Response of Gas Exchange to Soil Drought in Old P. kangdingensis
Cuttings of Section Tacamahaca Spach

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Abstract. In this paper, the cutting seedlings of P. kangdingensis, which age of 300-500 years old and diameter at breast height (DBH) was greater than 1m were used as experimental materials, and the anatomical structure of the leaves and stems of seedlings were studied by the method of paraffin section. The pot experiment was conducted by way of weighing to control water content in soil, that was to weigh the pot in every afternoon and supplement the consumption of water to keep it about 60% of the maximum field water capacity, and the control was kept about 80% of that. The water control test lasted for about 3 months. The water control test lasted for about 3 months, and gas exchange parameters were measured. The results showed that under drought treatment, the intercellular CO2 concentration (Ci), stomatal conductance (Gs), transpiration rate (Tr), net photosynthetic rate (Pn), water use efficiency (WUE) and stomatal limit (Ls) decreased significantly compared with the well-watered control, and there were significant differences in Pn, Gs, Tr and Ls. Observed reductions of Pn were mainly due to reductions in stomatal conductance, indicating that apart from stomatal closure, the photosynthetic apparatus was also partly inhibited.

Introduction

Poplar is characterized by wide distribution, strong adaptability, fast growth, easy breeding and wide use, so it also is considered as a good afforestation tree for papermaking, shelterbelt and short term rotation, and it has contributed significantly to forestry production and regional ecological management [1]. However, among woody plants, poplars are among the most droughts susceptible and their productivity is associated with large water requirements. Significant variability has been observed in morphological and physiological traits that are important in drought tolerance [2]. The geographical and climatic of Southwest China are complex and diverse, so that the genetic resources of Populus Cathay are very abundant and it is one of the natural distribution centers of poplar in China. Besides, the unique historical conditions also bring rich old poplar resources, which are the most authentic native tree species since they have survived for many years under the comprehensive effects of many ecological factors and human factors, and they are the best choice for afforestation to improve the local environment [3].

A detailed examination of the dynamic responses of leaf gas exchange to water deficit in relation to drought tolerance in old poplar grown in dry and wet season clearly environments such as the Southwest China has not been done. Therefore, this experiment took the cutting seedlings of the old tree of P. kangdingensis as material, and the pot experiment was conducted by way of weighing to control water content in soil to study the effect of drought stress on the gas exchange of P. kangdingensis cutting seedlings, and then discuss the physiological mechanism of old poplar to adapt to drought in the long growth process. It would provide theoretical support for selection of tree species and improvement of ecological environment.

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Materials and Methods

Plant Material and Cultured. In this experiment, the cutting seedlings, which selected from the *P. kangdingensis* grown in Southwest China, with age of 300-500 years old and diameter at breast height (DBH) was greater than 1m and were used as experimental materials. These old poplars are growing in south-west of China. The experiment was carried out in the automatic intelligent greenhouse in Southwest Forestry University. In the middle of October 2017, the branches which no diseases and pests, and growth uniformly were selected to cut into about 20cm per segment, then soaking in water with a proper amount of rooting powder and carbendazim for 14 hours, and then planted in the pots which filled with the homogeneous red soil. The drought treatment was started when the segments sprouted. During the experiment, the pests and diseases were observed and prevented every 10 days.

Drought Treatment and Experimental Design. Drought treatment began when the plants started sprout. Uniformly sized plants from each clone were selected and randomly divided into two groups. One group was well watered (control treatment) and the other group underwent successive periods of drought preconditioning treatment. Soil water content of control and drought treatment was maintained at 80% and 60% of field water holding capacity, respectively. Pot weighing method was used to control the soil water content, that is to weigh in the afternoon and supplement the consumption of water to keep it at the water level and maintain it for about 3 months, and then started to measure the relative parameters. The experiment was set up as a completely randomized design. For convenience, plants within each watering treatment (well-watered and drought preconditioned) were randomly placed on one bench within the greenhouse. The well-watered and drought-preconditioning treatments were switched between benches and plants within each treatment randomly rotated on the benches once a week.

