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BRIEF COMMUNICATION

# Photosynthesis and chlorophyll fluorescence responses of *Populus sibirica* to water deficit in a desertification area in Mongolia

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## Abstract

In the present study, photosynthetic traits and chlorophyll (Chl) fluorescence parameters of *Populus sibirica* grown under different irrigation regimes were investigated to estimate seedling growth and vitality for reforestation of a desertification area. According to our results, photosynthesis and Chl fluorescence were significantly affected by water deficit only under severe drought conditions.

*Additional key words*: land degradation; maximum quantum yield of PSII photochemistry; nonstomatal limitation; stomatal limitation.

Because of constant and rapid desertification, land productivity has been reduced due to water deficit stress (D´Odorico 2013). Mongolia is one of the countries that suffer from desertification. Even though the Mongolian government tried to plant tree seedlings between 1980 and 1990, a reforestation rate was very low because of many reasons (Tsogtbaatar 2004). One of the reasons was that research activities in the desertificated area were too academic to be applied in the field. In addition, field managers were not able to maintain aftercare due to the limited funding (Tsogtbaatar 2004). As water deficit is considered a primary limitation for plant growth in the desertification area (Chaves *et al*. 2003), investigation of effective irrigation regimes is very important for successful reforestation in Mongolia. Our study was carried out in order to investigate photosynthetic traits and Chl fluorescence parameter responses to different intensities of water deficit in *Populus sibirica* with the aim to suggest successful reforestation programs. *P. sibirica,* native species in Mongolia, is suitable for restoration in the desertification area in Mongolia because it is a fast growing and drought-resistant species (Tsogtbaatar 2004). However, only limited studies are available on the physiological response of *P. sibirica* to water deficit.

The experimental site was located in Elsen-Tasarkhai, the arid region in Mongolia, where the average temperature is 13.4°C and the annual precipitation is 185.5 mm. Two-year-old *P. sibirica* seedlings of a uniformed size were supplied from a nursery at Lun Som in Mongolia. In total, 81 seedlings were planted on May 2014 1.5 m apart from each other. Three different irrigation regimes, such as well watered (WW, 14 L per tree per week), moderate drought (MD, 5.6 L per tree per week), and severe drought (SD, 4 L per tree per week), were applied with a drip-irrigation system. Three replicates of each treatment (nine trees per a plot) were randomly assigned at the experimental site. The different irrigation treatments were applied for four months (from May to August 2014); this period is considered as the growing season in Mongolia. The survival and relative growth rates were measured twice during the growing season. The photosynthetic traits were analyzed using *LCi* (*ADC BioScientific Ltd.*, Furlong Way, Great Amwell, UK) equipped with a broad leaf chamber. Chl fluorescence parameters were measured

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*Abbreviations*: ABS – absorption energy flux; Chl – chlorophyll; $C_i$ – intercellular $CO_2$ concentration; CS – optical cross section; $DI_0$ – dissipation energy flux; *E* – transpiration rate; ET – energy flux for electron; $F_0$ – minimal fluorescence of the dark-adapted state; $F_J$ – fluorescence of the dark-adapted state at 2 ms; $F_m$ – maximal fluorescence of the dark-adapted state; $F_v/F_m$ – maximum quantum yield of PSII photochemistry ($\varphi_{PO}$); $g_s$ – stomatal conductance; LWP – leaf water potential; MD – moderate drought; NPQ – nonphotochemical quenching; OEC – oxygen-evolving complex; Phe – pheophytin; $PI_{ABS}$ – performance index; $P_N$ – photosynthetic rate; $Q_A$ – plastoquinone A; $Q_B$ – plastoquinone B; RC – reaction center; SD – severe drought; TR – energy flux for trapping; W – relative variable fluorescence at the time t between $F_0$ and $F_J$, $W = (F_t - F_0)/(F_J - F_0)$; WUE – water-use efficiency; WW – well watered treatment.

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using a *Pocket Plant Efficiency Analyzer* (*Hansatech Instruments Ltd*., Norfolk, UK). Leaf water potential (LWP) was determined by a pressure chamber (*Skye SKPM 1400*, *Skye Instruments*, Powys, Wales, UK). In each treatment, leaf photosynthetic and Chl fluorescence parameters were measured on three replicate plants using fully expanded, sunlit leaves on sunny, cloudless days. LWP was measured before dawn on three replicate trees using the fourth fully expanded mature leaf as counted from the shoot apex. In order to minimize the errors during procedure, the sample was kept within polythene bags with moistened tissue. Statistically significant differences among the mean values were tested by analysis of variance (*ANOVA*) at $p=0.05$ using *SPSS Statistics 22* for *Windows* software (*SPSS*, Chicago, IL, USA).

