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The Influence of Drought Stress on Seedlings of Four Deciduous Species, Common in Nurseries of the Semi-Arid Region of Iran

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ABSTRACT

Aim: With global warming and limited water resources in the world, especially in the arid lands of Iran, managing the production of forest seedlings in the country's nurseries is facing a particular challenge. In this regard, the study of drought-tolerant plants to select them in the mass seedling production programs can be helpful.

Material and methods: Potted seedlings of Persian oak (*Quercus brantii*), pistachio (*Pistacia atlantica*), common purple (*Cercis siliquastrum*), and black poplar (*Populus nigra*) under regular irrigation (100% field capacity) and drought-stressed (30% field capacity) conditions were examined over 120 days in the greenhouse of Bam Khorramabad Nursery.

Findings: In all species in stressed seedlings compared to control seedlings, the content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids decreased, but the content of proline and malondialdehyde (MDA) increased. Under stress conditions, MDA content in black poplar, common purple, Persian oak, and pistachio was 174, 121, 105 ,and 102% higher than under control seedlings, respectively, and this increase in black poplar over other species ranged from 93.5 to 176.5%.

Conclusion: This study confirms that black poplar has a lower resistance rate to drought stress among four tree species. Thus, due to their higher tolerance to water scarcity, Persian oak, pistachio, and common purple can be prioritized in the mass seedling production programs of nurseries in arid areas suffering from limited water resources.

Keywords: Chlorophyll, Drought stress, Malondialdehyde, *Pistacia atlantica*, Proline.

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Introduction

Today, in many regions of the world, excessive use of surface water and increasing dependency on non-renewable groundwater reserves is growing. Undoubtedly, due to this trend, a significant contribution from water supply for various uses in the future will face a problem [1]. Drought stress is one of the chief environmental stresses which happens for different reasons such as reduced rainfall, increased salinity, cold, high and low temperatures, and high intensity of sunlight [2]. Drought stress occurs when the available water in the soil is reduced, and atmospheric conditions cause continuous water loss by transpiration or evaporation. It leads to physiological and metabolic changes and negatively affects the growth and development of plants, and even causes the death of plant species [3, 4]. In general, the response of plants to drought stress depends on their genetic structure, species type, stress intensity, and plant growth stages [1,5]. Drought stress due to several non-stomatal factors leads to reduction or even stopping the photosynthetic pigment synthesis [6], decreased growth respiration, stomatal conductance, enzyme disturbance, and proline accumulation [7]. Photosynthetic pigments (such as chlorophylls and carotenoids) are important indicators of drought stress in many plant species, as decreasing changes of these pigments in drought stress conditions are more in drought-sensitive plants [3]. Amino acid proline regulates the osmotic and enzymatic activity and accumulates in plants exposed to drought stress [8].

In an investigation on *Pinus taeda*, a 40-fold increase was reported in proline concentration of drought-stressed seedlings compared to non-stressed seedlings [9]. In some studies, increased proline accumulation has been mentioned as an index of drought stress sensitivity of plant species [6,8,10]. Malondialdehyde (MDA) is the primary marker of lipid peroxidation in cell membranes, as in water deficit conditions, oxidative damage to cell membranes results in a rise in MDA content [8].

Numerous studies have been conducted about the effect of drought stress on pigment and biochemical traits of different forest species in various world regions [3,6,14]. Findings on *Lagerstroemia indica* seedlings showed that drought decreased chlorophyll and carotenoid contents but increased MDA and proline [2]. In other research, a higher accumulation of MDA and proline affected by drought stress was observed in seedlings of two Iranian oak populations *(Quercus brantii*) [11]. In seedlings of *Pinus massoniana*, lower contents of chlorophyll a and chlorophyll b and, in contrast, higher contents of proline and MDA were obtained in drought-stressed seedlings [10]. Findings on *Picea abies* seedlings revealed that plant pigments such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids decreased with increasing drought stress [12]. Likewise, a decrease in the content of chlorophyll and carotenoid of *Quercus acuminata* [13] and an increase in MDA *Cercis siliquastrum* [14] under the effect of drought stress was reported. In another study [15], drought stress led to a decrease of chlorophyll a, chlorophyll b, and total chlorophyll content and an increase of proline content in *Robinia pseudoacacia* seedlings. Evaluation of plant drought tolerance is possible by adopting strategies such as screening via the response of photosynthetic pigments and biochemical characters. However, mass production of tolerant plant species is considered a suitable approach for afforestation management in drought status [8].

