Photosynthesis and Chlorophyll *a* Fluorescence of Two Poplars under Water Stress

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In order to estimate drought tolerance in two species of *Populus*, *Populus alba* var. *pyramidalis* Bunge and *Populus nigra* L. var. *thevestina* (Dode), widely planted at the southern margin of the Taklimakan Desert, responses of net photosynthesis and chlorophyll *a* fluorescence to irradiance and water stress were examined under laboratory conditions. Results showed that *P. alba* exhibited stronger drought tolerance than *P. nigra*. A linear relationship between net photosynthetic rates (A_n) and electron transport rates (ETR) was found in both poplars under different irradiance and leaf water potentials. Net photosynthetic rates (A_n) in the two poplars significantly correlated linearly with the photochemical efficiency of the saturation light-adapted leaves throughout the range of leaf water potentials, suggesting that the leaf photochemical efficiency in saturation light-adapted leaves can be used to estimate leaf photosynthetic capacity and leaf water conditions in the two poplars within a magnitude of air temperature between 20 and 30°C.

Key words: chlorophyll *a* fluorescence, photochemical efficiency, photosynthesis, *Populus*, water stress

Populus alba var. *pyramidalis* Bunge and *Populus nigra* L. var. *thevestina* (Dode) are the most important poplar cultivars in the Xinjiang Region of northwestern China. The two poplars are widely used to establish windbreaks in the Xinjiang Region, which is located in the central area of the Eurasian continent, and is far away from oceans in every direction. The climate is characterized by very dry air and low precipitation. The growth of two poplar trees completely depends on the level of irrigation. We have observed the phenomenon in field that *P. alba* seemingly is of stronger drought tolerance than *P. nigra*, but until now, there has been no physiological evidence on the drought tolerance of the two species of poplars reported in the literature. It is of an important practical role to understand the effects of photosynthesis of the two poplars on water stress.

Plants face various environmental stresses in their distribution areas (Larcher, 1995). However, water stress is one of the most important stress factors to plant growth in arid and semi-arid areas. Although many studies on gas exchange characteristics and water relations of the main poplar cultivars under water stress have been done in North America and Europe (Schulte *et al.*, 1987; Bassman and Zwier, 1991; Dickmann *et al.*, 1992; Rhodenbaugh and Pallardy, 1993; Ridolfi and Dreyer, 1997) and in China (Liu *et al.*, 1993; Chen *et al.*, 1996a, b), most studies were carried out on poplars in humid and semi-humid regions of the world. We are not aware of any study on the photosynthesis and chlorophyll *a* fluorescence of the two poplar clones under either laboratory or field conditions.

This study associated gas exchange measurement with chlorophyll *a* fluorescence measurement to assess the response of photosynthesis to water stress in the two poplars. The objectives of this study were to: (1) assess responses of net photosynthetic rates and the photochemical efficiencies in *P. alba* and *P. nigra* to different leaf water potentials and drought

tolerance in *P. alba* and *P. nigra*; (2) compare the relationships of parameters between gas exchange measurement and chlorophyll *a* fluorescence measurement in *P. alba* and *P. nigra* under different water stress.

Materials and Methods

1 Plant materials

In early March 1998, hardwood cuttings (20 cm length, 5–8 mm diameter) of *P. alba* and *P. nigra* clones were selected from seedlings shoots grown in a greenhouse of the Silviculture Institute, Faculty of Agriculture, Shizuoka University (34°58'N, 138°24'E), Japan. The origin hardwood cuttings (20 mm length, 5–8 mm diameter) of *P. alba* and *P. nigra* clones were obtained in early March 1997 from the Cele Desert Research Station (38°22'N, 86°12'E), Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, located at the southern margin of the Taklimakan Desert. These hardwood cuttings were planted in 15-liter pots (0.05 m² × 0.3 m) filled with clay loam soil, cultured in a greenhouse under natural light, at the Silviculture Institute, Faculty of Agriculture, Shizuoka University, Japan, and kept well-watered and fertilized with a commercial nutrient solution (N : P : K = 5 : 10 : 5, Hyponex solution, Japan). Each pot was planted with one hardwood cutting. As seedlings grew for 6 months, reaching height of about 60–80 cm, leaf gas exchange and chlorophyll *a* fluorescence measurements were conducted. Four individuals with similar growth conditions for each poplar were selected to take measurements. The induction of plant water stress was undertaken by withholding irrigation. Each individual first measurement started after irrigation; then as irrigation was withheld, a series of measurements were conducted in the following days until the plant became permanently wilted. All experiments were conducted from September to early October 1998, in the Laboratory of Silviculture, Faculty of Agriculture, Shizuoka University, Japan.

There are two types of leaves in the adult trees of *P. alba*,

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long-shoot and short-shoot leaves, but one-year-old seedlings grow only long-shoot leaves. Therefore, our plant materials were long-shoot leaves of *P. alba* and the general leaves of *P. nigra*.

