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Physiological and biochemical responses of *Quercus brantii* seedlings to water deficit stress

Zolfaghari R.[1]*, Akbarinia M.[2]

1. Department of Forestry, Faculty of Agriculture and Forestry, Yasouj University, Yasouj, Iran
2. Department of Forestry, Faculty of Natural Resources, Tarbiat Modarres University, Noor, Iran

** Corresponding author's E-mail: zolfaghari@yu.ac.ir* (Received: Oct. 04. 2017 Accepted: March 12. 2018)

ABSTRACT

Water shortage is one of the most important environmental stresses in Mediterranean regions. Poor seedling quality may account for the failure of oak regeneration. To determine the best seed origin of *Quercus brantii*, we investigated on seedlings collected from 20 mother trees in the Zagros Mountain forests 700 to 2200 m altitudes above sea level. Seedlings from different altitudes were irrigated at 25%, 50%, 75% and 100% of field capacity (FC), from June through the end of August 2005, and then their growth, physiological and biochemical parameters were examined. The results showed that the activity of peroxidase (PO), superoxide dismutase (SOD), and amylase, as well as the rate of membrane lipid peroxidation and the content of lignin were not affected by water deficit stress. However, the survival and growth rates were reduced below 50% FC. Seedlings originated from lower altitudes had higher growth and survival rate than those from higher altitudes below 50% of FC. The seedlings grown under 50% FC had also high phosphorus and water soluble carbohydrate contents. In conclusion, the present study showed that the seedlings from lower altitudes, which their mother trees grown under warmer climate condition in growth season, were more resistant to water deficit due to higher root to shoot ratio, phosphorus and water soluble carbohydrate contents under water deficits.

Key words: Enzyme; Oak; Seed origin; Water soluble carbohydrate.

INTRODUCTION

Seedling stage is an important and usually a critical phase in the regeneration of woody plants under natural conditions. So, the risk of environmental stresses is very high at this stage. Mediterranean environment characterrized by important seasonal and episodic droughts (Tatarinov *et al.* 2016). Water availability is a limiting factor for the regeneration of oak and other woody plants (Aschmann 1984). In general, drought stress reduces plant growth (Clark *et al.* 2000).

Drought is a complex physico–chemical process in which many biological macromolecules and small molecules e.g. nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements are involved (Hong Bo shao *et al.* 2005). Some plants can tolerate increasing drought stress by decreasing osmotic potential or via accumulating solutes in the tissues (Morgan 1984).

Tyree & Jarvis (1982) suggested that low osmotic potential enhances the ability of plants to take water up from dry soil and its role is as important as the growth of root. Low soil moisture can also affect the rate of nutrient flow from the soil to the roots as well as alter the relative availability of nutrients in soil solution (Misra & Tyler, 1999). Furthermore, on dry sites a high K^+ level in the plants could increase seedling drought resistance. In Douglas-fir, *Pseudotsuga menziesii* Mirb. Seedlings, increased

K^+ concentration resulted in greater water-use efficiency (Haase & Rose, 1994). On the other hand, one of the earliest responses of plants to pathogens, wounding, drought, extremes of temperature or physical and chemical stress is the accumulation of reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen (Jiang & Zhang 2004). Antioxidant defense enzymes i.e., superoxide distumase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monod-ehydroascorbate reductase (MDAR) are of the system designed to minimize the concentrations of ROS. SOD catalyzes the superoxide into oxygen and hydrogen peroxide (Perry *et al.* 2011). Catalase and peroxidases can ellipse H_2O_2 (Peltzer *et al.* 2002).

So, POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blokhina *et al.* 2003). Hence with identification and quantification of morpho-logical and physiological characteristics of the seedlings in any environment, we can warrant plantation success (Blokhina *et al.* 2003).

Persian oak, *Quercus branti* Lindl., is one of the most important species in the Zagros mountains forests steppe, west of Iran. This forest is characterized by Mediterranean climate and extreme summer aridity (Fig. 1). Over 1.7 million ha of the Zagros forests has been destroyed since 1962 (Ghazanfari *et al.* 2004). *Q. branti* distributes on different altitudes (Heydari *et al.* 2013).

