



Higher soil salinity causes more physiological stress in female of *Populus cathayana* cuttings[☆]



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ABSTRACT

Dioecious plant species represent a major component of terrestrial ecosystems. Little is known about sex-specific responses to soil salinity. *Populus cathayana* Rehd, which is a dioecious, deciduous tree, was employed as a test species in our study. In a semi-controlled environment, physiological responses to salinity were investigated in male and female *P. cathayana* cuttings, which were subjected to two salt regimes: 0 and 80 mM NaCl added to the Hoagland's solution for one month growth. Relative to the control, the saline treatment significantly decreased net photosynthetic rate (P_n), transpiration (E), stomatal conductance (g_s), carotenoids (Caro), chlorophyll a (Chl a), total chlorophyll (TC) and catalase (CAT) activity, but increased Na^+ , Ca^{2+} , K^+ , malondialdehyde (MDA) content, superoxide dismutase (SOD) activity and carbon isotope composition ($\delta^{13}\text{C}$) in both sexes. Different sensitivity to saline conditions between males and females was detected. With higher soil salinity, females exhibited lower P_n , Chl a, TC, Chl a/b, Ca^{2+} , $\text{Ca}^{2+}/\text{Na}^+$, K^+/Na^+ , SOD and CAT activities but higher Na^+ and MDA content than males. However, there were no significant differences in these traits (except for SOD and CAT activities) detected in the control group. Our results indicated that males may be more tolerate to salinity than females, with females having lower gas exchanges, chlorophyll pigments, antioxidant enzyme activities, K^+/Na^+ ratio and water use efficiency (WUE) than males.

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1. Introduction

Soil salinity is a major abiotic stress affecting approximately 7% of the world's total land area [1]. It was reported that salinization of arable land may result in 30% land loss in the whole world within next 25 years and up to 50% by the middle of 21st century [2]. Higher soil salinity can have adverse effects on many biological processes and decreases the growth of plant. Many studies have reported that height growth, basal diameter, leaf area, leaf number and biomass accumulation are significantly decreased by an increase in salt concentration within plants of *Spondias tuberosa*, *Leucaena leucocephala*, *Prosopis juliflora* and *Rhynchosia minima* [3,4]. These effects were found to be associated with lower gas exchange rate [5,6] and less chlorophyll pigment content with increasing salt stress [3,7–9]. There is evidence that salt stress

affects the accumulation of ions and malondialdehyde (MDA) in plant cells. Salt-sensitive plants were detected to have more accumulation of Na^+ and MDA in leaves in comparison with the salt-tolerant plants when exposed to salt stress [10–13]. In addition, salinity affects water use efficiency by decreasing osmotic potential of soil solution, and disturbs antioxidant defense systems through inducing the production of reactive oxygen species (ROS) have also been demonstrated in some previous studies [6,14].

As an important component of terrestrial ecosystems, dioecious plant species comprising at least 6% of about the estimated 240,000 angiosperm species [15], and play a critical role in maintaining the stability of structure and function of ecosystems [16,17]. Because of different reproductive functions [18], morphological and physiological differences between male and female plants were thought to have arisen under stressful environments [19]. Many studies have demonstrated the adverse effects caused by soil salinity in angiosperm plants, but relatively few study investigate the sex-related physiological differences in response to salt stress. If the morphological and physiological traits of male and female individuals are significantly affected by salinity, the sex ratio, reproductive capacity and distribution of dioecious populations may change. Few

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studies have indicated that males having a better ability to restrain Na^+ transport from roots to shoots [20], and female cuttings of poplar are more sensitive to salinity and drought than males [21]. Some research has also reported that females have a lower tolerance to freezing for *Hippophae rhamnoides* [22], and that they suffer greater negative effects under drought stress in *Salix glauca*, *Populus cathayana*, *P. tremuloides*, *Silene latifolia* and *Hippophae rhamnoides* [23–25], and have more biomass accumulation at elevated CO_2 [16,26], as well as had a higher photosynthesis rate under increased temperature conditions [27]. However, further studies are needed to understand sex-related physiological and ecological responses caused by higher soil salinity in dioecious plants.

P. cathayana Rehd, a dioecious tree species, widely distributed in the northern, central and southwestern China. Because of its high survival rate and fast growth, it was chosen to be used as a test species in this study. Considering that photosynthesis rate, lipid peroxidation, antioxidant enzyme activities, ion accumulation, and water use efficiency are susceptible to higher soil salinity, we investigated these traits in male and female *P. cathayana* individuals grown under non-saline and saline environment, respectively. Because morphological and physiological differences between two sexes are considered to result from females typically investing more resource in reproduction [28,29], we hypothesized that the higher soil salinity may causes more physiological stresses in female of *P. cathayana* cuttings than in males.

