African Journal of Biotechnology Vol. 9(16), pp. 2333-2341, 19 April, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.949
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effects of rare earth elements on callus growth, soluble protein content, peroxidase activity and shoot differentiation of *Echinacea angustifolia* cultures *in vitro*

Lin Ma, Jun Zhao, Hongli Wu, Xiaohong Yuan and Dabin Hou*

School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China.

Accepted 29 March, 2010

The effects of lanthanum nitrate (La^{3+}) and cerium nitrate (Ce^{4+}) on *Echinacea angustifolia* callus growth and subculture were studied by the measurement of callus fresh weight, dry weight and time-course curve. The effects of La^{3+} on soluble protein content, peroxidase activity and shoot differentiation of callus were also investigated. Low concentrations of La^{3+} and Ce^{4+} (0.01, 0.1 and 1 mg/l) showed enhancing action on callus growth while the suppress effect was found at high concentration treatments (10 and 100 mg/l). The result from time-course experiment indicated that La^{3+} or Ce^{4+} showed promotion on callus growth after 15 days cultured and Ce^{4+} displayed a better effect than La^{3+}. Different proportion mixture of La^{3+} and Ce^{4+} at 1 mg/l in total showed stimulating action on callus growth and the optimum proportion was the mixture of 40% La^{3+} and 60% Ce^{4+}. Inhibitory effects were observed when the calli derived from the treatments with La^{3+} and Ce^{4+} were subcultured for the first generation. The dose-dependent effects of La^{3+} on soluble protein content, peroxidase activity and shoot differentiation were also noticed. The optimum concentration for callus growth and shoot differentiation was 0.1 mg/l and the green, loose calli with low percentage of brown callus were obtained at this concentration.

Key words: *Echinacea angustifolia*, rare earth elements, lanthanum, cerium, callus growth, soluble protein, peroxidase activity, shoot differentiation.

INTRODUCTION

Echinacea angustifolia, one of the three important medicinal plants native of North America, was introduced into China in the 1980s and is now widely cultivated around the country for commercial purpose. Extracts from root or the whole plant showed antioxidative, anti-inflammatory, free-radical scavenging, immunomodulating activity and has been widely used for a variety of ailments, such as common cold, toothache, coughs, sore throats, snakebite, and so on (Barrett, 2003; Hu and Kitts, 2000; Kindscher, 1989).

In vitro regeneration of *E. angustifolia* from different explants has been reported successfully. High-frequency shoot formation from seed, leaf and stem segment and direct somatic embryogenesis from hypocotyl explants made the rapid propagation easier (Goeckel et al., 1992; Harbage, 2001; Lakshmanan et al., 2002). Indirect somatic embryogenesis such as shoot and root differentiation from callus cultures is also an effective method for rapid propagation and there are many reports on other plant species. However, only one paper reported the shoot differentiation from anther-derived calli of *Echinacea purpure* (Zhao et al., 2006) and there is little information available in the literature about callus culture and growth of *E. angustifolia*.

Rare earth elements (REE) such as lanthanum, cerium, neodymium and europium have been reported to show biological effects on culture plant cell and tissue *in vitro*.

*Corresponding author. E-mail: hdb@swust.edu.cn. Tel/Fax: 0086-0816-6089523.

Abbreviations: **REE**, Rare earth elements; **MS**, Murashige and Skoog basal medium; **FW**, fresh weight; **DW**, dry weight; **POD**, peroxidase.

Low concentration of REE could promote cell growth, plantlet rooting and accumulation of secondary metabolism products (Chen et al., 2004; Lu et al., 2006; Luo et al., 2008). REE were also found to have stimulating or inhibitory effects on soluble protein content, antioxidant enzymes activity, nitrogen metabolism of *in vitro* culture leaf, shoot and root system (Song et al., 2004; Wan et al., 2004; Wang et al., 2007; Zhang et al., 2005). In recent years, several researchers have investigated the effects of REE on callus growth, chemical constituents and cell ultrastructure of callus (Lu and Chen et al. 1998; Lu and Lu et al. 1998; Ma et al., 2002; Ning et al., 2005; Yang et al., 2005), but little attention has been paid to their effect on callus differentiation. In this study, we investigated the effects of different concentration of lanthanum nitrate (La^{3+}) and cerium nitrate (Ce^{4+}) on *E. angustifolia* callus growth and subculture. La^{3+} and Ce^{4+} were applied into medium as single compound or mixed formation. Effect of La^{3+} on shoot differentiation from callus derived from leaf tissue was explored. The soluble protein content and peroxidase activity, which are generally considered as the important physiology parameters, were determined at different culture times.