Quercus brantii (Persian oak), *Pistacia atlantica* (pistachio), *Cercis siliquastrum* (common purple), and *Populus nigra* (black poplar) are species their seedling production is common in forest nurseries of Iran, especially in western parts (Zagros region), which is

Figure1) Bam Khorramabad nursery, Lorestan Province, Iran.

faced water shortage problems. So, several million seedlings of these tree species are used annually in programs for the restoration of forest habitats and the development of parks and green spaces in various parts of the country. The study of biochemical mechanisms of seedlings produced in forest nurseries can be important in selecting drought-resistant species and the realization of optimal irrigation management. However, according to the recent literature, there is insufficient information about the effects of drought stress on the biochemical responses of the above four tree species. Thus, the main question of the present investigation is as follows: What is the response of biochemical traits of the studied species to drought stress?

Material & Methods

For the present study, two-year-old uniform seedlings (stem height of 40 ± 5 cm and root collar diameter of 8 ± 2 mm) of Persian

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oak, pistachio, purple and black poplar (72 seedlings of each species) were investigated in Khorramabad nursery, western Iran, (Lan 48° 23′ 34″, Lat 33° 27′ 45″, 1390 m a. s. l.). Drought stress was applied as two levels of watering regimes, 100% field capacity (regular irrigation or control) and 30% field. capacity (FC) with three replications. The experiment was conducted in a greenhouse under controlled conditions, including a light period of 12 hours, a mean temperature of 23 ± 7 °C, and optical photosynthetic radiation of 1200 μmol m^{-2} S^{-1} for 120 days period.

To determine soil field capacity and amount of water required for pots at each level of watering regime, the method of Zarik et al. [16] was performed. At the end of the experiment period, plant pigments including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid (in $mg.g^{-1}$ fresh weight) were measured using the method of Arnon [17]. MDA content was determined following the procedure of Heath and Packer [18]. Free proline content was measured as described by Bates et al. [19].

Data analysis was performed using SPSS software ver. 26 (SPSS Inc., Chicago, IL, USA). The normality and homogeneity of variance of the data were determined using the Kolmogorov-Smirnov test and Levene test, respectively. The effect of drought stress and plant type was evaluated using two-way ANOVA. Duncan's multiple range test ($p = 0.05$) was used to compare means. Chlorophyll a was compared by Student's t-test in two irrigation levels in each species.

Findings

According to Table 1, the effect of irrigation regime and species type and the interaction effect of irrigation regime × species type on chlorophyll b, carotenoids, proline, and MDA were significant. Chlorophyll a was only affected by the irrigation regime (Table 1). Irrigation level and the interaction of irrigation regime × species type significantly affected the chlorophyll a/b and total chlorophyll. The total chlorophyll/carotenoid ratio was influenced by species type and the interaction of irrigation regime × species type (Table 1).

In each plant species, drought stress (30% FC) as compared with control (100% FC) resulted

Table 1) Two-way ANOVA of irrigation level and species type on measured variables.

Variables	Irrigation level		Species type		Irrigation level × species type	
	F-value	Significant	F-value	Significant	F-value	Significant
Chlorophyll a	218.138	0.000*	1. 239	0.328^{ns}	1. 112	0.373^{ns}
Chlorophyll b	191.890	0.000*	3. 454	0.042*	7. 405	0.002*
Chlorophyll a/b	18.300	0.001*	0. 810	0.503^{ns}	3. 850	0.030*
Total Chlorophyll	630. 825	0.000*	1. 780	0.191^{ns}	5. 414	0.009*
Carotenoid	285. 441	0.000*	16. 525	0.000*	6. 875	0.003*
Total Chlorophyll/ carotenoid	0. 850	0.370^{ns}	10. 240	0.001*	4. 850	0.014*
Proline	357. 751	0.000*	50. 338	0.000*	36. 395	0.000*
MDA	218. 403	0.000*	34. 656	0.000*	10. 461	0.000*

* and ^{ns} symbols indicate significance at the 5% level and no significance, respectively.

