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Full Length Article

Physiological and Lateral Root Anatomy Changes Improve Drought Tolerance and Adaptation to Recovery in *Cercis glabra*

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Abstract

Cercis glabra Pamp. is a tree species discovered in recent years, with high ornamental value, but its tolerance to drought stress is not clear. A study was conducted to analyze drought resistance of *C. glabra*. The results showed that antioxidant activities (SOD, POD, and CAT and MDA), including soluble protein, soluble sugar and free proline content in *C. glabra* leaves was significantly increased. Moreover, aerenchyma and Casparian strip were formed at lateral roots (3 cm from root apex) of *C. glabra* under drought stress at 8 days. Nonetheless, these physiological and biochemical changes also increased in *C. glabra* leaves including aerenchyma and Casparian strip formed at lateral roots upon re-watering. These results implied that *C. glabra* has tolerance to drought stress and capability to recover to some extent and adapt to drought stress by improving the activities of antioxidant enzymes, accumulating osmolytes and anatomical changes in lateral roots.

Keywords: *Cercis glabra*; Drought stress; Antioxidant enzyme activity; Lipid peroxidation; Osmotic adjustment substances; Aerenchyma; Casparian strip

Introduction

Drought stress is a serious impediment to the growth of trees (Lie *et al.* 2018). But trees adapt to drought stress by physiological responses, such as improving the activity of antioxidant enzyme and accumulating osmotic substances in leaves (David–Schwartz *et al.* 2019; Golmohammadi *et al.* 2019). Leaves are an important organ of the plant to produce organic matter by photosynthesis (Yuan *et al.* 2012) and very sensitive to drought stress (Farooq *et al.* 2009; Zhang *et al.* 2018). The increasing of reactive oxygen species (ROS) is a typical response of trees leaves to drought stress (Ren *et al.* 2016), and acts as a secondary massager to elicit a defensive response of the plant (Choudhury *et al.* 2017; Farooq *et al.* 2019). ROS (oxygen ions, peroxides, and free radicals) is formed in the normal metabolism of trees (He *et al.* 2019), and during drought stress, ROS levels increase rapidly resulting in oxidative damage to lipids, such as Ferula assafoetida and *Lycium ruthenicum* Murr. (Guo *et al.* 2018; Mohammadi *et al.* 2019). And antioxidant enzymes have a key role in ROS detoxification (Dubey *et al.* 2017; Zhang *et al.* 2017). Activities of antioxidant enzymes in plants under drought stress are usually regarded as indicators of tolerance of drought stress (Farooq *et al.* 2017; Li *et al.* 2019). The SOD, POD, and CAT activities increase, causing ROS scavenging under drought stress (Esposito *et al.* 2018; Brito *et al.* 2019). At the same time, leaf growth may be inhibited by a slight reduction in the water potential of tissues, too (Farooq *et al.* 2014; Plesa *et al.* 2019). Some trees can accumulate osmotic regulatory substances (soluble sugar, soluble protein, proline, and other solutes) to maintain the balance of cell osmotic potential under drought stress (Ivanov *et al.* 2019) including *Hippophae Rhamnoides* L. and poplar (Fernandez and Jacinto 2019; Han *et al.* 2019).

In addition, the plant roots are also sensitive to drought stress and resist by morphological changes. The roots of some trees also form Casparian strips and aerenchyma to adapt to drought stress, such as poplars and *Avicennia marina* (Kordyum *et al.* 2019; Rodriguez-Zaccaro and Groover 2019).

Cercis glabra Pamp. from the Fabaceae family is a leguminous tree and is one of the species with excellent ornamental value (Nadler *et al.* 2012; Jia and Manchester 2014). *C. glabra* is tall and straight, tree crown resembles umbrella form, after flowering first, grow leaf, when flowering full tree is amaranth or pink, the flower-like violet butterfly, quite moving. *C. glabra* can appreciate flowers in spring, heart-shaped glossy leaves in summer, reddish-brown fruit pods in autumn and straight trunk in winter. Garden plants with high drought resistance in arid and semi-arid areas are of great significance. This study analyzes the drought resistance of *C. glabra*, reveals the mechanism and adaptive capacity of *C. glabra* to drought stress, and provides a theoretical foundation for the promotion and application of *C. glabra*.

