rare earth elements (REEs) not only on cell growth and flavonoid accumulation of *Tetrastigma hemsleyanum* suspension cells but also on the isoenzyme patterns and activities of related enzymes were studied in this paper. There were no significant differences in enhancement of flavonoid accumulation in *T. hemsleyanum* suspension cells among La^{3+}, Ce^{3+}, and Nd^{3+}. Whereas their inductive effects on cell proliferation varied greatly. The most significant effects were achieved with 100 μM $Ce^{3+}$and Nd^{3+}. Under treatment over a 25-day culture period, the maximal biomass levels reached 1.92- and 1.74-fold and the total flavonoid contents are 1.45- and 1.49-fold, than that of control, respectively. Catalase, phenylalanine ammonia-lyase (PAL), and peroxidase (POD) activity was activated significantly when the REE concentration range from 0 to 300 μM, whereas no significant changes were found in superoxide dismutase activity. Differences of esterase isozymes under REE treatment only laid in expression level, and there were no specific bands. The expression level of some POD isozymes strengthened with increasing concentration of REEs within the range of 50–200 μM. When REE concentration was higher than 300 μM, the expression of some POD isozymes was inhibited; meanwhile, some other new POD isozymes were induced. Our results also showed REEs did not directly influence PAL activity. So, we speculated that 50–200 μM REEs could activate some of antioxidant enzymes, adjust some isozymes expression, trigger the defense responses of *T. hemsleyanum* suspension cells, and stimulate flavonoid accumulation by inducing PAL activity.

Keywords *Tetrastigma hemsleyanum* · Suspension cells · Rare earth · Metabolism · Isozyme

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Abbreviations

REEs	Rare earth elements
PAL	Phenylalanine ammonia-lyase
SOD	Superoxide dismutase
NAA	Naphthalene acetic acid
EST	Esterase
FW	Fresh weight
CAT	Catalase
ROS	Reactive oxygen species
POD	Peroxidase
6-BA	6-Benzyladenine
DW	Dry weight

Introduction

Tetrastigma hemsleyanum Planch. ex Franch is a rare and endangered medicinal plant in China; the main bioactive constituents of which are flavonoids. The plant has been shown not only to treat hepatitis, rheumatic arthritis, and high fevers and regulate immune functions [1], but also to suppress a variety of cancers such as carcinoma of the lung, liver, stomach, and leukemia by inducing tumor cell apoptosis [2]. Because of overexploitation, environmental deterioration, and difficult cultivation, wild *T. hemsleyanum* plants are on the verge of extinction. To conserve resources and lower the production costs, suspension cultures are being explored as a very promising alternative for the rapid production of the bioactive components of this plant. Our research group has obtained the suspension cell system of *T. hemsleyanum*. The main problems encountered in the in vitro culture of *T. hemsleyanum* are low biomass and low flavonoid content [3].

Many biotic and abiotic elicitors have been widely used to stimulate the production of secondary metabolites in

various plant cell cultures, for example, yeast extract [4], fungal elicitor [5], heavy metal salts [6], and so on. However, most elicitors decreased the cell biomass while stimulating secondary metabolites. In recent years, rare earth elements (REEs) have been explored for the elicitation of secondary metabolites [7, 8]. In addition, quite a lot of studies have shown that suitable concentrations and types of REEs could affect many plant physiological activities, from enhancing the chlorophyll content and improving the photosynthetic rate [9–11] to increasing the plant biomass and absorption of nutrients [12–14], thus promoting plant growth and cell proliferation distinctly [15, 16]. Moreover, REEs could increase the protective enzyme activity [17], maintain the structural stability of cell membranes, and induce plant resistance against diseases and a poor environment [18]. In general, an appropriate amount of REEs not only promotes the production of secondary metabolites, but also improves plant growth and plant resistance against stress. Although numerous physiological effects of REEs on plants have been known for some time, the essentiality of REEs on plants is still largely unknown despite the efforts of many researchers.

