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The Physiological Mechanism of *Populus euphratica* Adapting to Drought Stress

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Abstract

P. euphratica is well known for its high drought tolerance. It is the few tree species that forms natural forests in the semiarid area, which mainly distributes in Takla Makan Desert of Xinjiang in China. In order to study the mechanism of drought tolerance of *P. euphratica*, we investigated, sampled and analyzed the trees in Takla Makan Desert of Xinjiang. The results showed that the stomatic resistance increased as well as transpiration decreased in leaves of *P. euphratica* under drought stress. Compared with control, under serious drought stress the contents of K^+ and Ca^{2+} increased 0.43 and 1.99 times, respectively, but there is no change in the contents of Mg^{2+} and Na^+. The proton-pumping activities of plasma membrane bound H^+-ATPases hardly differed between leaves from control and middle drought stress grown *P. euphratica* plants. However, under serious drought stress the proton-pumping activities decreased. These results indicating that *P. euphratica* adapts to drought stress by various way, such as decreasing transpiration, absorbing ion selectively, reducing ATP consuming.

Keywords: *Populus euphratica*, Drought stress, Plasma membrane H^+-ATPase, Transpiration, Ion contents

1. Introduction

Drought was a mainly problem to the plants, more than 1/3 of lands are affected worldwide by drought stress. The limitation of growth and productivity of most plant species by drought outweighs other environmental stresses [1]. There was about 2/3 arid and semiarid area in China, especially in northwest, drought was a main factor that influenced the growth of forestry. Furthermore, the desert spread was speeded by drought, about 3.857×105 km^2 salt-affected soils in north China [2].

Euphratica, one of the oldest species of *Populus* in *Salicaceae*, is the sole species of the genus naturally growing in desert. Its distribution ranges from the northern Africa, Middle East to the northwest of China [3, 4]. The total area of *P. euphratica* forestry is about 64.87×10^4 hm^2 in world, 90% of which distributes in China and middle part of Asia [5]. Distribution of *P. euphratica* is mainly in Takla Makan Desert of Xinjiang in China [6, 7]. Severe environmental stresses in these semiarid areas, mainly drought and salinity, limit growth and productivity of most plant species [8].

Chen et al found that *P. euphratica* increased the proline content under drought stress [9]. Although *P. euphratica* showed the ability for drought tolerance, but its growth is correlated to environmental water condition [10]. *P. euphratica* displayed a rise of ABA content with the decrease of water table [11]. In suspension culture, compared with control (without PEG) the growth of *P. euphratica* cell showed no change in the medium containing 25% (W/W) PEG indicating its ability for drought stress [4]. The physiological background underlying drought tolerance of *P. euphratica* is still poorly understood. The aim of this study was, to gain new information about putative strategies to cope with drought stress. In the present study we investigated changes in plasma membrane H^+-ATPase activity, transpiration and various ions content in *P. euphratica* in Takla Makan Desert of Xinjiang in China in response to drought stress.

2. Materials and Methods

2.1. Plant materials

Mature *P. euphratica* trees (Figure 1), naturally growing along the Takla Makan Desert of Xinjiang in China, control (41°10'19"N, 84°14'03"E, 914 m elevation), middle stress (40°58'55"N, 84°14'42"E, 917 m elevation) and serious stress samples (40°46'31"N, 88°17'33"E, 923 m elevation) were sampled in May 2005. The sampled plants were 6-8 m in height, healthy and free of infections. Leaves were harvested within four different distances from the ground, placed in pre-wetted cotton gauze and subsequently placed on ice and stored at -80 °C until further analysis.

Figure 1. Pictures showing the sampling trees of control (A), middle drought stress (B) and serious drought stress (C).

2.2 Transpiration and stomatic resistance measurement

The transpiration of *P. euphratica* leaves was measured using Li-1600 apparatus. The sample trees were described as above.

