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EFFECTS OF PROGRESSIVE SOIL WATER DEFICIT ON GROWTH, AND PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *POPULUS EUPHRATICA* IN ARID AREA: A CASE STUDY IN CHINA

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Abstract

The aim of this study was to investigate the responses of *Populus euphratica* seedlings under a short-term soil water deficit. To mimic natural conditions in which drought stress develops gradually, stress was imposed by subjecting plants to a gradual decrease of soil water content for a period of 21 d. We studied growth, physiological and biochemical responses to progressive soil water deficit of potted *Populus euphratica* seedlings at outdoors. Results showed that, in 6 d of water withholding, the soil moisture content decreased to a slight drought stress level, and it reached a severe drought stress level after 15 d of water withholding in July. In the process of soil water declining from saturated to severe drought levels, the increasing soil water deficit resulted in decreases in the height, stem base diameter, number of lateral branches. Leaf predawn water potential decreased after 15 d of withholding irrigation. After 21 d of withholding irrigation, actual photochemical efficiency of photosystem II (PSII) in light-adapted leaves and photochemical quenching coefficient decreased, respectively; the peroxidase activity, the content of chlorophyll a and chlorophyll b decreased. There were no significant changes in proline, malondialdehyde content, chlorophyll a/b value and superoxide dismutase activity.

Key words: Water, Response, Seedling, Arid.

Abbreviations: AK: available potassium; AN: available nitrogen; AP: available phosphorus; Chl a: chlorophyll a; Chl b: chlorophyll b; CK: well-watered control treatment; D: water deficit treatment; F_m': maximum fluorescence yield of the light-adapted state; F_o: initial fluorescence yield in the dark; F_o': initial fluorescence in the light; F_m: maximum fluorescence in the dark; F_s: steady state fluorescence of specific time; g_s: leaf stomatal conductance; MDA: malondialdehyde; OM: organic matter; PAR: photosynthetically active radiation; POD: peroxidase; Pro: proline; PSII: photosystem II; ΦPSII: actual photochemical efficiency of PSII in light-adapted leaves; q_P: photochemical quenching coefficient; SOD: superoxide dismutase; TK: total potassium; TN: total nitrogen; TP: total phosphorus; ψ_p: leaf predawn water potential.

Introduction

Drought stress is one of the main environmental constraints that severely affect plant growth and development. It has been estimated that currently about 28% of the earth's land areas are too dry for plant production. Owing to changing patterns of precipitation, episodes of drought are increasing and are expected to continue to increase in the future. This requires a better understanding of the mechanisms of resistance and adaptation to drought. In an arid environment, water is a primary limiting factor for plant growth and spatio-temporal dynamics (Baghalian *et al*., 2011). During their lifetime, trees are susceptible to a wide range of environmental and chemical stressors that can result in tree decline. In particular, drought is a major limiting factor for seedling survival, especially during the initial phase of growth and establishment in field conditions (Rennenberg *et al*., 2006). To cope with drought, approaches are required which may alleviate drought stress on seedlings or trees in drought-affected areas and enable the establishment of forest plantations. Understanding the physiological and biochemical mechanisms that provide drought tolerance is very important in terms of developing selection and breeding strategies for the establishment of forests. Especially, research into the effects of water-deficit may provide valuable information about the various strategies of the plant intended to remove or reduce the harmful effects of water-deficit in soil on plant tissue.

Populus euphratica Oliv., as a predominant species in desert riparian forest ecosystems, is found in the arid and semi-arid deserts of Mid-Asia. It plays an important role in maintaining the ecosystem function and protecting oases from sandstorms (Thomas *et al*., 2006). It is not only a vulnerable species among the first group of 388 endangered or rare plants in China, but also an important forest genetic resource in urgent need of protection worldwide. It is an ideal material for studying the adaptation responses of tree species to various environmental conditions. China has the largest range and number of *P. euphratica* in the world. However, over recent decades, about half of the natural *P. euphratica* forest has disappeared due to water scarcity and the impact of man-made destructions in China. Most of the studies of *P. euphratica* have mainly focused on one of the physiological processes, such as photosynthesis, water usage, and response to drought stress in adult *P. euphratica* (Chen *et al*., 2012). Few studies, however, have been conducted to investigate the response to water deficit regarding *P. euphratica* seedlings under experimental potting conditions in the greenhouse (Dong *et al*., 2012). However, in these laboratories and greenhouse studies, very rapid and severe stressors are applied according to the soil moisture threshold levels suggested by Hsiao (Hsiao, 1973). Moreover, the stress under experimental conditions was not the same as that induced under field conditions, where drought stress usually develops much more slowly. It is well known that a steep stress gradient may have a different effect on the plant than slowly increasing drought stress, which gives the plant time to react and adapt to the stress by different mechanisms. Different responses can be observed in the photosynthetic processes when a plant is exposed to water deficit that is induced either slowly or rapidly (Silva & Arrabaça, 2004). Therefore, additional focus is needed for an integrative analysis of the physiological and biochemical activity of *P. euphratica* that was affected by a progressive soil water deficit under a natural environment. Thus, we characterized the growth, predawn water potential, stomatal conductance, and some chlorophyll fluorescence parameters when two-year-old *P. euphratica* seedlings were exposed in the outdoors to progressive soil water deficit in the lower reaches of the Tarim River. Answers to these questions will help to improve understanding of *P. euphratica* adaption to drought stress in arid areas, and further provide the scientific basis for effective methods of protecting *P. euphratica* forests.