MATERIALS AND METHODS

Callus induction and subculture

Young leaves of *E. angustifolia* were collected from three month old seed-germinated plants grown at greenhouse. Fresh leaves were surface-sterilized by soaking with 0.1% $HgCl_2$ for 10 min and shaking continually. Leaves were subsequently washed five times with sterile water under aseptic conditions. For callus initiation, the leaf margins were removed. Leaf sections (0.5 × 0.5 mm) were inoculated on callus induction medium with the adaxial surface. The callus induction medium was composed of MS basal medium (Murashige and Skoog, 1962) supplemented with 6-benzylamino-purine (1 mg/l), α-naphthaleneacetic acid (1 mg/l), vitamin B_6 (1 mg/l) and sucrose (3% w/v). All chemical regents used were AR grade. The medium was adjusted to pH 5.8 before adding agar (8 g/l) and then sterilized by autoclaving at 121°C for 20 min. All Erlenmeyer flasks (100 ml containing 20 ml medium) consisting of 8 - 10 leaf explants were maintained in dark conditions at 25 ± 2°C for 10 days and then moved to light conditions with a 12 h photoperiod of 25 μmol/m^2 ·s at 25 ± 2°C for 20 days. The calli induced from leaf explants were subcultured for proliferation on fresh MS medium at light conditions every 25 days. After 4 or 5 times subculture, the calli with stable growth and same morphological characteristics were screened for later experiments.

REE and treatment

Lanthanum nitrate hexahydrate ($La(NO_3)_3·6H_2O$, 98%, AR) and cerium nitrate hexahydrate ($Ce(NO_3)_4·6H_2O$, 99%, AR) obtained from Chengdu Kelong Chemicals Regent Plant (Chengdu, China) were used to provide the La^{3+} ion and Ce^{4+} ion for the test in this study, respectively. Stock solutions of 100 mg/l REE were prepared in distilled water. The suitable volume of REE solution was added into culture medium before pH adjustment and sterilization at 121°C. On treatments with La^{3+} or Ce^{4+} individually, five concentrations ranged from 0.01 to 100 mg/l were applied, while on treatments with mixed REE, different proportion of La^{3+} and Ce^{4+} was added simultaneously into the medium to give total concentration of 1 mg/l. The medium without REE was used as the control. For every flask of culture experiments, 0.5 ± 0.02 g fresh weight callus were inoculated. The culture temperature and light condition were the same as callus subculture described above. Two experiments with three replicates at each treatment were carried out.

Measurement of callus growth

The fresh calli from the five flasks of different treatments at 30 days after cultured were measured as fresh weight (FW), then dried at 60°C in an oven till constant weight and measured as dry weight (DW). The measurements were repeated twice. At time-course experiments, the growth of calli (FW and DW) was measured at different intervals from triplicate. The measured values at different time point were converted to the FW or DW of 1 g inoculated callus.

Determination of soluble protein content and peroxidase activity

One gram of callus (FW) from the treatments of different concentrations of La^{3+} after cultured for 30 days was ground using a glass rod in mortar at an ice bath. The extraction was carried out with 5 ml of Tris-HCl buffer at pH 8.0. Extract was centrifuged (5,000 g, 15 min) at 4°C and the residue was extracted again with the same method. The combined supernatant was used for quantification determination. Soluble protein content was determined according to Bradford (1976). Bovine serum albumin was used as the control. Peroxidase (POD) was extracted and determined with the method described by Wakamatsu and Takahama (1993). The determination was done at 25, 30 and 35 days after culture and triplicate samples were analyzed at each time.

Shoot differentiation

Subcultured calli of about 4-5 mm in size derived from the culture without REE were transferred onto fresh MS basal medium supplemented with 1 mg/l naphthaleneacetic acid, 1 mg/l 6-benzylaminopurine, 1 mg/l VB_6 and different concentrations of lanthanum nitrate (0, 0.01, 0.1, 1, 10 and 100 mg/l). About 45 to 50 calli in 10 flasks were used for each treatment and three replicates were applied. Number of differentiated shoots and brown calli were recorded 40 days after cultured.

RESULTS AND DISCUSSION

Effects of La^{3+} and Ce^{4+} on callus growth of *E. angustifolia*

To determine the effects of La^{3+} and Ce^{4+} on callus growth of *E. angustifolia*, FW and DW of callus were measured after a 30-day growth period on MS medium. Calli treated with lower concentration of La^{3+} or Ce^{4+} (0.01, 0.1 and 1 mg/l) showed a significant increase in FW as compared to the control calli (Figure 1A).

However, an obvious reduction in FW was observed in calli treated with higher concentration of REE (10 and 100 mg/l). Callus treated with 0.1 mg/l La^{3+} or Ce^{4+} showed the highest FW among the other REE treatments