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Materials and Methods

Plant materials and drought stress

The study site was Yangtze University, Jingzhou City (30°21′N, 112°8′E) in Hubei Province, China. The same size of annual seedlings of *C. glabra* were collected in December 2017 from Xingshan District, Hubei province, China. One seedling was cultivated per pot (20 cm in diameter and 16 cm in height) on 16 December 2017. Each pot was filled with the same amount of soil and humus (3:1) mixture. Drought stress treatment was initiated on 9 May 2018. The two-factor completely random design was adopted in the experiment. The experiment consisted of control and drought treatment groups. Repeat 4 times for each treatment. The soil moisture content was controlled by weighing method, weighing once a day, calculating the soil relative humidity, water after weighing, keeping the soil relative humidity of control and drought treatment at 75 and 25% respectively. After 8 days of drought, watering kept the soil moisture of drought treatment at 75%. At 0, 4, 8 days of drought and 4 days of re-watering, physiological parameters are measured in leaves of control and drought treatment groups, and complete lateral roots of control and drought treatment were preserved with FAA fixator (Kumar and Nautiyal 2017).

Enzyme assays and Lipid peroxidation

Samples of 0.2 g fresh leaves were taken, homogenized with 5 mL 50 mm phosphate buffer (pH 7.8), crushed with a mortar and pestle, and centrifuged at 10000×g and 4ºC for 20 min. The enzyme activity and malondialdehyde content were determined by the supernatant. Superoxide dismutase (SOD) activity was determined by the method of Giannopolitis and Ries (1977). The 3.0 mL reaction mixture contained 0.2 mL enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 m*M* EDTA, 13 m*M* methionine, 1.3 m*M* riboflavin, and 63 m*M* NBT. The test tube was exposed to 78 mmol photons s^{-1} m^{-2} for 10 min. And absorbance of the test tube was recorded at 560 nm. A unit of SOD activity was defined as the amount of enzyme required to inhibit the 50% NBT reduction rate at 560 nm. The catalase (CAT) and peroxidase (POD) activity were determined by Chance and Maehly methods (1955). For POD, the oxidation of alcohol in callus was determined by increasing the absorbance at 470 nm for 1 min. 3 mL of the reaction mixture contained 0.2 mL enzyme extract, 40 m*M* H_2O_2 and 20 m*M* callus alcohol. For CAT, the decomposition of H_2O_2 was determined by reducing the absorbance at 240 nm for 1 min. The 3 mL reaction mixture containing 0.2 mL enzyme extract, 15 mm H_2O_2 and 50 mm phosphate buffer (pH 7.0). Lipid peroxidation was determined by estimating malondialdehyde (MDA) (Esterbauer and Cheeseman 1990). To determine the content of malondialdehyde, 3.0 mL reaction mixture containing 2 mL 0.5% thiobarbituric acid and 1.0 mL supernatant. The mixture was heated at 100ºC for 30 min, and then cooled rapidly in an ice bath. After centrifugation at 10000 × g for 10 min, the sample absorbance was determined at 450, 532 and 600 nm using a blank containing all reagents. The MDA content of the sample was calculated using the formula: C (nmol/g) = $(6.45 (A_{532} – A_{600}) – 0.56 A_{450}) × 5/0.2$

Osmotic adjustment

A 0.1 g sample of fresh leaves were taken and ground in an ice-cold mortar and pestle containing potassium phosphate buffer (50 m*M*, pH 7.5). The homogenates were centrifuged at 4℃ and 10000 × g for 10 min, supernatant was collected. Soluble proteins were determined by method of Bradford (1976). Soluble sugars were analyzed by method of Yemm and Willis (1954). The concentration of proline in leaves was determined by method of Li (2000). A mixture of 0.2 g fresh samples and 5 mL of sulfosalicylic acid was boiled in water for 25 min at 100℃ for bathing, and then centrifugated at 3000×g for 10 min. The 6.0 mL reaction mixture containing 2 mL acidic inhydrin, 2 mL glacial acetic acid and 2 mL supernatant, and the mixture was boiled in water for 25 min at 100°C. After cooling, 4 mL of toluene was added, and the absorbance of the extracts was determined at 520 nm.

Observation of root anatomy

Under atomic microscope, the root hair zone (3 cm from root apex) was sectioned by freehand sectioning. Sections were stained with toluidine blue for aerenchyma, phloroglucinol-HCl for lignin, Sudan red 7B for suberin lamellae, and berberine hemisulfateaniline blue for Casparian strips (Yang *et al.* 2014).