2.3 Leaves ion contents measurement

Ions content was quantitated using AAS (Shimadzu, Kyoto, Japan). The collected 0.5g leaves were added to 5 ml of a HNO3 / HClO4 (5:1) solution. Afterwards, they were heated at 300 °C until white smoke got visible. Finally, the digestion solution was diluted in 5 ml of distilled water for ions measurement.

2.4 Plasma membrane isolation and purification

Plasma membrane vesicles were prepared from the *P. euphratica* leaves using aqueous two-phase partitioning according to the method described by Palmgren et al. [12] and Larsson et al. [13]. All procedures were carried out at 4 °C. The resulting plasma membrane pellets were immediately frozen in liquid nitrogen and stored at –80 °C.

2.5 Plasma membrane H^+-ATPase hydrolytic and proton pumping activity

ATP hydrolytic and proton pumping assays were carried as described in Yan et al. [14].

2.6 Protein quantification

Protein content was determined by the dye-binding method of Bradford [15] using bovine serum albumin as a standard.

2.7 Data analysis

Significant differences between samples were calculated by using the Student's t test. Variation is indicated by ± SD.

3. Results and Discussion

3.1 Description of sampling area

The study areas belong to the warm temperate zone displaying a dry continental climate. The mean annual precipitation is 65.6 mm while the annual potential evaporation is 2024 mm. The air temperature has large daily fluctuations with an annual maximum temperature of 41 °C and the minimum being as low as –25 °C. The northwest blows frequently throughout the entire year. The relative air humidity usually does not exceed 50%. From above pictures we can seen that there are many kinds of plant species existing in the control sampling area, but few other plants in the middle and serious drought stress area, indicating *P. euphratica* is a relative drought tolerant plant.

3.2 Transpiration and stomatic resistance analysis

Pore plays an important role in the plant metabolism, such as photosynthesis and transpiration [16]. The factors including light intensity, transpiration and soil water, especially soil water content which affect the transpiration [17].

In the present study we investigated the changes of transpiration and stomatic resistance of *P. euphratica* to cope with drought stress. Our results demonstrate that under drought stress stomatic resistance increased (Figure 2B) as well as transpiration decreased (Figure 2A) in leaves of *P. euphratica*. Obviously, *P. euphratica* can adapt drought stress via adjusting the stomatic resistance and reducing water loss.

Fig.2 Transpiration (A) and stomatic resistance (B) of control and drought stress samples.

3.3 Effects of drought stress on the ion contents of *P. euphratica* leaves

An additional osmosis stress was imposed upon the *P. euphratica* trees under drought stress. To prevent osmosis stress, inorganic ions or organic compound are accumulated in plants [18-20]. In order to study the effect of drought stress on the ions content, we investigated several major ions between control and drought stressed *P. euphratica* leaves. No difference of Na^+ and Mg^{2+} could be measured in the control and salt-alkali stress leaves (Figure 3A, D). When exposed to serious drought stress conditions, a slight increase of K^+ was observed in the *P. euphratica* leaves (Figure 3B). In contrast, there was an obvious increase of Ca^{2+} content under middle and serious drought stress conditions (Figure 3C). These results demonstrate that some kinds of ions can be absorbed selectively in *P.* euph*ratica* leaves under drought stress

