Ecotoxicity Of Rare Earth Elements

Soudek P.[1,*], Petrová Š.[1], Landa Lekuberria J. [1], Tomášková I.[2] And Vaněk T.[1]

[1]Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, v.v.i., Rozvojová 263, 165 02 Prague 6 – Lysolaje, Czech Republic

[2]Czech University of Life Sciences Prague, Department of Genetics and Physiology of Forest Trees, Faculty of Forestry and Wood Sciences, Kamýcká 1176, CZ-165 21 Praha 6 – Suchdol, Czech Republic

*corresponding author: Soudek P.

e-mail: soudek@ueb.cas.cz

Abstract The effect of REE on seed germination was followed in white mustard (*Sinapis alba* L.). The effect of REE on viability of suspension culture of *Arabidopsis thaliana* was also tested. Toxicity of Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, at five different concentrations (in the range 0.05 - 5 mM) was tested by standard ecotoxicity test. The root length of white mustard was measured after 72-h incubation. The elongation inhibition, EC_{50} value and slope values were calculated. The exposure of suspension culture of Arabidopsis thaliana took 96 h under dark condition. The tolerance of suspension cultures to REE was assessed using the reduction of 2,3,5-triphenyltetrazo-lium chloride (TTC). The results showed that REE toxicity decreased in the order: Lu > Er > Yb > Sc > Tm > Y > Ce > Ho > La > Nd > Pr > Dy > Gd > Tb > Eu > Sm.

Keywords: heavy metals, phytoremediation, accumulation, stress, rare earth elements (REE)

1. Introduction

Rare earth elements (REEs) are a group of 15 chemical elements in the periodic table, specifically the lanthanides. Two other elements, scandium and yttrium, have a similar chemistry and toxicology to the lanthanides, are commonly found in the same mineral assemblages, and are often referred to as REEs (EPA, 2012).

Despite the fact that their distribution in the Earth's crust is poor and their solubility and bioavailability are low, these metals are a potential environmental problem. They are widely used in electrical and electronic devices used for green and communication technologies. The devastation of the environment has already occurred greatly during their extraction. It can also be assumed that they will get back to environment after the end of the life cycle of the products they contain.

REE toxicity for human is relatively well documented (EPA, 2012; Hirano and Suyuki, 1996). Less information is about plant toxicity and the plant's ability to metals bio-concentration and thus bring them into the food chain. Li *et al*. (2013) to assess the risks arising from the consumption of vegetables containing REE should not lead to an exceedance of the estimated daily intake that is already harmful to human health for adults and children. It seems that REE transfer to plants is not very significant. Liang *et al*. (2005) reports that the REE content in wheat seeds was 3-4 orders lower than in soil. Availability depends on the physico-chemical properties of the soil. It can be increased, for example, by the addition of EDTA (Lihong *et al*., 1999). Root uptake is not the only way how REEs can get into the plant. Chua *et al*. (1998) has shown that the application of cerium onto the surface of the leaves of sugarcane results in its rapid distribution to different parts of the plant.

One of the other ways REEs can get into the soil is the intensive use of fertilizers enriched with REE in China (Zhang and Shan, 2001). At low concentrations REEs have a promoting effects on seed germination, growth of roots, total biomass accumulation, production of secondary metabolite and absorption of minerals and metals for medicinal plants (Chunhong *et al*., 2013).

Some REE toxicity studies on living organisms have been performed. Most of these works either test only one of the elements (Oral *et al*., 2010; Saitoh *et al*., 2010; Qu *et al*., 2004; Barry and Meehan, 2000) or used to test a variety of animal species, such as e.g. *Caenorhabditis elegans* (Zang *et al*., 2006), *Tenebrio molitor* (Zhao *et al*., 2005), *Holotrichia parallela* (Li *et al*., 2006) or *Daphnia carinata* (Barry and Meehan, 2000).

The aim of the present work was to evaluate the effect of REE on the germination of white mustard (*Sinapis alba* L.) seeds and on viability of suspension culture of *Arabidopsis thaliana* L.

2. Material and Methods

2.1. Plant material and chemicals

Heavy metals ions (Sc^{3+}, Y^{3+}, La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+} and Lu^{3+}) were obtained from chloride salts (Aldrich, Alfa Aesar, Acros Organics, VWR International, Laborchemie Apolda GmbH). Tested concentrations were 0.05, 0.1, 0.5, 1 and 5 mM of each metal.

