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Effect of rare earth elements on growth and antioxidant metabolism in *Lemna minor* L.

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Abstract — *Lemna minor* is frequently used in bioremediation processes to remove nutrients and contaminants from waste water. In this work the response of *L. minor* to treatments with lanthanum nitrate and with a mix of several light rare earth elements (REE) nitrates was investigated. Preliminary results indicate that *L. minor* shows an overall good tolerance to the presence of REE in the media. Toxic effects were observed after prolonged exposition to high concentration of REE. An increase in ascorbate and glutathione content as well as in ascorbate peroxidase, dehydroascorbate reductase and ascorbate free radical (AFR) reductase activity was observed in treated plants.

Key words: ascorbate, bioremediation, glutathione, lanthanum, *Lemna minor*, rare earth elements.

INTRODUCTION

Rare earth elements (REE) are members of Group IIIB in the Periodic Table that share physical and chemical properties due to a similar external electronic configuration. In addition to industry applications, REE are widely applied in agriculture and forestry in China, where REE enriched fertilizers are currently used for soil and foliar applications to enhance crop production (Hu *et al.* 2004). Because of the widespread application of REE to soil and crops, interest and concern about their biological effects have been increasing. A number of positive physiological responses in plants, including faster development, larger roots, greener foliage and better fruit colour in different species have been reported (Chen *et al.* 2000), but negative effects have been reported too (Hu *et al.* 2002; Nardi *et al.* 2004). Results of field trials and laboratory studies are still contradictory and sometimes inconsistent and many questions about mechanisms of REE effects, accumulation and toxicity are still open. Antioxidants metabolites and enzymes are cell defenses against reactive oxygen species produced by cell metabolism. Among them, ascorbate and glutathione are involved in a network of reactions, well known as the ascorbate-glutathione cycle. This cycle together with other enzymatic reactions catalyzed by superoxide dismutase, catalase and peroxidase, prevent the accumulation of toxic levels of the H_2O_2 and other reactive oxygen species (ROS) in cell compartments (Mittler 2002). In plants treated with REE the increase of superoxide dismutase, catalase and peroxidase is also reported (Fashui *et al.* 2000; Fashui 2002; Nardi *et al.* 2004). The aim of this work was to investigate the effect of treatments with lanthanum nitrate and a mix of different light REE nitrates on the antioxidant metabolism in common duckweed (*Lemna minor* L.).

MATERIALS AND METHODS

REE nitrate solution (La 13.9 g/l, Ce 45.9 g/l, Pr 3.63 g/l, Nd 0.021 g/l, pH 3, NO_3^-/Cl^- 7:1) (RE) was prepared exposing a light REE enriched chloride mixture (supplied by *Inner Mongolia Sanjili Rare Earth Materials* Co. Ltd.) to concentrate nitric acid solution and then dissolved in MilliQ water. Lanthanum and potassium nitrate solutions were prepared with a commercial reagent. Concentrations were determined by optical inductively coupled plasma spectrometry (ICP) and by ionic chromatography.

Lemna minor L. plants were maintained in 20 liters capacity trays and fed with Knop's solution

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(SAEGER 1925) under glasshouse conditions. Fresh Knop solution was added every three days to maintain solution level. 4 days before experiments were started, plants were placed in Petri dishes containing MilliQ water, washed and transferred in Knop solution at 24°C under white light (14-h photoperiod, 20 µmol m^{-2} s^{-1}) to acclimatize.

Batches of 4 Petri dishes were incubated with 20 ml of lanthanum nitrate and RE 5 and 10 mM, respectively. MilliQ water and potassium nitrate 15 and 30 mM were used as a control. After 2 and 5 days of treatment, whole plant batches (0.3 g) were collected and used for assays. The roots were washed with water, observed and photographed using a Leica DMLS stereo microscopy with tungsten lighting. Dry weight, ascorbate and glutathione content and cytosolic ascorbate (ASC) peroxidase, dehydroascorbate (DHA) reductase and ascorbate free radical (AFR) reductase activity were determined as reported by TOMMASI *et al.* (2001). Protein assay was performed according to BRADFORD (1976) using bovine serum albumin as a standard. Native-PAGE of ASC peroxidase and DHA reductase were performed on the cytosolic fraction according to TOMMASI *et al.* (2001). All experiments were repeated at least six times.

RESULTS

No differences between treated and control plants were observed after 2 days of treatment and no significant variations of dry weight, ascorbate and glutathione redox state were observed. Therefore, only data concerning 5 days treatment are reported.

