



Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice



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HIGHLIGHTS

- Low-level combined La(III) and acid rain treatment improved photosynthesis in rice.
- High-level combined La(III) and acid rain treatment inhibited photosynthesis in rice.
- Maximal effects of La(III) and acid rain on photosynthesis happened at booting stage.
- Change in photosynthesis in rice depended on stomatic and non-stomatic factors.

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ABSTRACT

Rare earth elements (REEs) pollution and acid rain are environmental issues, and their deleterious effects on plants attract worldwide attention. These two issues exist simultaneously in many regions, especially in some rice-growing areas. However, little is known about the combined effects of REEs and acid rain on plants. Here, the combined effects of lanthanum chloride (LaCl₃), one type of REE salt, and acid rain on photosynthesis in rice were investigated. We showed that the combined treatment of 81.6 μM LaCl₃ and acid rain at pH 4.5 increased net photosynthetic rate (P_n), stomatic conductance (G_s), intercellular CO₂ concentration (C_i), Hill reaction activity (HRA), apparent quantum yield (AQY) and carboxylation efficiency (CE) in rice. The combined treatment of 81.6 μM LaCl₃ and acid rain at pH 3.5 began to behave toxic effects on photosynthesis (decreasing P_n , G_s , HRA, AQY and CE, and increasing C_i), and the maximally toxic effects were observed in the combined treatment of 2449.0 μM LaCl₃ and acid rain at pH 2.5. Moreover, the combined effects of LaCl₃ and acid rain on photosynthesis in rice depended on the growth stage of rice, with the maximal effects occurring at the booting stage. Furthermore, the combined treatment of high-concentration LaCl₃ and low-pH acid rain had more serious effects on photosynthesis in rice than LaCl₃ or acid rain treatment alone. Finally, the combined effect of LaCl₃ and acid rain on P_n in rice resulted from the changes in stomatic (G_s , C_i) and non-stomatic (HRA, AQY and CE) factors.

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1. Introduction

As demand for high-tech devices and green technologies rises, rare earth elements (REEs) have been widely used in defence, refining, electronics, clean energy, medicine, environmental protection, agriculture, animal husbandry, etc. (Hu et al., 2004). It is estimated

that the global demand for REEs will rise for years (Schüler et al., 2011). The wide application and driving demand of REEs have resulted in their continuous accumulation in environments (Hu et al., 2006). In China, Australia, Japan, and Germany, for example, the average content of REEs in soil is 197.67, 104.30, 97.57 and 15.48 mg kg⁻¹, respectively, and the maximum content is 700 mg kg⁻¹ (Ni, 1995; Hu et al., 2006). It has been long observed that the suitable accumulation of REEs can improve the yield and quality of living organisms (including crops and animals), whereas excess accumulation of REEs affects the agricultural soil environment, crop growth, and human health (Weltje et al., 2002; Liu et al., 2012). Therefore, it is urgent to elucidate how REEs act on crops to ensure food quality and safety, human health and

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environmental safety. Investigators have invested a great deal of effort into studying the effect mechanisms of REEs on morphology, growth and yield of crops at the physiological and biochemical level (Weiping et al., 2002; Redling, 2006; d'Aquino et al., 2009; Zhang et al., 2013). These studies focus on photosynthesis (Zhang et al., 2013), the antioxidant system (d'Aquino et al., 2009), substance metabolism (Weiping et al., 2002), and other processes (Redling, 2006). Photosynthesis is the foundation for growth, development and yield formation of plants; thus, frequent changes to photosynthesis is a key toxic mechanism of stress in plants (Liu et al., 2013; Nakamura et al., 2013; Yang et al., 2013). Previous studies showed that low concentration of lanthanum chloride (LaCl_3) increases the chlorophyll content, Hill reaction rate, Mg^{2+} -ATPase activity, stomatic activity, photosynthetic rate and dry material accumulation of tobacco seedlings and broad bean seedlings, whereas high concentration of LaCl_3 decreases them (Chen et al., 2001; Xue and Yang, 2009). Moreover, low concentrations of LaCl_3 improve, and the high concentrations of LaCl_3 destroy the structure of chloroplast (Peng and Zhou, 2009).

Acid rain is a critical global environmental issue and causes concern worldwide. It has been reported that during 1993–2007, the average pH of acid rain in southern China ranges from 3.8 to 4.5 (Wu et al., 2006). A rainfall of pH 2.54 in China was reported in 2012 (Hangzhou Municipal Environmental Protection Bureau, 2013). It is well known that acid rain exerts deleterious effects on the photosynthesis of plants, such as decreases in Hill reaction activity, chlorophyll content and chlorophyll fluorescence parameters, and then the photosynthetic rate (Sun et al., 2012). The simultaneous occurrence of REEs pollution and acid rain pollution in many regions (Wei et al., 2001) has led to the new environmental issue of combined pollution by REEs and acid rain. In contrast with the vast literature about the effects of REEs or acid rain alone on photosynthesis, few reports have been published thus far regarding the study of the combined effect of REEs and acid rain on plants. Recently, we preliminarily observed that the combined pollution of acid rain and lanthanum ion [La(III)], one type of REE, inhibits the growth and chlorophyll fluorescence reaction of soybean seedlings (Wen et al., 2011). However, crop growth is based not only on external pollution but also its growth stages. Studies on stress response suggest that there are differences in crop resistance to stress at different growth stages (Kumar et al., 2013). These differences present challenges to the precise evaluation of the toxic effects of pollutants on crops. Thus, it is important to investigate the combined effects of REEs and acid rain on crops throughout the whole growth stage, which is relevant research that is rarely reported.