Materials and Methods

Treatments and experimental design: The experiment was conducted at the Ecology and Restoration Monitoring Test Station located in the lower reaches of the Tarim River during April to August of 2010. Two-year-old *P. euphratica* seedlings that were uniform in crown width, root radius, height, and trunk diameter were submitted to water deficit treatments for a period of 21 d. The average height was 51.9 cm. The pot was a PVC tube, 30 cm in diameter and 50 cm high, seated on a plastic plate under the PVC tube bottom. The potting soil was obtained from local uncultivated farmland. The basic nutrient content of the soil is shown in Table 1, and the field capacity of the soil was 29.47% (w/w). The seedlings were transplanted on April 7, 2010. A single seedling was planted in each pot, and 24 pots were planted. The seedlings were grown in well-watered conditions (watered one time per week until soil water drainage occurred from the bottom of the PVC tube). Plants were acclimated to growth chamber conditions for 3 months before treatments were imposed. There was no rainfall during the experimental period, and no fertilizer was used. The experiment was done at outdoor with a temperature between 25°C and 40°C, and day relative humidity between 18.78% and 34.68%, between 8:00-20:00. The highest air temperature and relative humidity appeared between 16:00-18:00. The photosynthetically-active radiation (PAR) displayed a single peak profile, and the peak was about 1557μmol $m^{-2} \cdot s^{-1}$ at 16:00.

After 3 months, 8 uniformly developed seedlings were selected for water deficit experiments starting July 18 and ending August 6. The seedlings were randomly divided into two groups. The experiment consisted of well-watered control (CK) and water deficit (D) treatments. For the well-watered control treatment, plants were watered every 2 d to completely saturate the soil in the pot (until water drained from the bottom of the PVC tube) in order to obtain non-limiting soil water conditions. Water deficit treatment was imposed by withholding irrigation for 21 d. Each treatment had four pots which were randomly placed. A single-plant per pot was considered a replicate (there were four replicates). At the beginning of the experiment, all pots were saturated with water and allowed to drain freely from the bottoms of the containers. During the period of experiment, an automatic weather station (*ICT, International,* Sydney, Australia) was set up at the study site on a mast approximately at crown height. Temperature and PAR were recorded every 10s.

Evaluation of soil water status and different parameters of the seedlings: Soil water content (gravimetric) was measured with an oven-drying method for 0-10, 10-20, 20-30, and 30-40 cm layers over the course of the experiment to evaluate soil water availability. Growth, physiological and biochemical parameters in the plants were estimated. Except for biochemical parameters, the other parameters were measured several times, and the measurements were done before every irrigation for the seedlings, respectively. In all cases, three or four seedlings per treatment were used for experimental purposes. All of the measurements were taken in the fixed tree in each treatment.

Growth, physiological and biochemical parameter measurements: Characteristics of plant growth were monitored by measuring stem length (from substrate surface to the top of seedling) and diameter (at the base), and by counting the lateral branches. The relative increase in stem length, stem diameter, and number of branches was calculated. The height, stem base diameter, and number of lateral branches per plant were recorded several times during the experimental period, respectively.

Chlorophyll fluorescence has been used as a nondestructive and noninvasive means of quantifying damage to the leaf photosynthetic system of deciduous and evergreen trees. Chlorophyll fluorescence was measured with a portable modulated fluorometer (*Mini-Pam, Walz,* Ulm, Germany). Leaves were tagged to ensure that the same leaves were measured throughout the experiment period. Red light (intensity < 0.1μmol $m^2 \cdot s^{-1}$ PAR) was taken as the measuring-light, and a saturation light pulse of 0.8 s in duration (intensity > 10,000 μmol $m^2 \cdot s^{-1}$ PAR) was supplied by the inner halogen lamp. Chlorophyll fluorescence of 10 randomly selected healthy and mature leaves per seedling (five from the top of the crown, five in the center), with three individuals measured per treatment, were measured on clear days from 08:00-20:00 at 2 h intervals to obtain the actual fluorescenc at specific time in light-adapeted leaves (F_s) and maximum fluorescence in light-adapted leaves (F_m'). The initial fluorescence yield (F_o) and maximum fluorescence yield in the dark (F_m) were measured before dawn and at midday after shading with a black cloth for 20 min. The minimal fluorescence of light-adapted leaves (F_o'), actual photochemical efficiency of photosystem II (PSII) in light-adapted leaves (φPSII), and photochemical quenching coefficient (q_p) were calculated using the following formula, respectively (Zhu *et al.*, 2013):

$$F'_0 = F_0 \Big/ \left(F_V/F_m + F_0/F'_m \right) \quad (1)$$

$$F_v = F_m - F_o \quad (2)$$

$$\varphi PSII = (F'_m - F_s)/F_m' \quad (3)$$

$$q_p = (F_m' - F_s)/(F_m' - F_o') \quad (4)$$

Table 1. Basic soil properties in the experiment (Mean ± SD, n=3).