According to our results, there were no significant differences in the survival rate; it was 100% for all treatments. However, the relative growth rate, LWP, photosynthesis, and Chl fluorescence were affected by the water deficit treatment. The relative growth rate decreased by 41.4 and 49.2% in the MD- and SD-stressed *P. sibirica* seedlings, respectively. As the water deficit increased, the relative growth rate significantly decreased. Many studies have suggested that relative growth rate decreases because of lowered photosynthetic $CO_2$ assimilation (Tezara *et al.* 2002, Li *et al.* 2011, Xu *et al.* 2015). LWP also significantly decreased under SD compared with WW, but there was no difference between the MD and WW treatment. As LWP is considered as a parameter to assess the severity of water deficit, our results were in agreement with other studies (Flexas and Medrano 2002, Tezara *et al.* 2002). The photosynthetic rate ($P_N$) declined under SD compared with MD and WW, whereas there was no significant difference between MD and WW (Fig. 1*A*). $P_N$ decreased significantly by 24.7% under SD. The transpiration rate (*E*) decreased with increasing water deficit (Fig. 1*B*). *E* decreased significantly by 18.1 and 42.0% under conditions of MD and SD, respectively. As a result, the water-use efficiency (WUE) increased in SD compared with that under WW. Regarding drought stress, most plants attempt to increase WUE or maintain water *via* stomatal closure that could restrict the supply of $CO_2$ for photosynthesis and is considered as a primary reason for $P_N$ reduction (Flexas *et al.* 2000, Yordanov *et al.* 2000). Furthermore, as the water deficit increased the stomatal conductance ($g_s$) slightly declined. This was consistent with results of previous studies (Flexas and Medrano 2002, Tezara *et al.* 2002, Li *et al.* 2011). The intercellular $CO_2$ concentration ($C_i$) increased significantly by 17.7% under SD conditions (Fig. 1*D*). This indicated that $P_N$ reduction was not induced by the limitation of $CO_2$ supply by stomatal closure but by inhibition of $CO_2$ fixation. Increased $C_i$ could be induced by photorespiration or by reduced activities or contents of enzymes, which are involved in the Calvin cycle during the dark reaction of photosynthesis (Yordanov *et al.* 2000, Tezara *et al.* 2002, Chaves *et al.* 2003, Galmés *et al.* 2011). Increased photorespiration was induced by limited $CO_2$ supply, and it could prevent photodamage. Otherwise, $P_N$ reduction with increased $C_i$ could result from the limitation in mesophyll conductance or the nonstomatal limitation, such as the reduced carboxylation efficiency and restricted PSII functions (Van Heerden *et al.* 2007, Wang *et al.* 2012). In our present study, Chl fluorescence parameters were used for evaluating electron transport in the light reaction of photosynthesis. Both Chl fluorescence transients (the JIP-test, Chl *a* fluorescence rise at O, J, I, and P steps) and Chl fluorescence parameters of *P. sibirica* were determined after 30 min of full dark adaptation. Polyphasic OJIP fluorescence indicates the redox reactions of plastoquinone A ($Q_A$) and plastoquinone B ($Q_B$) from PSII to PSI. The O-J phase indicates the reduction reaction of $Q_A$ from pheophytin (Phe), which is considered as the primary electron acceptor in the reaction centers (RCs). The J-I phase indicates the imbalance between reduction and reoxidation of $Q_A^-$. The I-P phase indicates rapid reduction of the plastoquinone pool around PSI (Stirbet *et al.* 1998, Kalaji *et al.* 2014). From the OJIP transient, the J-P phase decreased as the water deficit increased to SD. However, the only significant difference was observed at the P-step in SD (Fig. 2*A*). The fluorescence signal at the P-step decreased significantly by 37.3% under SD. This indicated inhibition in the reduction of PQ pool and the electron transport acceptors around PSI (Kalaji *et al.* 2014). The Chl fluorescence transient was normalized between $F_0$ and $F_J$ (at 2 ms) in order to evaluate the K-step (at about 300 µs), which is considered as a sensitive parameter, especially under drought stress (Strasser *et al.* 2004, Wang *et al.* 2012). In the normalized K-step curves, which were described as the ratio W(MD, SD)/ W(WW) = ΔW, where W is $(F_t - F_0)/(F_J - F_0)$, the K-peak was observed under SD (Fig. 2*B*). The K-peak indicated the inactivation of the oxygen-evolving complex (OEC) which resulted in the imbalance of electron flow from RCs to Phe (Strasser *et al.* 2004, Oukarroum *et al.* 2007). The maximum fluorescence signal ($F_m$) decreased significantly at SD as already observed in the OJIP transient (Fig. 2*A,C*). Reduced $F_m$ at SD indicated enhanced nonphotochemical quenching (NPQ) as heat (Yordanov *et al.* 2000). This suggested that PSII was protected from excess energy and photodamage effectively by NPQ in *P. sibirica*. The maximum quantum yield of PSII photochemistry ($\varphi_{PO} = F_v/F_m$), which is widely considered as a sensitive indicator of environmental stress, decreased significantly by 15.8% at SD (Fig. 2*C*). $F_v/F_m$ of $C_3$ plants ranges between 0.83 and 0.85 under optimal conditions, but it declines in unfavorable environment (Johnson *et al.* 1993, Van Heerden *et al.* 2007, Xu *et al.* 2010). Even though the performance index ($PI_{ABS}$) is considered as a sensitive indicator for drought stress (Oukarroum *et al.* 2007, Van Heerden *et al.* 2007, Rathod *et al.* 2011), it showed little changes in the present study. The effects of water deficit on the specific energy flux per RCs or effective absorption cross section (CS) in MD and SD were normalized with ones of WW, which was