White mustard *Sinapis alba* L. (Maryna-C) seeds (Forestina Ltd., Czech Republic) were used for germination test. All heavy metal substances were dissolved in distilled water which contained 2 mM $CaCl_2$ x

$2H_2O$, 0.5 mM $MgSO_4$ x $7H_2O$, 0.8 mM $NaHCO_3$ and 0.08 mM KCl (according to ČSN EN ISO 7346) (all chemicals from Penta, www.pentachemicals.eu) . The pH was adjusted to 7.6 by 0.1 M solution of NaOH.

Cell suspension cultures of *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) were grown on V4 medium (Heller, 1953) supplemented with the auxins naphtaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) (both of 1 mg/L) and sucrose (30 g/L). For the experiment, 100 mL of suspension was cultivated in 200-mL Erlenmeyer flasks in a horizontal shaker (150 rpm) in dark at 24 °C.

2.2. Semichronic toxicity test

The seeds were placed in plastic dishes of 10 cm diameter with a layer of a filter paper on the bottom. Seventeen seeds were equally placed into each dish on the surface of filter paper and 5 mL of tested aqueous solution with heavy metal was added. Each treatment had five replicates. The exposure took 72 h under dark condition at 25 °C. Then the root lengths were measured and inhibition values of root elongation were calculated according to formula:

$$I = (Dc - Dt)/ Dc$$

where I is an inhibition of root elongation in %; Dc is an average length of root in control conditions (without heavy metal treatment) [mm] and Dt is an average length of root grown under tested metal concentration [mm].

2.3. Viability test of cell cultures

Cultures were pre-cultivated for one day before treatment, when fresh weight (FW) of the biomass amounted to approximately 1.5 g. The exposure took 96 h under dark condition at 24 °C. Each treatment had three replicates. The tolerance of suspension cultures to REE was assessed using the reduction of 2,3,5-triphenyltetrazo-lium chloride (TTC) to water-insoluble red formazan as a measure of cell viability. A 0.8% stock solution of TTC was prepared in 66.7 mM phosphate buffer (pH 7.5) and stored at 4 °C. Cell culture were filtered, washed by 5 ml phosphate buffer (66.7 mM, pH 7.5), and 0.5 g of fresh biomass was transferred to an Falcon tube containing 10 ml TTC stock solution and mixed. After 24 h incubation in the dark at room temperature, cells were pelleted by centrifugation in a centrifuge for 2 min, rinsed in sterile water and pelleted again. An 30 ml volume of ethanol was added to the cell pellets and the suspension was extracted 48 h in the dark. Absorbance of the extracts was read at 485 nm.

2.4. EC_{50} calculation

EC_{50} is an effective concentration where 50 % of tested organisms give significant response to tested compound. For the calculation, the nonlinear regression with the bottom and upper maximum (0 and 100, respectively) was used. The data were processed by software GraphPad Prism (GraphPad Software, Inc., San Diego, California, USA) with the output to MS Excel.

2.5. Statistics

Statistical analysis was performed based on STATISTICA (StatSoft, Inc., Tulsa, Oklahoma, USA) program.

3. Results and Discussion

The highest inhibition effect of root elongation was found for lutetium (EC_{50} = 0.0612 mM) and the lowest for samarium (EC_{50} = 0.3014 mM). The range of obtained EC_{50} values was wide and difference between maximum and minimum of one order was found (Table 1). The REEs are subdivided into Light Rare Earth Elements (LREE) and Heavy Rare Earth Elements (HREE) on the base on electron configuration or atomic weight. Some authors divide REE into three groups (LREE, MREE and HREE) (Samson and Wood, 2005) but cannot agree where the boundaries between groups. In our table we used the distribution by METALL RARE EARTH LIMITED, which slightly corresponds to the REE toxicity in the germination test. The phytotoxicity of rare earth elements (REEs) is still poorly understood. D'Aquino *et al*. (2009) pre-soaking seed of *Triticum durum* for 2, 4 and 8 h with La^{3+} and mixture of REEs nitrate (La^{3+} 100.07 mM, Ce^{3+} 327.57 mM, Pr^{3+} 25.76 mM, Nd^{3+} 0.14 mM, Gd^{3+} 0.006 mM). They found inhibition of seed germination at higher concentrations (1 and 10 mM) after 2 and 4 h pre-soaking, while at low concentrations (0.01 and 0.1 mM) the seed

