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Populus yunnanensis males adopt more efficient protective strategies than females to cope with excess zinc and acid rain

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HIGHLIGHTS

- Sexual differences in poplar's zinc (Zn) and acid rain (AR) tolerance were studied.
- Males showed a lower level of H_2O_2 and $O_2^{\cdot-}$ than females under excess Zn and Zn+AR.
- Males synthesized more biochemical molecules than females under excess Zn and Zn+AR.
- Males have a greater potential tolerance than females to enrich Zn.
- Males possess more a effective self-protection mechanism than females to excess Zn.

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ABSTRACT

Dioecious plants show sexually different responses to environmental stresses. However, little is known about the dimorphic morphological and physiological responses to soil pollution. To investigate sex-related adaptive responses of *Populus yunnanensis* seedlings when exposed to excess zinc (Zn), acid rain (AR) and their combination (Zn+AR), we analyzed growth parameters, Zn accumulation and allocation, photosynthetic capacity and biochemical responses under different treatments. Results revealed that both excess Zn and Zn+AR have a negative effect on plant growth. Males have a greater potential than females to enrich Zn. The photosynthesis limitation could be attributable to a lower stomatal conductance, photosynthetic nitrogen use efficiency and nitrate reductase activity induced by Zn accumulation. Overproduction of reactive oxygen species was detected, and females showed higher levels of H_2O_2 and $O_2^{\cdot-}$ than did males under excess Zn and Zn+AR. In addition, indicators related to plant injury showed expected increases and exhibited sexual differences. Males synthesized more biochemical molecules, such as proline and non-protein thiol, showing a stronger defense capacity in responses to either excess Zn or Zn+AR. Taking into account the Zn accumulation and the resulting injuries in plants, we suggest that excess Zn causes sex-related adaptive responses and males possess a more effective self-protection mechanism, Zn-stressed individuals suffering from AR did not show notable aggravation or alleviation when compared to damages induced by excess Zn alone.

1. Introduction

Populus yunnanensis is a dioecious, widely distributed plant, which is a dominant woody species in high elevation but low latitude areas of southwestern China, especially in Yunnan and Sichuan provinces. It plays a key role in forestry production and environmental protection. However, this region is abundant in lead–zinc and copper–zinc mines, which may bring on plants long-term exposure to harmful stresses, such as excess zinc (Zn). The effect of excess Zn on growth has been studied in many plant species (Di Baccio et al., 2003; Shi and Cai, 2009; Sagardoy et al., 2010). In general, excessive Zn accumulation in plants can reach toxic levels (Broadley et al., 2007). Toxicity symptoms usually become visible at $[Zn]_{leaf} > 300$ mg Zn kg^{-1} dry weight (DW) (Chaney, 1993; Broadley et al., 2007). However, the toxicity threshold can be highly variable even within the same species (Chaney, 1993). Some plant species can accumulate large amounts of Zn, even more than 10000 mg Zn kg^{-1} DW, without any symptoms of toxicity (Baker and Brooks, 1989). Excess Zn inhibits plant growth and development, leads to a lower biomass accumulation (Di Baccio et al., 2003, 2011; Sagardoy et al., 2009; Adams et al., 2011), disequilibrates the uptake and redistribution of mineral nutrition (Adams et al., 2011; Di Baccio et al., 2011), and disturbs metabolic processes, such as photosynthesis and transpiration (Di Baccio et al., 2009; Xu et al., 2010).

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In addition to Zn stress, acid rain (AR) may be another serious threat to *P. yunnanensis*. Due to rapid industrialization, AR emerged as an important environmental problem in southern and southwestern China in the late 1970s, especially in some forested areas previously thought to be pristine (Larssen et al., 2006). The main distribution area of *P. yunnanensis* is within this region with serious AR. Previous studies have reported that AR badly damages plant growth and causes forest decline (Bian and Yu, 1992). Negative changes in plants include foliar necrosis, branch dieback (Fan and Wang, 2000), damaged cell membrane systems, lower photosynthetic capacity and disordered metabolism (Liu et al., 2011). However, little is known about the effects of AR on the native dioecious *P. yunnanensis*.

While plants' responses to Zn or AR have been well documented, few studies have investigated the combined effects of these two factors, especially in dioecious plants, which are an important component of terrestrial ecosystems, representing nearly 6% of angiosperm species (Renner and Ricklefs, 1995). Recent studies have revealed that dioecious plants exhibit sexual differences in responses to single or multiple stressors, and females usually show a lower tolerance capacity when compared with males (Chen et al., 2010a, 2011). Therefore, also knowledge of sex-related adaptive responses to excess Zn and AR in the native dioecious *P. yunnanensis* is important. In this study, we employed *P. yunnanensis* as a model species to evaluate the effects of excess Zn, AR and their combination (Zn+AR) on sex-related morphological changes and physiological responses. We hypothesized that (i) excess Zn causes sex-related adaptive responses; (ii) AR aggravates damages induced by excess Zn and increases differences in sex-related adaptive responses.