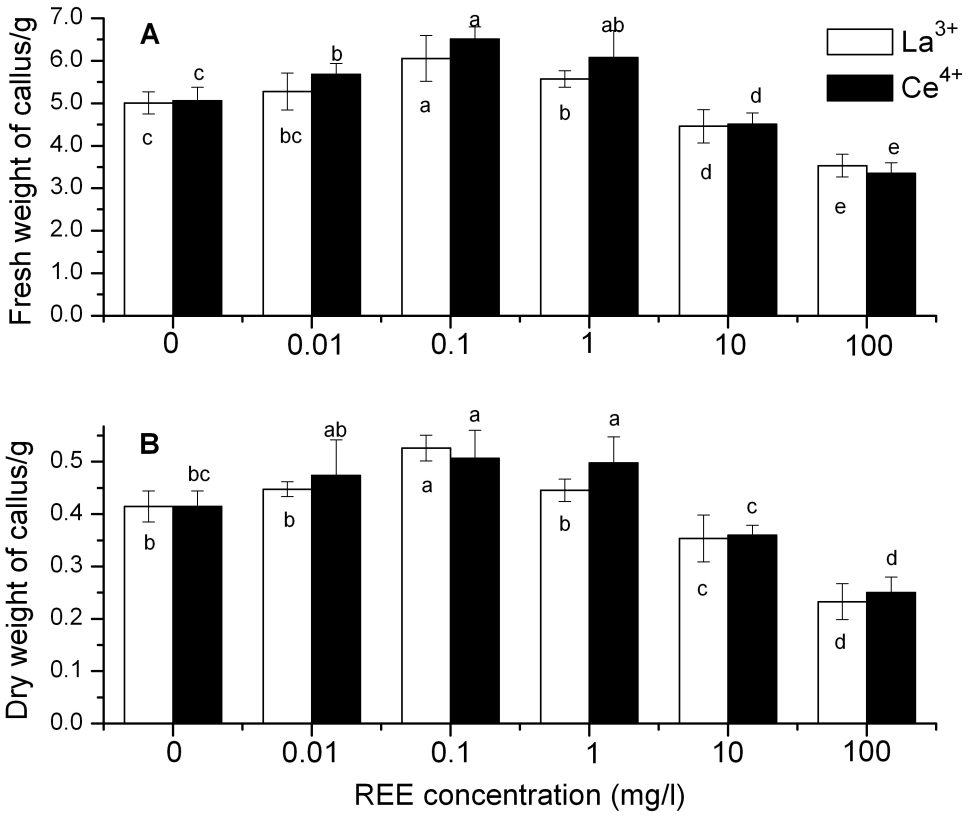

Figure 1. Effects of different concentrations of La^{3+} and Ce^{4+} on fresh weight (A) and dry weight (B) of *E. angustifolia* callus after 30 days cultured. The same letter at the same series columns indicates there was no significant difference among the treatments according to the LSD Test at 5% level. Bar at each value represents the standard deviation of mean.

and the control (Figure 1A). The same trend of REE effects on callus DW was observed (Figure 1B), but there was no significant difference between the treatments at 0.01 or 1 mg/l of La^{3+} and the control as well as between the treatments at 0.01 mg/l of Ce^{4+} and the control. The promotion action on callus growth at low concentration of REE and inhibitory action at high concentration demonstrated dose-dependent effect. These results are in accordance with the reports published previously (Chen et al., 2004; Lu et al., 1998; Wu et al., 2001). However, Ma et al. (2002) reported that the different concentration of La^{3+} which ranged from 10^{-4} to 10^{-1} g/l all showed a promoting effect on callus FW of *Ginkgo biloba*.

Time-course of callus growth with REE treatment

Callus growth curve treated with 0.1 mg/l REE and the control without REE is shown in Figure 2. The effect of La^{3+} or Ce^{4+} on callus growth had a similar trend. Callus grew slowly during the first 10 days but hereafter developed with a rapid growth phase. Callus FW and DW of the control were higher than that of the treatments with REE on days 10 after being cultured because the addition of REE had a negative effect on cell growth during the lag phase (Yuan et al., 1998). On days 20 and 25, callus FW at treatments with La^{3+} or Ce^{4+} were significantly higher than those growing in the medium without REE. However, an obvious increase of callus DW treated with REE was observed on day 15. This was possibly due to promotion effect of REE on nutrient elements uptake and metabolism (Guo, 1999). Compared to lanthanum, cerium showed a strong stimulation effect on callus growth. This result is different from the previous report which found that the growth of *Cistanche deserticola* callus was much more sensitive to La^{3+} than to Ce^{3+} because La^{3+} has a larger covalent radius (Ouyang et al., 2003). The different valences of cerium ion might produce different effects on cell growth through changing the conformation of the oxidoreductase (Xiang and Ge, 2008).

Effects of the mixture of La^{3+} and Ce^{4+} on callus growth

Similar to treatment with La^{3+} or Ce^{4+} alone, the mixture of La^{3+} and Ce^{4+} remarkably promoted callus growth at

