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REVIEW

Effects of rare earth elements on growth and metabolism of medicinal plants

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Abstract The rare earth elements (REEs) are a set of 17 chemical elements. They include the lanthanide series from lanthanum (La) to lutetium (Lu), scandium (Sc), and yttrium (Y) in the periodic table. Although REEs are used widely in industry and agriculture in China for a long time, there has been increasing interest in application of REEs to medicinal plants in recent years. In this paper, we summarize researches in the past few decades regarding the effects of REEs on the germination of seeds, the growth of roots, total biomass, and the production of its secondary metabolites, as well as their effects on the absorption of minerals and metals by medicinal plants. By compilation and analysis of these data, we found that REEs have promoting effects at low concentrations and negative effects at comparatively high concentrations. However, most studies focused only on a few REEs, *i.e.*, La, cerium (Ce), neodymium (Nd) and europium (Eu), and they made main emphasis on their effects on regulation of secondary metabolism in tissue-cultured plants, rather than cultivated medicinal plants. Advanced research should be invested regarding on the effects of REEs on yields of cultivated plants, specifically medicinal plants.

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1. Introduction

Rare earth elements (REEs) are a series of 17 chemical elements. They include the lanthanide series from lanthanum (La) to lutetium (Lu), scandium (Sc) and yttrium (Y) in the periodic table[1]. They are called as "rare earth elements" because most of them were isolated as oxides from rare minerals in the 18th and 19th century[2]. The total world reserves are an estimated 100 million metric tons of rare earth oxide (REO) and the regions having major ore reserves are China (43%), commonwealth of Independent States (19%), United States (13%), Australia (5.2%), India (1.1%), Canada (0.94%), South Africa(0.39%) and Brazil (0.08%)[3]. With more than 40% of total REEs reserves in the world, China owns the largest rare earth deposit (Baiyun Obo rare earth ore, Baotou, Inner Mongolia), and is a major exporter of REEs[4]. REEs are used widely in industry, such as in lighter flints, in carbon arc lighting, used as iron and steel additives, for glass polishing and ceramics, in rechargeable batteries, cell phones, and as car catalytic converters as well[5]. They have also been applied generally in agriculture for more than 30 years in China.

REEs have shown interesting biological effects on plants. They can exert positive or negative physiological effects on plants depending on the dosage and other conditions. In recent years, there is increasing interest in application of REEs to medicinal plants, as it is able to affect the growth and/or development of such plants directly or indirectly. Application of adequate REEs can promote the germination of seeds and roots development, increase plant biomass, and improve the quality of fruiting bodies. There is a certain critical level for absorption of REEs in plants, and these elements can improve growth and development of plants when supplied at a suitable concentration. However, if the concentration of REEs exceeds the optimum level, they can inhibit the plant growth and even cause mortality[6]. This review summarizes the researches of REEs in the last few decades effecting the germination of seeds, the growth of roots, total biomass, and the production of secondary metabolites, as well as its effects on the absorption of minerals and metals by medicinal plants.

2. Effects on the seed germination of medicinal plants

The seed germination rate (SGR) is a standard parameter to reflect the quality of seeds. REEs at appropriate concentration can affect the germination of seeds, which have been proved by a large number of published papers. One of the papers reported that treatment with REEs improved seed vigor, water absorption and cytoplasmic membrane permeability of seeds of a medicinal plant during the seed imbibition and germination. In addition, the oxygen evolution rate of the REEs-treated seeds was greater than those of non-REEs-treated ones, indicating greater metabolic activity and more energy for growth[7]. It was also reported that the appearance of low concentration (1, 3, or 5 mg/L) of neodymium cation (Nd^{3+}) significantly ($P<0.05$) promoted seed germination of *Cassia obtusifolia*, whereas the Nd^{3+} at a concentration higher than 5 mg/L inhibited its germination. Finally, the optimum concentration of Nd^{3+} was set as 3 mg/L. Besides, there was a synergistic effect of Nd^{3+} with a burdock fructooligosaccharide[8]. Similarly, Nd^{3+} at a concentration of 6 mg/L had a remarkable effect on the germination of the seed of *Astragalus*, stronger than that of the burdock oligosaccharide or NaCl. The SGR of *Astragalus* treated with 6 mg/L Nd^{3+} solution was 40–42 %, higher than 9–15 % of the control[9]. Many different concentrations of Nd^{3+} (10, 50, 80 and 100 mg/L) were also compared to evaluate the stimulation to the seed germination of *Angelica sinensis*, and the results showed that the seeds treated with 80 mg/L Nd^{3+} exhibit the highest germination rate of 57%, which was 22% higher than that of the control[10].

