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Bioaccumulation and effects of lanthanum on growth and mitotic index in soybean plants

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ABSTRACT

Rare earth elements such as lanthanum (La) have been used as agricultural inputs in some countries in order to enhance yield and improve crop quality. However, little is known about the effect of La on the growth and structure of soybean, which is an important food and feed crop worldwide. In this study, bioaccumulation of La and its effects on the growth and mitotic index of soybean was evaluated. Soybean plants were exposed to increasing concentrations of La (0, 5, 10, 20, 40, 80, and 160 μM) in nutrient solution for 28 days. Plant response to La was evaluated in terms of plant growth, nutritional characteristics, photosynthetic rate, chlorophyll content, mitotic index, modifications in the ultrastructure of roots and leaves, and La mapping in root and shoot tissues. The results showed that the roots of soybean plants can accumulate sixty-fold more La than shoots. La deposition occurred mainly in cell walls and in crystals dispersed in the root cortex and in the mesophyll. When La was applied, it resulted in increased contents of some essential nutrients (i.e., Ca, P, K, and Mn), while Cu and Fe levels decreased. Moreover, low La concentrations stimulated the photosynthetic rate and total chlorophyll content and lead to a higher incidence of binucleate cells, resulting in a slight increase in roots and shoot biomass. At higher La levels, soybean growth was reduced. This was caused by ultrastructural modifications in the cell wall, thylakoids and chloroplasts, and the appearance of c-metaphases.

1. Introduction

Rare earth elements (REEs) are a homogenous group of 17 chemical elements on the periodic table with similar physical and chemical properties. There are 15 lanthanide elements plus scandium and yttrium (IUPAC, 2005). Although they are called rare, these elements are naturally present in the environment, with the exception of promethium (Brioschi et al., 2012). Some REEs are almost as abundant in the environment as elements such as Cu and Zn that have been studied much more; the most scarce REEs, lutetium (Lu) and thulium (Tm), are actually more abundant in the Earth's crust than Cd and Se (Tyler, 2004). Currently, REEs have high prices and are widely used in the high-tech industry. Some of the most important uses are automotive catalytic converters, fluid cracking catalysts in petroleum refining, phosphors in flat panel displays permanent magnets and rechargeable batteries for hybrid and electric vehicles, generators for wind turbines, space-based satellites, and numerous medical devices (Humphries, 2012; Long et al., 2010). In addition, REEs have been used in agriculture in China for the last 30 years in order to enhance yield and improve crop quality (Liu et al., 2012a; Wang et al., 2012; Zeng et al., 2006). In fact, China was the first country in the world to apply commercial REE-fertilizers to crops. It is estimated that REE fertilizers area applied to about 5% of the total agricultural land in China (Hu et al., 2004; Wang et al., 2008). In recent years, agricultural use of REE-fertilizers has extended to countries such as Korea, Japan, Australia, Switzerland, and the Philippines (Redling, 2006; Wang et al., 2008).

Previous studies showed that low concentration of La can improve physiological processes and plant growth. At low concentration, La increased net photosynthetic rate and stomatic

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conductance in rice plants (Wang et al., 2014); reduced injuries caused by enhanced UV-B radiation in soybean seedlings (Yang et al., 2014); and, increased growth, soluble sugar content, and vitamin C levels in Chinese cabbage (Ma et al., 2014). Unfortunately, the effects of REEs on plant development remain contradictory and obscure (Liu et al., 2013; Skovran and Martinez-Gomez, 2015; Thomas et al., 2014). Many of the contradictions found in studies reporting the effects of REEs on plant growth are due to different vegetative growth stages and the different concentrations applied. Therefore, is important to know how to correctly determine the beneficial and harmful effects of La, as well as the concentration limits between these contrasting situations. Moreover, studies reporting these limits for beneficial and harmful La concentrations in plant are rare (Liu et al., 2013). A cellular parameter that may contribute to a better understanding of plant growth is the mitotic index, which is important in order to determine the root growth rate due to cell proliferation. This parameter is directly correlated to cell division frequency (D'Aquino et al., 2009) and is therefore essential for elucidating the effects of La on plant growth and development.

Soybean is widely consumed throughout the world (Yang et al., 2014). It is an economically important crop, and Brazil is among the largest producers. In general, soybean crops receive large amounts of phosphate fertilizers, especially in tropical countries like Brazil. This may introduce considerable levels of REEs in crops and soils (Turra et al., 2011). Consequently, a better understanding of the effects of REEs on soybean growth is key. The aim of this study was to evaluate La bioaccumulation, plant growth, and the mitotic index of soybean plants in response to La treatment. The effects of La on photosynthetic rate and chlorophyll content, as well as on nutrients levels, and analysis of transmission electron microscope (TEM) and mapping the distribution of La in soybean tissues by scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS) were also examined.