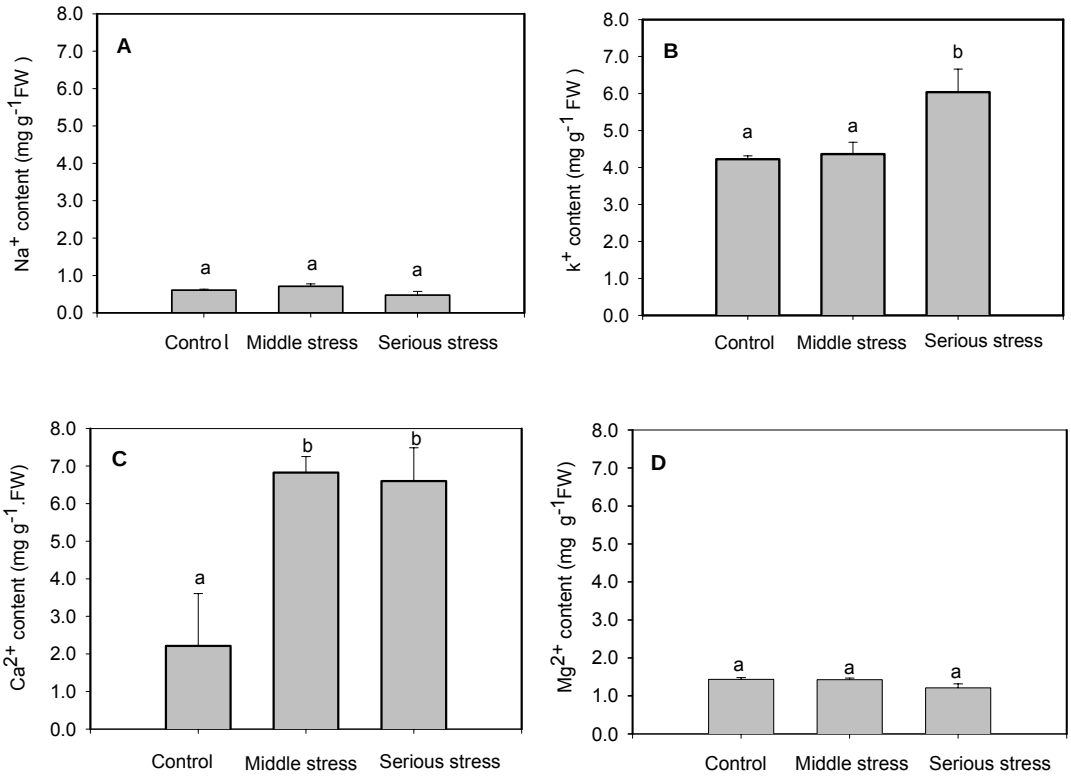

Fig. 3 Contents of Na^+ (A), K^+ (B), Ca^{2+} (C) and Mg^{2+} (D) in *P. euphratica* leaves

3.4 Plasma membrane vesicles purity examination

For H^+-ATPase assay, plasma membrane vesicles were isolated and purified from *P. euphratica* leaves using aqueous two-phase partitioning. To examine

the purity of these vesicles preparations, the H^+-ATPase hydrolytic activity was assayed in the absence and presence of a number of inhibitors. As can be seen in Table 1, the H^+-ATPase hydrolytic activity was reduced by about 95% in the presence of 1 mM vanadate (Na_3VO_4, an inhibitor of the plasma membrane H^+-ATPase). On the other hand, the H^+-ATPase hydrolytic activity showed a negligible sensitivity to azide (NaN_3, an inhibitor of mitochondrial ATPase), nitrate (KNO_3, an inhibitor of vacuolar ATPases) and molybdate ($(NH_4)_2MO_4$, an inhibitor of nonspecific phosphatases). These results demonstrate that preparations are enriched in plasma membranes vesicles without any significant contamination from other cellular membranes.

Table 1. Effects of different inhibitors on H^+-ATPase hydrolytic activity.

Treatment	Total hydrolytic activity (nM Pi min^{-1} mg^{-1} protein)	Relative activity (%)	Investigation of
Inhibitor (0 mM) + Brij 58 (0.05 %)	412.38 ± 13.55 B	100	Basic activities
Na_3VO_4 (1 mM) + Brij 58 (0.05 %)	21.09 ± 3.69 A	5.11	P-ATPase inhibition
KNO_3 (50 mM) + Brij 58 (0.05 %)	405.18 ± 20.39 B	98.25	V-ATPase inhibition
NaN_3 (1 mM) + Brij 58 (0.05 %)	406.37 ± 21.43 B	98.54	F-ATPase inhibition
$(NH_4)_2MO_4$ (0.1 mM) + Brij 58 (0.05 %)	410.22 ± 19.46 B	99.48	Phosphatase inhibition