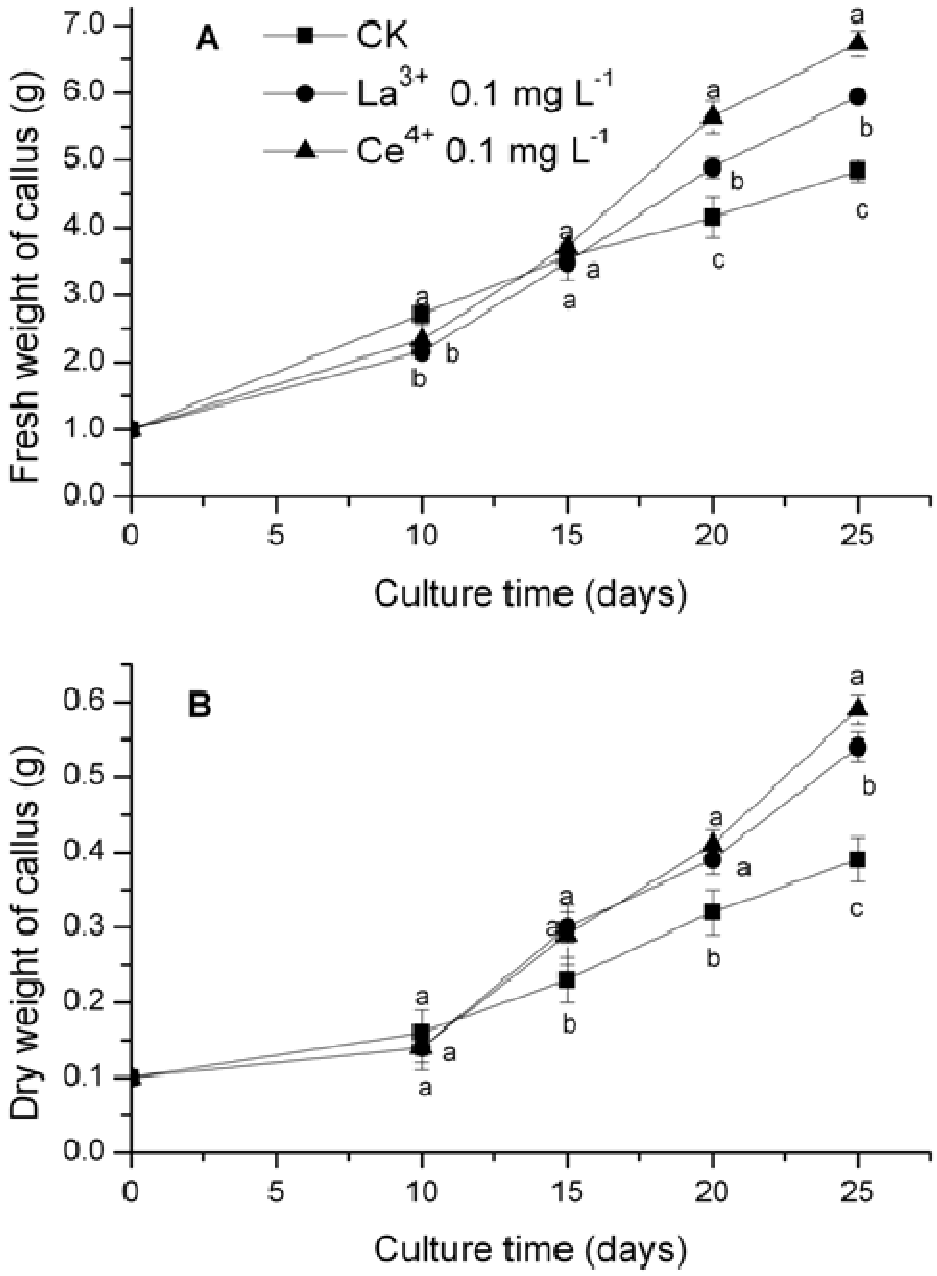

Figure 2. Time-course curve of fresh weight (A) and dry weight (B) of *E. angustifolia* callus treated with 0.1 mg L^{-1} La^{3+} and Ce^{4+}. The same letter at the lines of same time indicates there was no significant difference among the treatments according to the LSD Test at 5% level. Bar at each data point represents the standard deviation of mean.

the total concentration of 1 mg/l (Table 1). When La^{3+} (0.4 mg/l) and Ce^{4+} (0.6 mg/l) were mixed into MS medium, the highest fresh weight (7.07 g) and dry weight (0.55 g) were obtained, respectively. Several researchers have reported the effects of the mixture of REE on callus or cell growth of medicinal plants (Chen et al., 2004; Ge et al., 2006; Ouyang et al., 2003; Yuan et al., 2002), but the composition of the mixture of REE used in these studies was extremely different from each other.

Callus growth of subculture from the culture treated with REE

The calli cultured in the medium added with 0.1 and 1 mg/l of La^{3+} and Ce^{4+} were subcultured onto new MS medium without addition of REE after 25 days. The callus FW and DW were determined on days 10, 15, 20 and 25 and the result is shown in Figure 3. Calli from treatment with REE could grow normally as the control but the

Table 1. Effect of the mixture of La^{3+} and Ce^{4+} at different proportion on callus growth of *E. angustifolia*.

Concentration of REE (mg/l)		Growth of callus (g)	
La^{3+}	Ce^{4+}	Fresh weight	Dry weight
0	0	4.59 ± 0.32 c	0.36 ± 0.05 c
0.3	0.7	5.59 ± 0.71 b	0.45 ± 0.05 b
0.4	0.6	7.07 ± 0.79 a	0.55 ± 0.06 a
0.5	0.5	5.64 ± 0.56 b	0.45 ± 0.03 b
0.6	0.4	6.95 ± 0.62 a	0.53 ± 0.11 ab
0.7	0.3	5.93 ± 0.77 b	0.48 ± 0.10 ab

The data is shown as mean ± SD from two experiments with triplicate at each treatment. Values followed by the same letter in the same column were not significant according to the least significant difference test at 5% level.

