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Physiological responses of *Populus sibirica* to different irrigation regimes for reforestation in arid area

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ABSTRACT

This study was conducted in Elsen tasarkhai, an arid region in Mongolia, to investigate the physiological response of *Populus sibirica* to different irrigation regimes and to suggest optimal irrigation intervals for successful reforestation in Mongolia. Two-year-old *P. sibirica* seedlings were planted, and three different irrigation regimes (well-watered (WW), irrigation every 2 days; moderate drought (MD), irrigation every 5 days; severe drought (SD), irrigation every 7 days) were applied. Although the survival rate was unaffected by the applied water deficit intensity, the relative growth rate was significantly reduced in SD in accordance with a decline in carbon assimilation. Although the total chlorophyll content also decreased as the water deficit increased, the chlorophyll b (Chl b) and total carotenoid (Car T) levels increased to dissipate the excess energy. Increased quantum efficiencies in the SD condition were caused by the enlargement of the reaction center (RC) antenne, indicating increased of excess energy dissipation. Therefore, *P. sibirica* showed resistance to a certain degree of water deficit intensity (MD and SD). The irrigation regime of SD appeared to be adequate for *P. sibirica* based on survival rate and physiological traits. These results might enlighten the development of effective irrigation systems in arid area.

1. Introduction

Desertification in Mongolia has proceeded rapidly; 72% of Mongolia territory is in danger of desertification, and more than half of that area is classified as a very fragile ecosystem (MNET, 2010). To combat desertification, the Mongolia government has planted more than 20 million seedlings since the 1980s. Even though planting trees is a more effective way to combat desertification than livestock exclusion or planting shrubs, reforestation area covers less than 15% of the deforestation area (Tsogtbaatar, 2004; Miyasaka et al., 2014). Tsogtbaatar (2004) pointed out that the main factors of low reforestation rate are a lack of skilled labor, limited financial support, and studies that are too academic to apply in real field conditions.

Populus spp., members of a diverse and widely distributed genus, can adapt to various environmental conditions (Yin et al., 2005a, 2005b; Lei et al., 2006). Because *Populus* spp. are fast growing and demonstrate easy to control breeding and vegetative propagation, they play important economic and environmental roles in different countries (Mao et al., 2008). *Populus* spp. also tolerate a certain degree of drought (Kang et al., 1996; Yin et al., 2005a, 2005b; Mao et al., 2008). Therefore, *Populus sibirica*, as a native species in Mongolia, has been the main species used for reforestation. However, even though *P. sibirica* is a main species for reforestation, only a few studies have focused on physiological and ecological traits of *P. sibirica*.

Drought, a multidimensional abiotic stress that controls plant growth (Yordanov et al., 2000; Ferdousee et al., 2010), is a main constraint for the growth of trees especially in desertified areas (Kang et al., 1996; Yin et al., 2005b; Xu et al., 2010). Drought inhibits the growth rate and productivity of plants with various reasons (Yin et al., 2005a; Maes et al., 2009). Furthermore, limited photosynthesis caused by drought alters carbon allocations for maintaining water potential as high as possible (Roland et al., 2015). Plants can maintain their high water potential by minimizing water loss or maximizing water uptake

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Abbreviations: φE_0 ($\varphi E_0 = ET_0 / ABS$), probability of an absorbed exciton that can move an electron beyond Q_A^-; φPO, ($\varphi PO = F_V / F_m$), maximum quantum yield of PSII photochemistry; ψ_0 ($\psi_0 = ET_0 / TR_0$), efficiency of a trapped exciton that an electron can move into the electron transport chain further than Q_A; ABS/CS, the ratio of absorbed electrons per CS; ABS/RC, the ratio of absorbed electrons per RC; Car T, total carotenoids; C_i, internal CO_2 concentration; CS, cross-section; Chl a, chlorophyll a; Chl b, chlorophyll b; DRGR, root collar diameter relative growth rate; DI_0/CS, the ratio of dissipated excited energy per CS; DI_0/RC, the ratio of dissipated excited energy per RC; *E*, transpiration rate; ET_0/CS, the ratio of electron transport per CS; ET_0/RC, the ratio of electron transport per RC; F_0, the minimum chlorophyll fluorescence of the open state PSII center; *gs*, stomatal conductance; HRGR, height relative growth rate; LWP, leaf water potential; P_N, leaf net CO_2 assimilation rate; PI_{abs}, performance index; Q_A, plastoquinone A; Q_B, plastoquinone B; RC, reaction center; SEM, scanning electron microscopy; SLA, specific leaf area; TR_0/CS, the ratio of trapped electrons per CS; TR_0/RC, the ratio of trapped electrons per RC; Total Chl, total chlorophyll; WW, well-watered.

