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BOTANY

Lanthanum content and effects on growth, gas exchanges, and chlorophyll index in maize plants

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ABSTRACT. Rare earth elements, such as lanthanum (La), have been applied to agriculture via fertilizers, aiming to increase the productivity and crop quality, such results observed mainly in China. However, the knowledge about the effect of La on maize growth, as well as for other species, despite the growing interest it is still limited. The aim of this study was to evaluate the effects of La on maize growth, La content, photosynthetic rate and chlorophyll content in maize plants in response to La treatment (0, 25, 50, 100, 150, 300 and 600 μM) in nutrient solution for three weeks. The plants were placed in geminated pots using a split-root technique. One of the pots in the geminated set was filled with a complete nutrient solution without La, while another was filled with a nutrient solution without phosphorus but containing different concentrations of La. It was verified that roots of maize plants can accumulate approximately sixty percent more La than shoots. Moreover, low La concentrations stimulated an increase in chlorophyll index, resulting in a slight increase in shoot biomass. At higher levels, La didn't reduce growth but caused a decrease in both photosynthetic rate and chlorophyll index.

Keywords: crops; photosynthesis; rare earth elements; *Zea mays*.

Teor de lantânio e efeitos no crescimento, trocas gasosas e teor de clorofila em plantas de milho

RESUMO. Elementos terra rara, como lantânio (La), são aplicados à agricultura via fertilizantes, com o objetivo de aumentar a produtividade e a qualidade das culturas agrícolas, fato observado principalmente na China. Entretanto, o conhecimento sobre o efeito de La no crescimento do milho, bem como para outras culturas agrícolas, ainda é limitado apesar do interesse crescente. O presente estudo objetivou avaliar o efeito da aplicação de La sobre o crescimento, teor de La, fotossíntese e teor de clorofila em plantas de milho, expostas a concentrações crescentes de La (0, 25, 50, 100, 150, 300 e 600 μM) em solução nutritiva por três semanas. As plantas foram colocadas em vasos geminados usando uma técnica de raiz divisória. Um dos potes no conjunto geminado foi preenchido com solução nutritiva completa que não continha La, enquanto outra foi preenchida com solução nutritiva sem fósforo, mas contendo diferentes concentrações de La. Foi verificado que as raízes das plantas de milho podem acumular sessenta por cento mais La quando comparado à parte aérea. Além disso, as baixas concentrações de La estimularam o aumento do teor de clorofila, resultando em um aumento na biomassa da parte aérea. Em concentrações mais elevadas, a aplicação de La não reduziu o crescimento das plantas de milho, mas causou diminuição na taxa fotossintética e no índice de clorofila.

Palavras-chave: culturas agrícolas; elementos terras raras; fotossíntese; *Zea mays*.

Introduction

Rare earth elements (REE) are a group of seventeen chemical elements in the periodic table that exhibit similar chemical and physical properties (International Union of Pure and Applied Chemistry [IUPAC], 2005). Current uses of REE include automotive and petroleum refining catalysts, rechargeable batteries for hybrid and electric vehicles, nd numerous medical and electronic devices (Alonso et al., 2012, Chakhmouradian & Wall, 2012; Massari & Ruberti, 2013). In addition, these elements have been applied to agriculture, aiming to increase the productivity and quality of agricultural crops, a practice primarily observed in China (Xiong, 2000), where commercial fertilizers are enriched with REE (Pang, Li, & Peng, 2013). Studies also show that REE are present in phosphate fertilizers (Otero, Vitória, Soler, & Canals, 2005;

Turra, Fernandes, & Bacch, 2011) due to their presence in phosphatic rocks, which are used in fertilizer production (Al-Thyabat & Zhang, 2015; Ramos et al., 2016).

Finding ways to increase crop productivity is a constant concern among all of those involved in production chains. It is highly probable that the Brazilian agriculture industry is indirectly applying REE in the long term via phosphate fertilizers. In general, the increase in production due to the use of REE is not conclusive, making it necessary to elucidate their mechanisms in plants (Skovran & Martinez-Gomez, 2015). In this way, it is evident that the functions and physiological and biochemical pathways of these elements in plants are still uncertain. Therefore, it is important to determine the effects (beneficial or harmful) and the safe concentration limits of REE, primarily with respect to species of agricultural importance.