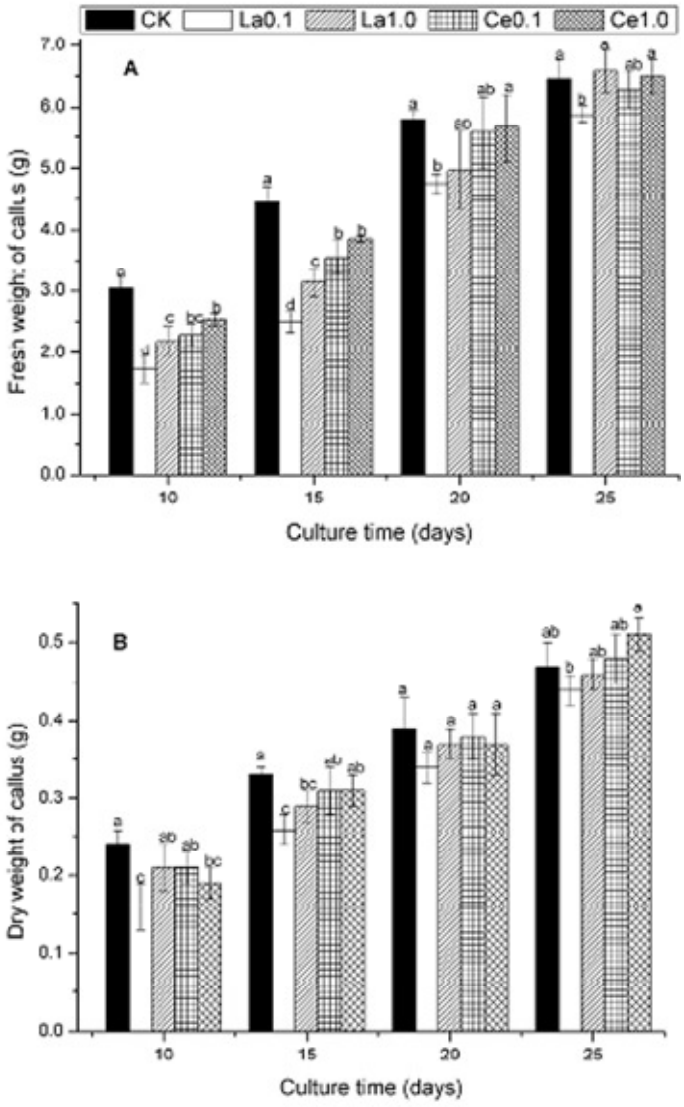

Figure 3. Effects of La^{3+} and Ce^{4+} on fresh weight (A) and dry weight (B) of the subcultured callus. Calli derived from the treatments with 0.1 mg L^{-1} La^{3+} (La0.1), 1 mg L^{-1} La^{3+} (La1.0), 0.1 mg L^{-1} Ce^{4+} (Ce0.1), 1 mg L^{-1} Ce^{4+} (Ce1.0) and the control without REE (CK) were subculture onto fresh MS basal medium for the first generation. The same letter at the columns of same time indicates there was no significant difference among the treatments according to the LSD Test at 5% level. Bar at each value represents the standard deviation of mean.

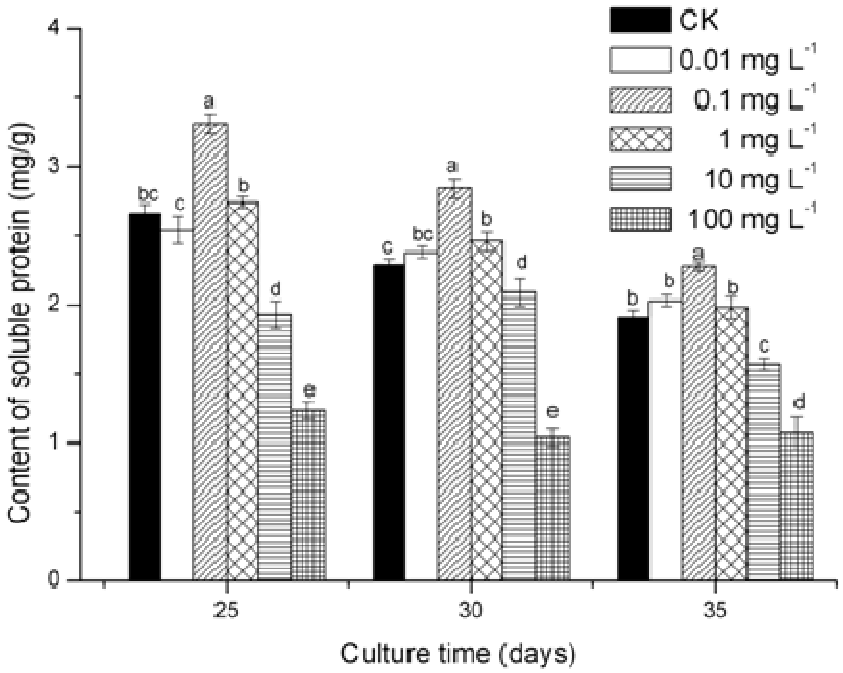

Figure 4. Effect of different concentrations of La^{3+} on soluble protein content of *Echinacea angustifolia* callus. The same letter at the columns of same time indicates there was no significant difference among the treatments according to the LSD Test at 5% level. Bar at each value represents the standard deviation of mean.

biomass was lower than that of the control at the first 20 days. On days 25, only the DW of treatment with 0.1 and 1.0 mg/l Ce^{4+} were higher than that of the control (Figure 3B) and there was no significant difference on callus FW between the treatments with REE and the control (Figure 3A). Our result is similar to previous report on *Coptis chinensi* (Lu et al., 1998). It is not clear how the REE affect callus growth during subculture. A full understanding for a long term effects of REE on subculture of callus needs continuous subculture for many times.