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(Chaves et al., 2003). To minimize water loss, stomata close during the daytime, and leaf thickness increases (Fernàndez and Reynolds, 2000; Bussotti, 2008). In addition, leaf morphologies, including a thickened trichome layer, leaf rolling, steep leaf angle maintained by heliotropism, and reduction in the density and length of stomata, can change to minimize water loss (Maes et al., 2009; Mehri et al., 2009; Doheny-Adams et al., 2012). To maximize water uptake, plants develop root systems based on carbon allocation to increase the root/shoot ratio until it reaches a threshold appropriate for the present degree of drought intensity (Yin et al., 2005b; Xu et al., 2010). However, limitation of carbon assimilation produces excess energy that induces harmful oxidative stress. Chlorophyll content is decreased to limit the amount of energy absorbed, and the plant increases the amount of energy dissipated to protect its photosystem via non-photochemical or photochemical quenching (Neidhardt et al., 1998; Lei et al., 2006; Wu et al., 2008; Jaleel et al., 2009; Karatas et al., 2014). Therefore, all drought responses involve dissipation of excess radiation, even though plants have various responses that depend on their own strategies and the intensity of the drought (Yordanov et al., 2000; Chaves et al., 2003). Thus, drought responses are not mutually exclusive, but are combined within a plant (Chaves et al., 2003).

Hartwell et al. (2010) have confirmed that the current irrigation scheme for *Populus* spp. and *Salix gooddingii* in the desert environment is over irrigated. Establishing an irrigation system to supply enough water has proven to be challenging. Therefore, our main objective is to investigate the physiological response of *P. sibirica* to different water deficit intensities in order to suggest an optimal irrigation interval for effective and successful reforestation in Mongolia.

2. Materials and methods

2.1. Field site

The experimental site was in Elsen tasarkhai, Mongolia (N47°19′37.60″, E103°42′16.30″). The study was conducted for three months from June to August 2015, which is considered a whole growing season in that area. During the experimental period, the coldest and warmest monthly mean air temperatures were in January (−17.0 °C) and in July (19.8 °C). The precipitation during the study period was 118.4 mm. The soil type is sandy, as determined by the micro-pipet method (Miller and Miller, 1987), and the soil pH of soil ranged from 9.45 to 9.63. Although the exchangeable sodium percentage and sodium absorption ratio were calculated to determine the salinity at the experimental site, that area did not contain saline soil, sodic soil, or saline–sodic soil.

2.2. Experimental design

Two-year-old *Populus sibirica* seedlings of a uniform size were selected from a nearby nursery in Lun Soum, Tuv Aimag and planted with 1.5 m spacing in 2014. Three replicates of each treatment, nine seedlings per each replicate, were randomly assigned across the experimental site. The total number of seedlings was 81. Three different water regimes (well-watered (WW), irrigation every 2 days, 14 L tree^{-1} week^{-1}; moderate drought (MD), irrigation every 5 days, 5.6 L tree^{-1} week^{-1}; severe drought (SD), irrigation every 7 days, 4 L tree^{-1} week^{-1}) were applied with a drip irrigation system during the experimental period.

2.3. Growth rate measurement (HRGR, height relative growth rate; DRGR, root collar diameter relative growth rate)

The height of 81 seedlings was measured from ground level to the highest part of seedling using a measuring tape, and root collar diameter of 81 seedlings was measured at 2 cm above the ground using a digital caliper (Mitutoyo Corporation, CD-15DC, Kawasaki, Japan) twice during the growing season. The relative growth rate (RGR) was calculated as follows:

$$\text{RGR}\left(\text{cm}\cdot\text{days}^{-1}\right) = [(\text{2nd growth parameter}) - (\text{1st growth parameter})] / \text{number of days} \tag{1}$$

2.4. Specific leaf area

The specific leaf area (SLA), an indicator of leaf thickness, was calculated using the ratio of dry leaf mass to leaf area ($\text{cm}^2\cdot\text{g}^{-1}$). Nine fully expanded sunlit leaves were collected from each of at least nine plants per plots in July. Leaf area was measured with Photoshop CS6 (Adobe System, San Jose, CA, USA), and the dry weight was measured after drying at 70 °C for 72 h.

$$\text{SLA}\ (\text{cm}^2\cdot\text{g}^{-1}) = \text{leaf area}\ (\text{cm}^2)/\text{leaf dry weight}\ (\text{g}) \tag{2}$$

2.5. Stomatal length and stomatal density

The stomatal length and density were determined using scanning electron microscopy (SEM) images. The sample leaves were collected around noon and dried naturally for five days in July. After returning to Korea, the samples were mounted on metal stubs and coated with gold with sputter coater (LEICA EM AC200, Leica Microsystems, Austria). The SEM images were produced using a field emission scanning electron microscope (FESEM, AURIGA, Carl Zeiss, Germany). The length was calculated with Photoshop CS6, and the number of stomata was counted for the stomatal density.

