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Original Research

Anatomy and ultrastructure adaptations to soil flooding of two full-sib poplar clones differing in flood-tolerance

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ABSTRACT

Flooding stress always depressed plants survival and growth in flood-affected areas. To explore anatomy and ultrastructure adaptations to hypoxia stress, two full-sib poplar clones differing in flood-tolerance LS1 (flood-tolerant) and LS2 (flood-susceptible) were compared for waterlogging effects on them. The two clones LS1 and LS2 originated from *Populus deltoides* cv. *Lux* ex. *I-69/55* × *P. simonii*. Morphological, ecophysiological and growth parameters, as well as anatomy and ultrastructure characteristics of their seedlings were subjected for 15 days to flooding treatment, followed by a three-day drainage and recovery stage. Results showed that flooding stress adversely influenced all characteristics aforementioned in all flooded plants. Compared with LS1, LS2 suffered clearly more severe flood injury during hypoxia and slower recovery ability after drainage, illustrated by morphology, biomass accumulation, gas exchange, chlorophyll fluorescence, root metabolism, and relative membrane permeability and malonaldehyde content of roots. Correspondingly, more pronounced anatomy and ultrastructure damages in leaves and roots were found in flooded LS2 as well, including palisade cell deformation in leaves, as well as serious lysis of cortical parenchyma cells and decompositions of nucleus and organelles in roots. Our results showed that morphological, ecophysiological and growth responses to soil flooding paralleled their anatomy and ultrastructure adaptations in leaves and roots. Stable intercellular and intracellular structures in leaves and roots, especially in the latter, helped the flood-tolerant clone behaved better than the flood-susceptible clone. Roots suffered more severe anatomy and ultrastructure injury than leaves under hypoxia stress. The flood-tolerant clone kept a stable cross-section anatomy with normal aerenchyma and ultrastructure in roots, which enable plant-internal aeration so that maintain aerobic respiration and basic root activities under flooding condition. The flood-susceptible clone was prone to lost normal cross-section anatomy under soil flooding caused by serious cortical parenchyma cell lysis and ultrastructure destruction, which resulted in root disorganization and dysfunction.

1. Introduction

Flooding is a worldwide natural phenomenon, which frequently happen in floodplains and arable farmlands. In these areas, flooding of varied durations and intensity often greatly affects ecological environment and plants growth adversely. China has about 6.6×10^5 km^2 flooded lands, occupying 6.6% of its total area, and mainly distributed in the middle and lower valleys along the Yangtze and the Yellow rivers (Wang et al., 2002). Poplar, a fast-growing and flood-tolerant species, has been adopted as one of the most important multipurpose afforestation trees in these flood-affected areas of China (Du et al., 2012). However, flooding stress still negatively affected its wood production and ecosystem significantly (Peng et al., 2013). Therefore, figuring out its flood-tolerance mechanism and breeding more flood-tolerant poplar clones are of significance for afforestation and ecosystem protection in these areas. Morphological, physiological, ecophysiological and growth characteristics of poplar under soil flooding stress were well characterized in previous literatures, accompanied with few molecular transcriptional regulation, but seldom focused on its anatomical and ultrastructural adaptations (Bejaoui et al., 2016; Kreuzwieser et al., 2009; Yu et al., 2015). Anatomical results can provide a view on type, quantity, and arrangement of cells, as well as intercel-

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lular structure of a certain organism (Atanackovic et al., 2012; Moog, 1998; Suralta and Yamauchi, 2008). Ultrastructural analysis principally demonstrate cell component changes, including type, quantity, structure and arrangement of organelles, as well as starch accumulation etc. (Longstreth and Borkhsenious, 2000). Survival and biomass accumulation of plants under flooding stress are closely related to functions of leaves and roots (Kreuzwieser and Rennenberg, 2014; Striker and Colmer, 2016). Thus, their anatomical and ultrastructural responses to soil hypoxia are important for elucidating mechanism of poplar flood-tolerance (Herrera et al., 2009). In the present study, we investigated anatomical and ultrastructural adaptations of leaves and fine roots to soil flooding stress in two full-sib poplar clones differing in flood-tolerance.

2. Materials and methods

2.1. Plant materials and experiments

The study was performed in Huazhong Agricultural University, Wuhan, China (30°28′N, 114°21′E). The region has a warm temperate climate, with a yearly mean temperature of 16.3 °C, 1269 mm of rainfall, and an annual average of 240 frost-free days. Most rainfall occurs in June ~ August.