Esterase (EST) plays an important role in primary metabolism of plant cells; the increase in EST activity usually shows the more vigorous related metabolic activity, which is favorable to the growth and development of cells [19]. The synthesis of secondary metabolites in plants is widely believed to be part of the defense and stress responses of plants. Plant defense reactions can be triggered by elicitors and antioxidant defense systems are based on enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) [20], so these enzymes are key enzymes involved in secondary metabolites.

Thus far, there are some papers related to the change of antioxidase activity in plant seedlings under REE treatment [21, 22], but few studies have focused on the influence of REEs on isoenzyme expression related to metabolism. In particular, there is scarce research directed at the change of isoenzymes in cultured cells in vitro under REE treatment. Analysis of isoenzyme variation has proven to be particularly useful in research on the metabolic regulation of organisms. Thus, further investigation of the impacts on isoenzyme expression may be significant in clarifying the regulatory mechanism of REEs.

In the present study, we studied the variability in activity and isoenzyme profiles of these key enzymes involved in primary and secondary metabolites under REE treatment in suspension cells of *T. hemsleyanum.* The aim of the study was to improve flavonoid production and cell growth of *T. hemsleyanum* cells under the induction of the suitable REE elicitors. More generally, such studies may contribute to the efforts to reveal the REE mechanism of promoting plant cell growth and secondary metabolism.

Materials and Methods

Chemicals

The reagents used for electrophoresis were purchased from Sigma-Aldrich Co. Ltd. (USA): Ammonium persulfate, Tetramethylethylenediamine, Acry, and Bis. $NdCl_3$, $Ce(NO_3)_3·6H_2O$, $La(NO_3)_3·6H_2O$ and others were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). The chemicals and reagents used in this research were analytical grade

Cell Lines and Culture Conditions

Callus were inducted from the young stems of *T. hemsleyanum* in MS medium, supplemented with 0.2 mg/L naphthalene acetic acid (NAA), 2.0 mg/L 6-benzyladenine (6-BA), 30 g/L sucrose, and 8 g/L agar. The green–yellow callus was transferred into liquid MS containing 1.5 mg/L NAA, 1.0 mg/L 6-BA, and 30 g/L sucrose. The pH of the medium was adjusted to 5.8 before autoclaving. The inoculum density was approximately 60 g fresh weight per liter of the medium. The culture was incubated in a rotary shaker (150 rpm) under a 16-h photoperiod and at a temperature of 24±2 °C. Autoclaved REE solution was added to the culture at day 10. The control group was inoculated with an equal volume of sterile double-distilled water. Samples were collected after another 15 days. The sampling time was defined through a preexperiment, as the logarithmic phase of *T. hemsleyanum* suspension cells was at the period of the 10th to 18th day [3].

Determination of Cell Growth and Total Flavonoids

Cell growth was determinated according to Peng et al. [3]. After 15 days of growth, the fresh weight of each sample was measured. The growth ratio (in percent) of each culture was obtained by dividing the difference between the fresh weight at the end and at the beginning of the growth period by the fresh weight at the beginning of the growth period. The cells were separated by filtration and then dried at 60 °C under vacuum to constant weight to obtain the dry cell weight. The total flavonoids were extracted and determined by the method of Zhishen [23]. The total flavonoid concentration was calculated by dividing the content of total flavonoids by the dry cell weight (in milligram per gram dry weight (DW)).

Enzyme Extraction and Assay

Suspension culture tissue (2 g) was ground in 1 ml of ice-cold 0.1 M Tris–HCl extraction buffer (pH 6.5) containing 70 mM 2-mercaptoethanol and 5 % polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 rpm for 15 min

at 4 °C, and the clear supernatants were collected for the determination of enzyme activity and the analysis of isoenzymes. The activity of enzymes was denoted in units/fresh cell weight ($U g^{-1}$ FW).