2.6. Leaf water potential

To investigate the leaf hydration, the leaf water potential (LWP) was determined using a pressure chamber (Skye SKPM 1400, Skye Instruments, Powys, Wales, UK). The LWP was measured as many times as every 3 h in a day in July. Samples were collected from the fourth fully-expanded mature leaf as counted from the shoot apex with three replications. To reduce errors during the procedure, the leaves were carried to the chamber quickly, using humidified polyethylene bags to minimize water losses.

2.7. Gas exchange measurement

The gas exchange measurements (P_N, photosynthesis rate; *gs*, stomatal conductance; *E*, transpiration rate; C_i, internal CO_2 concentration) were determined using an LCi (ADC BioScientific Ltd., Furlong Way, Great Amwell, UK) equipped with a broad leaf chamber. The leaf photosynthetic parameters were measured in the morning, between 8:00 and 11:00, on three replicated plants using fully expanded, sunlit leaves on sunny, cloudless days in July.

2.8. Chlorophyll fluorescence parameters

The chlorophyll fluorescence parameters and OJIP transient were measured using a Pocket Plant Efficiency Analyzer (Hansatech Instruments Ltd., Norfolk, UK) on plants that were dark-adapted for 30 min while gas exchange parameters measurement. The chlorophyll fluorescence parameters and OJIP transient were also measured at the fourth leaf from the apex between 9:30 and 11:00 am with nine replicates. The chlorophyll fluorescence parameters were calculated according to Strasser et al. (2000 and 2004). Then, the quantum efficiencies per reaction center (RC) and cross-section (CS), the extracted fluorescence parameters and vitality indexes were normalized to the WW condition, which was set as 1.0.

2.9. Chlorophyll and total carotenoid contents

The chlorophyll contents (Chl a, chlorophyll a; Chl b, chlorophyll b; Total Chl, total chlorophyll; Car T, total carotenoids) were calculated by the method of Arnon (1949). The leaves were transported to Korea using an ice box to keep them fresh, and all procedures were performed within 24 h of picking. Fresh leaf samples of 0.1 g were extracted using 10 ml of 80% acetone solution at 4 °C for 7 days. The solution's optical density was determined using an Epoch Microplate Spectrophotometer (Synergy-BioTek, Winooski, VT, USA) at 663 nm, 647 nm, and 470 nm.

$$\text{Chl a}\left(\text{mg}\cdot\text{L}^{-1}\ \text{FW}\right) = 12.7\times A_{663} - 2.69\times A_{645} \tag{3}$$

$$\text{Chl b}\left(\text{mg}\cdot\text{L}^{-1}\ \text{FW}\right) = 22.9\times A_{645} - 4.68\times A_{663} \tag{4}$$

$$\text{Total Chl}\left(\text{mg}\cdot\text{L}^{-1}\ \text{FW}\right) = 20.2\times \text{A645} + 8.02\times \text{A663} \tag{5}$$

$$\text{Car T}\left(\text{mg}\cdot\text{L}^{-1}\ \text{FW}\right) = 1000\times A_{470} - 1.82\times \text{Chl a} - 85.02\times \text{Chl b} \tag{6}$$

(A, absorbance; pigment concentration calculated as $\mu g\cdot ml^{-1}$ of extract).

2.10. Data analysis.

All statistical analyses were performed using SPSS Statistics 22 for Window software (SPSS, Chicago, IL, USA). Statistically significant differences among the mean values were tested by One-way ANOVA test with Duncan's Multiple Range Test at $p = 0.05$.

