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Effects of rare earth and acid rain pollution on plant chloroplast ATP synthase and element contents at different growth stages

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HIGHLIGHTS

- Acid rain and La^{3+} affected rice chloroplast ATPase activity.
- Changes in the ATPase activity were related to ATPase gene transcription level.
- Acid rain and La^{3+} affected rice chloroplast functional element contents.
- Rice at different growth stages had different responses to La^{3+} and acid rain.

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ABSTRACT

Combined rare earth and acid rain pollution has become a new environmental problem, seriously affecting plant survival. The effects of these two kinds of pollutants on plant photosynthesis have been reported, but the micro mechanisms are not very clear. In this research, we studied the effects of lanthanum [La(III), 0.08, 1.20 and 2.40 mM] and acid rain (pH value = 2.5, 3.5 and 4.5) on the ATPase activity and gene transcription level and the functional element contents in rice leaf chloroplasts. The results showed that the combined 0.08 mM La(III) and pH 4.5 acid rain increased the ATPase activity and gene transcription level as well as contents of some functional elements. But other combined treatments of acid rain and La(III) reduced the ATPase activity and gene transcription level as well as functional element contents. The change magnitude of the above indexes at rice booting stage was greater than that in seedling stage or grain filling stage. These results reveal that effects of La(III) and acid rain on ATPase activity and functional element contents in rice leaf chloroplasts are related to the combination of La(III) dose and acid rain intensity and the plant growth stage. In addition, the changes in the ATPase activity were related to ATPase gene transcription level. This study would provide a reference for understanding the microcosmic mechanism of rare earth and acid rain pollution on plant photosynthesis and contribute to evaluate the possible environmental risks associated with combined La(III) and acid rain pollution.
One sentence summary: The effects of La(III) and acid rain on activity and gene transcription level of rice chloroplast ATPase and contents of functional elements were different at different growth stages.

1. Introduction

Rare earth elements (REEs) are heavily exploited and widely used due to their unique physical and chemical properties (Zhang and Shan, 2001; Hu et al., 2006; Loell et al., 2011a, 2011b; Delgado et al., 2012; Hao et al., 2015). Especially, in China, REEs have been used in agricultural fertilizers for a long time, resulting in their accumulation in environment (Zhang and Shan, 2001; Hao et al., 2015). For example, the average concentrations of REEs in

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water, suspended particles and sediments of the Sidaosha river in China' Baotou were 3826 μg L^{-1}, 31524 mg kg^{-1} and 30461 mg kg^{-1}, respectively (Liang et al., 2014). And REEs have been detected in soils at concentration as high as 27549.58 mg kg^{-1} in China's Baiyun Obo Mining Area (Hao et al., 2015). In other developing or developed countries, REEs are widely applied in many fields such as military and industry (e.g. metallurgical, petrochemical, glass ceramic), leading to their accumulation in terrestrial environments (Hu et al., 2006; Loell et al., 2011a, 2011b; Delgado et al., 2012). Indeed, the average concentrations of REEs have been reported to reach as high as 316 mg kg^{-1} (Cape Verde) or as low as 32 mg kg^{-1} (Denmark) in non-mining areas across the world (Ramos et al., 2016). Many studies have shown that REEs in environment can affect plant survival in various ways (Paola et al., 2007; D'Aquino et al., 2009; Thomas et al., 2014). Moreover, REEs in plants can accumulate in the bones and livers of animals and humans through the food chain, thereby threatening the health and safety of humans (Chen et al., 2001; Zaichick et al., 2011; Migaszewski and Gałuszka, 2015). The environmental risks of REEs have attracted academic concerns (Paola et al., 2007; D'Aquino et al., 2009; Liang and Wang, 2013; Sun et al., 2013; Wang et al., 2014a; Xia et al., 2017; Zhang et al., 2017).

Acid rain pollution is one of the most serious global environmental problems facing mankind today (Larssen et al., 2006). China, a largely agricultural country, has become the third largest zone of acid rain exposure in the world, after North America and Europe (Menz and Seip, 2004; Fang et al., 2013). From 1993 to 2007, the average pH of acid rain in southern areas of China has ranged from 3.8 to 4.5 (Wu et al., 2006). In 2012, a very acidic rainfall, with a pH of 2.54, occurred in China (Hangzhou Muncipal Environmental Protection Bureau, 2013). The impacts of acid rain on plants have attracted wide attention (Menz and Seip, 2004; Larssen et al., 2006; Sun et al., 2016). REEs and acid rain pollution often appear in the same time and space, therefore effects of combination of these two pollutants on crops have become new concerns for agricultural and environmental safety. Previous studies have reported that combinations of acid rain and lanthanum [La(III)] can affect antioxidant system (Liang and Wang, 2013), root phenotype (Sun et al., 2013), nitrogen assimilation (Zhang et al., 2017), and nitrate reductase transcription (Xia et al., 2017) in soybean seedlings. Similarly, studies have found that this combination of stressors affects cytosol free calcium concentrations in horseradish roots (Zhang et al., 2016). Photosynthesis has irreplaceable scientific value as a basis for measuring the impact of environmental pollution on ecosystems (Farquhar et al., 2003; Larsen et al., 2007; Evers et al., 2010). Although it has been reported that combination of acid rain and a certain dose of La(III) can affect photosynthesis and yield in some plant species, the mechanism of action of this toxic response is not clear (Wang et al., 2014b). Therefore, the questions that will be addressed in this study are: (1) How does exposure to a combination of acid rain and REEs affect plant photosynthesis? (2) What are the relationships between different physiological functions in the presence of the combined pollution?

