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ORIGINAL ARTICLE

Does drought stress induce physiological mechanisms in *Celtis caucasica* L. Seedlings?

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ABSTRACT
Drought stress is an important environmental factor inhibiting plant growth. The examination of physiological reaction of different species to the drought stress is useful to recognize effective mechanisms in drought tolerance and choosing the suitable species for plantation in dry lands. In order to determine the resistance of Celtis caucasica and understand the physiological mechanisms in response to the drought condition, an experiment was designed in a completely randomized block with one factor (irrigation), at five levels (1, 3, 5, 7, and 9 days) with five replications chlorophyll fluorescence parameters (Fv/Fm, Fm and F0) and pigments content (total chlorophyll, chlorophyll a, and chlorophyll b) of leaves. The results indicated that drought stress induced a significant reduction at five percent level in maximum photochemical efficiency of photosystem II (Fv/Fm) and maximum fluorescence (Fm) while the minimum fluorescence (F_0) was not significantly affected by drought. With increasing the intensity of drought, chlorophyll content in leaves was increased. Maintaining the leaf chlorophyll content under stress conditions, is one of the physiological indicators of stress resistance concluding that C. caucasica would be one of the most suitable species for afforestation plans in arid and semi-arid regions.
Key words: *drought stress, Celtis caucasica, chlorophyll fluorescence, proline*

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INTRODUCTION

Drought stress is an important environmental factor that affects plants growth, productivity, photosynthesis and changes plants metabolism [23], [24]. Drought stress is one of the most important environmental inhibitor for photosynthesis via stomatal closure [13].In this condition, decreasing photosynthesis is related to disturbing the biochemical processes of the photosynthetic apparatus. Photosystem II is the most sensitive part of the photosynthetic systems and plays a critical role in plants reaction to environmental stresses [13]. Damage to photosystem II is frequently the first symptoms of stress in a leaf [23].

Plant physiologists have suggested chlorophyll fluorescence as a sensitive indicator of stress condition in plants. The measurement of chlorophyll fluorescence in natural environment is a useful technique to understand the tolerance of photosynthetic apparatus of the environmental stress [23]. This technique is nondestructive and can be just as operative as the gas exchange techniques to disclose differences between drought tolerant and susceptible species [25]. There have been many fluorescence parameters so that it is not possible to give an extensive review of all of these here. F_0 (minimum fluorescence from dark adapted leaf is the level of fluorescence when primary quinine electron acceptors of PS II (Q_A) are maximally oxidized (PS II centers are open), F_m (Maximum fluorescence from dark adapted leaf is the

level of fluorescence when Q_A is maximally reduced (PSII centers are closed), F_v(variable fluorescence from dark adapted leaf shows the ability of PSII to perform primary photochemistry (photoreduction of Q_A) and F_v/F_m.The values of F_v/F_m in dark adapted leaves indicates the potential yield of the photochemical reacting in PSII used as a practical alternative to monitor the photosynthetic performance [15]. The optimal values of F_v/F_m in healthy leaves of most plant species are about 0.832 [6] and the lower values show the exposure of plants to stress. This is the consequence of photo damage to PSII reaction centers, and the progression of quenching process [23], [9]. Literature reviews show that there are differences between physiological responses of plants to drought stress. These differences are related to species type and to some extent, experimental conditions. In the present research, *C.caucasica* was employed to study the photosynthetic responses to the drought stress. It is commonly used as an urban tree because of its ability to withstand drought and tolerate in urban environments. The aims of this study were: (i) to evaluate the effect of drought stress on the chlorophyll fluorescence parameters (ii) to find the relationship between chlorophyll contents and drought tolerance in *C. caucasica* and, (iii) assessing the vitality of *C.caucasica* in different irrigation regimes for planting in semi-arid areas.

MATERIAL AND METHODS

Materials and Experimental design

This study was conducted in the Alborz Research Station in southwest slope of Alborz mountain in Iran (latitude 35° 48´N, 50° 54´E and 1300 m a.s.l) with a semi-arid climate where means annual temperature and rainfall are 13.7 ° C and 230 mm, respectively.

The seeds were collected from 7km in the road of Chaloos-Nojan with elevation of 1450m and in April, 2011, they were sawn in plastic pots (15×40 cm) containing an equal mixture (2:1:1) of clay, sand and organic fertilizer. Pots were irrigated every day for about one year. No artificial lighting was used in this study excluding natural light from the sun. Regular management was conducted until the seedlings were about 50 cm height. Twenty five seedlings were selected randomly and classified to five groups of treatments. Drought experiment started in August 2012. Measurements were performed on 1, 3, 5, 7 and 9 days after drought. Soil was fully drenched before the experiment.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (PAM 2500 WalzGermany) on three fully expanded leaves of five pots per treatment. Leaves were adapted to darkness for 30 minutes by attaching light clips to the leaf surface. All measurements carried out every day, from 10 AM to 14 PM.