Statistical analysis

All data were analyzed by S.A.S. 9.1 software, and significant differences were compared by Duncan's new complex range method. The minimum significant differences of multiple comparison tests were used to determine the significance differences at the 0.05 level between treatments. The standard error (SE) was calculated and is shown in tables and figures by Excel 2016. The photos were marked by Photoshop 6.0.

Results

Activity of antioxidant enzymes

Compared with control treatment, the activities of SOD and CAT in *C. glabra* increased by 19.3 and 65.4% under drought at 4 days, and the activities of SOD, POD, and CAT increased by 20.0, 33.1 and 55.5% under drought at 8 days, and by 31.6, 105.1 and 18.7% upon re-watering at 4 days (*P*

Table 1: Effect of drought stress on antioxidant enzyme activities of *Cercis glabra*

Treatment	SOD (U/g Fr. Wt.)		POD (U/g Fr. Wt.)		CAT (U/g Fr. Wt.)	
	control	drought	control	drought	control	drought
DS 0 d	248.13 ± 8.72bc	255.53 ± 1.01bc	9.94 ± 0.86d	10.58 ± 1.06d	153.60 ± 9.55e	155.03 ± 5.60e
DS 4 d	220.07 ± 6.24d	262.45 ± 1.27b	11.71 ± 1.32d	12.91 ± 0.21cd	195.35 ± 6.77d	323.02 ± 9.80b
DS 8 d	261.22 ± 4.33b	313.40 ± 3.48a	16.69 ± 1.20c	22.22 ± 1.83b	269.02 ± 11.42c	418.35 ± 10.42a
RW 4 d	242.31 ± 2.90c	318.85 ± 8.74a	13.13 ± 1.01cd	26.93 ± 1.53a	278.92 ± 4.29c	331.06 ± 6.47b

DS: Drought stress, RW: Re-watering. Data were shown as means ± SE. Different letters in the table indicate significant differences between treatments ($P < 0.05$)

< 0.05) (Table 1). At 0–8 days, SOD, POD and CAT activities of *C. glabra* all increased significantly under drought stress ($P < 0.05$). After 4 days of re-watering, POD activities increased, and CAT activities decreased significantly ($P < 0.05$).

Lipid peroxidation

Compared with control treatment, the MDA contents increased by 61.9 and 33.2% under drought treatment at 4 and 8 days, and by 29.7% under re-watering treatment at 4 days ($P < 0.05$) (Fig. 1). At 0–8 days, MDA content in *C. glabra* increased gradually under drought, and then decreased significantly under re-watering at 4 days ($P < 0.05$).

Soluble sugar, soluble protein and free proline content

Compared with the control treatment, the soluble sugar content increased by 13.0 and 35.6% under drought at 4 and 8 days ($P < 0.05$) (Fig. 2A). At 0–8 days, soluble sugar content in *C. glabra* increased gradually under drought, and then decreased significantly under re-watering treatment at 4 days ($P < 0.05$). Compared with the control treatment, the soluble protein contents increased by 27.6 and 95.6% under drought at 4 and 8 days, and by 204.3% under re-watering at 4 days ($P < 0.05$) (Fig. 2B). At 0–8 days, soluble protein content in *C. glabra* increased gradually under drought. Compared with the control treatment, the free proline content increased by 58.0 and 266.7% under drought at 4 and 8 days, and by 13.5% under re-watering at 4 days ($P < 0.05$) (Fig. 2C). At 0–8 days, free proline content in *C. glabra* increased gradually under drought, and then decreased significantly under re-watering treatment at 4 days ($P < 0.05$).