The chloroplast ATP synthase (ATPase, CF_1-CF_0) is the main functional substance of photosynthesis, and it plays an important role in the light reaction and assimilation force synthesis (Yamori et al., 2011). The activity of this compound directly affects photosynthetic productivity and ultimately affects the synthesis of assimilation products, and the growth and development of plants (Taiz and Zeiger, 2010). Ca, Mg, Fe, Cu, Zn and other metal elements are also involved in chloroplast membrane structure, electronic transmission in photosynthetic light reactions, ATP synthesis, and antioxidant systems (Marín-Navarro et al., 2007; Dobrowolska et al., 2011; Qu et al., 2012; Trotta et al., 2012). Changes in the contents of these metals in chloroplasts will therefore affect chloroplast structure and the normal process involved in light and dark reactions (Dong et al., 2013; Chen et al., 2016). La(III), the first element of lanthanide in the periodic table, commonly has a high content in soil (Svon and Schmidhalter, 2005). Rice is a common grain crop, and most of its production have been used for human consumption (West et al., 2014). Therefore, we investigated the effects of La(III) mixed with acid rain pollution on both the activity and gene transcription of chloroplast ATPase and the concentrations of functional elements in rice chloroplast at different growth stages. The aim is to understand the toxicological mechanism of combined La(III) and acid rain pollution on plant photosynthesis, to evaluate the potential environmental risks associated with La(III) present in soil and acid rain pollution.

2. Materials and methods

2.1. Solution preparation

According to the current status of acid rain pollution in China (Wu et al., 2006; Hangzhou Muncipal Environmental Protection Bureau, 2013) and the average REEs contents in soils of areas around the world (Dutta et al., 2016), three pH values of acid rain (pH 4.5, 3.5 and 2.5) and three concentrations of $LaCl_3$ solutions (0.08, 1.20, 2.40 mM) were used in this study. The control rain, simulated acid rain solutions, the modified nutrient solution (pH 5.5) and La(III) solutions were prepared according to our previously published methods (Wang et al., 2014a; Hu et al., 2016).

2.2. Crop culture and treatment

Rice seeds were surface-sterilized in $HgCl_2$ (0.1%) solution for 10 min to eliminate pathogenic microorganisms and then germinated at 28 °C in an incubator. When the seeds were germinated, they were sown and grown in a sterilized sand bed. When the second leaf appeared, the rice plants were used for experimental treatments in a greenhouse (relative humidity: 70–80%; temperature: 25 ± 3 °C; day/night cycle: 16/8 h; light intensity: 1000 μmol m^{-2} s^{-1}). The rice plants were transplanted individually into 16 pots filled with the control substrate (vermiculite and pearlite, 1:1, v/v, the modified rice nutrient solution was added to maintain the substrate's water content at approximately 60%) or $LaCl_3$ substrate (vermiculite and pearlite, 1:1, v/v, treated with $LaCl_3$ solutions) (Wang et al., 2014b; Hu et al., 2016). The KH_2PO_4 (1 mM, 30 mL) was evenly sprayed on the foliage every other day to provide the necessary inorganic phosphate for the rice plants (Wang et al., 2014a; Gupta et al., 2017). The rice plants were irrigated with 300 mL of control rain or simulated acid rain every 3 d. All treatments were replicated 5 times. At the seedling stage (15 d after the experimental treatments), booting stage (60 d after the experimental treatment) and filling stage (80 d after the experimental treatment), the fresh leaves from the third leaf position (the functional leaves) were collected for the determination of experimental indicators.

2.3. Extraction of chloroplasts and determination of chlorophyll content

Fresh leaves were rinsed with deionized water and ground with chloroplast extraction buffer (1.00 mM $MgCl_2$, 0.08 mM K_2HPO_4, 1.00 mM $MnCl_2$, 2.00 mM EDTA, 0.01 M KCl, 0.05 M HEPES-KOH, 0.30 M sorbitol) to form a slurry (Hu et al., 2016). The slurry was then filtered through 4 layers of gauze, and the filtrate was centrifuged at 800 × *g* relative centrifugal force (RCF) and 4 °C for 5 min. The supernatant was centrifuged at 1500 × *g* RCF for 5 min, and chloroplasts formed the precipitate. The chloroplast