3. Effects on root of medicinal plants

The root system is one of most important organs for plant. It absorbs water and nutrients and synthesizes organic compounds. The growth and physiological activities of the root system directly affect the growth and physiology of the whole plant. REEs help the growth of the root system by promoting the formation of adventitious roots and affecting cell differentiation and root morphogenesis. When different concentrations of La $(NO_3)_3$ and $Eu(NO_3)_3$ were added to the rooting medium of cultures of *Eriobotrya japonica* (loquat) *in vitro*, $La(NO_3)_3$ (1.0–3.0 μM) and $Eu(NO_3)_3$ (2.0–3.0 μM) significantly increased the rooting rate and root fresh weight, promoted root elongation, and enhanced the activities of peroxidase and nitrate reductase. The effects of $La(NO_3)_3$ were greater in terms of promoting root elongation and enhancing peroxidase activity, while those of $Eu(NO_3)_3$ were greater in terms of increased root fresh weight and increased activity of nitrate reductase[11]. A research on the effects of La, Ce and Nd on *Dendrobium densiflorum* showed that root regeneration was affected more obviously by REEs alone rather than by a combination of REEs with the plant growth regulator indole-3-butyric acid, and that roots treated with indole-3-butyric acid only exhibited the lowest regeneration rate. The effect of Nd^{3+} was stronger than those of La and Ce, with the root regeneration rate 100% and the mean number of roots per seedling 23.9 when treated at the optimum Nd^{3+} concentration of 4.0 mg/L[12].

Several studies reported that low concentrations of La^{3+} promote root vigor, but at higher concentrations, La^{3+} exhibited an inhibitory effect. When supplied at 10 μM, La^{3+} promoted root vigor of *Huperzia serrata*, with the roots of treated plants showing a 1.7-fold increase in vigor compared with that of the control. When supplied at 50 and 100 μM, La^{3+} had negative effects[13]. Adding 5 μM Nb^{3+} to the medium significantly increased the rooting rate of stem sections of *Dendrobium densiflorum*, which was possibly related to an Nb^{3+}-induced increase in the level of endogenous indole-3-acetic acid[14]. In a study on the effect of cerous nitrate on rapid propagation of *Dioscorea zingiberensis*, Ce^{3+} at 1–15 mg/L promoted the formation of seedling root tissue. The strongest effect was observed with 5 mg/L Ce^{3+}, whereas it inhibited root growth at a higher concentration[15].

4. Effects of REEs on biomass of medicinal plants

4.1. *Effects on aboveground yields of medicinal plants*

Chlorophyll, a green pigment produced by all green plants, is vital for photosynthesis by which plants transform light energy into chemical energy. Therefore, photosynthesis is extremely important for plant growth. Studies showed that appropriate concentrations of REEs can increase the chlorophyll content of leaves and helps plant growth. For *Ginkgo biloba,* treatment with 100 mg/L rare earth increased the leaf yield by 11.64% compared with the control group. In contrast, a higher concentration (400 mg/L) decreased the leaf yield by 19.3%[16].