Chlorophyll content

To assess chlorophyll content, fresh and mature leaves (0.1g) were extracted with 10 ml of 80% acetone and centrifuged at 4000 rpm for 15 min. The absorbance was read spectrophotometric ally at 654 and 663 nm and calculated for chlorophyll a, chlorophyll b, and total chlorophyll according to [3].

Statistical Analysis

The normality of data was assessed using the Kolmogorov-smirnov test and the homogeneity of variances was determined using the Leven's test. The average of parameters was compared using Duncan or Games Howel test.Data analysis was performed using SPSS 17.0.

RESULTS AND DISCUSSION

Chlorophyll fluorescence

As can be seen in Table 1, maximum photochemical efficiency of PSII (F_v/F_m) and maximum fluorescence (F_m) in *C. caucasica* decreased significantly after 5 days drought. During the experiment, the lowest amount of F_v/F_m reaching 0.254 after 9 days withholding water.

Table 1. Fo, Fm and Fv/Fmvalues in*C. caucasica* L. under different watering regimes. Data were expressed as mean ± SE

Treatment	F_0 Mean+SE	F_m Mean+SE	F_v/F_m Mean+SE
Control	1.704±0.107 a	6.583±0.088 a	0.748±0.008 a
3d	1.607±0.109 a	6.272±0.204 a	0.740±0.008 a
5d	1.686±0.110 a	5.872±0.287 a	0.695±0.111 b
7d	1.731±0.127 a	6.023±0.251 a	0.685±0.020a b
9d	1.927±0.166 a	3.236±0.416 b	0.254±0.066 c

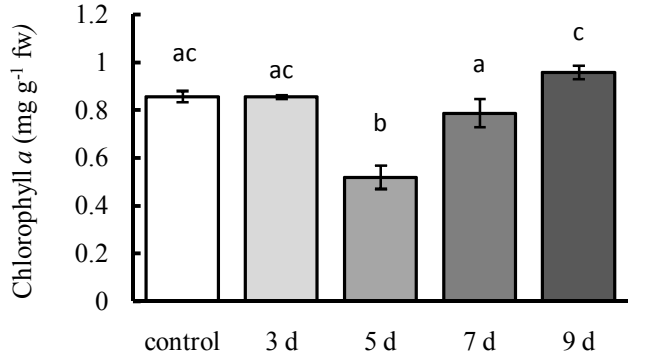
Note: Data in the same column followed by different small letters are significantly different at 0.05 level of significance.

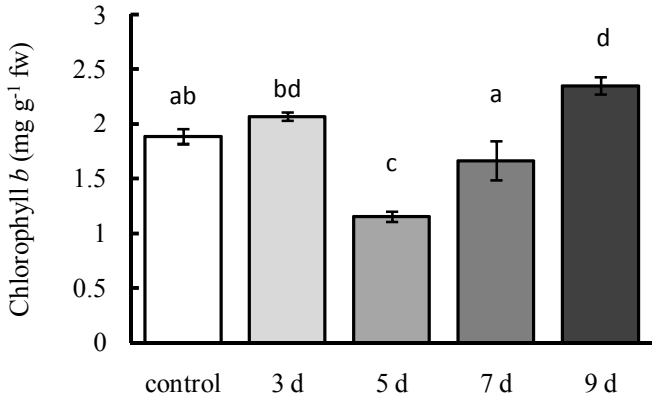
The amount of F_m in 9 days drought has been significant differences with other treatments with the measure of 3.236 (µmol m^{-2} s^{-1}). The minimal fluorescence (F_0) was constant throughout the entire course of the experiment. Most studies showed that under drought conditions, the reduction of photosynthesis associated with disorder in biochemical reactions[14]. Drought damages the reaction center of photosystem II [8], [9], [20]. Hence the reduction of the maximum photochemical efficiency of photosystem II (F_v/F_m) and fluorescence changes in the time interval is used as a criterion of tolerance and stress resistance[12]. We found out that the maximum photochemical efficiency of photosystem II (F_v/F_m) and maximum fluorescence (F_m) had a downward trend with increasing the drought stress. This is in agreement with [4] who reported the reduction of F_v/F_m and F_m in seedlings of *Pinushalepensis*, *Quercuscoccifera* and *Quercus ilex*. We also showed that *C. caucasica* had significant differences in the ratio of F_v/F_m since 5 days and in F_m since 9 days after drought. The high level of F_v/F_m and F_m indicated the high amount of photosynthesis. The F_v/F_m represents the electron transport capacity of photosystem II [10]. That has high correlation with quantum yield of net photosynthetic [2]. According to Alidib et al., [1] decrease of efficiency of photosystem II centers to consumption of photons, exhibited the measure of photo inhibition under drought stress conditions. The decrease in F_m revealed that the lower oxidation of Q_A under drought conditions and reduced photochemical reactions.