	OM ($g\cdot kg^{-1}$)	TN /($g\cdot kg^{-1}$)	TP ($g\cdot kg^{-1}$)	TK /($g\cdot kg^{-1}$)	AN ($g\cdot kg^{-1}$)	AP ($g\cdot kg^{-1}$)	AK ($g\cdot kg^{-1}$)
Soil sample	16.33±1.69	1.05±0.12	0.99±0.11	17.7±0.41	71.68±29.72	86.38±18.96	222.62±43.75

At the same day, stomatal conductance (g_s), one of the gas-exchange parameters, was taken on the same leaves used for chlorophyll fluorescence, was automatically recorded for 10 leaves of each individual plant tested from 8:00-20:00 at 2 h intervals, with three individuals measured per treatment, by the dynamic plant stomata meter (AP4, Delta-T, Cambridge, UK), every 3-6 days. Leaves in the plant were directly measured without harm, rather than being picked before measurement. Moreover, the stomatal conductance was measured three times for each leaf, and their mean value was used.

Predawn (6:00) leaf water potential (ψ_p) was measured by a Dew Point Microvolt-meter (HR-33T, Wescor Company, Logan, USA), every 3-6 days. Five healthy and grown leaves from the well-lit portion of the canopy of each individual plant were picked, immediately sliced at the center, and placed in the C-52 sample chamber of the Dew Point Microvolt-meter to measure ψ value, and their mean value was used for each individual plant tested. The leaf water potential was calculated by ψ / -7.5 (Zhu *et al.*, 2011).

At the end of the experimental period (21 days of water withholding), after measuring the growth parameter, chlorophyll fluorescence parameters, g_s and ψ_p, fresh leaves per plant were picked and packed with gauze and put into liquid nitrogen, respectively. The biochemical parameters were measured using different methods, respectively. For enzyme extraction and assays, 0.2 g of fresh leaves were ground with a mortar and pestle in 4 mL of the solution containing 50 mM phosphate buffer (pH7.0), 1% (w/v) polyvinylpyrrolidone (PVP) under low temperature maintained by ice-tray and centrifuged at 15000 rpm for 15 min at 4 °C, and the supernatant was collected for enzyme assays. SOD activity and activity of POD (EC1.11.1.7) were determined following Rajput *et al.* (2015). Pro content was determined following Bates *et al*. (1973). MDA content was estimated following Heath and Packer (Heath Packer 1968). Chlorophyll was extracted from 0.5 g of fresh leaf material for 72 h in the dark at 4°C, using acetone (80%). After incubation, the extract was read at 645 and 663 nm with an Uvikon 940 spectrophotometer with spectral slit width 1.8 nm. The following parameters were calculated: Chl *a* = 12.7A663 – 2.69A645, and Chl *b* = 22.9A645 – 4.68A663.

Statistical analysis: Data analysis was performed using SPSS 13.0 (SPSS, Chicago, Inc., USA). Data were subjected to one-way analysis of variance (ANOVA) to determine significant differences among the treatments. Differences were discriminated using an LSD test. Significant results were assumed for $p \leq 0.05$. The graphs were produced with SigmaPlot 9.0 (SPSS, Inc., USA).

Results

Soil water change and drought grade: At the end of the experiment (21 d of water withholding), compared to the control, soil water content in the water deficit treatment decreased along the imposed drought period. Based on field capacity (29.47%), we have considered the existence of distinctness decreasing degree at different soil layers. Specifically, in the initial part of the experiment (0-6 d of water withholding), surface soil water content of the drought treatment dropped significantly, and deep layer soil water content reduced slightly. In the middle of the experiment (7-15 d of water withholding), surface soil water content decreased slowly, but deep soil water content decreased sharply. In the late stage of the experiment (16-21 d of water withholding), soil water content made no difference at the surface, and the deep layer declined slowly. At each layer, soil water content on the 6 d of water withholding was significantly different than that at the 21 d of water withholding ($p<0.05$, n=3) (Fig. 1.).

In the course of drought, the relative water contents (the percentage of soil water content relative to field capacity, w/w) of water deficit treatment after 6, 15, and 21 d of water withholding were 69.79%, 31.59%, and 25.69%, respectively. It is generally considered slight drought when relative soil moisture is between 60%-70%, moderate drought when it is 40%-60%, and severe drought when it is below 40% (Hsiao, 1973). Thus, in the hot summer with a temperature between 25°C and 40°C along the lower reaches of the Tarim River, soil water content in the pots was under the slight and severe drought stress level, at the 6 and 15 d, respectively, of water withholding after saturation irrigation.

Effect of water deficit on growth of *P. euphratica* seedlings: During the experimental period, the seedlings growths under water deficit treatment were inhibited due to soil water deficit. There were a few leaves (3-5) turning yellow by the end of the experiment. The height of the seedlings of the water deficit group increased by 1 cm in the initial part of the experiment (0-6 d), while in the control group it increased by 3.4cm (Fig. 2a.). In the middle and later stage (7-21 d), the height of the seedlings of the water deficit and the control group increased by 0.3 cm and 3.4 cm, respectively. The seedlings of the water deficit treatment did not have lateral branches (Fig. 2b.); whereas, those of the control group did. The basal stem diameter did not increase in the water deficit group, while it increased slightly in the control group (Fig. 2c.).