It is probable that the influences of lanthanum (La) on cells are numerous and complex, including ionic charge and homeostasis or binding to aromatic rings and phospholipids (Liu & Hasenstein, 2005). Hypotheses that support the beneficial effects of REE on plant metabolism include stimulation of the antioxidant system and increase in nutrient absorption (D'Aquino, Pinto, Nardic, Morganad, & Tommasi, 2009). Recently, it was verified that La influences the mitotic index of soybean plants, increases photosynthetic rate and chlorophyll index, and develops cell cycle abnormalities that benefit biomass production (Oliveira et al., 2015). La was also introduced as a calcium antagonist, since the ionic radii of La and Ca are similar, and La competes for sites with which Ca would normally bind (Hu, Ding, Chen, Wang, & Dai, 2002; Xie et al., 2002; Wang et al., 2016). In addition, studies show that La and cerium (Ce) can bind to the porphyrin of chlorophyll, forming La-chlorophyll or Ce-chlorophyll (Hong, Wei, & Zhao, 2005). Many of the contradictions found in these studies are due to the stage of vegetative growth and the different concentrations applied. Therefore, it is important to determine the real effects of La, as well as the concentration limits between these contrasting situations.

Brazil stands out as one of the world's largest maize producers, with the crop being widely cultivated and possessing high environmental adaptability. The maize crop also receives a large amount of phosphate fertilizer, which may indirectly carry REE, such as La. This can introduce considerable levels of REEs into soils and crops. Consequently, elucidating the effects of REEs on plant growth and development is of fundamental importance. The aim of this study was to verify the effect of La on maize growth, La content, photosynthetic rate, and chlorophyll index of maize plants in response to La treatment.

Material and methods

The experiment was conducted under greenhouse conditions, where upon maize seeds (2B604Hx hybrid) were sowed in vermiculite. After 14 days, the seedlings were transplanted for acclimatization to pots containing Hoagland nutrient solution (Hoagland & Arnon, 1950) at 40% of their ionic strength and later transferred to geminated pots using a split-root technique. One of the pots in the geminated set was filled with a complete nutrient solution without La, while another was filled with a nutrient solution without phosphorus but containing different concentrations of La. In the pots containing La, phosphorus was not added in order to avoid precipitation of these two elements, and to keep La constantly available for absorption (Ding, Liang, Zhang, Yan, & Zhang 2005). The maize plants were exposed to the following La concentrations: 0, 25, 50, 100, 150, 300 and 600 μM, with 4 replicates of each being employed. After three weeks of La exposure, the photosynthetic rate (A), transpiration (E) and stomatal conductance (g_s) were determined using an infrared gas exchange analyser – IRGA (LI-6400XT Infra Red Gas Analyzer, Li-Cor, Nebraska, USA). The evaluations were conducted between 8:00 and 10:00 for all four replicates of each La concentration. In addition, an indirect measurement of chlorophyll content was performed using the portable chlorophyll, SPAD-502 (Konica Minolta, Tokyo, Japan).

The roots and shoots (of the pots containing La) were harvested separately and washed in distilled water and dried in an oven with forced air circulation at 60°C until reaching constant weight. Each part was subsequently ground in a knife mill (Willey type), and 500 mg was weighed for the analytical measurements of La. Samples were digested using the alkaline fusion technique, and the La quantification was performed through ICP-MS, essentially as described previously (Oliveira et al., 2015). A certified reference material (AquaticPlant-BCR670, Institute for Reference Materials and Measurements, IRMM, Geel, Belgium) was included for quality control. Blank and certified reference samples were along with every batch of fusion. The mean La concentration was 481 ± 0.23 mg kg^{-1} in the standard reference material and 487 mg kg^{-1} the value found in the certified reference material (AquaticPlant-BCR670). Thus, the mean recovery in the certified samples (98.8%) has reliable analytical data accuracy for La analysis.