After subcultured for more than 30 days, some calli at 1.0 mg/l La^{3+} or Ce^{4+} treatment appeared color changed from green to pale green. The brown callus was also observed firstly in treatment with REE after being cultured for 40 days but the same case did not appear in the control.

Effect of La^{3+} on soluble protein content

Figure 4 shows the effects of different concentration of La^{3+} on the soluble protein content of callus at three culture times. Compared to the control, treatment with 0.1 mg/l La^{3+} showed a significant increase and almost no obvious influences were found at the concentration of 0.01 and 1 mg/l. In contrast, the soluble protein content decreased markedly at high concentration treatments and the difference between 10 or 100 mg/l and the control was significant. These results are similar to the early published report which also showed a dose-dependent effect (Li et al., 2001; Wan et al., 2004). However, another paper reported that the high concentration of REE up to 320 mg/l had no negative effect on soluble protein content of aloe leaf cultured *in vitro* (Zhang et al., 2005). A decreased trend of the soluble protein content was observed when the culture time increased due to the fact that the most vigorous growth of callus was at 25 days after incubation.

Effect of La^{3+} on POD activity

As shown in Figure 5, La^{3+} addition at 0.01 and 0.1 mg/l significantly increased the POD activity of callus and the addition at 1 mg/l had only a slight effect. In agreement with the results determined on soluble protein content, the high concentrations of La^{3+} (10 and 100 mg/l) also showed an inhibitory action on the POD activity. Similar results that REE enhanced the POD activity of root (Hong et al., 2005; Song et al., 2002, 2004), shoots (Wang et al., 2007), leaf (Zhang et al., 2005) and callus (Ge et al., 2006) cultured *in vitro* at low concentration have already been reported. As one of the antioxidative enzymes, peroxidase plays an important role in plant growth and development and is considered as an indicator of growth (Wolter and Gordon, 1975). The POD activity enhanced by La^{3+} is possibly related to partial replacement of the

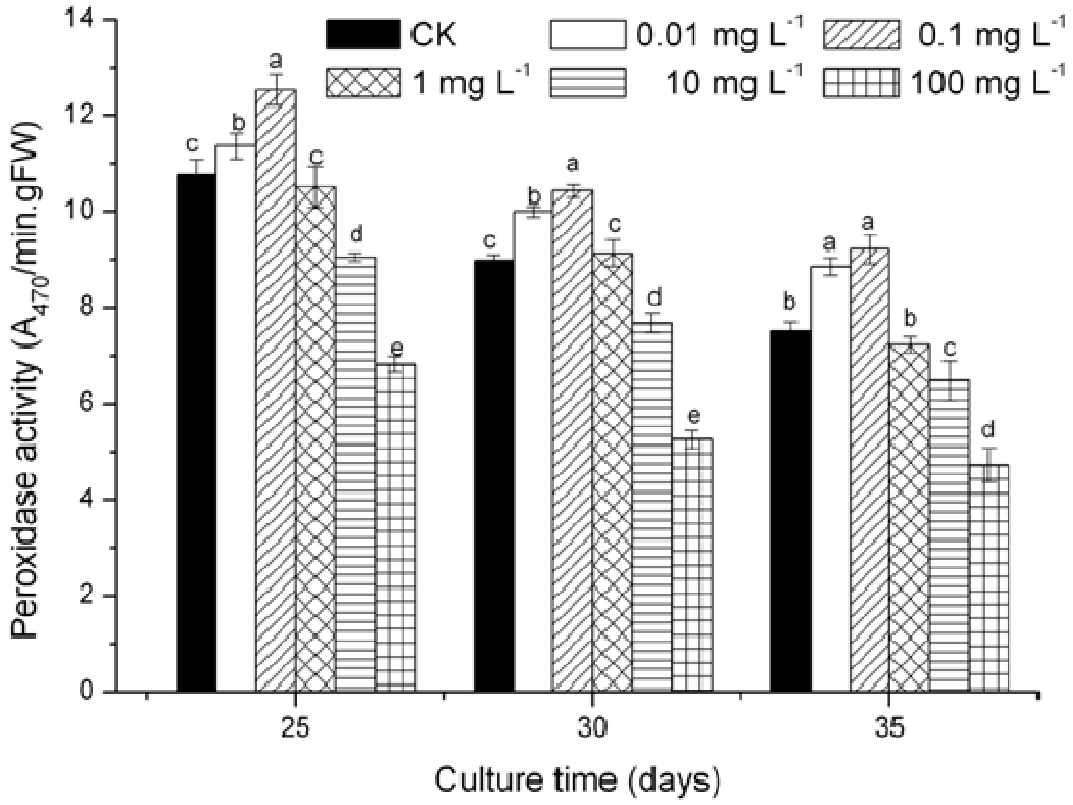

Figure 5. Effect of different concentrations of La^{3+} on peroxidase activity of *Echinacea angustifolia* callus. The same letter at the columns of same time indicates there was no significant difference among the treatments according to the LSD Test at 5% level. Bar at each value represents the standard deviation of mean.

metals in POD by La^{3+} (Wang et al., 2003).