POD activity was determined by the change in absorbance at 460 nm during incubation of the extracts at 25 °C with 10 mM guaiacol and 1 mM H_2O_2 in 0.05 M sodium acetate buffer (pH 5.4) in a total volume of 3 ml. An enzyme unit (U) represented a 0.1 increase in absorbance (460 nm) per second. This was based on the method of Donald [24], with some modifications.

CAT activity was determined by the change in absorbance at 240 nm during incubation of the extracts at 25 °C with 10 mM H_2O_2 in 0.05 M phosphate buffer (pH 7.8) in a total volume of 3 ml. An enzyme unit (U) represented a 0.1 decrease in absorbance (240 nm) per minute. This was based on the method of Wang [25], with some modifications.

Phenylalanine ammonia-lyase (PAL) activity was determined according to the method of Peng [26]. An enzyme unit (U) represented a 0.1 increase in absorbance (290 nm) per minute. SOD activity was determined according to the method of Beauchamp and Fridovich [27].

EST and POD Isoenzyme Analysis

Polyacrylamide gel electrophoresis in a discontinuous buffer system was used to study the patterns of EST and POD isoenzymes. Table 1 shows the separating gel and the stacking gel. The electrode buffer was Tris–glycine (pH 8.3). The electrophoretic separations were performed at 4 °C. The initial current was 20 mA and was adjusted to 40 mA when 0.2 % bromophenol blue entered the separation gel. The duration of the electrophoresis time was 5–6 h. The sampling amounts of the crude enzymes for EST and POD isoenzyme analysis were 30 and 5 μg of total protein, respectively.

EST was stained according to Mandak [28]. The gel stained for POD activity was immersed for 2–5 min in 200 ml of 0.07 % ascorbic acid solution containing 2 g benzidine (dissolved in 18 ml acetic acid) and 1 ml of 30 % hydrogen peroxide (added before use). The gel was then submitted for analysis in a Bio-Rad Gel Doc XR+ imaging system (Bio-Rad Co. Ltd., USA).

Table 1 Separating gel and stacking gel in isoenzyme electrophoresis

Reagent	Separating gel solution (ml)	Stacking gel solution (ml)
30 % Acry–0.9 % bis	8	0.65
1 mol/L Tris–HCl buffer (pH 8.8)	5.2	0
1 mol/L Tris–HCl buffer (pH 6.8)	0	0.5
Distilled water	12.6	2.8
10 % Ammonium persulfate	0.12	0.04
Tetramethylethylenediamine	0.03	0.006

Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as means±standard deviation (SD). The data were subjected to one-way analysis of variance in SPSS 17.0 for Windows (SPSS Inc., USA), and the significance between treatments was assessed by Tukey's test at $P<0.05$.

Results

Effect of Different Concentrations of REEs on Cell Proliferation and Flavonoid Accumulation

Table 2 shows that the optimal dosages of each REE had a clear positive influence on cell growth and total flavonoid yield. There was a steady increase in cell growth with increasing concentrations of REEs when the concentration ranged from 0 to 100 μM. When the concentration of REEs was higher than 100 μM, the stimulation was weaker. REEs even restrained cell proliferation markedly at 300 μM concentration. Cell growth was markedly increased by 100 μM Nd^{3+}, Ce^{3+}, and La^{3+} by 92.3, 74.2, and 49.1 %, respectively, compared to the control and the differences between them were significant ($P<0.05$).

REEs caused a prominent increase flavonoid content with increasing concentrations of REEs when their concentration ranged from 0 to 200 μM. When the concentration was higher than 200 μM, the flavonoid content was lower. The total flavonoid content was markedly increased by 200 μM Nd^{3+}, Ce^{3+}, and La^{3+} by about 55, 58, and 41 %, respectively, compared to the control. The total flavonoid content in the 100-μM REE treatment group was slightly lower than that in the 200-μM treatment group, and the difference between them was not significant, but the fresh weight growth rate of the former was significantly higher than that of the latter. Thus, in terms of flavonoid production, the optimal concentration of REEs was 100 μM.