Effect of water deficit on ψ_p, g_s, φPSII and q_p of PSII: During the experimental period, for seedlings of the water deficit treatment, the continuous increase in the soil water deficit resulted in lower ψ_p values (Fig. 3.). In contrast, the control group seedlings'ψ_p had slight fluctuation, which showed no water deficit stress. The difference in the ψ_p between the water deficit group and the control group was increased as the duration of water withholding increased. At the 3rd d of water withholding, there was a difference of -0.26 MPa between the two groups; at the 15th d, the difference became -1.85 MPa. In response to progressive decreases in soil water, very remarkable changes were found in ψ_p, indicating poor water status in the seedlings of the water deficit group.

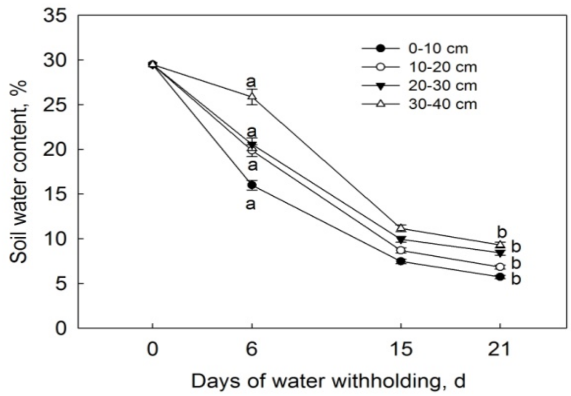

Fig. 1. Variations of soil water contents in the drought group during the experimental period.

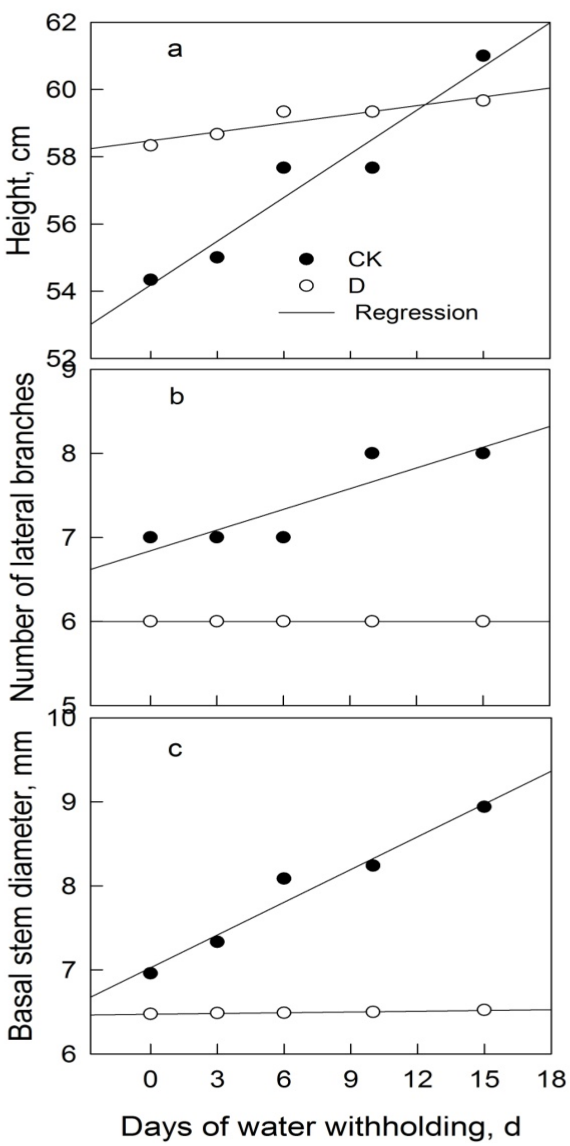

Fig. 2. Variations of height (a), number of lateral branches (b) and basal stem diameter (c) of *P. euphratica* seedlings under well-watered (CK) and water deficit (D) during the experimental period.

Because air temperature changes among different days, comparisons of g_s were made between the water deficit group and the control group at the same measuring time on 6 August (the 21st d of water withholding for the drought group) to avoid deviations resulting from the differences in air temperature (Fig. 4.). A variance analysis indicates that there were significant differences in g_s at all times of the day. The g_s in the control group displayed an increasing and then decreasing trend, and the highest g_s was about 243.50mmol m^{-2}s^{-1} at 12:00; whereas, in the water deficit group, it displayed a declining and then slightly increasing change, and the highest g_s was about 103.00 mmol m^{-2}s^{-1} at 8:00. This indicates that the g_s was largely reduced when the soil water content dropped from field water capacity to severe drought stress. The g_s in the water deficit group was lower than that in the control group, indicating that severe soil drought resulted in stomatal closure, which reduced the air exchange capacity of the seedlings.

The progressive soil water deficit also affected the energy transition and metabolism of *P. euphratica* during photosynthesis period, as well as the chlorophyll fluorescence of PSII. Because PAR varies among different measuring times, comparisons were made of the φPSII and the q_p between the water deficit group and the control group at the same time on August 6 (the 21 d after water withholding for the drought group) to avoid deviations resulting from the differences in PAR.