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Table 2) Comparison of means (± standard deviation) (mg. g$^{-1.}$ FW) of variables measured as affected by drought stress and species type

Variables	100% FC				30% FC			
	Quercus brantii	Pistacia atlantica	Cercis siliquastrum	Populus nigra	Quercus brantii	Pistacia atlantica	Cercis siliquastrum	Populus nigra
Chlorophyll a	0.92±0.06*	0.81±0.06*	0.97±0.09*	0.94±0.13 *	0.56±0.04	0.48±0.17	0.42±0.05	0.39±0.02
Chlorophyll b	0.96±0.16b	1.35±0.18a	0.89±0.13b	0.95±0.14b	0.49±0.08c	0.24±0.09d	0.28±0.08cd	0.31±0.10cd
Total Chlorophyll	1.89±0.10b	2.16±0.19a	1.86±0.06b	1.90±0.02b	0.92±0.07c	0.67±0.08d	0.70±0.12d	0.76±0.17c
Cartenoid	0.63±0.07b	0.42±0.04d	0.75±0.05a	0.54±0.07c	0.26±0.05e	0.19±0.02f	0.26±0.04e	0.23±0.04e
Chlorophyll a/b	0.99±0.21bc	0.61±0.09c	1.13±0.29bc	1.02±0.31bc	1.16±0.12bc	2.14±0.70a	1.58±0.45ab	1.49±0.42ab
Total chlorophyll/ carotenoid	3.03±0.35bc	5.12±0.20a	2.49±0.21c	3.55±0.42b	3.63±0.76b	3.59±0.55b	2.86±0.99bc	3.32±0.10bc
Proline	0.60±0.07d	0.59±0.11d	0.58±0.03d	0.66±0.07d	0.90±0.04c	0.98±0.08bc	1.06±0.05b	1.75±0.10a
MDA	4.95±1.20d	6.19±1.66d	6.42±0.90d	10.08±1.36c	9.98±1.96c	12.66±2.68b	14.21±1.77b	27.59±3.02a

* In chlorophyll a, row indicates a significant difference between two irrigation levels of each species, using the T-test. Using the Duncan test, different English lowercase letters in each row indicate significant differences among eight water deficit-species type treatments.

Table 3) Amount and percentage increase in proline and MDA content (mg. g^{-1}.FW) of each species between two field capacities, and percentage increase in *Populus nigra* compared to other species.

Variables	Tree species	FC 100%	FC 30%	Increase of amount between two levels	Percentage of increase of 30% FC over 100% FC	Increase of the amount of Fc 30% of P. nigra over other species (%)
Proline	*Populus nigra*	0.66	1.75	1.09	165	-
	Cercis siliquastrum	0.58	1.06	0.48	82	65.1
	Pistacia atlantica	0.59	0.98	0.39	66	78.6
	Quercus brantii	0.60	0.90	0.30	50	94.4
MDA	*Populus nigra*	10.08	27.59	17.51	174	-
	Cercis siliquastrum	6.42	14.21	7.79	121	93.5
	Pistacia atlantica	6.19	12.66	3.79	105	117.9
	Quercus brantii	4.95	9.98	5.03	102	176.5

in a decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, and an increase in proline and MDA (Table 2). Under drought stress conditions, the lowest concentration of chlorophyll b and carotenoid content (0.24 and 0.19 mg.g^{-1}. FW, respectively) and the highest value of chlorophyll a/b (2.14) were recorded in *Pistacia atlantica*. Also, the lowest content of total chlorophyll allocated to *Pistacia atlantica* and *Cercis siliquastrum* (0.67 and 0.70 mg.g^{-1} FW, respectively) and the highest amount of total chlorophyll/carotenoid ratio obtained for *Quercus brantii* and *Pistacia*

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atlantica (3.63 and 3.59, respectively). The highest values of proline and MDA (1.75 and 27.59 $mg.g^{-1}$.FW, respectively) were observed for *Populus nigra* (Table 2). In well-irrigated treatment, the highest amount of chlorophyll b, total chlorophyll, and total chlorophyll/carotenoid belonged to *Pistacia atlantica* (1.35 and 2.16 $mg.g^{-1}$.FW and 5.12, respectively) (Table 2).

The increased rate of proline in stressed seedlings with 30% field capacity compared to well-irrigated seedlings in *Populus nigra*, *Cercis siliquastrum*, *Pistacia atlantica*, and *Quercus brantii* was 165%, 83%, 66%, and 50%, respectively (Table 3). This increase for the proline of the above species was 1.09, 0.48, 0.30, and 0.39 $mg.g^{-1}$.FW, respectively. Also, under drought stress, the percentage increase of MDA level in *Populus nigra* seedlings compared to *C. siliquastrum*, *P. atlantica*, and *Q. brantii* was 93.5%, 117.9%, and 176.5%, respectively (Table 3).