Effect of Different Concentrations of REEs on POD, CAT, SOD, and PAL Activity

In view of their more efficient stimulation, Ce^{3+} and Nd^{3+} were selected for further experiments. Figure 1 shows that the effect of REEs on enzymes activity was consistent with the hormesis effect (low-dose stimulatory and high-dose inhibitory response). SOD activity did not differ significantly between the treated group and the control when REEs

Table 2 Effect of REEs on cell proliferation and flavonoid content of *T. hemsleyanum* suspension culture

Concentration	La^{3+}		Ce^{3+}		Nd^{3+}	
	Fresh weight growth rate (%)	Total flavonoid content (mg/g DW)	Fresh weight growth rate (%)	Total flavonoid content (mg/g DW)	Fresh weight growth rate (%)	Total flavonoid content (mg/g DW)
0 μM (CK)	166.2±10.44 a	30.6±2.57 a	166.2±10.44 a	30.6±2.57 a	166.2±10.44 a	30.6±2.57 a
10 μM	178.4±7.12 a	32.34±1.73 a	180.4±12.11 a	35.1±2.82 a, b	180.3±12.17 a, d	35.8±1.22 b
50 μM	200.1±9.01 b	37.7±1.75 b	230.8±11.11 b	38.0±1.68 b	293.7±10.71 b	38.4±1.47 b
100 μM	247.8±13.36 c	39.1±1.72 b	289.6±9.17 c	45.7±2.50 c, d	319.6±10.43 c	44.4±2.84 c
200 μM	140.5±7.14 d	37.0±2.88 b	180.2±11.10 a	48.5±1.76 c	187.6±10.24 d	47.5±3.12 c
300 μM	79.0±7.84 e	37.1±2.22 b	85.7±12.80 d	43.5±3.88 d	98.1±7.92 e	45.4±4.71 c

Different English letters in the same column indicate a significant difference through pairwise comparison by LSD multiple comparison test ($P<0.05$)

were added within 0–300 μM. PAL, CAT, and POD were distinctly activated when the REE concentration was within the range of 0–200 μM. CAT activity did not change with the REE concentration basically. However, PAL and POD activities were higher with the increase of the REE concentration. CAT, SOD, and PAL activity decreased significantly and dropped below that in the control when the REE concentration was higher than 300 μM, which indicated that, similarly to heavy metal ions, REEs could inhibit the enzyme system of plants when their concentration is beyond the critical value.

Effect of Different Concentrations of REEs on the POD and EST Isoenzyme

Our above-mentioned results showed that the impact of REEs on POD activity was significant and dose dependent. Therefore, research into the influence of REEs on POD and EST isozymes would be helpful in revealing the antioxidation and metabolic regulation mechanism of REEs.

Figure 2 shows that the changes in the strip numbers and their color depths were consistent with the changes in their activity. There were no real fundamental distinctions in the