2 Methods

A Mini-cuvette gas exchange system (CMS-400, Heinz Walz GmbH, Effeltrich, Germany) was used to measure the photosynthesis of the leaves. The light source was composed of a fiber illuminator (FL-400, Heinz Walz GmbH, Effeltrich, Germany) and special fiber optics (400-F, Heinz Walz GmbH, Effeltrich, Germany). Portions of leaves of *P. alba* and *P. nigra* were enclosed in the gas exchange cuvette, in which air temperature, relative air humidity and light intensity were controlled. The portion of leaf was traced on paper before gas exchange measurements, and the traced paper area was determined with the Delta-T Image Analysis (Dias, Delta-T Devices, Ltd., England).

Leaf water potential (ψ) was measured by a H-33T microvoltmeter with C-52 Chamber (Wescor, Inc., Logan, Utah, USA). Three discs replicates per leaf sample were used to determine leaf water potential. All experiments of gas exchange were measured at an air temperature of 25°C, ambient CO₂ concentration of about 350 $\mu\text{mol mol}^{-1}$ and air to leaf vapor pressure deficit (VPD) of 8–9 hPa (about 70% relative air humidity). The saturating irradiance was over 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The measurements of chlorophyll *a* fluorescence of leaves were made with a portable pulse-modulated chlorophyll fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). Most measurements of gas exchange and chlorophyll *a* fluorescence were conducted at the same time by fixing the fibre optic cable in the cuvette of Mini-PAM photosynthesis measuring system, while some measurements of chlorophyll *a* fluorescence were made alone. The photochemical yield was termed as $(F_m' - F)/F_m'$ or $\Delta F/F_m'$; photosynthetic electron rates (ETR, $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$) were calculated according to the formula: $\text{ETR} = 0.84 \times 0.5 \times \text{PPFD} \times (F_m' - F)/F_m'$; and non-photochemical quenching was termed as $\text{NPQ} = F_m/(F_m' - 1)$. The definition of every parameter is described in Mini-PAM handbook of operation (Heinz Walz GmbH, Germany, Edition 1, 1996), Bjorkman and Demmig-Adams (1994), White and Critchley (1999). In all experiments, leaf dark-adapted time was over half of an hour.

Results

Response curves of the net photosynthetic rates to different leaf water potentials were obviously different (Fig. 1) in the two poplars, and among different individuals in the same poplar. In order to maintain more information on response of photosynthesis of *P. alba* and *P. nigra* to different leaf water potentials, the light response curves of two poplars under different leaf water potentials were completely shown in Fig. 1. However, as the light response curves (*A/Q*) of *P. alba* under leaf water potentials between -1.5 and -2.5 MPa could not be captured in our experiment, the light response

Fig. 1 Light response of net photosynthetic rates (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) of (a) *P. alba* and (b) *P. nigra* at various leaf water potentials (-MPa) along different PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements were performed at 25°C, 70% of relative air humidity and ambient CO₂ concentration for potted plants. The leaf water stress was induced by withholding water.

Fig. 2 Electron transport rates (ETR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of (a) *P. alba* and (b) *P. nigra*, Non-photochemical quenching (NPQ) of (c) *P. alba* and (d) *P. nigra* at various leaf water potentials (-MPa) under different PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements were performed at 25°C, 70% of relative air humidity and ambient CO₂ concentration for potted plants. The leaf water stress was induced by withholding water. Small bar denotes the standard error (SE). Some SEs are too small to display.

curves (*A/Q*) of *P. alba* were mainly measured under good water condition and under water stress (Fig. 1a). The light response curves (*A/Q*) of *P. nigra* were captured under well-distributed leaf water potentials from -0.99 to -1.72 MPa

Fig. 3 Net photosynthetic rates (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) of (a) *P. alba* and (b) *P. nigra*, at various leaf water potentials (-MPa) under saturating PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Photochemical efficiency ($\Delta F/Fm'$) of (c) *P. alba* and (d) *P. nigra* at various leaf water potentials for dark-adapted leaves (Δ , Yield-d) and saturating light-adapted leaves ($\square$, Yield-s). Measurements were performed at an air temperature of 25°C, 70% of relative air humidity and ambient CO_2 concentration for potted plants. The leaf water stress was induced by withholding water.

(Fig. 1b). The A_n of *P. nigra* declined to near zero as leaf water potentials decreased to about $-1.7 \sim -1.8$ MPa, whereas A_n of *P. alba* declined to near zero until leaf water potentials probably reached about -3.0 MPa. As leaf water potentials in *P. alba* were -2.54 ± 0.18 MPa, the A_n at saturating light was $3\text{--}4 \mu\text{mol m}^{-2} \text{s}^{-1}$; as leaf water potentials in *P. nigra* were -1.72 ± 0.26 MPa, the A_n at saturating light was $5\text{--}6 \mu\text{mol m}^{-2} \text{s}^{-1}$ respectively.