Inhibitors such as NaN_3, KNO_3 and $(NH_4)_2MO_4$ were added to the incubation medium to test the contamination of endoplasmic membrane and soluble phosphate enzymes, respectively. The azide-and nitrate-sensitive ATPase hydrolytic activities were analyzed at pH 7.5. Vanadate-sensitive ATPase activity was measured at pH 6.5. Changes due to the inhibitor in ATPase hydrolytic activity of membrane vesicles was calculated based on the activity of the boiled-membrane control at each assay pH. Values are means of five replicates ± SD. Significant differences for P≤0.01 are marked by different letters.

3.5 Plasma membrane H^+-ATPase hydrolytic activity

Previous studies showed that the plasma membrane H^+-ATPase activity was decreased under water stress [21]. Gong et al. [22] reported that the H^+-ATPase activity was reduced in leaves of wheat under drought stress. The activity was changed in the callus of wheat after PEG6000 treatment [23]. The plasma membrane H^+-ATPase acts as a primary transporter hydrolyzing ATP and pumping protons out of the cell. Changes of hydrolytic activities in control and drought stress *P. euphratica* leaves were investigated. Results from our experiments showed that the plasma membrane H^+-ATPase hydrolytic activity was increased by about 21% in the middle drought stress leaves, but decreased about 25% under serious drought stress (Table 2).

Table 2. Plasma membrane H^+-ATPase hydrolytic activity of control and drought stress leaves.

Samples	Total hydrolytic activity (nM Pi min^{-1} mg^{-1} protein)	Relative activity (%)
Control	406.72 ± 15.36 b	100.00
Middle drought stress	492.67 ± 23.78 c	121.13
Serious drought stress	308.63 ± 26.26 a	75.88

H^+-ATPase hydrolytic activity of membrane vesicles was calculated based on the activity of the boiled-membrane control at pH 6.5. Values are means of five replicates ± SD. Significant differences for P≤0.05 are marked by different letters.

3.4 Plasma membrane H^+-ATPase proton pumping activity

H^+-pumping activity of these membrane vesicles was characterized to demonstrate their proton transport competence. In Figure 4, after initiation of H^+-pumping by adding Mg^{2+} and ATP, there was a rapid quenching in the vesicles. As expected, this established pH gradient completely collapsed after addition of the protonphore CCCP. Nearly no difference was observed between the control and middle drought stress leaves vesicles, but decreased obviously under serious drought stress.

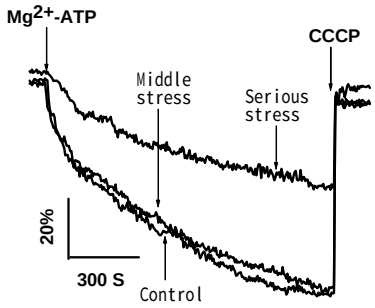

Figure 4. Changes of H^+-pumping activity measured in plasma membrane vesicles using acidine orange (AO) as the pH-probe. ΔpH was established by the activity of the plasma membrane H^+-ATPase and was measured as a decrease (quench) in fluorescence of the pH-sensitive probe AO. The reaction was initiated by adding 20 µl of a 5 mM mixture of MgSO4 and ATP (pH 6.5), subsequently incubated at 25 °C for 4 min and finally stopped by adding 10 µl of 2 mM CCCP, a protonophore.

4. Conclusion

The physiology mechanism of *P.* euphratica responses against drought stress can be found that are defined by a range of adaptations. In the present study we investigated changes of some physiologies in *P. euphratica* leaves in response to drought stress. We show that *P. euphratica* adapts to drought stress can by decreasing transpiration, absorbing ion selectively, reducing ATP consuming.

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