Effect of La^{3+} on shoot differentiation

Low shoot differentiation rate, less than 10% which was different from the report on anther culture by Zhao et al. (2006), was obtained from either the control or treatments with La^{3+}, but the stimulation effect of La^{3+} for differentiation at low concentration (0.01 and 0.1 mg/l) was still observed. Optimal concentration of La^{3+} for shoot differentiation was 0.1 mg/l and the difference between this concentration and the control was significant. No shoot differentiation was found at treatment with 100 mg/l La^{3+} and the differentiation rate of 0.67% was recorded at 10 mg/l concentration (Table 2). Most of the calli from the control and treatments with 0.01 and 0.1 mg/l La^{3+} exhibited normal growth with green and loose callus. No difference of the percentage of brown calli could be found among the three concentrations. When the concentration of La^{3+} increased, the callus color changed from green to pale green, brown and dark brown, and the morphology of callus changed from loose to watery. In agreement with the change of color and morphology, the percentage of brown calli increased from 8.36 at 1 mg/l La^{3+} to 85.56 at 100 mg/l La^{3+} (Table 2).

Our results showed that La^{3+} added into the differentiation medium had a significant influence on organogenesis. Hai et al. (2006) also observed the enhance effect of low concentration of REE (from 0.5 to 3.0 mg/l) on plantlet induction from callus of wheat anther culture. Ning et al. (2005) reported that the addition of 1.2 mg/l REE in induction medium of callus exhibited a higher differentiation rate than the addition REE in the differentiation medium. REE was considered to play a similar role as the plant hormone (Lu et al., 1997) and also thought to be analogous to Ca, especially to La, which was nicknamed 'super calcium' (Brown et al., 1990). La^{3+} may affect shoot differentiation through acting on the plant endogenesis hormone and influencing the function of calcium.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Xiong Tan for providing the seeds of *E. angustifolia* and to Ms. Ying Zhong for help on cultivating the plant in green-house.

Table 2. Effect of lanthanum nitrate on shoot differentiation and callus morphology.

Lanthanum nitrate (mg/l)	Percentage of shoot differentiation (%)	Percentage of brown calli (%)	Morphology of callus
0	5.56 ± 1.0 bc	1.41 ± 1.2 d	green, loose
0.01	6.97 ± 0.9 b	1.36 ± 1.2 d	green, loose
0.1	9.70 ± 0.9 a	3.48 ± 1.2 d	green, loose
1	4.86 ± 1.2 c	8.36 ± 2.3 c	pale green, loose
10	0.67 ± 1.2 d	40.43 ± 4.1 b	brown, watery
100	0.00 ± 0.0 d	85.56 ± 2.9 a	dark brown, watery

Calli were cultured on MS basal media supplemented with 1 mg/l α-naphthaleneacetic acid, 1 mg/l 6-benzylaminopurine, 1 mg/l VB_6 and different concentration lanthanum nitrate. Data were recorded after 40 d of culture. Results represent mean ± SD of three replications, each with 40 – 50 calli. Values with different letters in each column were significantly different (LSD multiple comparison, $p < 0.05$).

REFERENCES

Barrett B (2003). Medicinal properties of *Echinacea*: A critical review. Phytomedicine, 10: 66-86.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brown PH, Rathjen AH, Graham RD, Tribe DE (1990). Rare earth elements in biological systems. In Gschneidner, et al. (eds) Handbook on the Physics and Chemistry of Rare Earths, Elsevier Sciences Publisher B.V., New York, pp. 423-453.

Chen SA, Zhao B, Wang X, Yuan X, Wang Y (2004). Promotion of the growth of *Crocus sativus* cells and the production of crocin by rare earth elements. Biotechnol. Lett. 26: 27-30.

Ge F, Wang X, Zhao B, Wang Y (2006). Effects of rare earth elements on the growth of *Arnebia euchroma* cells and the biosynthesis of shikonin. Plant Growth Regul. 48: 283-290.

Goeckel C, Wawrosch C, Leonhardt W, Kopp B (1992). Micropropagation of *Echinacea angustifolia*. Planta Med. 58: 626-627.

Guo BS (1999). Recent research advance of rare earth in the field of biology. Chin. Rare Earths 20: 64-68. (In Chinese)

Hai Y, Kang MH, Guo JZ, He N, Zhang D (2006). Effects of REA and genotypes on the green wheat plantlet induction frequency. Acta Agri. Boreali-Sinica 21: 34-36. (In Chinese)

Harbage JF (2001). Micropropagation of *Echinacea angustifolia*, *E. pallida*, and *E. purpurea* from stem and seed explants. Hort. Sci. 36: 360-364.

Hong F, Song W, Wan Z, Yu M, Yu J, Liu J, Sheng Y, Xi Q (2005). Effect of La (III) on the growth and aging of root of loquat plantlet *in vitro*. Biol. Trace Elem. Res. 104: 185-191.

Hu C, Kitts DD (2000). Studies on the antioxidant activity of *Echinacea* root extract. J. Agric. Food Chem. 48: 1466-1472.

Kindscher K (1989). Ethnobotany of purple coneflower (*Echinacea angustifolia* , Asteraceae) and other *Echinacea* species. Econ. Bot. 43: 498-507.