Discussion

Plant adaptation to water scarcity is formed through different physiological and biochemical responses. These collective responses lead to an adjustment in the growth rate of plants as an adaptive response for survival [20]. Similar to the findings reported on *Pinus massoniana* [10], *Picea abies* [12], *Robinia pseudoacacia* [15], and *Pistacia atlantica* [21], the four species of our study responded to increased drought stress with reductions in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid. In well-irrigated treatment (100% FC), the highest amount of carotenoid was related to *C. siliquastrum* seedlings, and the highest content of chlorophyll b and total chlorophyll was recorded for *Pistacia atlantica* seedlings. Chlorophyll is a major component of chloroplasts and an important indicator of oxidative stress. It plays a critical role in photosynthesis and declines as drought stress increases [8]. Under drought stress, the increasing levels of radicals and other ROSs (oxidative stress) undermine chloroplasts, leading to chlorophyll degradation and reduction of membrane permeability [12]. These combinations disturb chlorophyll and decline pigments production [15,22,23]. The other possible reason behind the reduction in chlorophyll content under water deficit conditions is enhanced activities of chlorophyllase (chlorophyll-degrading enzyme) [2,10] The ratio of total chlorophyll to carotenoid was not affected by drought stress in our study, as indicated by the uniform sensitivity of both pigments to drought stress.

Carotenoids are responsible for scavenging singlet oxygen; hence, plant carotenoid levels have been suggested to measure their tolerance. Drought stress caused a substantial loss of photosynthetic reaction centers. The carotenoid loss was expected since carotenoids are mainly associated with photosynthetic reaction centers [11,23,24].

Similar to results on *Pinus eldarica* [24], *Cercis siliquastrum* [14], *Quercus brantii* [11], and *Lagerstroemia indica* [2], in the current investigation, drought stress caused an increase of proline in all tested plant species. Also, the maximum proline accumulation observed in stressed seedlings of *P. nigra* was more than that in the other three species. Proline is a regulator of osmotic potential and a protective agent for the structure of organs and membranes of plant cells [11]. The rate of proline accumulation in plant cells depends on plant species and stress intensity [25].

Consistent with the findings on *L.indica* [2], in the current investigation, the carotenoid content was reduced in stressed seedlings compared to control seedlings of all the examined species. Basically, in plants, to increase adaptation to drought stress, part of the carotenoid is decomposed and

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converted to abscisic acid, reducing the content of carotenoids in the plant [26]. This is while the carbon use efficiency and increased production of ethanol and lactate under water deficit diminish the synthesis of carotenoids and chlorophyll [14]. It may also be stated that the production of ROSs under drought stress has deleterious effects on carotenoid content, especially in high concentrations. The accumulation of these compounds, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, causes the destruction and decomposition of carotenoids [2,27].

Similar to reports made on *Q. acutissima* [13] and *C. siliquastrum* [14], our findings showed that the MDA of all studied species was related directly to drought status. Also, the highest amount of MDA was obtained in *P. nigra* seedlings under drought stress. In general, the common response of plants to oxidative stress is associated with lipid membrane peroxidation or the increase of the content of MDA [11]. In other words, under stress, overproduction of ROSs causes oxidative damage, which ultimately leads to plant death. In other words, under stress, overproduction of ROSs causes oxidative damage, which ultimately leads to plant death. Since the increase of MDA content is directly connected to the degree of sensitivity of plant species [28,29,30,31], it can be noted that in our study, *Populus nigra* is more sensitive against drought stress than other species due to the higher content of MDA.

Conclusion

Generally, findings of the present study show a decrease in photosynthetic pigments rate and an increase in proline and MDA content in all studied species under drought conditions. Under water deficit, a substantial increase of MDA in *P. nigra,* compared to *Q. brantii*, *P. atlantica*, and *C. siliquastrum*, was detected. Thus, it is suggested that in arid regions of the country, in forest nurseries facing water shortage, mass seedling production of *P. nigra* to be less prioritized than *Q. brantii*, *P. atlantica*, and *C. siliquastrum* due to its low tolerance to water scarcity.

Conflict of Interest: The author states that there are no conflicts of interest regarding the publication of this manuscript.

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