Table 1. Values of EC_{50} [mM] and slope for REE from germination test (LREE, MREE and HREE)

	Sc	Y	La	Ce	Pr	Nd	Sm	Eu
EC_{50}	0.1403	0.1837	0.2121	0.1927	0.2296	0.2146	0.3014	0.2801
slope	0.6254	0.7237	0.7673	0.9429	0.9504	1.023	1.198	1.095

	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
EC_{50}	0.2495	0.2507	0.2454	0.2104	0.0846	0.1583	0.0899	0.0612
slope	1.082	1.001	1.163	1.076	0.6508	0.8289	0.5807	0.6083

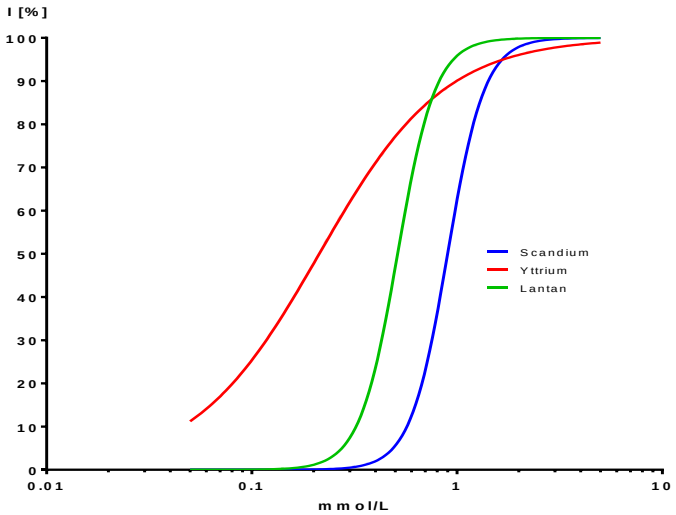

Figure 1. Effect of some REE on viability of suspension culture of *Arabidopsis thaliana*

germination was already inhibited after 8 h. The exposure–response relationships of three native Canadian plant species (*Asclepias syriaca*, *Desmodium canadense* and *Panicum virgatum*) and two commonly used crop species (*Raphanus sativus* and *Solanum lycopersicum*) to the REEs lanthanum, yttrium and cerium were tested by Thomas *et al*. (2014). Only *D. canadense* (Y) and *A. syriaca* (Ce low pH) exhibited effects at doses that could be measured in the natural environment, but only at high concentrations in the majority of cases. Specific example of the phytotoxicity of four rare earth oxide nanoparticles, nano-CeO_2, nano-La_2O_3, nano-Gd_2O_3 and nano-Yb_2O_3 on seven higher plant species (radish, rape, tomato, lettuce, wheat, cabbage, and cucumber) were investigated in the study of Ma *et al*. (2010). A suspension of 2000 mg L^{-1} nano-CeO_2 had no effect on the root elongation of six plants, except lettuce. On the contrary, 2000 mg L^{-1} suspensions of nano-La_2O_3, nano-Gd_2O_3 and nano-Yb_2O_3 severely inhibited the root elongation of all the seven species. All this papers show us, that the ecotoxicity of REEs is relatively low and correspond with our results.

The slope is important because it gives us information about intensity of the toxicity of the tested compound. A lower slope value indicates a slow decrease of toxic effect. When two compounds with the same EC_{50} value are compared, higher potential risk for the environment is caused by the compound with a lower decrease of toxic effect (lower slope) (Soudek *et al*., 2010). The results show that REE with higher toxicity to germinating white mustard seeds has a lower slope value and, therefore, a slow decrease of the toxic effect.

Viability tests of *Arabidopsis thaliana* suspension cultures show similar results to toxicity tests (Fig. 1). E.g. calculated EC_{50} values for yttrium was 0.2130 mM. The measurements of this experiment are not finished yet.

4. Conclusions

Our results showed a high diversity in the response of white mustard and *Arabidopsis* to the presence of REE. We found wide differences in toxicity. The results showed that REE toxicity decreased in the order: Lu > Er > Yb > Sc > Tm > Y > Ce > Ho > La > Nd > Pr > Dy > Gd > Tb > Eu > Sm. We show that the toxicity test to be a useful tool for the selection of plants suitable for phytoremediation purposes.

Acknowledgments

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