All results were analysed using analysis of variance (ANOVA), and submitted to regression analysis at a 0.05 significance level of probability using probability using Sisvar 5.3 (Build77) statistics software (Ferreira, 2011), and the graphs were made in the SigmaPlot Programme (version12.5, SystatSoftware, Chicago, IL,USA).

Results and discussion

Lanthanum caused a significant increase in shoot biomass yield at lower rates, and decreased shoot biomass was not noted at higher levels of La (Figure 1A). The La concentrations of 25 and 50 μM led to an increase in both maize biomass and chlorophyll index, although increases in photosynthetic rate, transpiration and stomatal conductance were not verified. Emmanuel, Anandkumar, Natesan, and Maruthamuthu (2010) observed an increase in biomass production and length in maize plants submitted to concentrations of La. Xie et al. (2002) also observed that low concentrations of La promoted higher growth and production in rice plants, which is observed because La can regulate the physiological activities of the plant, such as the production of superoxide dismutase (SOD) and peroxidase (POD) enzymes. The activities of such enzymes as SOD, peroxidase, ascorbate, carotenoids and flavonoids could have been stimulated by La, helping to control reactive oxygen species, thereby reducing the oxidative stress affecting the membranes of several organelles and, in this way, promoting plant growth (Wang et al., 2011).

Lanthanum content in maize roots and shoots increased significantly when plants were exposed to increasing concentration of La (Figure 1B). There was no presence of La in the roots that developed in the geminated pot without addition of this element. In general, La content was higher in roots than shoots; only 3% of La was translocated into the shoots (Figure 1C). These results are consistent with others observed in prior studies, which report that the REE tend to remain in the roots, and less than 5% is translocated into the shoots (Hu et al., 2002). Similar results have been reported with low REE concentration in the shoot (Oliveira et al., 2015). The lower La content in the shoots may be associated with the presence of apoplastic barriers in the roots. Diatloff, Asher, and Smith (1995) reported that the Caspary bands in the root endoderm of the plant provide a barrier for the diffusion of La from the cortex apoplast to the vascular tissue, preventing the transport of the absorbed La from the roots to the shoots of the plants.

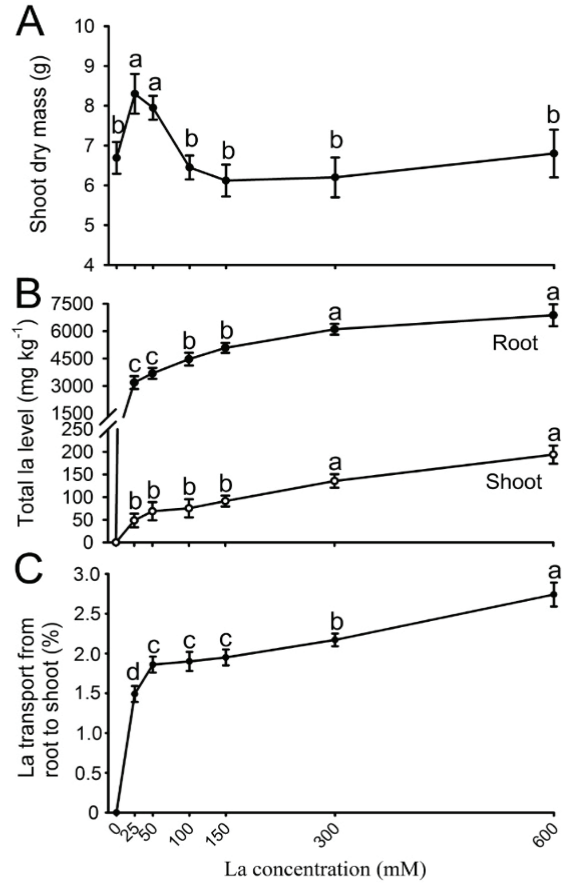

Figure 1. Variation in dry matter of shoot (A), in lanthanum index in shoots and roots (B) and translocation of La (C) in maize plants (*Zea mays*) submitted to La concentrations in the culture solution.