The rapid changes in fluorescence that occur during the rapid induction to a peak have long been attractive for detecting differences in photosynthetic performance in plants. On immediate exposure to light, fluorescence rises to the minimum level of fluorescence, termed F_0 level, which is the fluorescence level obtained when the PSII reaction centers are in the open state, i.e., capable of photochemistry since Q_A, the primary quinone acceptor of PSII, is maximally oxidized. The results indicated that the minimum fluorescence in *C.caucasica*was not significantly affected by drought in different treatments. This result is in agreement with findings of [11] and [22]. Since F_0 values are related to chlorophyll fluorescence of PSI receptors [2], [27] and considering that non-significant difference of F_0 between irrigation regimes, it seems the receptors of chlorophylls had almost a similar efficiency between irrigation regimes.

Chlorophyll content

With the drought process, Chlorophyll a, Chlorophyll b, and total Chlorophyll in *C. caucasica* had significant upward trend. Chlorophyll a, Chlorophyll b, and total Chlorophyll in 9 days drought were 1.1, 1.2, 1.2 times, respectively, in compare to that of the control.





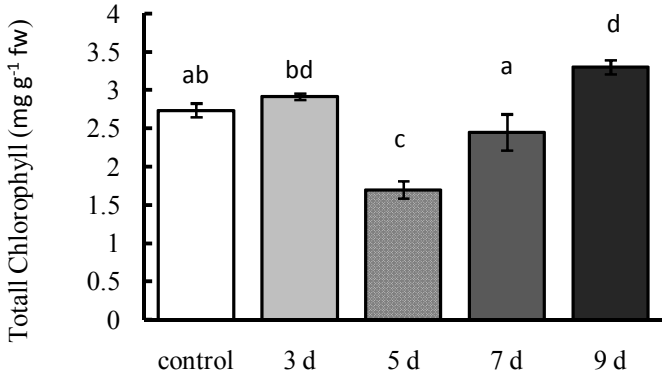


Fig.1. Effects of different watering regimes on chlorophyll *a*, chlorophyll *b,* and total chlorophyll of *C. caucasica* leave. Values are mean ± SD and bars indicate standard error.

Chloroplast pigments play an important role for the light absorption and conversion in the photosynthesis process. Generally in most plants the contents of photosynthetic pigments (chlorophyll) are reduced with increasing drought [7]. Research showed that the chlorophyll content in plants with low resistance is decreased with increasing the drought [17], [4]. which is in contradiction with our results. In our experiments, chlorophyll content of *C. caucasica* increased with drought deepening. Guan *et al* [16] found that total chlorophyll content in Moslach chinensis increases with decreasing soil water content. The increase in chlorophyll content may be associated with the decrease of leaf water content during the drought [19]. This may compensate the reducing leaf area under drought stress. Increase in chlorophyll content on one hand and reducing leaf area on the other can be attributed to the maintenance of photosynthetic rate under drought stress [24]. Drought stress damages the reaction centers of photosystem II [8], [9]. In our study, the F_v/F_m value decreased with increasing the levels of stress. This decrease was very small until 7 days after drought, reflecting *C. caucasica* seedlings has a strong ability to tolerate drought stress.

We concluded that *C. caucasica* seedlings are resistant to drought stress. *C. caucasica* seedlings can increase its chlorophyll content and on the other hand the decline rate of the photochemical efficiency of photosystem II is very small. Since the drought damages the photosynthetic apparatus via reducing the capacity of electron acceptance, it has a negative impact on the photochemical efficiency of photosystem II [22]. This indicates the higher photosynthetic efficiency of *C. caucasica* under drought conditions. The results of chlorophyll fluorescence confirm that a significant increase in chlorophyll content under stress enhances the excitation capacity of photosystem II being one of the physiological indicators of stress resistance. *C. caucasica* would be one of the most suitable species for afforestation plans in arid and semiarid regions.

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