The photosynthetic electron transport rates (ETR) showed

Fig. 4 Relationships between net photosynthetic rates (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and photochemical efficiency ($\Delta F/Fm'$) under saturation PPFD in (a) *P. alba* and (b) *P. nigra*. Measurements were performed at an air temperature of 25°C, 70% of relative air humidity and ambient CO_2 concentration for potted plants.

significant differences (t -test, $p < 0.05$) for the two poplars under different leaf water potentials (Fig. 2 a,b). Higher leaf water potentials responded with higher ETR values (Fig. 2 a,b). There were large differences for non-photochemical quenching (NPQ) in the two poplars under different leaf water stress (Fig. 2 c, d). Higher leaf water potentials responded with lower NPQ, whereas lower leaf water potentials responded with higher NPQ. In both poplars, NPQ increased with the increase of PPFD under different leaf water potentials (Fig. 2 c,d).

Figure 3 (a, b) shows the relationships between values of A_n and leaf water potentials of two poplars under saturating light. The A_n of *P. nigra* showed a sharp decline when leaf water stress decreased near -1.5 MPa. The A_n in the leaves of *P. alba* decreased smoothly with the decline of leaf water potentials, compared with leaves of *P. nigra*. The photochemical efficiency ($\Delta F/Fm'$) of dark-adapted leaves under different leaf water potentials was similar for the both poplars, and showed slight decreases with decreasing leaf water potentials (Fig. 3 c, d). But the photochemical efficiency ($\Delta F/Fm'$) of saturation light-adapted leaf decreased significantly with the decrease of leaf water potentials (Fig. 3 c, d).

There were positive linear relationships between A_n and photochemical efficiency under saturation light for the two poplars (Fig. 4 a, b). The slopes of regression equations were different for the two poplars, and the slope value (Fig. 4 a) of *P. alba* was lower than that of *P. nigra* (Fig. 4 b).

$$A_n(Pa) = 0.0869x + 0.4436 \quad A_n(Pn) = 0.1079x - 0.3878$$

$$R^2 = 0.9826, P < 0.01 \quad R^2 = 0.9657, P < 0.01$$

$$A_n(Pa) = 0.0591x + 1.4336 \quad A_n(Pn) = 0.0978x - 4.3071$$

$$R^2 = 0.6318, P < 0.01 \quad R^2 = 0.7349, P < 0.01$$

Fig. 5 Relationships between net photosynthetic rates (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and electron transport rates (ETR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). (a) Relationships between the net photosynthetic rates (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and ETR of *P. alba* (Pa) and *P. nigra* (Pn) under good water conditions and different PPFD; (b) Relationships between net photosynthetic rates (A_n (sat. light), $\mu\text{mol m}^{-2} \text{s}^{-1}$) and ETR of *P. alba* (Pa) and *P. nigra* (Pn) under different leaf water potentials (including good water condition and water stress) and saturation PPFD. Measurements were performed at 25°C, 70% of relative air humidity and ambient CO_2 concentration for potted plants.

Leaf net photosynthetic rates (A_n) linearly correlated with electron transport rates (ETR) in both poplars under different PPFD and leaf water potentials (Fig. 5a, b). Figure 5a shows the relationships between A_n and ETR in both poplars under good water conditions and different PPFD, whereas Fig. 5b shows the relationships between A_n and ETR in both poplars under saturation PPFD and different leaf water potentials. Under good water conditions and different PPFD, the ratios of A_n to ETR were higher than that under saturation PPFD and different leaf water potentials in both poplars (Fig. 5a, b).

Discussion

Rhodonbaugh and Pallardy (1993) reported that net photosynthetic rate declined steeply as leaf water potentials declined below -1.0 MPa, and net photosynthetic rates approached zero when leaf water potentials reached about -1.5 MPa for three *Populus* clones. Bassman and Zwier (1991) reported that five poplar clones decreased sharply in net photosynthetic rate with a decrease of xylem pressure potentials, and irreversible wilting appeared between $-1.2 \sim -2.4$ MPa. Our results showed that A_n decreased sharply when ψ_1

reached about -1.5 MPa in *P. nigra* (Figs. 1b, 3b), whereas the decrease of A_n in *P. alba* was not so steep with the decrease of leaf water potentials. *P. alba* can maintain a certain degree of photosynthesis under water stress (Figs. 1a, 3a), which reflected a good adaptation of *P. alba* to arid environments. However, the findings indicated the two poplars have a good drought tolerance relatively, compared with results in literature (Rhodonbaugh and Pallardy, 1993; Bassman and Zwier, 1991), because the original environment of the two poplars is an arid region. *P. alba* showed stronger drought tolerance than *P. nigra*, whose findings were consistent with the phenomenon observed in the field survey.

