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Effects and mechanisms of the combined pollution of lanthanum and acid rain on the root phenotype of soybean seedlings

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HIGHLIGHTS

- Combined effect of La^{3+} and acid rain exerted inhibition on soybean root phenotype.
- Inhibition of combined effect on root were stronger than the single pollution.
- Combined effect of La^{3+} and acid rain on soybean root phenotype depended on growth.
- Disturbance of mineral nutrients caused inhibition of root growth.

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ABSTRACT

Rare earth pollution and acid rain pollution are both important environmental issues worldwide. In regions which simultaneously occur, the combined pollution of rare earth and acid rain becomes a new environmental issue, and the relevant research is rarely reported. Accordingly, we investigated the combined effects and mechanisms of lanthanum ion (La^{3+}) and acid rain on the root phenotype of soybean seedlings. The combined pollution of low-concentration La^{3+} and acid rain exerted deleterious effects on the phenotype and growth of roots, which were aggravated by the combined pollution of high-concentration La^{3+} and acid rain. The deleterious effects of the combined pollution were stronger than those of single La^{3+} or acid rain pollution. These stronger deleterious effects on the root phenotype and growth of roots were due to the increased disturbance of absorption and utilization of mineral nutrients in roots.

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1. Introduction

Rare earth elements (REEs) refer to 17 elements in the periodic table, including scandium, yttrium, and 15 lanthanide elements. REEs have useful physical and chemical properties that enable their applications in functional materials, catalysts, luminescent materials, ceramics, and medicines (Gordon et al., 2004; Rodewald et al., 2007; Zhao et al., 2008). At suitable concentrations (for example, 0.08 Mm La^{3+} for soybean seedling (Wen et al., 2011)), REEs are also used in agriculture to improve the yield and quality of crops as well as the production of animal in many countries, including Australia, Switzerland, Philippines, and China (Pang et al., 2002; Hu et al., 2004; Redling, 2006). The industrial and agricultural uses of REEs are increasing and predicted to increase further in the next few decades (Schüler et al., 2011). Thus, concern on the accumulation of REEs in soils is growing (Sun et al., 1996; Wang et al., 2001; Tyler, 2004), and the increase in REEs level in the environment caused by anthropogenic activities has become a global environmental issue (Gu et al., 2001). In China, Australia, Japan and German, for example, the average content of REEs in soil is 197.67, 104.30, 97.57 and 15.48 mg kg^{-1}, respectively (Xiong, 1995). The accumulation of REEs in soils inevitably affects plant growth (Hu et al., 2004; Kobayashi et al., 2007; d'Aquino et al., 2009; Ma et al., 2010), prompting many researchers to focus on the effects of REEs ions on plants and the relevant mechanisms (Tyler, 2004; Zeng et al., 2006; Guo et al., 2008; Wang et al., 2008, 2010a; Oral et al., 2010). For example, some researchers have found that plant growth is positively affected by low-concentration REEs but negatively affected by high-concentration REEs. These effects are called "hormesis effects" (Diatloff et al., 1995, 2008; Calabrese and Baldwin, 2002; Song et al., 2002; Wang et al., 2008; d'Aquino et al., 2009; Ma et al., 2010). The effect mechanisms of REEs on plants have also been demonstrated by analyzing the changes in the activities of metabolic enzymes (Song et al., 2002), the structure and function

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of membranes (Zheng et al., 2000, 2002b; Yang et al., 2012a,b), the protective system (d'Aquino et al., 2009), hormonal balance (Liu et al., 2008), Ca^{2+} and K^+ channels (Liu and Hasenstein, 2005; Kobayashi et al., 2007; Wang et al., 2010b), genetic materials (Wang et al., 1999), etc.

Acid rain is a global environmental issue (Rodhe et al., 2002; Wai et al., 2005), and the average pH value of acid rain in China is from 3.0 to 4.5 (State Environmental Protection Administration of China, 2005; Li et al., 2010). It is well known that acid rain exerts deleterious effects on the phenotype and physiological characteristics of various plants (Belesky and Fedders, 1995; Singh and Agrawal, 2008; Kováčik et al., 2011; Liu et al., 2011a,b; Huang et al., 2012; Shukla et al., 2013; Wang et al., 2013). These effects induce changes in the plant population structure and the plant community functions (Singh and Agrawal, 2008; Liu et al., 2011b). Acid rain can also cause the acidification of surface waters and soils, inhibiting plant growth (Larssen and Carmichael, 2000; Larssen et al., 2006). The deleterious effect mechanisms of acid rain on plants have been explained from proton effect, ion effect, photosynthesis effect, free radical effect, etc. (Huang et al., 2004; Singh and Agrawal, 2008).