2. Materials and methods

2.1. Plant materials

A total of 30 male and 30 female trees were collected from 15 populations covering the whole distribution region of *P. yunnanensis* in their natural habitats (Meigu, 28°18′N and 103°06′E, Sichuan Province, China). The mean altitude, annual rainfall and annual temperature in the area are 2300 m, 1115 mm and 10.1 °C, respectively. The cuttings were planted in 10 L plastic pots filled with 8 kg homogenized soil in March 2011. The properties of the soil used in this study were as follows (based on kg^{-1} dry soil): pH 7.1, organic carbon 18.1 g, total N 1.8 g, available phosphorus 2.5 g, total potassium 18.55 g, organic matter 23.4 g, and the total Zn content 110 mg. After sprouting and growing for about 2 months, healthy male and female cuttings about 40 cm high were replanted (one cutting per pot) and kept in a greenhouse located in the Chengdu Institute of Biology, the Chinese Academy of Sciences. A total of 80 male and 80 female individuals with a similar crown size and an equal height were chosen and used for the study.

2.2. Experimental design and treatments

The experimental layout was completely randomized with three main factors (sex, Zn and AR). Therefore, there were eight treatments in total. Each treatment included twenty cuttings. Five replicates with four cuttings each were used to minimize sampling errors. In the Zn treatment, deionized water containing 20 mM $ZnSO_4{\cdot}7H_2O$ was evenly added to the pots every 3 d during the first 2 weeks of the treatment, and the final Zn level reached about 5 g Zn kg^{-1} dry soil, which is comparable to the soil Zn content of some lead–zinc mines present in the Yunnan province, China. In the parallel treatment, plants were sprayed with simulated AR (pH 4.0, 50 mL per plant) every 3 d during the first 2 weeks (Larssen et al., 2006). The simulated AR was prepared with solution of H_2SO_4 and HNO_3 in the ratio of 5:1 by chemical equivalents, which represents the average ion composition of rainfall in southern China (Fan, 1993). In the treatment to reveal the Zn and AR interaction, plants were first watered by 20 mM $ZnSO_4{\cdot}7H_2O$ solution and then sprayed with simulated AR (pH 4.0, 50 mL per plant). Control plants received equal quantities of deionized water. All treatments lasted for 90 d, from 25 May to 23 August, 2011.

2.3. Plant growth and morphological responses

At the end of the experiment, plants were harvested and divided into roots, stems and leaves. Biomass samples were dried (80 °C, 48 h) to constant weight and weighted. The root/shoot dry mass ratio (R/S) was calculated. Total numbers of leaves (TNL) were counted and leaf areas were determined by a Portable Laser Area Meter (CI-203; CID, Camas, WA, USA).

2.4. Zinc and nitrogen in plant compartments

Dried samples were ground and passed through a 20-mesh screen. The total Zn content was analyzed by flame atomic absorption spectroscopy (Igartua et al., 2000), and the total N content of leaves was determined by the semi-micro Kjeldahl method as described by Kost and Boerner (1985).

2.5. Gas exchange

Gas exchange was measured using the fourth fully expanded and intact young leaves near the shoot apex of each plant. Net CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) were measured with Li-Cor 6400, a portable photosynthesis measuring system (Li-Cor Inc., Lincoln, NE, USA). The gas exchange was measured in the morning (08:00–11:00) using the following conditions: leaf temperature, 25 °C; leaf-to-air vapor pressure deficit, 1.5 ± 0.5 kPa; photosynthetic photon flux (PPF), 1400 $\mu mol\ m^{-2}\ s^{-1}$; relative air humidity, 50%; and ambient CO_2 concentration, 350 ± 5 $\mu mol\ mol^{-1}$. The ratio of A to the leaf nitrogen content (on area basis in $g\ m^{-2}$) in the leaves was regarded as the photosynthetic nitrogen use efficiency (PNUE).

2.6. Nitrate reductase activity

The nitrate reductase (NR) activity was determined as described by Scheible et al. (1997) and Zhao et al. (2009b). Leaf material (0.5 g) was ground with liquid nitrogen and suspended in extraction buffer, containing 100 mM HEPES-KOH (pH 7.5), 1 mM EDTA, 10% (v/v) glycerol, 5 mM dithiothreitol, 0.1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, 20 μM FAD, 1 μM leupeptin, 5 μM Na_2MoO_4, and 1% PVP. After centrifugation at 10000 g, 4 °C for 20 min, the supernatant was used for the NR activity determination. The NR activity was measured by mixing 1 volume of extract with 5 volumes of prewarmed (25 °C) assay buffer (100 mM HEPES-KOH, pH 7.5, 5 mM KNO_3, and 0.25 mM NADH). The reaction was started by the addition of the assay buffer, incubated at 25 °C for 30 min, and then stopped by adding 0.1 M zinc acetate. After 20 min, the tubes were centrifuged at 13000 g for 5 min. The nitrite produced was measured at 520 nm by adding 1 mL of 1% (w/v) sulfanilamide in 3 M HCl plus 1 mL of 0.02% (v/v) N-(1-naphthyl)-ethylenediamine in distilled water.

2.7. Relative electrolyte leakage

Relative electrolyte leakage (REL) was measured as described by Zhang et al. (2011). Fifteen fresh leaf discs (0.5 cm in diameter) were incubated in tubes with 10 mL of deionized water at 25 °C for 12 h.