At all measuring times, there was a significant difference in φPSII values between the water deficit group and the control group except at 20:00, in which the control group seedlings had a higher φPSII value (Fig. 5.). Particularly, in the same group, φPSII values were similar at 8:00 and 20:00. The control group had a small amplitude (0.44-0.78), while the water deficit group had a larger amplitude (0.24-0.73). In addition, the curves were U-shaped, which indicates progressive soil drought had not destroyed the photosynthetic system. This is because the φPSII value at 20:00 could fully recover, despite of the actual photochemical efficiency of the water deficit group seedlings declining under drought stress at 8:00.

Variance analyses indicated that there was a significant difference in q_p between the water deficit group and the control group at all monitoring times except at 20:00, and the seedlings in the drought group had a lower q_p (Fig. 5.). This indicates that severe soil drought caused the portion reduction in absorption ability of PSII antenna pigment in the seedlings; this resulted in the weakening of photochemical activity in the PSII reaction center and, ultimately, led to the reduction in photosynthesis. In addition, leaf q_p in both groups displayed U-shaped daily curves, which indicates progressive soil drought had not destroyed the photosynthetic system for the q_p at 20:00 could fully recover, despite of the q_p of the water deficit group seedlings declining under drought stress at 8:00. The trough appeared at 14:00, and the maximum appeared at 8:00 and 20:00. However, the control group had a small amplitude (0.44-0.78), while the water deficit group had a larger amplitude (0.23-0.73), suggesting that the seedlings of the control group were more stable in terms of photochemical activity. This also suggests that progressive soil water deficit should affect photosynthetic activity in the seedlings.

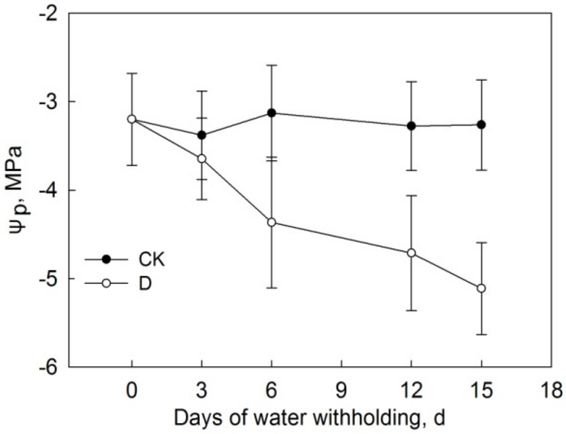

Fig. 3. Variations of ψ_p of *P. euphratica* seedlings under well-watered (CK) and water deficit (D) during the experimental period.

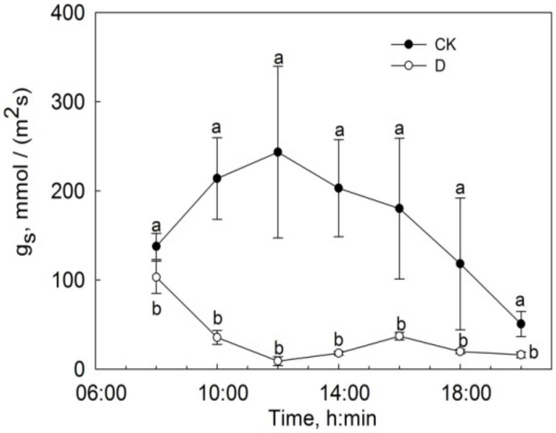

Fig. 4. Diurnal changes of g_s of *P. euphratica* seedlings under well-watered (CK) and water deficit (D) after 21 days of water withholding.

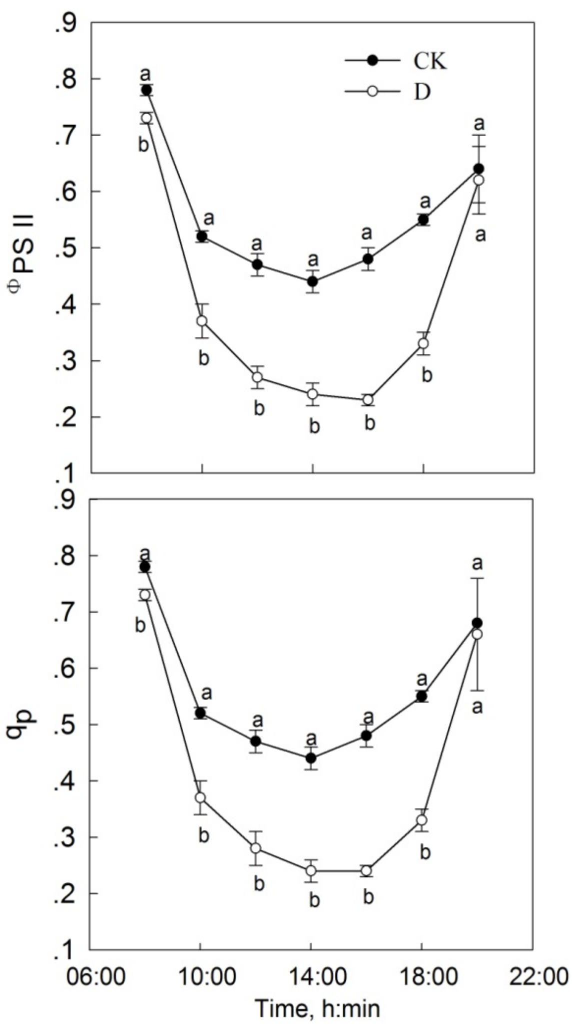

Fig. 5. Diurnal changes of φPSII and q_p of *P. euphratica* seedlings under well-watered (CK) and water deficit (D) after 21 days of water withholding. Different letters indicated significant differences ($p<0.05$) between CK treatment and D treatment by the LSD test.