Gas Exchange Measurements. Gas exchange measurements were made using a CIRAS-3 (PP SYSTEMS, US). Measurements were made at saturating PPFD (1,100 μmol m⁻² s⁻¹), 26°C, CO₂ 380 μmol mol⁻¹ and greenhouse RH of approximately 40%. Photosynthetic rate (A, μmol CO₂m⁻² s⁻¹), stomatal conductance (gs, molH₂O·m⁻²·s⁻¹), intercellular CO₂ concentration (Ci, μmol mol⁻¹), water use efficiencies (WUE) and limiting value of stomata (Ls) were recorded and analyzed using an infrared gas analyzer. WUE and Ls were calculated by following equations:

\[
WUE = \frac{P_n}{T_r}
\]

\[
Ls = 1 - \frac{C_i}{C_a}
\]

\[
C_a: \text{environmental CO}_2 \text{concentration (μmol/ mol)}
\]

Statistical Analysis. Statistical analyses were performed using SPSS 10.0 software (SPSS Inc. Chicago, USA). One-way ANOVA (Bonferoni post-hoc test) at P < 0.05 was used to test whether there were significant differences for each treatment, and the results from this test are displayed as letters associated with the mean.

Results

Effects of Drought Stress on Leaf Gas Exchanges of the Old Poplar Cutting. Most Photosynthesis indices of *Tacamahaca* Spach were reduced on the effect of drought treatment. As for the net photosynthetic rate (Pn) of poplar leaves, it was decreased by 50.72% under drought stress, showing significant difference between control and drought treatment (P<0.05) (Fig. 1A).

Stomatal conductance indicates the degree of stomatal opening, affecting photosynthesis, respiration and transpiration. Stomata can adjust its own opening size according to the changes of environmental conditions and make the plant obtain the most CO₂ and loss less water. As shown in Fig. 1B, under drought stress, the stomatal conductance of poplar leaves decreased by 39.24% compared with the control. The difference reached a significant level (P<0.05), indicating that the leaves of the poplar seedlings could reduce the limited water transpiration and maintain their own growth by inhibiting the opening of the stomata.
Transpiration rate (Tr) is also known as transpiration intensity. As showed in Fig. 1C that transpiration rate of poplar leaves decreased by 84.5% under drought stress, and the difference was very significant (P<0.05). The change trend of Gs and Tr showed that the transpiration rate was mainly determined by the stomatal conductance (Gs), when the stomatal conductance (Gs) was bigger, and the transpiration was faster. Therefore, once the plant suffered stress, the stomatal conductance would be closed, and then the transpiration rate was reduced, so that the water loss of leaves was diminuend of soil water evaporation.

Intercellular CO$_2$ concentration (Ci) refers to the concentration of CO$_2$ in cells of leaves. The main factors affecting were stomatal conductance (Gs), the stomatal opening degree, and the CO$_2$ external source. As shown in Fig. 1E, compare with control, the CO$_2$ concentration in the leaves decreased by 23.53% but the difference was not significantly (Fig. 1E), which may be the result of the decrease of Gs. Water use efficiency (WUE) and stomata limiting value (Ls) showed the same change trend under drought stress (Fig. 1D, F).

**Figure 1.** Finite Effects of drought stress on leaf gas exchange of old poplar cutting

Different letters represent significant differences (p<0.05)

**Discussion**

It is a well-established fact that photosynthesis is closely related to growth and production in most plants, and plant responses and adaptation to biotic and abiotic stresses is reflected in changes in their gas exchange [4, 5]. Therefore, gas exchange measurements made in this study permitted
estimation of various parameters of photosynthesis.

Stomata play a significant role in growth and survival in environments with high evaporative demands or low soil water conditions. Under water-deficit conditions, stomatal closure is a short-term mechanism preventing leaves and shoots from reaching damagingly low water content in most plant species since plant biomass accumulation is dependent on the rate and duration of CO2 assimilation, by regulating water loss and carbon gain, the stomata effectively regulates plant productivity. Thus, declines in net photosynthesis (Pn) parallel declines in gs under drought conditions. Stomatal limitation of Pn is accepted as one of the main limitations to plant productivity in dry-land ecosystems [6].

In present study, the net photosynthetic rate (Pn) of cuttings from old P. kangdingensis decreased significantly under drought stress, and the response of Gs, Ci, WUE, Ls and Ci to water stress was generally similar to the pattern displayed by Pn. However, under water stress, the relative reduction in Pn and Ls was more than that in other factors. Stomatal closure is one aspect in the whole response of the plant to drought, which includes also morphogenetic and metabolic processes. Observed reductions of Pn in the present study were mainly due to reductions in stomatal conductance (fig.1), indicating that apart from stomatal closure, the photosynthetic apparatus was also partly inhibited. Some studies reported consistent measurements of increased Ci of cowpea under drought [7, 8]. The Ci values we have reported here were also found to be consistent in the experiment. However, some studies have shown that because of non-uniform stomatal closure during drought, Ci may be over estimated under ambient CO2 concentration. Other studies have also shown that the decline of CO2 uptake could be dependent on stomatal closure and on non-stomatal components [9, 10].

Acknowledgments

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Reference

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