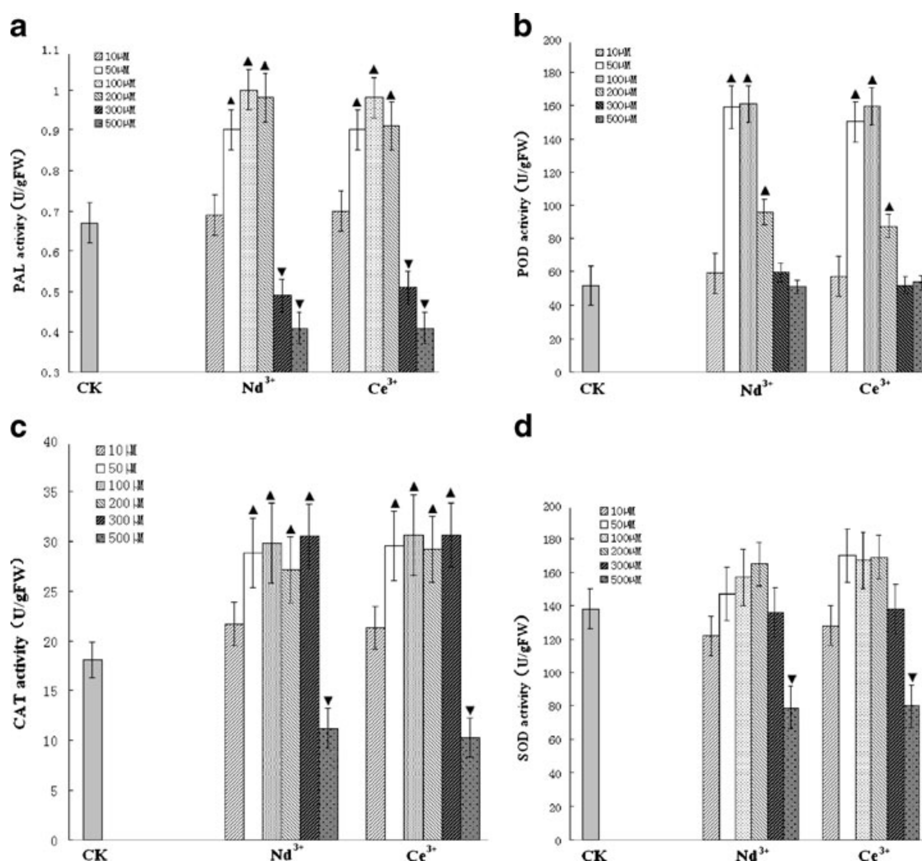

Fig. 1 Effect of REEs on PAL (**a**), POD (**b**), CAT(**c**), and SOD (**d**) activity in *T. hemsleyanum* suspension culture. *Black up-pointing triangle* indicated a significant increase and *black down-pointing triangle* indicated a significant decrease compared with the control by LSD multiple comparison test ($P<0.05$). The *vertical bars* denote ±SD, $n=3$

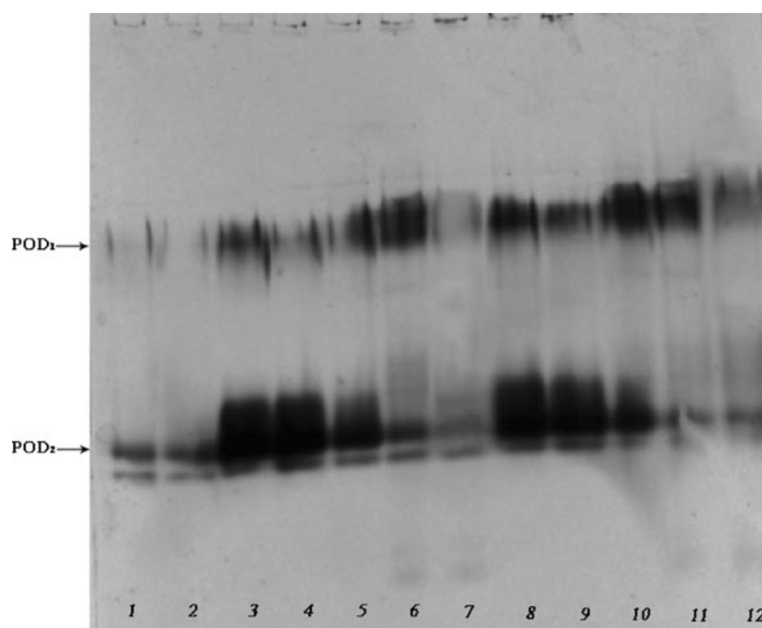

Fig. 2 Electrophoresis banding patterns of POD in suspension cells of *T. hemsleyanum*. *Lanes 1–12* were control, 10 μM Nd^{3+}, 50 μM Nd^{3+}, 100 μM Nd^{3+}, 200 μM Nd^{3+}, 300 μM Nd^{3+}, 500 μM Nd^{3+}, 50 μM Ce^{3+}, 100 μM Ce^{3+}, 200 μM Ce^{3+}, 300 μM Ce^{3+}, and 500 μM Ce^{3+}treatments, respectively

POD isozyme patterns between the control and the 10-μM REE treatment group. The difference in the 50–200-μM treatment group was mainly in the intensity of the bands; no special bands appeared. The enzymatic expression levels of POD_1 and POD_2 were significantly increased. The expression of POD_1 continued to intensify when the concentration of REEs ranged from 200 to 300 μM, and at the same time, the expression of POD_2 weakened. Some new bands emerged between POD_1 and POD_2 when the concentration of REEs ranged from 300 to 500 μM.