3. Results and discussion

As many studies have reported that plants change their metabolism in responses to abiotic and biotic stress in ways that could enhance their acclimation to unfavorable conditions (Rivero et al., 2014), the survival rate of *P. sibirica* was not affected in this study (data not shown). However, different responses to the different water deficit intensities were observed. To balance carbon fixing and water containment under drought conditions, the plants demonstrated a trade-off between growth and physiological responses (Fernàndez and Reynolds, 2000). As a result of water deficit, HRGR was limited, as indicated in many studies (Fig. 1A) (Fernàndez and Reynolds, 2000; Achten et al., 2010). Limiting HRGR was linked with biomass allocation, and it might be advantageous under drought conditions because it reduces resource requirements (Fernàndez and Reynolds, 2000; Chaves et al., 2003). A decrease in SLA was also linked to biomass allocation and the acclimation of photosynthesis. Under the drought conditions in this study, SLA decreased (Fig. 2) because of high mesophyll tissue or elevated leaf thickness (Fernàndez and Reynolds, 2000; Yin et al., 2005a; Bussotti, 2008; Padilla et al., 2009; Kwon and Woo, 2015). However, Hartwell et al. (2010) reported that the average SLA value for *Populus* spp. was 80.8 $cm^2\ g^{-1}$ which is the SLA value measured under the SD treatment in this study. Thus, the SLA increased under the MD and WW treatments rather than decreased under the SD treatment.

Regarding the stomatal morphology in response to drought, the pore width was significantly decreased only in the SD condition (Table 1). Doheny-Adams et al. (2012) observed that drought reduced the stomatal size because the maximal stomatal conductance was adjusted by reducing the stomatal size. However, the water deficit treatment could not affect stomatal density because the stomatal density was determined before the initiation of drought treatment (Table 1) (Mehri et al., 2009).

A decreased LWP under drought condition was observed in this study, which is similar to other studies (Fig. 3) (Jiang and Huang,

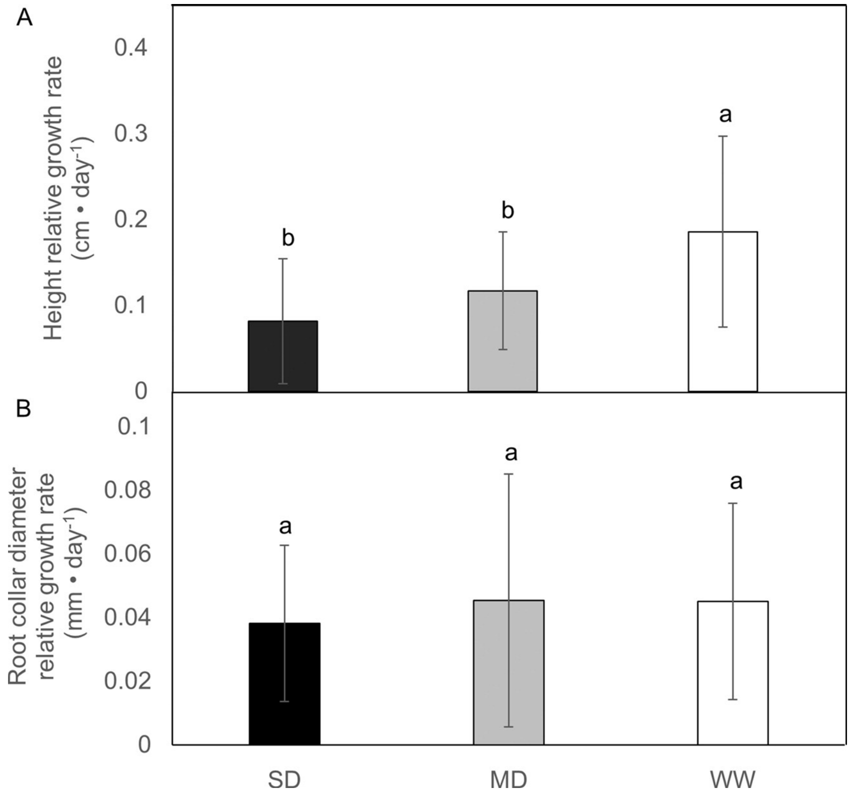

Fig. 1. The effect of water-deficit on the height relative growth rate (A) and root collar diameter relative growth rate (B). *Different letters* on the bars indicate significant differences (Duncan's Multiple Test at $p < 0.05$). SD — severe drought, MD — moderate drought, WW — well watered.