Effect of water deficit on biochemical parameters: The content of chlorophyll a, chlorophyll b, Pro, MDA, and the activity of SOD and POD of the seedlings have shown different responses to soil water deficit. The content of chlorophyll a, chlorophyll b, and the activities of POD of the water deficit group (the 21st d of water withholding) were significantly lower than that of the control group ($p<0.05$, $n=3$). However, the activity of SOD of the water deficit group was not significantly different from that of the control group, and the chlorophyll a/b value could not change as the content of chlorophyll a and b reduced equally under drought in the water deficit group. Furthermore, analysis of variance demonstrated that no obvious difference existed between the water deficit group and the control group in content of the MDA and Pro (Table 2).

Discussion

The responses elicited vary according to developmental stage, severity, and duration of the stress. For potted *Jatropha curcas* seedlings grown in a climatic chamber with a day/night temperature of 28±2 ˚C / 20±4 ˚C, and day/night relative humidity of 35±5% / 75±5%, soil water deficit categories are: (1) non-to-mild stress (100-70% FC; day 0-9); (2) mild-to -moderate stress (70-30% FC; day 10-20); and (3) severe stress (30-15% FC, day 21-28) of water withholding after saturation irrigation (Sapeta *et al.*, 2013). However, in the hot summer with a temperature between 25 °C and 40 °C, and day relative humidity between 18.78% and 34.68% along the lower reaches of the Tarim River, soil water content in the pots was under slight and severe drought stress level after 6 and 15 d of water withholding, which indicates that plants in extremely arid zones are more vulnerable to drought stress.

The combined effect of soil water deficit and high atmospheric evaporative demand that frequently occur during summer time in arid regions can be detrimental. The availability of adequate water supply is primarily essential for the plants' metabolism to complete its life cycle with optimum growth and productivity. Tolerance to water-deficit conditions is a complex trait achieved by plants through coordinated action of physiological, biochemical, and molecular adaptations. Stomata of plants are the controlling structure for transpiration and gas exchange with the outside world. Normally, the first symptom of drought stress becomes evident at the stomatal level. Stomatal limitations are often thought to be short-term responses to drought stress. For young *Carapa guianensis* plants in water-stress, when predawn leaflet water potential (between 4:30 and 5:30) reached around -2.5 MPa, stomatal conductances were significantly ($p<0.05$) decreased from 239.2 mmol $m^{-2}s^{-1}$ (7:00) to 48 mmol $m^{-2}s^{-1}$ (9:00), and it remained about 10.1 mmol $m^{-2}s^{-1}$ from 11:00 (Kaliene *et al.*, 2013). After 30 d of withholding water, stomatal conductances of “Chemlali” olive trees decreased from 38±1 mmol $m^{-2}s^{-1}$ to 17±1 mmol $m^{-2}s^{-1}$, and decreased from 56±1 mmol $m^{-2}s^{-1}$ to 18±2 mmol $m^{-2}s^{-1}$ in “Chetoui” olive trees (Guerfel *et al.*, 2009). In the current study, stomatal conductances of seedlings in the water deficit treatment decreased from 103.0 mmol $m^{-2}s^{-1}$ (8:00) to 7.33 mmol $m^{-2}s^{-1}$ (12:00) (the 21st d of water withholding) and it was under -34.43 mmol $m^{-2}s^{-1}$ after 12:00. However, it increased from 137 mmol $m^{-2}s^{-1}$ (8:00) to 243 mmol $m^{-2}s^{-1}$ (12:00), and it was over 56.76 mmol $m^{-2}s^{-1}$ before 20:00 in the control treatment (Fig. 4.). These reverse changes indicated that the water deficit induced the stomata closure instead of opening in order to avoid leaf dehydration by sustaining transpiration. Especially, there was a large difference in stomatal conductance from 12:00 to 16:00 between the control group and the water deficit group, which may indicate a synergistic effect of both soil drought and atmospheric drought on the stomatal conductances in *P. euphratica* seedlings.