Anatomical structure of roots

Drought stress promoted the formation of aerenchyma and Casparian strip in the lateral roots of *C. glabra* (Fig. 3). Compared with control, lignification and suberization were not observed in the cortex in the root hair area of lateral roots in *C. glabra*, but the lateral roots not only formed aerenchyma in the cortex but also formed Casparian strip in the endodermis under drought treatment at 4 and 8 days and upon re-watering at 4 days. At 0–8 days, aerenchyma of *C. glabra* lateral root gradually expanded, which continued

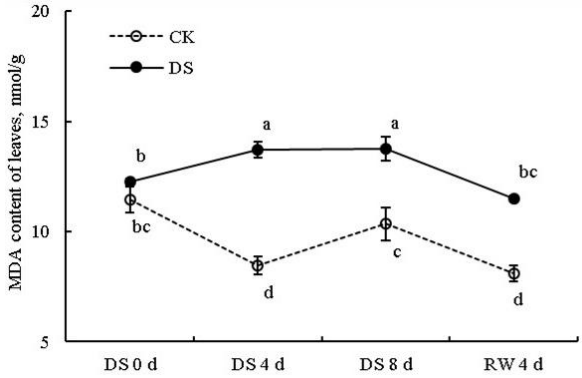

Fig. 1: MDA content in leaves of *Cercis glabra* seedlings under drought stress. CK: Control treatment, DS: Drought stress, RW: Re-watering. Data were shown as means ± SE. Different letters in the figure indicate significant differences between treatments ($P < 0.05$)

under re-watering treatment at 4 days; the Casparian strip gradually formed under drought stress, and then became more apparent under re-watering at 4 days.

Discussion

The SOD, POD, and CAT are important antioxidant enzymes in trees (Li *et al.* 2019). The SOD catalyze the conversion of reactive oxygen species to H_2O_2, while POD and CAT convert H_2O_2 to H_2O and O_2 (Esposito *et al.* 2018). The antioxidant enzymes in tree leaves are reported to generally increased under drought stress, such as olive (Brito *et al.* 2019). In this study, the SOD, POD, and CAT activities in the leaves of *C. glabra* were significantly higher than control treatment under drought treatment at 8 days, indicating that *C. glabra* could resist drought stress by increasing the activities of SOD, POD, and CAT, as reported for olive (Brito *et al.* 2019). After 4 days of re-watering, the activities of SOD, POD, and CAT in *C. glabra* were significantly higher than the control, indicating that *C. glabra* still had higher antioxidant ability to resist drought stress. Likely, increase in antioxidants activities of *C. glabra* gradually increased with the deepening of stress, which was different from *L. ruthenicum* (Guo *et al.* 2018). The MDA is one of the lipid peroxidation products of cell membranes, and its content can reflect the degree of trees injury (Mohammadi *et al.* 2019). The higher MDA content in present study shows that the higher degree of damaged of *C.*

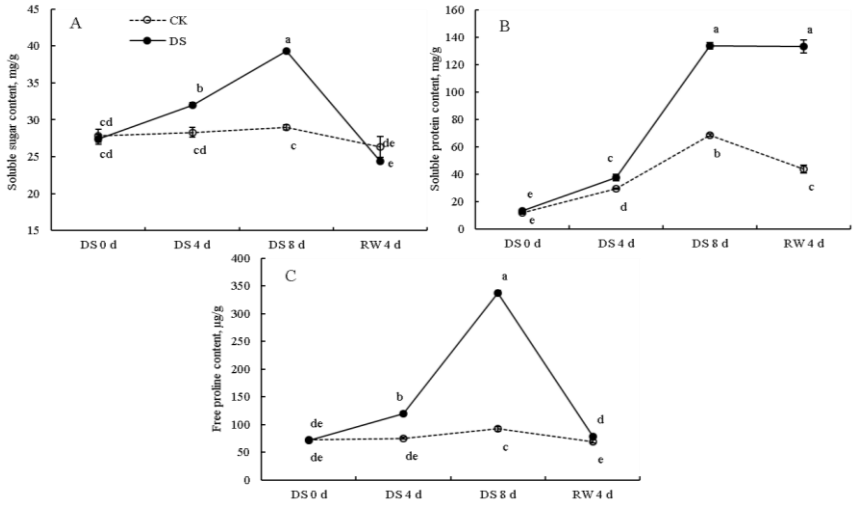

Fig. 2: Osmotic substances content in leaves of *Cercis glabra* seedlings under drought stress. CK: Control treatment, DS: Drought stress, RW: Re-watering. Data were shown as means ± SE. Different letters in the figure indicate significant differences between treatments ($P < 0.05$)