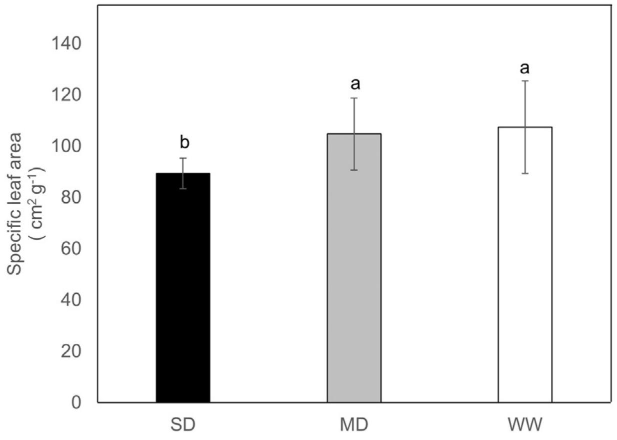

Fig. 2. Effect of water deficit on specific leaf area of *Populus sibirica* (n = 3). *Different letters* on the bars indicate significant differences (Duncan's Multiple Range Test at $p < 0.05$). SD — severe drought, MD — moderate drought, WW — well watered.

Table 1

Effects of water deficit on stomatal density and pore width in *Populus sibirica* leaves. Values are mean ± standard deviation (n = 3). *Different letters* indicate significant differences (Duncan's Multiple Range Test at $p < 0.05$). SD — severe drought, MD — moderate drought, WW — well watered.

Treatment	Number of stomata × 700	Pore width (mean in μm) × 7000
SD	194.70 ± 7.21a	1.06 ± 0.25b
MD	149.03 ± 22.03a	1.61 ± 0.10a
WW	192.29 ± 32.52a	1.61 ± 0.25a

2001; Anjum et al., 2011). There were no significant differences in LWP at 4:00, 10:00, and 13:00 among treatments (p-value = 0.084, 0.056, 0.063, respectively). Except those times, the LWP in SD trees was significantly smaller than in MD and WW trees. In response to the drought, plants maintained their water potentials through osmotic adjustment with osmolytes, such as proline (Sharma et al., 2011; Karatas et al., 2014). Lei et al. (2006) suggested that osmotic adjustment could be a good strategy against water deficit especially for young seedlings which did not develop a root system.

Because more than 90% of plant water loss is due to transpiration, plants should maintain a balance between carbon assimilation and transpiration (Chaves et al., 2003). In line with the results of many other studies (Yin et al., 2005a, 2005b; Wu et al., 2008; Lang et al., 2013), the reduction of P_N was observed under the drought conditions (SD) in the present study (Fig. 4A). Although there was no significant difference in stomatal conductance, it decreased as the water deficit increased (Fig. 4B). The decreased in E show stomatal limitation, in agreement with many other studies (Fig. 4C) (Flexas and Medrano, 2002; Chaves et al., 2003; Flexas et al., 2006; Li et al., 2011; Xu et al., 2013). Stomatal closure is an immediate reaction to drought and could restrict the supply of CO_2 for photosynthesis (Flexas et al., 2000; Yordanov et al., 2000; Xu et al., 2010). An increase in C_i was observed under the water deficit condition (SD) (Fig. 4D). The photorespiration and reduction of enzymes or content included in the dark reaction of photosynthesis was responsible for the increased C_i under the drought condition (Neidhardt et al., 1998; Tezara et al., 2002; Chaves et al., 2003; Galmés et al., 2011). The reduction in P_N with increasing C_i might be caused by mesophyll conductance limitation or non-stomatal limitation (e.g., reduction of carboxylation efficiency or PSII functions) (Tezara et al., 2002; Van Heerden et al., 2007; Wu et al., 2008; Wang et al., 2012). Many studies have pointed out that the non-stomatal limitation prevailed in reducing the P_N under severe drought conditions (Tezara et al., 1999; Wu et al., 2008; Wang et al., 2012).