4.2. *Effects on yields of medicinal plant cell or tissue cultures*

The effects of REEs on callus induction were investigated by using explants of *Coptis chinensis* on 67-V medium containing different concentrations of Yb^{3+} and Eu^{3+}. The results showed that both 0.1 mg/L Yb^{3+} and 1 mg/L Eu^{3+} promoted callus growth, with the fresh weights of calli in the treated groups being significantly greater than that of the control ($P<0.01$). However, it inhibited callus growth and even resulted in callus death in case of higher concentrations. When calli were subsequently transferred to rare earth-free medium, the fresh weights of calli derived from media containing REEs were significantly lower than that of the control group ($P<0.01$). This result suggested that a lower concentration of REEs could promote callus growth, while lower concentrations of Yb^{3+} and Eu^{3+} both adversely affected subsequent callus sub-culture [17]. The effects of brassinosteroids, rare earth and potassium dihydrogen phosphate on the yield of fresh *Ganoderma lucidum* were studied in a $L_9(3^4)$ orthogonal experiment. The results showed that brassinosteroids highly significantly ($P<0.01$) and REEs significantly ($P<0.05$) increased the yields of *G. lucidum*, respectively. The yield of *G. lucidum* was highly increased up to 35% compared to the control at an optimal condition[18]. When 10 μmol/L Cr^{3+} was added to the medium of *Dendrobium huoshanense* cells, the cell mass increased from 0.501 to 0.649 g/L/d. After 24 days' culture, the dry weight of the protocorm-like bodies was 25.45 g/L, which was 20.2% greater than that of the control. However, the cell growth was severely inhibited ($P<0.01$) when the concentration of Cr^{3+} was increased to 80 μM[19]. Some researchers attempted to use REEs to regulate growth and differentiation of embryogenic callus of *Crocus sativus*. The results revealed that its growth was most obviously promoted by 0.04 mM La^{3+} with the propagation coefficient of 12, which was 1.48-fold greater than that in REE-free medium[10].

5. Effects on production of secondary metabolites in medicinal plants

In nature, plants are exposed to a variety of adverse factors, including microbial invasion and different damages. Plant body has to produce all kinds of secondary metabolites as a part of their resistance responses for defense. The biosynthesis of secondary metabolites is complex and diverse, which involves a series of metabolic pathways[20].

As REEs alter membrane permeability, so that the secondary metabolites are able to be secreted more rapidly into the medium. REEs also increase the production of secondary metabolites *via* promoting the transcriptions of essential biosynthetic genes[21].

5.1. *Regulation of secondary metabolites in medicinal plant cell cultures*

Over the past few decades, there are several researches regarding the effects of REEs on cell cultures of medicinal plants depending on the different dosages and other conditions. Addition of REEs promotes bio-synthesis of secondary metabolites, such as flavonoids, isoflavones and benzyl ethanol glycosides in medicinal plant cells[12,22,23]. In a study, lanthanum nitrate was added to cell cultures of *Catharanthus roseus*, leading to a dose-dependent accumulation of alkaloids. Lanthanum nitrate promoted accumulation of alkaloids at low concentration (20 ppm), but it had an inhibitory effect at a higher concentration (60 ppm)[24]. Moreover, La^{3+} was tested for elicitor-like effects on taxol production in suspension cultures of four different *Taxus* spp. cells. In *T. yunnanensis* cell cultures, La^{3+} (1.15–23.0 mM) stimulated taxol production, and the maximum stimulation was found at 5.8 mM La^{3+}, increasing the volumetric taxol yield by nearly three-fold, from 2.61 to 9.89 mg/L. However, at higher concentrations than 23 mM (*i.e.*, 23.1 and 46.2 mM), La^{3+} caused significant growth inhibition. When adding La^{3+} into the other three *Taxus* cell lines (an embryo, a leave and a stem cell of *T. chinensis*), only stem cells showed a significant effect on taxol production, increasing the volumetric yield by about three-fold [25]. Another paper reported that addition of 1 mg/L Eu^{3+} to the culture medium of *Rheum officinale* results in a 10-fold increase in emodin content and a 130-fold increase in chrysophanol content in the calli compared to their respective concentrations in control calli[26]. Addition of Eu^{3+} also affected the production of secondary metabolites of *Glycyrrhiza uralensis*, *e.g.*, a lower concentration of Eu^{3+} promoted callus growth and flavonoid production. This effect was most pronounced on medium containing 0.1 mg/L Eu^{3+}. At that concentration, the Eu^{3+} treated plant tissues contained 2.7-fold greater flavonoid content than the control, and a 4-fold increase in liquiritigenin content[27]. Similarly, compared with that of the control, adding Ce_2O_3 and $CeCl_3$ to the medium of *C. roseus* improved the production of raubasine by 11-fold and 9-fold, respectively, and adding Y_2O_3 and $NdCl_3$ improved the production of catharanthine by 30-fold and 25-fold, respectively[28]. It was also reported that Ce played a considerable role in increasing the content of intracellular polysaccharides. The highest production of polysaccharides (3.35 g/L) was achieved after 18 days' cell culture in medium containing Ce, and this level was 1.53-fold of the control[19]. In addition, Nd, La and Ce were reported to have positive effects on the cell growth of *Cistanche deserticola* and production of phenylethanoid glycosides (PeG) at proper concentrations. A mixture of rare earth elements (MRE, La_2O_3:CeO_2:Pr_6O_{11}:Sm_2O_3=255:175:3:1) showed the most remarkable effects. After 30 days' culture, 0.02 mM MRE gave the highest content (20.8%) and production (1.6 g/L) of PeG, which were 104% and 167% higher than those obtained in control (without rare earth elements)[23].