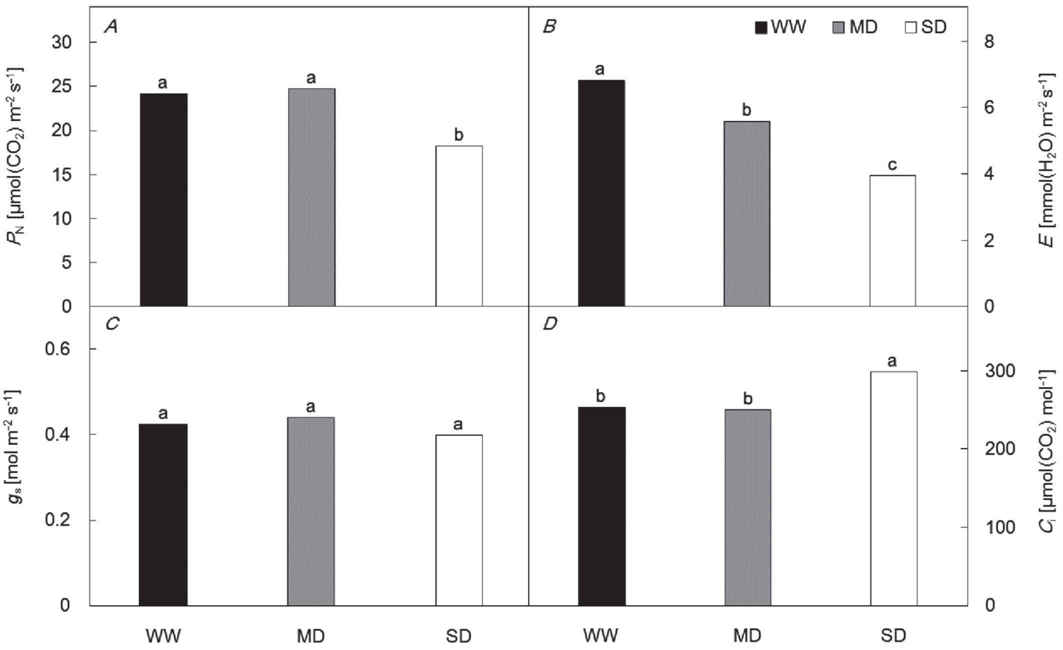

Fig. 1. Changes of net photosynthetic rate ($P_N$) (*A*), transpiration rate (*E*) (*B*), stomatal conductance ($g_s$) (*C*), and intercellular $CO_2$ contents ($C_i$) (*D*) in different drought treatments of *Populus sibirica* (*n* = 3). *Different letters* indicate significant difference ($P$<0.05). WW – well watered, MD – moderate drought, SD – severe drought.