Polyphasic OJIP fluorescence sensitively indicated a redox reaction of plastoquinone A (Q_A) and plastoquinone B (Q_B) from PSII to PSI (Strasser et al., 2000; Wen et al., 2005). In the OJIP test, a significant reduction in chlorophyll fluorescence was observed at the P-step (300 ms) (Fig. 5), which indicated that the inhibition involved the reduction of the PQ-pool and the reduction of the electron transport acceptors around PSI under the drought condition (SD) (Kalaji et al., 2014). At the K-step (200 μs), hidden between the O-step and J-step, a peak has usually been observed as a parameter sensitive to drought stress, however, it was not observed in this study (Strasser et al., 2004; Oukarroum et al., 2007; Wang et al., 2012). The increase in F_0 under the drought condition (SD) resulted from an accumulation of Q_B^- non-reducing centers at the PSII (Fig. 6B) (Lu et al., 1998). The maximum quantum yield of the PSII photochemistry ($\varphi PO = F_v / F_m$) of the C_3 plants ranged between 0.83 and 0.85 in comfortable conditions, but it decreased significantly in this study under the drought condition (SD) (Fig. 6B) (Van Heerden et al., 2007; Xu et al., 2010). The decrease in efficiency of trapped and absorbed excitons that can move an electron transport chain further than Q_A^- (ψ_0 and φE_0) was caused by the increase in trapped and absorbed excitation energy (Fig. 6B). The quantum efficiencies per RC and CS increased except for the ratio of electron transport (ET_0/RC and ET_0/CS) under the SD condition (Fig. 6A). The increase in both the ratio of absorbed electrons (ABS/RC) and the trapped electrons per RC (TR_0/RC) were caused by the enlargement of the antennae of the RCs, and the increases in the ratio of dissipated excited energy per RC and CS (DI_0/RC and DI_0/CS) indicated the enlargement of the excess energy being dissipated. Both of these responses have been considered as acclimation to drought

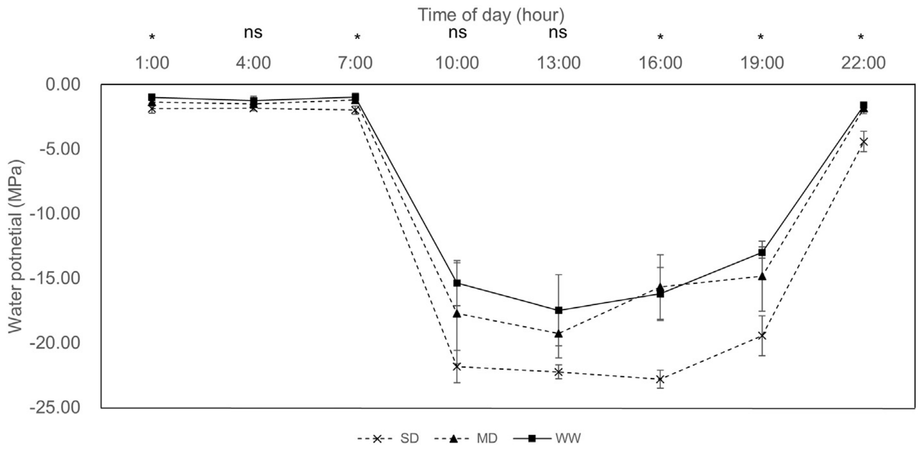

Fig. 3. Diurnal change in *Populus sibirica* leaf water potential in response to various water deficits after 43 days of treatment (n = 3). Differences are indicated by asterisks (significant) and ns (non-significant) (Duncan's Multiple Range Test at $p < 0.05$). SD — severe drought, MD — moderate drought, WW — well watered.