5.2. *Effects on the production of secondary metabolites in cultured medicinal plants*

When *G. lucidum* was treated with REEs, the fruiting bodies had greater contents of crude protein, crude fiber, ash, and a lower content of crude fat. This effect was most remarkable when the concentration of REEs arrived at 100–150 μg/g. Compared with the control, the increases of protein, fiber and polysaccharides were 6.55–6.56%, 0.91–1.96% and 4.00–11.29%, respectively, while the fat content was decreased by 4.15%. The greatest change was the ash content, with 5.50% increase when 25 μg/g REEs was applied. REEs also increased the amino acid content of plant tissues and changed the proportions of the various amino acids[29].

Different concentrations of REEs solutions (50, 100, 200 and 400 mg/L) were sprayed onto leaves of *G. biloba* seedlings to evaluate its effects on chemical composition of the leaf tissues. The 50 mg/L REEs solution significantly increased the contents of protein, soluble sugars and flavonoids by 33.0%, 29.0% and 48.6%, respectively. However, the 400 mg/L REEs solution significantly decreased the contents of protein, soluble sugars and flavonoids by 26.7%, 19.3% and 3.4%, respectively[16]. For *H. serrata*, REE La^{3+} promoted the huperzine A content (2-2.2-fold higher of the control) at 50 μM[14]. Similarly, La^{3+} (20–80 μM) promoted accumulation of artemisinin in *Artemisia annua*[30].

In general, REEs may stimulate cell growth and production of secondary metabolites at lower concentrations but become toxic to the cells at higher concentrations.

6. Effects on absorbing other minerals of medicinal plants

Plants need many mineral elements during the normal processes of growth and development. Specifically, they require high levels of the major inorganic elements (*e.g.*, N, P and K), moderate amounts of other nutrients (including atoms Ca, Mg and S), and other trace elements (including Mn, Cu, Zn, B, Mo and Cl), as well as beneficial elements (such as Se, Na and REEs)[31]. Scientists believed that REEs can regulate absorption of other mineral elements in plants[32]. When *G. lucidum* was cultivated with different concentrations of REEs (25, 50, 100, 150, 200 and 250 μg/g), change of the contents of other minerals was observed in the plant tissues. For example, the contents of Fe, Zn and Mg were increased by 2.40–3.98%, 1.54–1.81% and 0.70–3.12%, whereas that of Cu was decreased by 0.65–1.15%. The specific experimental data revealed that the REE content of the fruiting body increased only very slightly, even when higher concentrations of REEs were supplied to the plants. All fruiting bodies contain less than 0.02 μg/g REEs, and there was no significant difference observed in this value among the different REE concentrations[29]. REEs can also inhibit absorption of heavy metals. La^{3+}, for example, decreased the absorption of Pb^{2+} in plants grown under lead-polluted conditions[33,34].

7. Conclusions

REEs have a variety of effects on seed germination, growth of roots, total biomass accumulation, production of secondary metabolite and absorption of minerals and metals for medicinal plants. They have promoting effects at low concentrations while negative effects at comparatively high concentration. However, most studies have focused only on a few REEs (La, Ce, Nd and Eu) and made main emphasis on its effects of regulation of secondary metabolism in tissue-cultured plants, rather than cultivated medicinal plants. Further research in this area should be strengthened for the effects of REEs on yields of cultivated plants, specifically medicinal plants. Some other functions of REEs, for example, the ability to inhibit absorption of harmful minerals, may be a solution for cultivation of plants that tend to accumulate heavy metals (to levels that exceed the Chinese herbal medicine standard). Finally, the safety of REEs should be investigated in detail with in-depth studies on the effects of REEs on the environment, plants, humans and the ecology of complex ecosystems.

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