2. Material and methods

2.1. Plant materials and experimental design

Cv. BRSMG760SRR soybean seeds (*Glycine max* (L.) Merrill) were sown in a vermiculite substrate, grown in a greenhouse, and watered daily with double-distilled water. The 14-day-old seedlings were transplanted to 2 L pots containing Hoagland nutrient solution (Hoagland and Arnon, 1950) with 50% ionic strength. One week after transplantation, these plants were exposed to a nutrient solution containing La treatments ($La(NO_3)_3 \cdot 6H_2O$-Sigma–Aldrich, St. Louis, MO, USA). The nutrient solution was changed once each week. Throughout the experimental period, the nutrient solution underwent constant aeration, and pH was monitored and adjusted to 6.0 ± 0.2 by adding 0.1 M NaOH or HCl.

In order to avoid La precipitation in the nutritive solution, a La stock solution was prepared in complexed form as chelate (La-EDTA). This procedure was needed to keep La available for root uptake, since the addition of La to a regular nutritive solution containing P would cause lanthanum phosphate precipitation, as predicted by Visual Minteq (Gustafsson, 2012).

La was applied in seven concentrations: 0, 5, 10, 20, 40, 80, and 160 μM. After 28 days of La treatment, a total of 28 plants (7 La treatments with 4 replicates) were harvested individually, and roots and shoots were washed with running deionized water and dried in a forced-air drying oven at 60 °C until constant mass was reached.

2.2. Analysis of La and nutrient levels

For La analyses, roots and shoots (approximately 0.100 g) were fused with 1.4 g of lithium metaborate in a platinum crucible at 1000 °C in a fusion machine (Fluxer BIS, Claisse, Québec, Canada). After cooling, the resulting beads were dissolved in beakers containing approximately 50 mL of a solution containing 2.5% tartaric acid and 10% HNO_3. Each beaker was then transferred to a hot plate at 120 ± 20 °C. The beakers were stirred magnetically for complete solubilization. Samples were next transferred to 100 mL polypropylene volumetric flasks and the volume was completed with 2.5% tartaric acid solution and 10% HNO_3. A certified reference material (Aquatic Plant-BCR670®, Institute for Reference Materials and Measurements, IRMM, Geel, Belgium) was included for quality control. Blank and certified reference samples were analyzed along with every batch of fusion. La content in extracts was determined by inductively coupled plasma mass spectrometry (ICP-MS) (Model NexION 300D, Perkin Elmer, Waltham, MA, USA). La translocation from roots to shoot was estimated considering [(mg La in shoot/mg La in plant) × 100].

For macro and micronutrient analyses, dried tissue (500 mg) was weighed and digested with 4.0 mL of concentrated HNO_3 +2.0 mL of concentrated $HClO_4$ (Sigma–Aldrich, Saint Louis, MO, USA) at 120 ± 8 °C for 1 h and then at 220 ± 8 °C until $HClO_4$ fumes were observed. Total Ca, Mg, K, Cu, Mn, Fe, and Zn contents in the samples were determined using an atomic absorption spectrophotometer (PerkinElmer, San Jose, CA, USA); total S contents were determined using turbidimetry of barium sulfate; and total P was determined using a spectrophotometer to measure the colorometry of a phospho-molybdenum complex at 680 nm (Malavolta et al., 1997).

2.3. Gas exchange analysis

The gas exchange characteristics were analyzed before harvest using an infrared gas-exchange analyzer, IRGA (Li-6400, Li-Cor, Lincoln, NE, USA). Stomatal conductance (g_s), transpiration rate (E), and photosynthetic rate (A) were measured as follows: in the four replicates of each La treatment, three fully expanded leaves were selected at 9 a.m., and the density of the photosynthetically active photon flux was fixed in the device chamber at 1000 μ mol m^{-2} s^{-1}.

2.4. Chlorophyll content

In order to evaluate chlorophyll content, five fully expanded leaves were taken at random. Total and *a* and *b* chlorophyll were extracted from samples and quantified using 200 mg of plant material that was macerated with 10 mL of 80% acetone (v/v). Subsequently, the extracts were filtered through fiberglass and the volume was completed to 20 mL with 80% acetone. A spectrophotometer (Epoch spectrophotometer, Biotek, Winooski, VT, USA) was used at two wavelengths (663 and 645 nm) for maximum absorption of chlorophyll *a* and *b*, respectively. Chlorophyll concentrations were calculated using the extinction coefficients and equation given by Arnon (1949).

2.5. Mitotic analysis

The root tips were fixed in Carnoy solution (3:1 v/v, ethanol–glacial acetic acid) and hydrolyzed in 1N of HCl at 60 °C for 10 min. They were then squashed in a 1.5% propionic carmine stain in 45% acetic acid (Aksoy and Deveci, 2012). Slides were examined immediately. Approximately 1000 cells were counted for each La treatment. Four replicates were made for each La concentration. The analyses were performed with a microscope (Carl Zeiss Axio