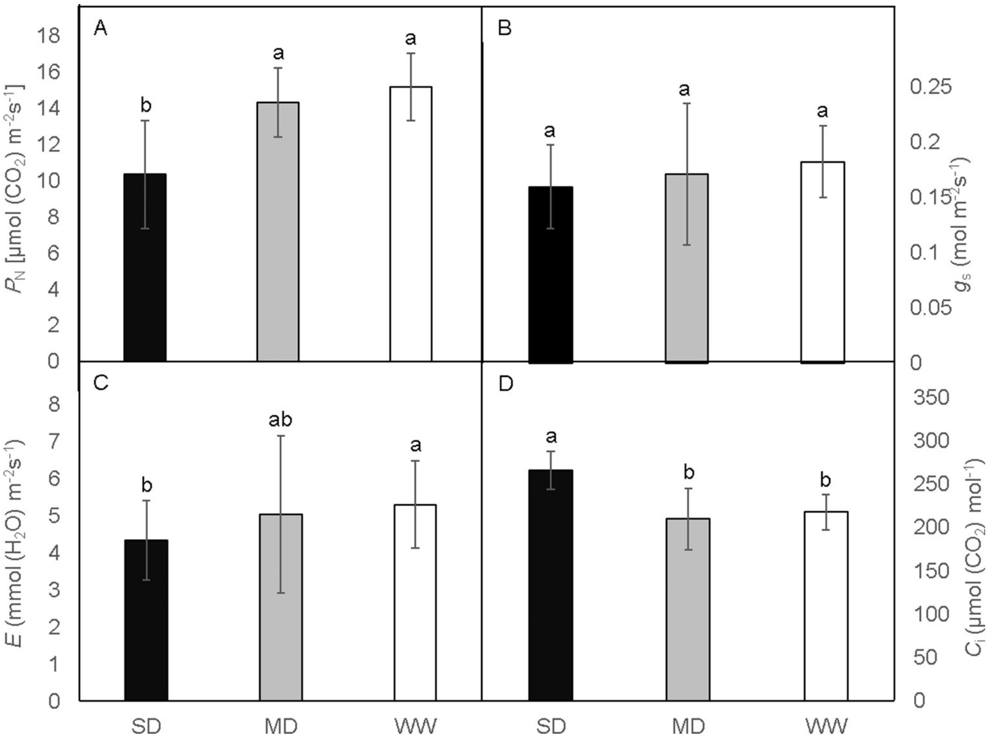

Fig. 4. Changes in net photosynthetic rate (P_N) (A), stomatal conductance (gs) (B), transpiration rate (E) (C) and intercellular CO$_2$ content (C_i) (D) in different drought treatments of *Populus sibirica* in 2015 (n = 3). *Different letters* on the bars indicate significant difference (Duncan's Multiple Range Test, $p < 0.05$). SD — severe drought, MD — moderate drought, WW — well watered.

(Van Heerden et al., 2007; Falqueto et al., 2010). The unchanged TR$_0$/RC and the ratio of trapped electrons per CS (TR$_0$/CS) resulted from a compensatory mechanism (Van Heerden et al., 2007).

According to the results of Lang et al. (2013), photosynthetic efficiency was restricted by photoinhibition but that can be limited by enhancing photorespiration and heat dissipation (Figs. 4 and 6). To avoid photoinhibition and enhance photosynthetic efficiency, plants decrease the size of their chlorophyll antennae in their photosystems and their chlorophyll content in response to drought (Neidhardt et al., 1998; Jiang and Huang, 2001; Wu et al., 2008). No minimization of antenna size was observed in this study (Fig. 6A). However, the Total Chl, Chl a / Chl b, and Car T contents significantly decreased as the drought intensity increased (Table 2). Many studies have indicated that reduced photosynthetic pigments are adaptations that plants use to protect themselves from photoinhibition and photodynamic damage (Neidhardt et al., 1998; Lei et al., 2006; Wu et al., 2008; Jaleel et al., 2009; Karatas et al., 2014). The decreased Chl a / Chl b indicates damage to the light-harvesting complex (Wu et al., 2008). According to the results of Lei et al. (2006), the reduced Car T content indicates that the water deficit in this study might have induced oxidative stress through accumulation of reactive oxygen species. However, the decrease in the Total Chl / Car T under the drought condition (SD) indicates that the amount of Car T increased compared to the Total Chl in response to drought. Because carotenoids play an important role as a lipid soluble antioxidant, precursor in signaling and as an accessory pigment to protect photochemical process and change under the drought, carotenoids affect plants' drought tolerance (Yordanov et al., 2000; Jaleel et al., 2009).

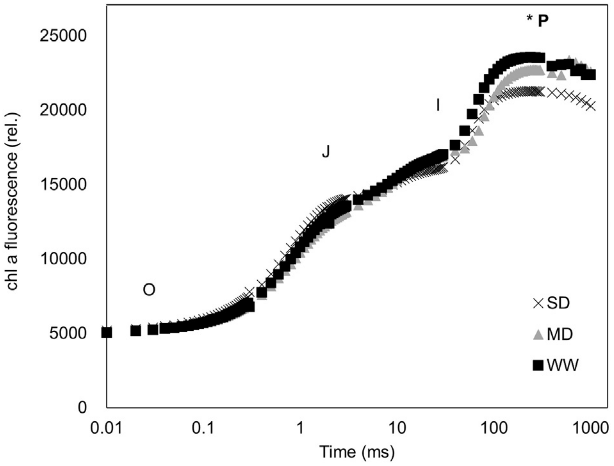

Fig. 5. The polyphasic chlorophyll fluorescence transient curves from leaves of *Populus sibirica*, plotted on a logarithmic time scale under different water deficit intensities. Asterisks indicate significant differences with Duncan's Multiple Range Test at $p < 0.05$ (n = 3). O-step — at 50 µs, K-step — at about 200 µs, I-step — at 30 ms, P-step — at 300 ms, SD — severe drought, MD — moderate drought, WW — well watered.