Figure 3 shows that the differences of EST isozymes under REE treatment only laid in expression level, and there were no specific bands. EST activity could be improved with an increase in the expression level of EST_1–EST_4 when the REE concentration was within the range of 0–100 μM, whereas at a higher concentration of 200 μM, each spectrum signal weakened distinctly, indicating a decrease in the expression level. At this time, the cells might begin to be poisoned by REEs. EST expression was kept to a minimum in all treatments when the REE concentration was 500 μM, so that the spectrum signal near the cathode was almost undetectable. The results showed that EST activity was highest under 50–100 μM REE treatment. The present conclusion agrees with the earlier

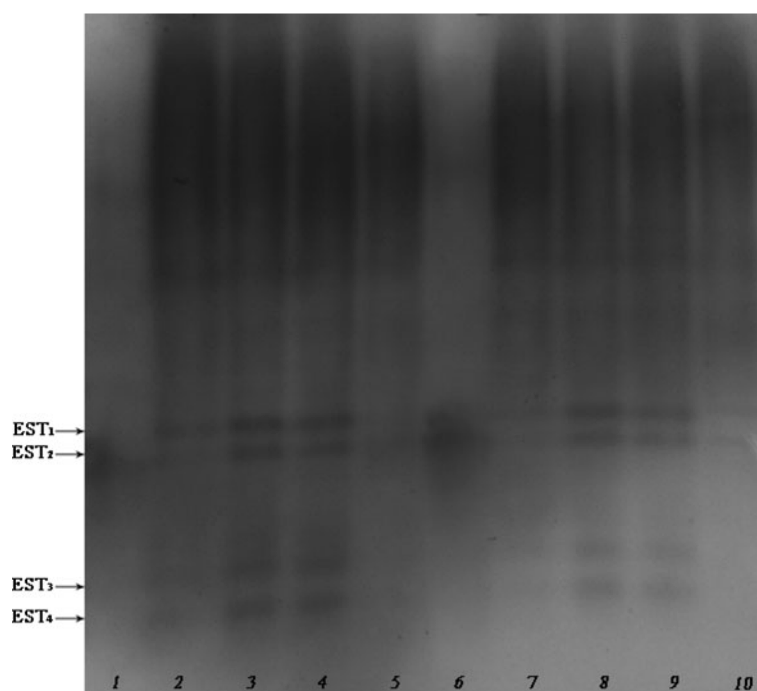

Fig. 3 Electrophoresis banding patterns of EST in suspension cells of *T. hemsleyanum*. *Lanes 1–10* were 500 μM Nd^{3+}, 200 μM Nd^{3+}, 100 μM Nd^{3+}, 50 μM Nd^{3+}, control, 500 μM Ce^{3+}, 200 μM Ce^{3+}, 100 μM Ce^{3+}, 50 μM Ce^{3+}, and 10 μM Ce^{3+} treatments, respectively

supposition that 50–100 μM REEs are clearly capable of promoting cell proliferation.