There was a decrease in photosynthetic rate, beginning at 50 μM of La (Figure 2A). There was no significant difference in transpiration (Figure 2B), while for stomatal conductance, there was a reduction at intermediate concentration applied (Figure 2C). The relative chlorophyll index showed a significant increase at 25 and 50 μM of La and a reduction at higher La concentrations together with chlorosis in the leaves. According to Xiong (2000), supplying REE to plants could increase the intensity of photosynthesis and the net photosynthetic rate by 11-31%, depending on whether a mixture of REEs or individual elements is added. Zhou et al. (2011) evaluated gas exchange in *Salvia miltiorrhiza* with increasing La concentrations and observed that La increased the photosynthetic rate, stomatal conductance, transpiratory rate and biomass. For soybean plants, Oliveira et al. (2015) also reported an increase in photosynthetic rate and chlorophyll content in the presence of 5 and 10 μM of La. It is believed that this phenomenon occurs because REE may influence gas exchange, especially stomatal conductance, raising the photosynthetic rate due to a greater assimilation of CO_2 for the photosynthetic process (Wang, Wang, Zhou, & Huang, 2014). In *Juglans nigra*, a reduction of photosynthetic activity was observed with increasing concentrations of La (Nicodemus, Salifu, & Jacobs, 2009), which was also observed in this study. As mentioned before, there was a slight increase of the biomass at the lowest concentration (25 μM) which correlated with the corresponding photosynthetic rate and chlorophyll index. However, when the concentration exceeds 50μM, the inverse response was observed, with the reduction of the biomass, probably due to the reductions in the gas exchanges and consequently, the reduction of photoassimilates.

The effects of REE on photosynthesis are also related to the development of chloroplasts, chlorophyll content and enzymatic activity, since La may participate in the degradation of chlorophyll, an essential pigment to the photosynthetic process. Several authors assume that chlorophyll content and photosynthetic rate can be regulated by REE by increasing the light absorption efficiency, regulating the excitation energy distribution of photosystems I and II, and promoting Hill reaction activities (Liu, Wang, & Chen 2012). An increase in these variables could also be attributed to several photosynthetic processes, including the stimulation of photochemical activities, gas exchanges and CO_2 fixation (Zhang, Li, Guo, & Tezuka, 2009). According to Hu, Richter, Sparovek, and Schnug (2004), the effects of REE application on photosynthetic rate, chlorophyll content, and photosynthetic enzyme activity are the primary reasons for increased wheat yield. However, since the results observed in the present study indicated a reduction of photosynthetic rate and a small increase in chlorophyll index, La concentration was most likely not efficient in maximizing photosynthetic processes.