2. Materials and methods

2.1. Plant materials and experimental design

Cuttings of *P. cathayana* were collected from lateral branches of 30 different mature trees (30 genotypes) sampled in 10 populations, including 15 males and 15 females, naturally occurring in riparian sites in western Sichuan Province. The cuttings were planted in spring and rooted in an experimental field of China West Normal University, in Nanchong (106°04'E, 30°48'N), Sichuan Province, China in 2009. Annual rainfall, air temperature, and relative humidity in the cultivated site are range between 980–1150 mm, 15.8–17.8 °C and 76.0%–86.0% respectively. After sprouting and growing for about 2 months, 30 male and 30 female healthy cuttings with approximately the same size and height were chosen and replanted (one cutting per pot) in 12-liter plastic pots containing 10 kg homogenized local purplish soil (Udorthent) (soil water content, Na_2O , K_2O and CaO concentrations are 24.0%, 0.83%, 2.48% and 7.29% respectively). The replanted cuttings were grown for two weeks prior to being transferred to following saline treatment.

The experimental layout was completely randomized with two main factors (sex and saline treatment), and carried out in a well-ventilated greenhouse at the experimental field of China West Normal University for one month. Saline treatment was manually applied every three days by giving 1.5 liter full-strength Hoagland's solution containing 0 and 80 mM NaCl, and watering to excess and allowing excess solution to drain into dishes placed under the pots. Five replications, three cuttings in each replication were used to minimize random errors. During the experimental period, the average air temperature and relative humidity were 25.8 °C and 70%, respectively.

2.2. Gas exchange and chlorophyll pigment measurements

According to the sampling method of Xu et al. [25], five cuttings of each sex and treatment were randomly selected, and the fourth fully expanded and exposed leaf of each cutting was measured. Net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were

measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) under the following conditions: leaf temperature, 26 °C; leaf-to-air vapor pressure deficit, 1.5 ± 0.5 kPa; photosynthetic photon flux density (PPFD), $850 \mu\text{mol m}^{-2} \text{s}^{-1}$; and ambient CO_2 concentration, $370 \pm 10 \mu\text{mol mol}^{-1}$. The gas exchange parameters were measured three times (on July 21, 22 and 24, respectively) between 08:00 and 11:30 h.

Fresh leaves (0.2 g) collected from the fifth fully expanded leaf of male and female cuttings were ground into mash, soaked in an acetone and alcohol (1:1) mixture solution [30], and measured by using a spectrometer (APL UV-754, Shanghai, China). The absorbance of chlorophyll a, chlorophyll b and carotenoids was determined at 663, 645 and 470 nm respectively. Absorbance values were converted to concentrations (mg g^{-1}) as described by Arnon [31] and expressed per unit leaf fresh weight.

2.3. Relative water content (RWC) and malondialdehyde (MDA) content measurements

The sixth fully expanded leaf of male and female cuttings were cut and immediately weighed with an analytical balance for the field fresh weight. Then, whole leaves were floated on distilled water in the dark for 24 h to determine their turgid weight. Dry weights were obtained after wrapping the leaf in aluminium foil and oven-drying at 70 °C for 48 h. We calculated relative water content (RWC) (%) from fresh mass (FM), turgid mass (TM) and dry mass (DM) measurements on the whole leaf: $\text{RWC} = 100 \times (\text{FM} - \text{DM}) / (\text{TM} - \text{DM})$ [32].

MDA content was measured according to the method of Kramer et al. [33], which start reaction with thiobarbituric acid (TBA). Fresh leaf tissue (0.3 g) was homogenized in 6 ml of 10% (w/v) trichloroacetic acid (TCA), and centrifuged at 12,000g for 10 min. Then, 2 ml 0.6% TBA in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 15 min and then rapidly cooled in an ice bath. After centrifugation at 12,000g for 10 min, the absorbance of the supernatant at 450, 532 and 600 nm was determined with a spectrometer (APL UV-754, Shanghai, China). The MDA content (C) was calculated using the following formula: $C (\text{m}) = 6.45 (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \text{OD}_{450}$, where OD is optical density of the sample.

2.4. Superoxide dismutase (SOD) and catalase (CAT) activity measurements

Superoxide dismutase (SOD) and catalase (CAT) activity were measured according to a modified method as described by Lei et al. [34]. Fresh leaf samples (0.3 g) were ground in a pre-chilled mortar with a little quartz sand, and the ground samples were homogenized in an ice bath in 6 ml sodium phosphate buffer (SOD pH 7.8, CAT pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and 0.1 mM ethylenediaminetetraacetic acid (EDTA), and then extracted. The homogenate was centrifuged at 12,000g for 20 min. All of the above were performed at 4 °C. The supernatants were collected and used for assays of enzyme activity.

SOD activity was determined by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) as described by Giannopolitis and Ries [35]. The reaction mixture (3 ml) contained 1.5 ml of Na-phosphate buffer (pH 7.8), 0.3 ml of 50 mM methionine, 0.6 ml of 750 m NBT, 0.3 ml of 0.1 mM EDTA, 0.3 ml of 20 mM riboflavin and 0.1 ml of enzyme extract. The reaction was started with the addition of riboflavin and carried out for 30 min under irradiance of $170 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by a white fluorescent lamp. The absorbance at 560 nm was determined with a UV spectrophotometer (APL UV-754, Shanghai, China), and the extract volume causing 50% inhibition of NBT

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