4. Conclusion

In this study, *P. sibirica* in the SD condition was limited in its growth and assimilation of carbon via the stomatal aperture. Furthermore, *P. sibirica* avoided photodamage through a decline in chlorophyll content, which could limit the absorbed energy, and photochemical quenching, which could dissipate the excess energy. All of these responses to drought were combined to help the plants cope with the drought condition. In addition, those responses indicate that *P. sibirica* has a certain degree of resistance to drought (MD and SD). Even though the growth of *P. sibirica* slowed under the applied drought conditions, it overcame those conditions through photochemical quenching. Therefore, the optimal irrigation regime in this study is an irrigation rate of 4 L tree^{-1} week^{-1} (every 7 days). Current irrigation regimes in Mongolia should thus be adjusted to longer intervals to minimize the need for irrigation in reforestation areas, based on the response of *P. sibirica* to longer irrigation intervals.

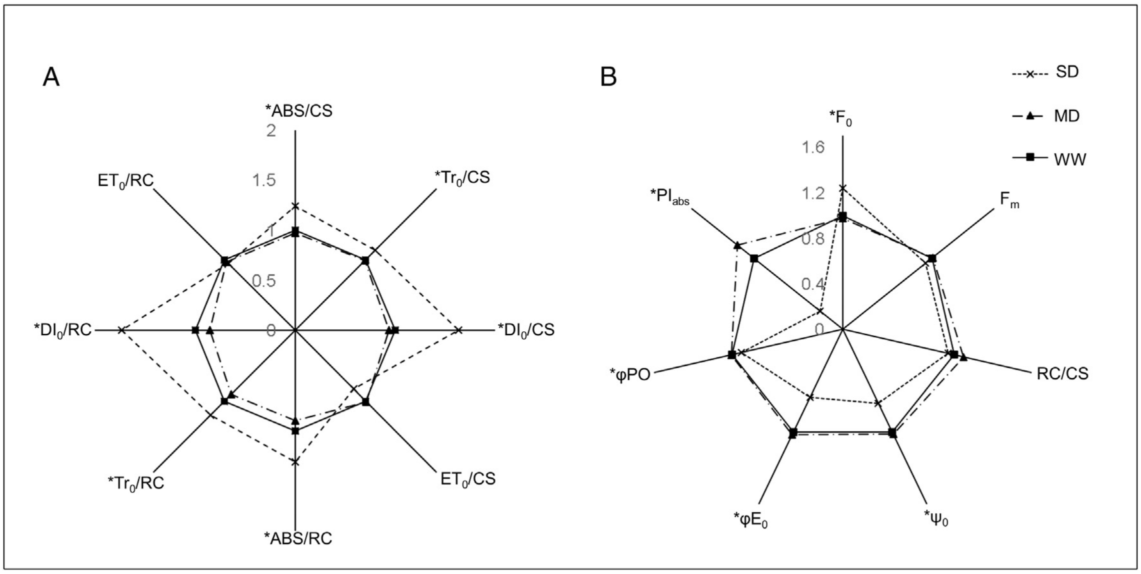

Fig. 6. Changes in the specific energy fluxes per reaction center (RC) and per cross-section (CS) in different drought treatment conditions of *Populus sibirica* (A). Effects of drought stress on F_0, F_m, RC/CS, ψ_0, φE_0, φPO and PI_{abs} of *Populus sibirica* in different drought treatments conditions (B). Asterisks indicate significant differences with Duncan's Multiple Range Test at $p < 0.05$ (n = 3). SD — severe drought, MD — moderate drought, WW — well watered.

Table 2
Changes in total chlorophyll content (Total Chl, μg ml^{-1} FW), total carotenoid content (Car T, μg ml^{-1} FW), ratio of chlorophyll a to chlorophyll b (Chl a / Chl b), and ratio of total chlorophyll and carotenoid contents (Total Chl / Car T) under different water deficits. *Different letters* indicate significant differences (Duncan's Multiple Range Test, $p < 0.05$, n = 3). SD — severe drought, MD — moderate drought, WW — well watered.

Treatment	Total Chl	Chl a	Chl b	Car T	Chl a / Chl b	Total Chl / Car T
SD	47.57 ± 0.77c	36.53 ± 1.05c	11.05 ± 0.30c	11.51 ± 0.59c	3.31 ± 0.18b	4.14 ± 0.15c
MD	99.13 ± 0.37a	79.00 ± 0.29a	23.15 ± 0.15a	20.23 ± 0.17a	3.28 ± 0.02b	4.90 ± 0.02a
WW	57.62 ± 0.29b	45.31 ± 0.16b	12.32 ± 0.13b	14.52 ± 0.03b	3.68 ± 0.03a	3.97 ± 0.01b

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