Plants treated for 5 days with lanthanum nitrate and RE 10 mM showed enlargement of root apexes (Fig. 1) as well as yellowing of leaves. No alterations were observed in control plants. Figure 2 shows total protein content and ASC peroxidase, DHA reductase and AFR reductase activities after 5 days of treatment with lanthanum nitrate and RE nitrate 5 and 10 mM, potassium nitrate 15 and 30 mM and MilliQ water. Protein content decreased in plants treated with lanthanum nitrate and RE, while an increase of ASC peroxidase, DHA reductase and AFR reductase activities was observed.

Increased activity of ASC peroxidase and DHA reductase in treated plants was also confirmed by native-PAGE, where more intense bands and a change in the number of the DHA reductase proteins were observed (data not shown).

Figure 3 shows total ascorbate and glutathione content (oxidized + reduced forms) after 5 days treatment with lanthanum nitrate, RE 10 mM, potassium nitrate 30 mM and MilliQ water. An increase of total ascorbate and glutathione content in treated plants was observed.

DISCUSSION

These preliminary results suggest that RE treatments, at tested concentrations, did not promote the growth of duckweed, and in the meantime it caused toxic effects after 5 days of exposure. Both treatments induced variations of the two major antioxidants, ascorbate and glutath-

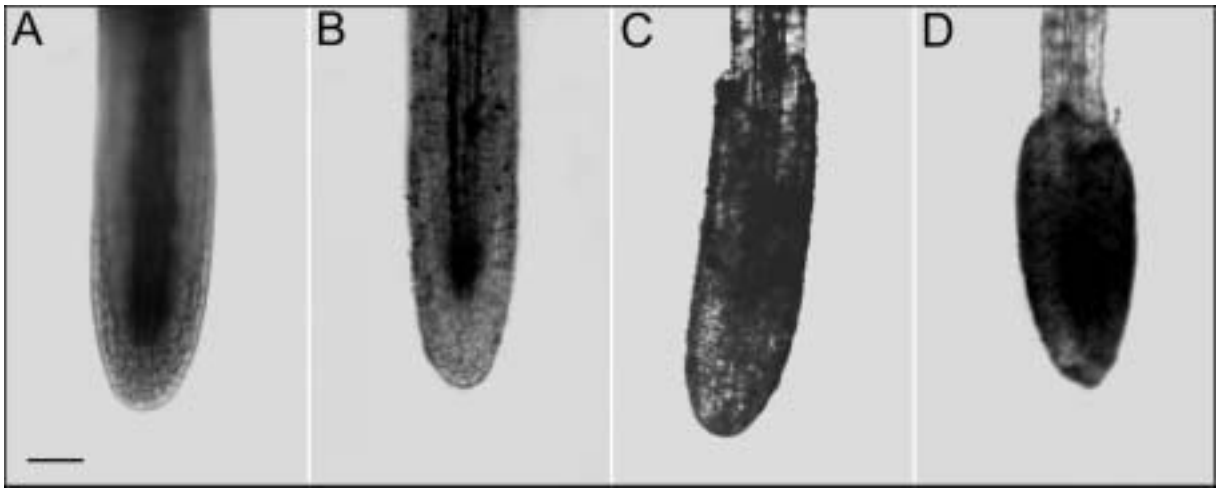

Fig. 1 — Roots of duckweed plants treated for five days with: water (A), KNO_3 30 mM (B), $La(NO_3)_3$ 10 mM (C), RE 10 mM (D). Bar = 1 µm