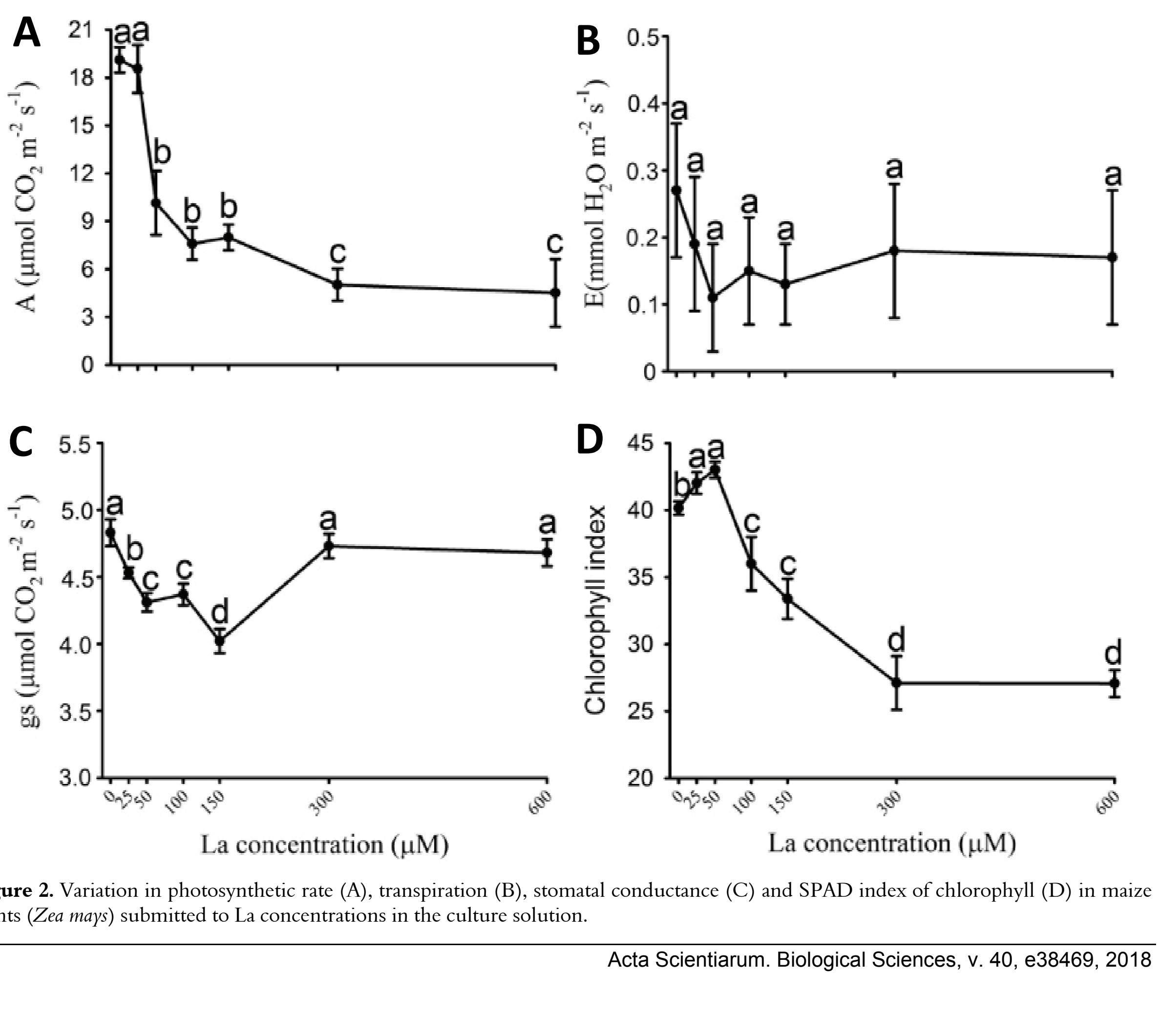

Figure 2. Variation in photosynthetic rate (A), transpiration (B), stomatal conductance (C) and SPAD index of chlorophyll (D) in maize plants (*Zea mays*) submitted to La concentrations in the culture solution.

The results of Maksimovi, Kastori, Putnik-Deli, and Borišev (2014) show that total leaf area, dry biomass, photosynthetic pigment concentration, and proline decreased, indicating that maize plants were under stress, especially when yttrium (Y) was applied. The decrease in chlorophyll index could be attributed to the disturbance in the biosynthesis of the pigment, the degradation due to lipid peroxidation (Wang, Shi, Liu, Wang, & Qiao, 2012), or the damages caused by the entry of REE into the chloroplast (Fu et al., 2014). In the present study, the other factor that may have contributed to the reduction of photosynthesis was the reduction of the chlorophyll index, an essential pigment to this metabolic process. Kopsell (2011) and Wang et al. (2011) also observed that La and Ce reduced the chlorophyll content in *Hydrilla verticillata*. However, different results were shown by Hong, Wei, and Zhao (2002), where a La concentration of 500 μg mL^{-1} increased the growth, chlorophyll index, photosynthetic rate, and uptake of nitrogen and phosphorus in spinach plants. Thus, when the photosynthetic rate, transpiration, and chlorophyll are impaired, they could be associated with plant growth.

Conclusion

The application of La up to 25 μM in the nutritive solution shows a slight increase in maize growth, accompanied by the stimulated photosynthetic rate, as well as the chlorophyll index. At higher levels, La did not reduce the growth but caused a decrease in both photosynthetic rate and chlorophyll index in maize plants.

These results provide important information in terms of La effects on maize growth. Further studies are needed to consolidate such information and it is suggested that the researches focus at concentrations up to 25 μM.

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