Discussion

Effect of REEs on Cell Proliferation and Secondary Metabolite Accumulation

In this work, the ionic radius of La^{3+}, Ce^{3+}, and Nd^{3+} decreased in turn and the stimulation of flavonoids showed no obvious difference, but the promotion of cell proliferation was obviously enhanced. The promotion of *Arnebia euchroma* (Royle) Johnst cell growth by Ce^{3+} and Nd^{3+} has previously been found to be superior to that by La^{3+} [29], which is consistent with our result. However, Ozaki et al. [30] reported that the uptake of REEs declined with a decrease in the ionic radius. As REEs perform its role by being absorbed into plant cells [31], the conclusion of Ozaki et al. contradicted our result. It is probably because that the REEs used in the experiment of Ozaki et al. were all heavy REEs except for Ce^{3+}, while those used in our experiment were all light REEs. Light REEs and heavy REEs differ in their ion radius, solubility, and the ability to bind to chlorophylls [32]. These differentiations between them may lead to the differences in absorption features, for example, the absorption and transition efficiency of light REEs is much higher than that of heavy REEs in wheat seeds [33].

Cistanch deserticola cells have been found to be more sensitive to La^{3+} than to other REEs [34]. *Saussurea medusa* cells have been found to be more sensitive to Ce^{3+} than to La^{3+}, mixture of REEs, and Nd^{3+} [35]. However, the mixture of REEs showed the most remarkable effects on the cell growth of *A. euchroma* and production of shikonin derivatives [7]. The contradictory findings may be due to the fact that the specific absorption of REEs varies in different plants and in different growth environments [36]. The significance of the stimulation also depends on the type of cells and the culture growth stage [37]. Therefore, treatment with REEs showed different effects. So, it should not be simply said that the effect of REE stimulus varied with their ionic radius.

The results of the present study indicated that an appropriate amount of REEs had positive effects on cell growth and flavonoid accumulation, whereas high doses of REEs evidently had negative effects, which are consistent with previous reports [7, 34, 37, 38]. It was proposed that REEs affected the plant physiology by initiating the Ca^{2+} signal transduction system [22]. Others argued that REEs could influence the metabolism of plants by replacing or competing with the metal ions such as Ca^{2+} and Mg^{2+} at their low concentration for binding sites in cell membrane and further affect the function of some enzymes and cell membrane and enhance the absorption, utilization, and transformation of nutrients, thereby leading to the fast growth of plant cells [34, 39, 40]. When the REE concentration was high enough, they possessed some properties of heavy metal ions, combined with membrane proteins, and inhibited the enzyme activities [7]. Likewise, Yang et al. [41] found that Ce^{3+} at the low or high concentration could directly interact with the proteins on/in the plasma membrane of horseradish. The interaction improved or destroyed the structure of the proteins on/in the plasma membrane and thus promoted or decreased the intra-/extracellular substance exchange, and then, the growth of cells is accelerated or damaged.

Effect of REEs on the Related Enzyme Activities of Metabolism

Previous studies have shown that REEs enhanced root morphogenesis and the growth of *Saussurea involucrate* by acting as a mild abiotic stress to stimulate POD and SOD activities [42]. REEs have also been found to alleviate the oxidative damage induced by UV-B radiation and to reduce the content of reactive oxygen species (ROS) and MDA [43, 44]. Our results basically agree with the data reported by Shi et al. [45], which showed that low concentrations of La^{3+} (0.002–0.02 mM) promoted plant growth in cucumber seedlings but did not affect the activity of antioxidant enzymes, whereas higher concentrations (0.2−2 mM) stimulated these activities but suppressed plant growth. However, the maximum tolerant dose we found is different from that in the above-mentioned study; this is probably because the REE uptake and usage efficiency vary among seedlings and in vitro culture systems.

Yuan et al. [46] have shown that CAT and SOD activities in suspension cultures of *Taxus cuspidata* were activated by Ce^{4+}, whereas POD activity was restrained strongly. In the present study, no significant changes were found in SOD activity when REEs were added within 0–300 μM, but CAT and POD activity was activated significantly, and POD activity even amounted to threefold of that in control when the REE concentration was 100 μM, and the REE dosage also had a strong influence on POD activity. Our research found that SOD activity did not change significantly under REE treatment. However, the activities of CAT and POD were enhanced with an increase of REE concentration. The result of the current study is very similar to that of Wang et al. [47]; they reported that the activities of CAT, POD, and SOD in *Lepidium meyenii* shoots were enhanced to different extents by La^{3+}, Ce^{3+}, and Nd^{3+}. In conclusion, an appropriate concentration of REEs could activate the antioxidase activity and strengthen the ability of scavenging radicals in plant cells, but each antioxidase changes differently among various kinds of plant cells.