Water potential is one of the most important indicators of water status in plants. Early research led to the belief that when the soil dehydrates, the leaf water potential decreases (Schulze, 1993); however, this is not the case. *Juglans regia* and *Diospyros kaki* trees under severe water stress are still able to maintain higher leaf water potential; whereas, *Gleditsia sinensis* and *Diospyros lotus* are relatively low in leaf water potential under the same conditions (Shi *et al.,* 2009). The predawn leaf water potential of two-year-old olive trees (*Olea europaea L.,* cv. Coratina) decreased rapidly after 7 d of water deficit by withholding water, and it was equal to -6.0 MPa after 15 d of water deficit (Bartolomeo *et al.,* 2009), but it decreased to values below -1.5 Mpa after 15 d of withholding irrigation in two Poplar clone plants (Grazia *et al.,* 2012). Our results show that the predawn leaf water potential of the seedlings in the water deficit group declined along with the reduction of soil water content; it was equal to -5.11 MPa after 15 d of withholding irrigation (Fig. 3.), which suggests that soil water deficit led to serious water deficit in *P. euphratica* seedlings. According to these studies, although most plants decrease their water potential when they suffer water deficit stress, the drop in the degree of water potential is different.

The inhibitory effect of drought on photosynthetic activity has been widely described. Drought stress mainly damaged PSII of photosynthetic tissue. Maintaining PSII efficiency by reducing energy absorption can prevent oxidative damage and, hence, be adaptive in sites with prolonged periods of drought and intense radiation (Baquedano *et al*., 2006). Under well-watered conditions, φPSII of “Chemlali” and “Chetoui” olive trees were 0.16±0.03 and 0.22±0.01, respectively. After 30 d of withholding water, φPSII decreased by 24 % in “Chemlali” and by 34 % in “Chetoui”. After 30 d of withholding water, q_p of “Chemlali” decreased from 0.31±0.03 to 0.23±0.01, and it decreased from 0.42±0.03 to 0.32±0.04 for the “Chetoui” olive trees (Guerfel *et al*., 2009). In this study, φPSII and q_p were negatively affected (Fig. 5.). They had significantly reduced after 21 d of water withholding and the degree of their drop was the same. They decreased from 0.44 ± 0.01 to 0.24 ± 0.01, and 0.78±0.01 to 0.73±0.01 at 14:00 and 8:00 in the water deficit group, respectively, which suggests that the higher the temperature, the bigger the changes of the φPSII and q_p. The amplitude of their changes (0.24-0.73) in the water deficit group were larger than that (0.44-0.78) in the control group, which indicates that the seedlings’ photosynthesis was inhibited due to the progressive soil water deficit (Fig. 5.). However, there were no irreversible damages in PSII because the daily trend of φPSII and q_p was the same for both the control group and the drought group. Furthermore, there were reversibility changes of φPSII and q_p in leaves after 21 d of water withholding, which indicates the seedlings’ photosynthesis still working even under the most severe drought for no irreversible damages in PSII. However, if drought intensifies, the stomatal conductance may continue to fall, and irreversible damages may occur in the photosynthetic, which may led to poor growth, and even death for *P. euphratica* seedling. According to these studies, there are essential differences among different plants, such as olive trees and *P. euphratica* seedling, in terms of φPSII and q_p, which decrease when plants suffer water deficit stress; however, the degree of the drop of φPSII and q_p in different plants is different.

The syntheses of organic osmolytes, enzymatic, and non-enzymatic antioxidants together have been found to play an important role in the development of tolerance against water-deficit conditions. In Poplar leaves and roots, osmotically-active substances accumulate under water-limited conditions (Regier *et al*., 2009). After 15 d of withholding irrigation, free-proline increased significantly in two Poplar clone plants with respect to control plants (Grazia *et al*., 2012). Within a cell, SOD constitutes the first line of defense against reactive oxygen species, and MDA is an end product of the peroxidation of membrant lipids. Higher SOD activity was associated with better protection against water stress-induced oxidative injury. Under mild and/or moderate drought stress, some adapted species exhibit increases in activities of antioxidant enzymes, such as SOD and POD (Lima *et al*., 2002; Sajedi *et al*., 2012). The activity of SOD shows different trends with increasing soil water deficit, increasing first and then decreasing, decreasing first and then increasing, continually increasing (Pan *et al*., 2013; Li *et al*., 2010). It began to decline stress as the extension of drought stress under moderate drought stress (Fan *et al*., 2011). However, in the present study, the activity of SOD did not increase obviously after 21 d of withholding irrigation (Table 2), which implies three possible changes of SOD activity: keeping stable, increasing first and then decreasing, and decreasing first and then increasing. Furthermore, no obvious change may be due to a strong ability to adapt to drought stress, a shorter time of water withholding, or other factors which need further research. The activity of POD showed different trend with plant varies, increasing first and then decreasing (Kuang & Ge, 2010), continually increasing, or continually decreasing (Bacelar *et al*., 2006). Similar to water-stressed olive plants (Bacelar *et al*., 2006), the activity of POD decreased after 21 d of withholding irrigation (Table 2), which implies two possible changes of POD activity: continually decreasing, and increasing first and then decreasing. In the present study, the activity of POD decreased obviously which may be attributed to a short term of water deficit. Many factors can affect the enzyme activity, such as soil water, tree species, growth and leaf maturity of seedling. Therefore, it is not enough to evaluate the extent of the drought stress on the plant by one or two enzyme activity. Severe drought stress may cause damage to cells by inducing active oxygen production or by disrupting the scavenging systems that quench active oxygen and eliminate the detrimental effect (Van Breusegem *et al*., 1998). Under severe drought stress, leaf chlorophyll content often declines due to chlorophyll degradation (Burcu & Merve, 2014). However, drought stress did not reduce chlorophyll content, but led to decreased chlorophyll a/b ratio (Sapeta *et al*., 2013). In fact, an increase in Chla/b ratio during drought has been reported for several species (Liu *et al*., 2011). In our research, drought stress reduced chlorophyll content, but did not lead to decreased chlorophyll a/b ratio; this is the same as that in the well-watered conditions. Water deficit stress significantly increased Pro contents in the leaves of safflower (Qudsia *et al*., 2013); however, MDA contents decreased in *Populus nigra* L. (Regier *et al*., 2009). In *Sophoraviciifolia* seedling, Pro contents were not related to soil water (Wang *et al*., 2005). Accumulation of MDA in the cell is found to be an efficient determinant of stress-induced oxidative damage to peroxidation of membrane lipids affecting decreased growth rate and productivity of the crop plants exposed to different abiotic stresses. As responses to drought stress, there were significant increases in MDA in cotton leaves (Deeba *et al*., 2012). However, Gebrekirstos *et al.* (2006) asserted that MDA content does not necessarily represent the degree of membrane lipids peroxidation. In our research, the contents of Pro and MDA did not demonstrate an obvious change in the water deficit group after 21 d of water withholding (Table 2), which indicated three possible changes in their trends: keeping stable, increasing first and then decreasing, and decreasing first and then increasing. This may be attributed to the short term of water deficit which did not induce injury in the seedlings, or other factors which require further research. Since the test period was relatively short, further research is needed to determine physiological and biochemical responses of more than two years *P. euphratica* seedlings to water deficit lasting more than 21 days.