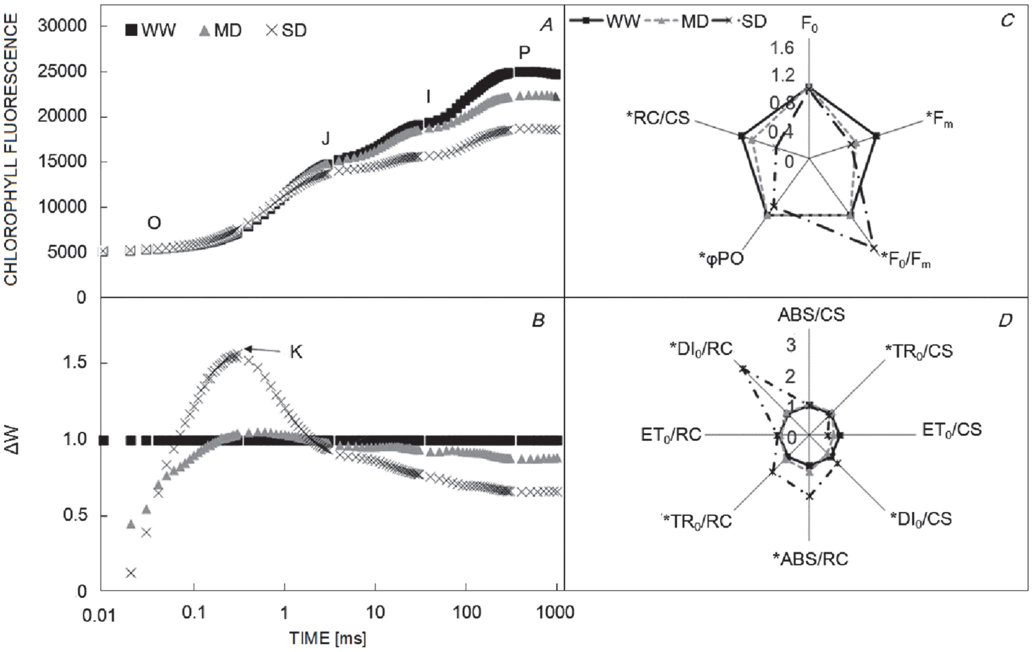

Fig. 2. The polyphasic chlorophyll fluorescence transient curves from leaves of *Populus sibirica* which is plotted on a logarithmic time scale (*A*). Changes in the amplitude of the K-step, which is expressed as the ratio ΔW = W (treatment)/W(control), W = ($F_t$ – $F_0$)/($F_J$ – $F_0$) (*B*). Effects of drought stress on $F_0$, $F_m$, $F_0/F_m$, $\varphi_{PO}$, and RC/CS in different drought treatments (*C*). The changes of the specific energy fluxes per reaction centers and per cross sections in different drought treatment (*D*). Asterisks indicate significant differences at $P$<0.05, *n* = 3. O-step – at 50 µs, K-step – at about 200 µs, J-step – at 2 ms, I-step – at 30ms, P-step – at 300 ms, WW – well watered, MD – moderate drought, SD – severe drought, $F_0$ – minimal fluorescence of the dark-adapted state, $F_m$ – maximal fluorescence of the dark-adapted state, $F_0/F_m$ – the ratio of minimal and maximum fluorescence, $\varphi_{PO}$ - the maximal quantum yield of PS photochemistry, RC/CS – active reaction center per cross section, ABS/RC – relative activities in absorption flux per reaction center, $TR_0$/RC – trapped energy flux per RC, $ET_0$/RC – electron transport flux per RC, $DI_0$/RC - dissipated energy flux per RC, ABS/CS - absorption flux per cross section, $TR_0$/CS - trapped energy flux per CS, $ET_0$/CS - electron transport flux per CS, $DI_0$/CS - dissipated energy flux per CS. WW (■) – well watered, MD (▲) – moderate drought, SD (×) – severe drought.

set as 1.0. Regarding the specific energy fluxes per CS, the ratio of trapped electrons per CS ($TR_0/CS$) decreased significantly by 8.7% at SD, and the ratio of dissipated excited energy per CS ($DI_0/CS$) increased significantly by 31.0% at SD (Fig. 2*D*). However, the ratio of electron transport per CS ($ET_0/CS$) was unchanged (Fig. 2*D*). ABS/RC, $TR_0/RC$, and $DI_0/RC$ increased significantly by 100, 69.4, and 209.5% under SD, respectively, but $ET_0/RC$ remained without changes (Fig. 2*D*). As the water deficiency increased, the number of open-state RCs in CS (RC/CS) was reduced; this is related to trapped excitation energy ($TR_0/CS$), which also decreased. Enhanced ABS/RC and $TR_0/RC$, which indicated the increasing antenna size of RCs, and increased $DI_0/CS$ and $DI_0/RC$, which indicated the dissipation of excessive energy, were acclimation responses to water deficit (Falqueto *et al.* 2010, Van Heerden *et al.* 2007). The unchanged $ET_0/RC$ was a result of compensatory mechanism of *P. sibirica* (Van Heerden *et al.* 2007). In conclusion, no differences were found between WW and MD in all parameters including the relative growth rate, LWP, photosynthesis, and Chl fluorescence, but under SD conditions, physical and physiological traits were significantly affected. However, *P. sibirica* showed the effective acclimation responses to water deficit *via* the dissipation of excessive energy. The reduced $P_N$ might be caused by inhibition of various enzyme activities in the Calvin cycle or by mesophyll limitations due to water deficit, but not by limitations of PSII activities. Therefore it is necessary to evaluate enzyme activities and their dependence on irrigation regimes to figure out the reason for reduction of photosynthesis and the relative growth rate. For reforestation, the survival rate is the most important parameter for evaluation of optimal irrigation regimes. From these results, *P. sibirica* exhibited resistance to water deficit applied in this experiment. Therefore, an irrigation pattern of 4 L per tree per week could be recommended for effective reforestation in the desertification area in Mongolia. Further studies of long-time irrigation regimes are needed in order to develop more economical and effective reforestation.

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