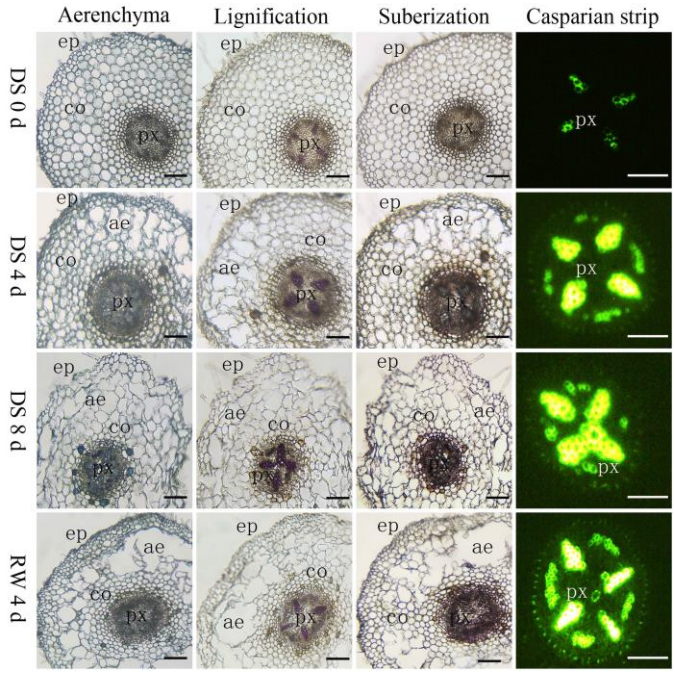

Fig. 3: Anatomical Structure of *Cercis glabra* seedlings lateral roots under drought stress.DS: Drought stress, RW: Re-watering, Ep: epidermis, Ae: aerenchyma, Co: cortex, Px: primary xylem. Ruler =50 μm

glabra under the drought stress, although the *C. glabra* scavenging ROS by increasing the activity of antioxidant enzymes, still can't remove excessive ROS and have been reported for *L. ruthenicum* (Guo *et al.* 2018). Upon re-watering, decrease in MDA content in *C. glabra* indicate the specie subjected to the relatively high degree of stress injury, which may be caused by too short re-watering time.

The soluble sugar, soluble protein, and proline are important osmotic regulators, which can maintain cell turbidity and prevent excessive plasma dehydration (Ivanov *et al.* 2019). Some plants increase these osmolytes concentration to resist drought stress, such as *H. rhamnoides* (Fernandez and Jacinto 2019). In this study, the accumulation of these osmolytes in *C. glabra* were significantly higher than the control under drought treatment at 4 and 8 days, indicating its drought resistance (Fernandez and Jacinto 2019; Han *et al.* 2019). While upon re-watering, decrease in soluble sugar and proline content of *C. glabra* decreased significantly indicate that *C. glabra* still had higher osmotic regulation ability to resist drought stress.

Aerenchyma can not only provide oxygen to plant roots in hardened soil but also transport harmful compounds such as ethanol upward (Kordyum *et al.* 2019). The Casparian strip is a lignified and suberized band thickening part of radial and transverse walls of cells (Rodriguez-Zaccaro and Goover 2019). On the one hand, it can prevent water from entering the vascular column from the cell wall and intercellular space of endodermis, and on the other hand, it can prevent the radial oxygen loss of plant roots. Some plants form aerenchyma and Casparian strips under drought stress, such as poplars and *A. marina* (Rodriguez-Zaccaro and Groover 2019). In this experiment, lateral root hair zone of *C. glabra* has formed the aerenchyma in the cortex and the Casparian strip in the endodermis under drought stress, indicating that lateral root of *C. glabra* seedlings can form aerenchyma and Casparian strip to resist drought stress, maybe because of soil hardening under drought treatment. The aerenchyma in the root hair area of the lateral roots of *C. glabra* gradually expanded, and the Casparian strip was more obvious under re-watering, possibly because the soil hardening was more serious due to watering.

Besides, lateral root endodermis not form lignification and suberization, only form the Casparian strip, which is different from red bayberry (Yang *et al.* 2011), maybe because the lignification and suberization of endodermis to a lesser degree and colors of secondary metabolites in endodermis are darker which covers red of lignification and suberization.

Conclusion

The drought stress caused damage to the physiological metabolism of *C. glabra*, and has a certain tolerance to drought stress by improving the activity of antioxidant enzyme and accumulating osmotic substances in leaves and forming aerenchyma and Casparian strip at lateral roots.

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