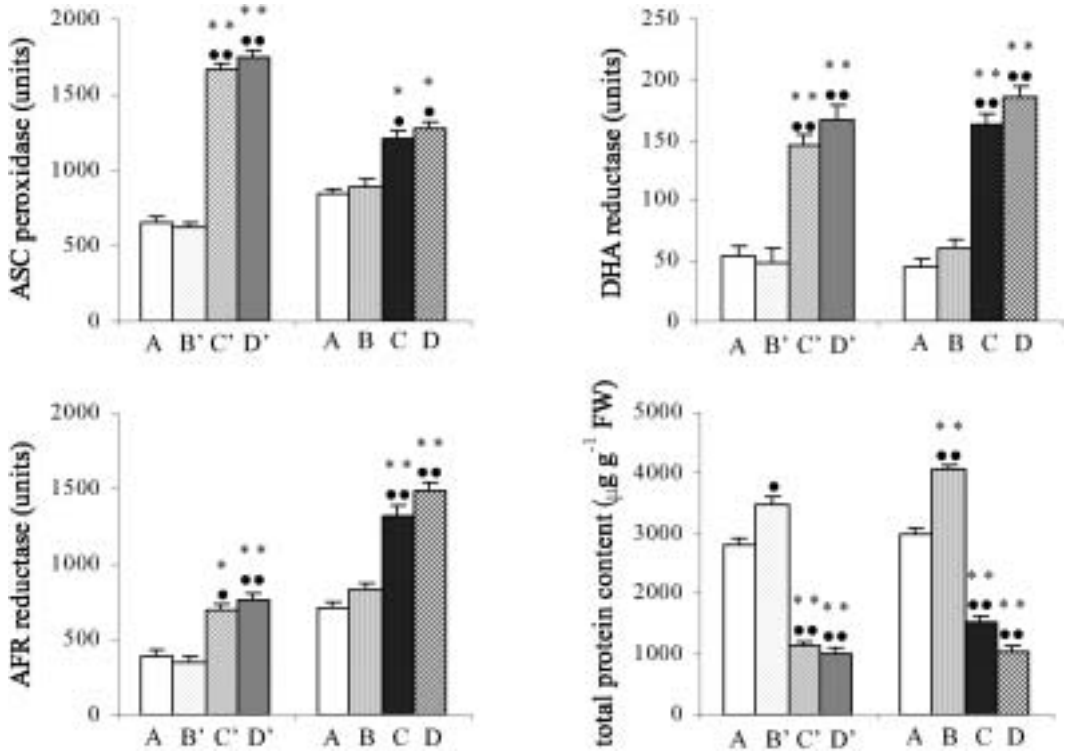

Fig. 2 — Activity of ASC peroxidase, DHA reductase, AFR reductase and protein content in duckweed plant treated for five days with: water (A), KNO_3 15 mM (B'), 30 mM (B), $La(NO_3)_3$ 5 mM (C'), 10 mM (C), RE 5 mM (D'), 10 mM (D). Activities are expressed in units; 1 unit = 1 nmol substrate metabolized mg^{-1} protein min^{-1}. The results are given as the mean values of six experiments ± SD; * and * * indicate values significantly different respect to the control in water by the Student's *t* test with P < 0,05 and 0,01, respectively; • and • • indicate values significantly different respect to the control in KNO_3 by the Student's *t* test with P < 0,05 and 0,01, respectively.

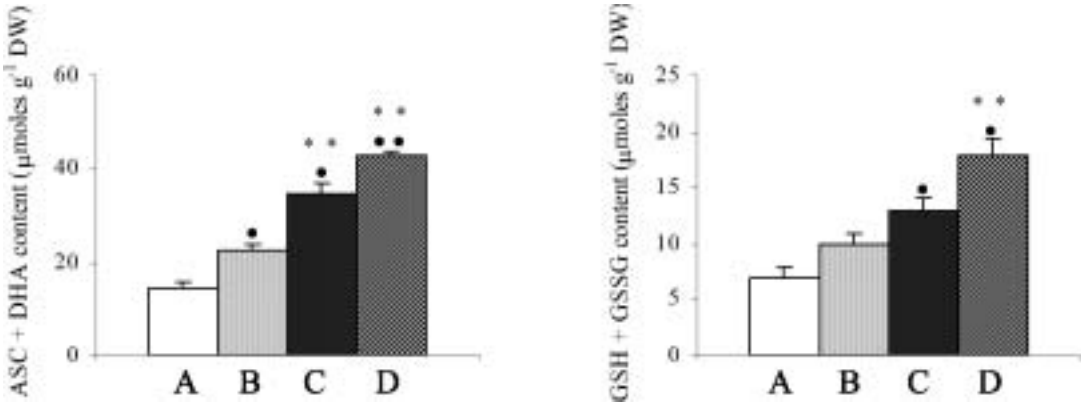

Fig. 3 — Ascorbate and glutathione content in duckweed plant treated for five days with: water (A), KNO_3 30 mM (B), $La(NO_3)_3$ 10 mM (C), RE 10 mM (D). The results are given as the mean values of six experiments ± SD; * * indicate values significantly different respect to the control in water by the Student's *t* test with P < 0,01; • and • • indicate values significantly different respect to the control in KNO_3 by the Student's *t* test with P < 0,05 and 0,01, respectively.

ione, as well as a remarkable increase of the activity of the enzymes involved in their metabolism. The increase of antioxidants induced by lanthanum and cerium nitrate treatment at low concentrations has been reported by FASHUI (2002) in aged *Oryza sativa* L., where ROS production was successfully controlled by the antioxidant stimulation and the final result was an improvement of aged seed germination. Similarly, antioxidant increase induced by lanthanum and REE nitrate treatment at low concentrations has been reported by NARDI *et al*. (2004) in *Triticum durum* Desf., where seedling growth was strongly inhibited by treatment, thus suggesting that antioxidant stimulation was not able to overcome massive ROS production and to prevent the oxidative stress induced by high concentration of lanthanum and REE. Therefore, plant response to REE seems to be dose- and species-dependent. Moreover antioxidant modulation in *L. minor*, induced by lanthanum and RE nitrate, may induce tolerance to REE exposition, but it is not able to avoid tissue damages due to prolonged treatments. This feature indicates that *Lemna minor* may be an useful tool for studying biological effects of RE in aquatic ecosystems.

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