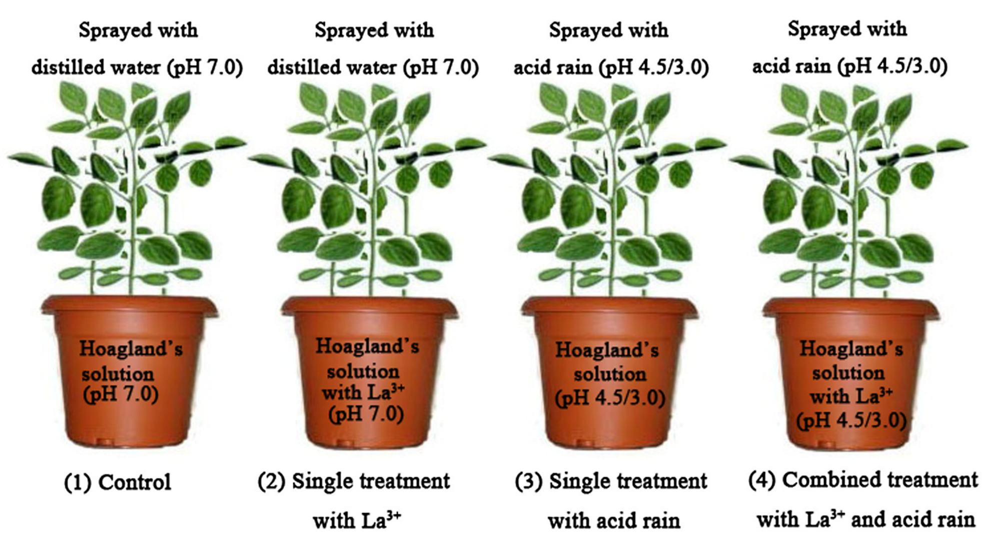
The simultaneous occurrence of REEs pollution and acid rain pollution in many regions (Wei et al., 2001) causes a new environmental issue that is called the combined pollution of REEs and acid rain. It is very important to investigate the combined effects of REEs and acid rain on plants, including the combined effect of low-concentration REEs (or the improvement concentration) and acid rain on plants, which is commonly ignored; and the combined effect of REEs and acid rain at the current and future pollution level. Our group has previously conducted a preliminary study on the effect of the combined pollution of lanthanum ion (La^{3+}) and acid rain on soybean seedlings, and discussed the effect mechanisms from the view of photosynthesis in leaves (Wen et al., 2011). Plant roots absorb nutrients and moisture from soil, and roots directly contact with soil that is the important compartment of the combined pollution of REEs and acid rain. Thus, the combined effects of REEs and acid rain on plant roots are important to be investigated, especially the effects on the root phenotype of plants because root phenotype is an important index for determining the response of plants to environmental changes (Fageria and Moreira, 2011). However, no study on the effects of the combined pollution of REEs and acid rain on the root phenotype of plants has been reported.

Soybean (*Glycine max* L.) is a consumed crop in the word and recommended for the use in phytotoxicity studies by the US EPA (2012). La is the first lanthanide element in the periodic table and is ubiquitous in soils (Tyler, 2004). This study investigated the combined effects of La^{3+} and acid rain on the root phenotype, root growth, mineral element contents in roots, and root activity of soybean seedlings. The objective was to understand the effects and mechanisms of the combined pollution of REEs and acid rain on plant roots.

2. Materials and methods

2.1. Plant materials and experimental designs

Soybean seedlings were cultured as described in our previous study (Wen et al., 2011; Sun et al., 2012). Sterilized seeds of soybean (Zhonghuang 25, Wuxi Seed Co., Ltd., China) were germinated and grown at 25 ± 5 °C, 300 μmol m^{-2} s^{-1} light intensity, and 16/8 h day/night cycles in a greenhouse. Fifteen-day-old seedlings were cultured in half-strength Hoagland's solution. Air supply during hydroponic cultivation was provided by an aquarium air pump in the medium solution. The nutrient solution was renewed every 3 d to stabilize the pH. Twenty-five-day-old seedlings with similar sizes were selected for the experiments. The La^{3+} solutions (0.08, 0.40, and 1.20 mM (Xiong, 1995; Wen et al., 2011)), acid rain solutions (pH 3.0 and 4.5 (State Environmental Protection Administration of China, 2005; Li et al., 2010)), and complex solution of La^{3+} and acid rain were prepared according to previously reported methods (Wen et al., 2011) with minor modifications. The acid rain stock solution at pH 1.0 was prepared with a solution of concentrated H_2SO_4 and HNO_3 in a ratio of 3.0:1 (v/v, by chemical equivalents) according to the general anion composition of rainfall in China (2007–2008) (Chen et al., 2010). Given the extremely low solubility of REE phosphate, 1 mM KH_2PO_4 in the Hoagland's solution (pH 7.0) was replaced by 1 mM KCl to avoid precipitation of REEs phosphate. This solution was called -P nutrient solution. The seedlings were subjected to 12 treatments, as shown in Scheme 1. First was the control treatment. Soybean seedlings were cultured in the -P nutrient solution (pH 7.0) and sprayed with distilled water until the drops began to fall. Second was the La^{3+} treatment. Soybean seedlings were cultured in the -P nutrient solution with La^{3+} (0.08, 0.40, and 1.20 mM, pH 7.0), and then sprayed with distilled water until the drops began to fall. Third was the acid rain treatment. Soybean seedlings were cultured in the acidic -P nutrient solution (pH 3.0/4.5) and sprayed with acid rain at the same pH with acidic -P nutrient solution (pH 3.0/4.5) on foliage until the



Scheme 1. Schematic of different treatments.

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