Lakshmanan P, Danesh M, Taji A (2002). Production of four commercially cultivated *Echinacea* species by different methods of *in vitro* regeneration. J. Hort. Sci. 77: 158-163.

Li J, Ma Z, Yuan Y, Sun A, Hu C (2001). Dynamic effects of cerium on syntheses of soluble protein and taxol in suspension culture of *Taxus chinensis* Var. *Mairei* cells. J. Rare Earth 19: 223-228.

Lu K, Chang Z, Chen B, Guo D, Zheng J, Wang K (1997). Hormone-like effect of rare earth elements on the plant. J. Beijing Med. Uni. 29: 289-291. (In Chinese)

Lu K, Chen B, Wang K, Guo D, Zheng J (1998). Effect of europium chloride on callus growth and cell ultrastructure of rheum. J. Chin. Rare Earth Soc. 16: 371-374. (In Chinese)

Lu P, Lu K, Zheng J, Guo D (1998). Effect of rare earth elements on callus growth of *Coptis chinensis*. J. Beijing Med. Uni. 30: 389-391. (In Chinese)

Lu Q, Yang Q, Zou H (2006). Effects of cerium on accumulation of anthocyanins and expression of anthocyanin biosynthetic genes in potato cell tissue cultures. J. Rare Earth 24: 479-484.

Luo JP, Wang Y, Zha XQ, Huang L (2008). Micropropagation of *Dendrobium densiflorum* Lindl. ex Wall. through protocorm-like bodies: effects of plant growth regulators and lanthanoids. Plant Cell Tissue Organ cult. 93: 333-340.

Ma YF, Shang FD, Xue GL (2002). Effects of La^{3+} on callus culture from young leaves of *Ginkgo biloba* L. J. Henan Uni. Nat. Sci. 32: 78-79. (In Chinese)

Murashige T, Skoog F (1962). A revised method for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum, 15: 473-497.

Ning SX, Gao N, Yang WX (2005). The effects of lanthanum nitrate on callus culture and differentiation of *Pteridium apnilinum*. Nat. Sci. J. Harbin Norm. Uni. 21: 92-95. (In Chinese)

Ouyang J, Wang X, Zhao B, Yuan X, Wang Y (2003). Effects of rare earth elements on the growth of *Cistanche deserticola* cells and the production of phenylethanoid glycosides. J. Biotechnol. 102: 129-134.

Song W, Gu F, Yu J, Shen Y, Qi Q, Liu J (2004). Effects of lanthanum on root growth and senescence of GF 43 (*Prunus domestica*) plantlet *in vitro*. J. Rare Earth 22: 696-700.

Song W, Hong F, Wan Z (2002). Effects of lanthanum element on the rooting of loquat plantlet *in vitro*. Biol. Trace Elem. Res. 89: 277-284.

Wakamatsu K, Takahama U (1993). Changes in peroxidase activity and in peroxidase isozymes in carrot callus. Physiol. Plant 88: 167-171.

Wan Z, Song W, Yu M, Hong F, Gu F, Su G, Zhao M (2004). Effects of neodymium on nitrogen metabolism of plantlet loquat *in vitro*. J. Rare Earth 22: 431-435.

Wang DF, Wang CH, Wei ZG, Qi HT, Zhao GW (2003). Effect of rare earth elements on peroxidase activity in tea shoots. J. Sci. Food Agric. 83: 1109-1113.

Wang YL, Wang XD, Zhao B, Wang YC (2007). Reduction of hyperhydricity in the culture of *Lepidium meyenii* shoots by the addition of rare earth elements. Plant Growth Regul. 52: 151-159.

Wolter KE, Gordon JC (1975). Peroxidases as indicators of growth and differentiation in aspen callus cultures. Physiol. Plant 33: 219-223.

Wu J, Wang C, Mei X (2001). Stimulation of taxol production and excretion in *Taxus* spp. cell cultures by rare earth chemical lanthanum. J. Biotechnol. 85: 67-73.

Xiang L, Ge ZQ (2008). Effects of different valences of cerium ion on conformation of horseradish peroxidase. J. Rare Earth 26: 857-863.

Yang S, Liu X, Guo D, Zheng J (2005). Effect of rare-earth element Eu^{3+} on callus growth and flavonoids content in *Glycyrrhiza uralensis*. Zhong Yao Cai 28: 533-534. (In Chinese).

Yuan X, Wang Q, Zhao B, Wang Y (2002). Improved cell growth and total flavonoids of *Saussurea medusa* on solid culture medium

supplemented with rare earth elements. Biotechnol. Lett. 24: 1889-1892.
Yuan YJ, Hu GW, Wang CG, Jing Y, Zhou YQ, Shen PW (1998). Effect of La, Ce on *Taxus cuspidata* cell growth, biosynthesis and release of taxol. J. Rare Earth 16: 300-306.
Zhang Z, Hu N, Liu X, Xu H (2005). Effect of rare earth on physiological-chemical characteristics and its growth of aloe in tissue culture. J. Rare Earth 23: 522-525.
Zhao FC, Nilanthi D, Yang Y-S, Wu H (2006). Anther culture and haploid plant regeneration in purple coneflower (*Echinacea purpurea* L.). Plant Cell Tissue Organ Cult. 86: 55-62.