Rice is an important food crop worldwide and is widely used in crop research (Liu et al., 2013; Nakamura et al., 2013; Niu et al., 2013; Yang et al., 2013). Moreover, rice commonly is grown in regions where REE pollution and acid rain simultaneously occur. La is the first lanthanide element in the periodic table and is ubiquitous in soils (Tyler, 2004). Here, the combined effects of LaCl_3 and acid rain on the net photosynthetic rate (P_n), stomatic conductance (G_s), intercellular CO_2 concentration (C_i), Hill reaction, apparent quantum yield (AQY) and carboxylation efficiency (CE) in rice throughout the whole growth stage were investigated. The objective of this research was to understand the combined effects of LaCl_3 and acid rain on photosynthesis in rice during the whole growth stage.

2. Materials and methods

2.1. Preparation of rice nutrient solution, LaCl_3 solution and simulated acid rain

Modified rice nutrient solution was prepared according to the ionic composition released by the International Rice Research

Institute (IRRI) (Yoshida et al., 1976). The full-strength modified nutrient solution had the following composition: 1.43 mM NH_4NO_3 , 0.51 mM K_2SO_4 , 1.00 mM CaCl_2 , 1.64 mM MgSO_4 , 9.47 μM MnCl_2 , 0.075 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 19.00 μM H_3BO_3 , 0.15 μM ZnSO_4 , 0.16 μM CuSO_4 , 36.00 μM FeCl_3 and 77.42 μM citric acid. SiO_2 was supplied as 1.67 mM with $\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$ in nutrient solution. The nutrient solution pH was adjusted to 5.5 by using a PHS-29A pH meter (Shanghai Anting Scientific Instrument Factory, Shanghai, China).

The control rain with a pH of 7.0 was prepared by adding Ca^{2+} , Na^+ , K^+ , Mg^{2+} , SO_4^{2-} , NO_3^- and Cl^- to deionised water, where the Ca^{2+} , Na^+ , K^+ , Mg^{2+} , SO_4^{2-} , NO_3^- and Cl^- contents were 0.83 μM , 1.32 μM , 0.15 μM , 5.34 μM , 0.36 μM , 0.64 μM , 0.47 μM , 0.69 μM and 1.80 μM , respectively. The ionic composition was derived from precipitation data in the southeast of China (Kong et al., 2012; Xie et al., 2012).

Based on the current content of REEs in Chinese soil and the pH of acid rain, three concentrations of LaCl_3 (81.6, 1224.5 and 2449.0 μM) and three pH values of acid rain (pH 4.5, pH 3.5 and pH 2.5) were used in this study. LaCl_3 solutions (81.6, 1224.5 and 2449.0 μM) were prepared by dissolving appropriate quantities of lanthanum chloride hexahydrate ($\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$, Sigma–Aldrich, USA) in modified nutrient solution without phosphate. The simulated acid rain at pH values of 4.5, 3.5 and 2.5 was prepared by adjusting the pH of control rain with the addition of concentrated H_2SO_4 and HNO_3 at a ratio of 1.1:1 (v/v, by chemical equivalents) (Kong et al., 2012; Xie et al., 2012).

2.2. Plant culture and treatments

Air-dried substrate (vermiculite and perlite, 1:1, v/v) was weighed and exactly 1.0 kg was added to each pot (diameter = 15 cm, height = 30 cm). A LaCl_3 solution (Sigma–Aldrich, USA) (81.6, 1224.5 and 2449.0 μM) was added to each pot. Modified rice nutrient solution was added to maintain the water content at approximately 60% before mixing the substrate thoroughly and equilibrating for 2 weeks. The substrate treated without and with LaCl_3 served as the control substrate and LaCl_3 substrate, respectively.

Rice seeds were surface sterilized in HgCl_2 (0.1%) solution for 10 min and rinsed with deionised water several times. The sterilized seeds were placed in dishes under-laid with three layers of moistened gauze and germinated in the incubator for 2 d. Germinated seeds were sown in a sterilized sand bed. At the stage of two leaves, the uniform healthy plants were transplanted into each pot filled with control substrate or LaCl_3 substrate. Pots were placed in a greenhouse at $25 \pm 3^\circ\text{C}$, with a light intensity of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, a day/night cycle of 16/8 h, and a relative humidity of 70–80%. Modified rice nutrient solution was used to irrigate the plants and to maintain the water content of substrate at approximately 60%. Meanwhile, rice plants were sprayed every 3 d with 300 mL of simulated acid rain per pot, and the control plants were sprayed with control rain at pH 7.0. The spray amount of acid rain and control rain was calculated according to the precipitation and evaporation in the southeast of China. Moreover, the modified nutrient solution was supplied every 3 d. All treatments were performed in five replicates, and 1 mM KH_2PO_4 was sprayed on the foliage every other day to apply the required inorganic phosphate to the plants. At the seedling stage (15 d after the spraying of acid rain), tillering stage (35 d after the spraying of acid rain), booting stage (60 d after the spraying of acid rain) and filling stage (80 d after the spraying of acid rain), the fresh leaves treated with or without LaCl_3 and acid rain were sampled for analyses.

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