Ishida *et al.* (1999) showed that a tropical tree exhibited higher A_n in early morning than at midday at a given ETR, suggesting a high rate of photorespiration at midday. Our results showed that the ratios of A_n to ETR in both poplars under different water conditions and saturation PPFD (above $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$, Fig. 5b) were less than those in both poplars under good water conditions and different PPFD (Fig. 5a), which showed the photorespiration rate is higher in both poplars under saturation PPFD than under different PPFD (from low PPFD changed gradually to saturation PPFD). Our results proved that the suggestion of Ishida *et al.* (1999) is appropriate to the two poplars in our study.

Flexas *et al.* (1999) reported that grapevines showed good correspondence between A_n and ETR under good water conditions and mild water stress, but severe water stress induced drastic down-regulation of photosynthesis and broke the above correspondence. Our results, through, showed a good linear relationship between A_n and ETR in both poplars throughout different PPFD and leaf water potentials (Fig. 5 a,b). Ishida *et al.* (1999) also showed that low A_n was the result of both a reduction in the photochemical process and an increase in stomatal limitation. Another of our experiments (submitted to another journal for review) showed that stomatal conductance was lower for air temperatures below 20°C and above 30°C than air temperatures between 20 and 30°C , whereas the two poplars exhibited similar stomatal conductance for air temperatures between 20 and 30°C . Although ratios of A_n to ETR in both poplars were different under different leaf water potentials and PPFD, the linear relationships between A_n and ETR existed in our results (Fig. 5 a, b). Therefore, we suggest that the leaf photochemical efficiency can be used to estimate quantitatively leaf photosynthetic capacity and leaf water condition in the two poplars in the range of air temperatures between 20 and 30°C .

Massacci and Jones (1990) reported that long-term water stress had a marked effect on both gas exchange and chlorophyll fluorescence quenching. Bjorkman and Dimmig-Adams (1994) suggested that the increase of NPQ induced by non-radiative dissipation was a photo-protective process. There were large variations of NPQ between well-watered leaves and water-stressed leaves in the two poplar clones (Fig. 2 c, d). NPQ in water-stressed leaves increased more considerably than in well-watered leaves with the increase of irradiance. At the approximate light saturation point of photosynthesis of

both poplars, the ETR and NPQ between well-watered and water-stressed leaves showed maximal differences in both poplars (Fig. 2), suggesting the ETR and NPQ values could be used to estimate the water conditions of the two poplars. At similar water stress levels (-1.72 and -1.78 MPa), the NPQ value of *P. alba* and *P. nigra* showed a relative large difference under saturation irradiance, and NPQ of *P. nigra* was higher than NPQ of *P. alba*, compared with the NPQ values at good water conditions (Fig. 2 c, d), suggesting that drought tolerance of *P. alba* is stronger than that of *P. nigra*.

Photochemical efficiencies in dark-adapted and saturation light-adapted leaves of the two poplars showed significant differences across a gradient of leaf water potentials (Fig. 3 c,d). Lu and Zhang (1999) reported that water stress shows no effect on the maximal quantum yield (dark-adapted) of PS II photochemistry. Our results showed that photochemical efficiency in dark-adapted leaves of the two poplars was stable relatively along gradients of leaf water potentials, but the leaf photochemical efficiency under saturation light decreased with the decrease of leaf water potentials (Fig. 3 c, d). These findings indicate that maximal quantum yield (dark-adapted leaves) can not be used to estimate plant leaf water conditions and photosynthetic capacity, but the photochemical efficiency in saturation light-adapted leaves can be used to estimate the leaf water condition. This phenomenon may be common in many kinds of plants.

Net photosynthetic rates (A_n) in the two poplars linearly correlated with photochemical efficiency in the saturation light-adapted leaves under different leaf water potentials (Fig. 4 a, b). These linear relations indicated that measurement of photochemical efficiency in saturation light-adapted leaves can be used to estimate A_n . Because these results were measured at an air temperature of 25°C in our experiments, and there was similar stomatal conductance in both poplars at air temperatures between 20 and 30°C (mentioned in the above paragraph), therefore, we suggest that the photochemical efficiency in saturation light-adapted leaves can be used to estimate leaf photosynthetic capacity and leaf water condition of plants in the range of air temperatures between 20 and 30°C .

This research was supported by the Japan Society for the Promotion of Science (CAS-9601), the Chinese Academy of Sciences and partly supported by the National Natural Foundation of China (39870154). We express our appreciation to Dr. Qingmin Han for his valuable discussion

during the preparation of this manuscript. Finally, we must thank the reviewers for their valuable comments on this manuscript, which was helpful for us in improving this manuscript.

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(Accepted June 8, 2001)