The phenylpropanoid metabolic pathway plays a very important role in plant secondary metabolism, which is a main mechanism of flavonoid formation [48, 49]. The key

enzyme in the first stage of the phenylpropanoid transition is PAL [50]. Previous studies have shown that PAL activity responds to various abiotic stimuli and therefore enhances the biosynthesis of flavonoid [51, 52]. Our results showed that 50–200 μM REEs clearly enhanced PAL activity, and the effect was more remarkable with the increase in concentration, which agreed with the observed influence of REEs on flavonoid content. To test the way in which REEs influence PAL activity, we conducted a pilot trial; different concentrations of Ce^{3+} and Nd^{3+} were added into the reaction solution for PAL enzyme activity determination, and the PAL enzyme activity of cells untreated by REEs was then determined. The results showed that 50–200 μM Ce^{3+} and Nd^{3+} had no activation function on PAL activity, instead the inhibitory effect was strengthened with the increase in the REE concentration (not shown). Thus, it is speculated that REEs did not directly influence PAL, but instead acted indirectly by regulating its metabolic pathway.

In light of these data and previous results, we speculated that 50–200 μM REEs could activate some of antioxidant enzymes, trigger the defense responses of *T. hemsleyanum* suspension cells, and stimulate flavonoid accumulation by inducing PAL activity, although more data are needed to support this hypothesis.

Effect of REEs on the Regulation of Related Isoenzyme Expression

Many studies have suggested that isoenzymes are closely related to the antistress quality and the synthesis of plant secondary metabolism products. EST and POD exist in all parts and at different development stages of plants. They participate in many important physiological activities. Their varieties play a major role in plant metabolism regulation and environmental stress response [53]. Some researches have shown that environmental factors can result not only in changes in EST and POD activity, but also in their isoenzyme expression, for example insect violation [54], disease [55], radiation [56], air temperature [57], and so on.

Previous studies have reported that no obvious changes were observed in the band number of CAT patterns of *Lemna minor* L. except for varying intensities under 5 mM REE treatment [21]. Similarly, in our research, no obvious changes were observed in the band number of POD and EST patterns except for varying intensities of some bands due to increasing concentrations of REEs when their concentration was within the range of 0–200 μM. However, several new POD bands emerged when more than 300 μM of REEs were supplemented. At the same time, some EST bands weakened and vanished. It might be because the response to stress was various with the sorts of enzyme. On the other hand, the researchers leading the above trial adopted seedlings in vivo rather than cultures in vitro as study object; suspension culture cells in vitro were more sensitive to the changed outside culture conditions. Moreover, *L. minor* L. itself was a "highly REE-tolerant" plant species. Treatments at concentrations up to 5 mM RE did not cause either visible symptoms on *L. minor* L. seedling or significant effects on ROS production and lipid peroxidation [21]; however, 200 μM REE had a clear negative influence on cell growth of *T. hemsleyanum* in our study.

In conclusion, along with the increase in the REE concentration, EST and POD activity initially increased and then later decreased. In the meantime, the isozyme expression was regulated. The expression quantity of some bands was increased. When REE concentration was higher than the critical value, they could make abiotic environmental stresses and inhibit the expression of some isozymes, and some new "protective enzymes" were induced to resist the damage, at the same time. This finding matched the typical stress reaction characteristics of plants. A plant will adopt all kinds of measures to improve its resistance so as to adapt to a poor environment. However, when the stress level surpasses the tolerance limit of the plant, its resistance measures are weakened, and the plant will eventually die.

Acknowledgments This work was supported by the Natural Science Foundation of Ningbo City (grant no. 2012A610179) and the New Shoot Talents Program of Zhejiang Province (grant no. 2010R433006).

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