A change in plant growth is a reflection of the apparent form of water stress. Water deficit alters plant growth rate depending on the intensity and duration of the stress. Drought reduced *Jatropha curcas* stem elongation, leaf emergence, and total leaf area (Sapeta *et al*., 2013). Long-term drought during one and two growing seasons, respectively, strongly affected the timing and number of flushes of oak plants (*Quercus robur* L), and negatively influenced subsequent growth (Nadine *et al*., 2012). Our results show that the increasing rate of *P. euphratica* seedling's height and stem base diameter declined as the soil water content decreased. Furthermore, the growth rate of the seedlings height in the later 15 d (7-21 d) was much slower than that in the initial stage of the experiment (0-6 d) in the water deficit treatment; this indicates that, in the natural environment in the lower reaches of the Tarim River, the *P. euphratica* seedlings can grow well at least within 6 d after one overflow irrigation, in 15 to 21 d, it can live, and over 21 d, it may wilting, which is quite useful for plantation management, especially for designing irrigation regimes, in extremely arid areas. When building a shelter forest, *P. euphratica* is the preferred species. Furthermore, in the case of high temperature and no rain, the *P. euphratica* must be watered every 21 d at least in seedling stage, but in the mature stage the time interval of water can increase during the period of planting. Since the test period was relatively short, further research is needed to determine how long it can survive under progressive soil water deficit.

Table 2. Biochemical parameters of *Populus euphratica* seedlings under well-watered (CK) and water deficit (D) after 21 days of water withholding. Values are the mean ± SD (n=3).

Treatments	Chl a / ($mg\cdot g^{-1}FW$)	Chl b ($mg\cdot g^{-1}FW$)	Chl a/b	POD ($unit\cdot g^{-1}FW\cdot min^{-1}$)	SOD ($unit\cdot g^{-1}FW\cdot min^{-1}$)	MDA (μg·g-1)	Pro (μg·g-1)
CK	0.90 ± 0.07a	0.26 ± 0.02a	3.46 ± 0.04a	1691.8 ± 140.2 a	394.35 ± 42.69a	1.08 ± 0.1a	163.32 ± 19.69a
D	0.66 ± 0.04b	0.19 ± 0.04b	3.47 ± 0.03a	1063.9 ± 106.1 b	369.02 ± 35.08a	1.04 ± 0.03a	231.71 ± 20.05a

Different letters indicated significant differences ($p<0.05$) between CK treatment and D treatment by the LSD test. Chl a: chlorophyll a; Chl b: chlorophyll b; POD: peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde; Pro: proline

Conclusions

The seedlings of *P. euphratica* here investigated showed a different response to water deficit. Our results show that, under a progressive soil water deficit for a period of 21 d, the seedlings height, stem base diameter, and number of lateral branches decreased. The continuous increase in the soil water deficit resulted in lower ψ_p values. There were significant differences in g_s at all times of the day of the 21st d of water withholding. The highest leaf stomata conductance was about 103.00 mmol $m^{-2}s^{-1}$mmol $m^{-2}s^{-1}$ at 8:00 after 21 d of withholding irrigation. At all measuring times of the day of the 21st d of water withholding, water deficit reduced φPSII and q_p at all monitoring times, except at 20:00, but increased their daily amplitude. Short-term soil water deficit did not induce changes in Pro and MDA content, chlorophyll a/b value or SOD activity, but led to decreased content of chlorophyll a, chlorophyll b, and the activities of POD. The results can provide guidance for *P. euphratica* conservation and recovery in arid areas.

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