Two full-sib family clones, LS1 (flood-tolerant) and LS2 (flood-susceptible), originated from *Populus deltoides* cv. *Lux* ex. *I-69/55* (flood-tolerant) × *P. simonii* (flood-susceptible), were adopted as the materials in our study (Du et al., 2012; Peng et al., 2013). Four-week-old stem saplings of LS1 and LS2 were transferred from sterile agar cultures into plastic pots (14cm × 10cm × 13 cm) containing mixture of vermiculite and culture soil (1:3, v/v) (Zhenjiang Peilei Development Co., LTD, China). The soil (pH6.0) consists of 2% ~ 5% of N, P_2O_5 and K_2O, together with more than 20% organic matter (dry weight). After transplantation, the seedlings were covered with a transparent plastic lid to maintain a high humidity level to facilitate survival. After survived, the plastic lids were removed, and all plants were watered with Hoagland solution weekly and tap water twice a week. All plants grown in a room with temperature of 25 ± 2 °C, under a photoperiod of 16 h light and 8 h dark with the light intensity of 400 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps, and 70% ~ 80% air humidity. All plants with height of 35 ~ 40 cm were randomly assigned to one of two water regime treatments for 18-day study (15 days of flooding followed by a 3-day recovery period): (1) watered (control, CK); (2) flooded (FL). The plants were divided into two groups of each treatment: the one was used for non-destructive growth and ecophysiological investigations, from the other samples were collected for anatomical, ultrastructural and physiological analysis. The first group comprised 15 plants of LS1 and LS2, respectively, for each treatment (5 blocks, 3 plants per block). In the other group 30 plants of LS1 and LS2, respectively, arranged randomly, were sampled. Pots of the control group had four drainage holes in the bottom. The plants were watered using tap water daily as needed to maintain soil moisture at field capacity. The flooded plants were flooded to a height of 10 cm above the soil surface in tanks.

After 15 days, the flooded plants were allowed to recover under control condition for 3 days after drainage to simulate natural environmental conditions. During flooding and recovery, ecophysiological investigations were executed before flooding and on the 1st, 2nd, 3rd, 7th, 15th and 18th day of treatment. Root metabolism, relative membrane permeability (RMP) and malonaldehyde (MDA) content of roots were determined at the 1st, 3rd, 7th, 15th and 18th day of flooding. Anatomy and ultrastructure of leaves and fine roots were investigated on the zero (non-flooding), 3rd, 7th and 15th day of flooding, respectively.

2.2. Measurement of leaf gas exchange and chlorophyll fluorescence

Leaf gas exchange and chlorophyll fluorescence in the 5th fully expanded and mature leaf from top of the stem of five plants per clone per treatment were measured between 09:00 and 11:00 h using a LI-6400 photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with a standard LI-COR gas exchange chamber. A 1500 μmol photons $m^{-2}s^{-1}$ light intense of illumination was provided by red diodes (6400-02 LED Source) and the gas flow rate was set as 500 μmol s^{-1}. Gas exchange measurement included net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (Gs), internal CO_2 concentrations (Ci), atmospheric CO_2 concentrations (Ca), together with the corresponding ambient environmental conditions, such as temperature, relative humidity and photosynthetically active radiation (PAR). Intrinsic water use efficiency (WUEi) was calculated as follows: WUEi = Pn/Gs (Mielke et al., 2005).

Chlorophyll fluorescence of leaves was measured from 20 plants (five plants per treatments per clone) after 20-min dark adaptation. The investigation was conducted using a LI-6400 fluorescence system (LI-COR Inc., Lincoln, NE, USA), including variable fluorescence (F_v), potential efficiency of primary conversion of light energy of PSII (F_v/F_m), and the ratio of variable fluorescence to initial fluorescence (F_v/F_o) etc.

2.3. Measurements of root metabolism, relative membrane permeability and malonaldehyde content of roots

Fine roots of plants were excised and washed twice for 1 min in demineralized water. Water from root surface was carefully swept off with filter paper. Thereafter, some of them were immediately used to analyze RMP, and the others were frozen immediately in liquid nitrogen, and kept at −80 °C until utilization. RMP and MDA content were measured with usual methods as described by Li (2000). For RMP measurement, the roots were put in test tubes containing 20 ml of deionized distilled water. The test tubes were vortexed for 5 s and the solution was assayed for initial electrical conductivity (EC_0). Subsequently, the same samples containing roots were boiled at 100 °C for 15 min to determine the resulting electrical conductivity (EC_1) of the water. Percent RMP was calculated as: RMP (%) = $EC_0/EC_1 \times 100$. Electrical conductivity was measured with 3173 portable conductivity meter (Jenco Instruments, Inc., USA). MDA concentration of homogenized roots was measured spectrophotometrically at the wavelength of 532 nm and 600 nm based on the reaction of MDA with thiobarbituric acid (TBA). Intensity of root metabolism (dehydrogenase activity) was determined spectrophotometrically at the wavelength of 485 nm based on triphenyl tetrazolium chloride (TTC) assay. All parameters mentioned above were measured by five plants per treatment per clone.

2.4. Measurement of plant biomass

At the end of ecophysiological study, 15 plants of LS1 and LS2 in each treatment were harvested, respectively. Roots, stems and leaves were subjected to 105 °C for 2 h, then dried at 60 °C to constant weight, and weighted, respectively. Root/aerial part ratio was calculated as follows: Root/aerial part ratio = root dry weight/(stem + leaf) dry weight. Total biomass was calculated as follows: total biomass = (root + stem + leaf) dry weight.

2.5. Light and electron microscopy studies

The 5th full expanded and mature leaf from the top of stem was used for anatomical and ultrastructural analysis. Same parts