Investigation of the tree populations' responses to environmental variable will give us a better understanding of their adaptive processes in different environments. With this knowledge, we are better equipped to design breeding programs, hence decreasing the effects of summer drought (Modrzynski & Eriksson 2002). In this study, we selected oak acorns from 20 mother trees at different altitudes (700-2200 m) and exposed them to different levels of water deficit. Hypothetically, the populations of *Q. branti* in lower altitudes origin are more resistant to water deficit.

Also, because of the large number of responses that can occur under water deficit condition, the aims of the present study were: 1) to assess the effect of water deficit stress on growth, nutrition, soluble carbohydrate accumulation and antioxidant system of *Q. branti* seedlings. 2) to compare the *Q. branti* seedlings from different altitudes with each other in relation to water deficit tolerance.

MATERIALS AND METHODS

Plant materials

Acorns of *Q. brantii* of 20 mother trees were collected from Zagros forests in Iran. The mother trees were located in three altitude classes from 700 through 2200m (700-1200m, 1200-1700m and 1700-2200m).

Acorns were sown in March 2005 in 200 cm^3 pots containing the Zagros forest soil modified by 20% sand in volume (45% sand, 30.7% silt, and 25.3% clay). Then seedlings were daily irrigated until June 2005.

The water deficit experiment was started in June 2005. Uniformed in height and viable seedlings were selected for this experiment and then those randomly divided into four irrigation level treatments.

Irrigation treatments

The soil field capacity (FC) was determined for 200 cm^3 pots containing local forest soil by weighting. Uniformed seedlings were subjected to four irrigation level treatments including 100% FC (control), 75%, 50% and 25% of FC in outdoor condition (n = 10 of each mother tree).

Seedlings were irrigated to field capacity every 2 days by weighting the pot plus plant at field capacity during the experiment (from June through end of August 2005).

All seedlings were harvested at the end of the experiment and divided into leaves, stem and roots. Leaves of the seedlings were frozen with liquid N_2 and kept at -80 °C for further experiments.

Each experiment was conducted using three seedlings from each mother tree.

Growth and survival rate measurements

Fresh weight of leaf, shoot and root were measured. Dry weight of the samples was also determined after oven-drying at 80 °C for 24 hours. The ratio of root/shoot and biomass of the plants were then calculated.

Survival rate (%) of the seedlings from different mother trees were measured by calculating difference numbers of alive seedlings at the beginning and end of the water deficit treatments.

Lipid peroxidation

The level of the peroxidation of membrane lipids was determined by measuring malondialdehyde (MDA) as final product of lipid peroxidation. Samples were homogenized in an aquatic solution of Trichloroacetic acid (TCA) (10% w/v). The homogenate was centrifuged at 15000 × g for 10 min and 1 ml of the supernatant was added to 1 ml of 2-Thiobarbituric acid (TBA) 0.5%. The mixture was incubated at 100 °C in a water bath for 30 min. and the reaction was ceased by ice-water bath. The absorbance was read at 532 nm followed by correction for the non-specific absorbance at 600 nm. The amount of MDA-TBA complex was calculated from the extinction coefficient of $155 mM^{-1}cm^{-1}$ (Vos *et al.* 1991).

Extraction and determination of antioxidant enzymes

Frozen samples (200 mg fresh weight) were homogenized in 3 ml Hydroxyethylpiperazine ethane sulfonic acid-KOH buffers (HEPES-KOH) (pH 7.8) containing 0.1 mM Ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 15000 × g for 15 min. All operations were performed at 4 °C. The supernatant was used for SOD activity (Giannopolitis & Ries 1977). Reaction mixture (3 ml) consisted of 50 mM HEPES-KOH buffer (pH 7.8), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 µM NBT, aliquots of enzyme extract and 1 µM riboflavin. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm. The protein content was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Peroxidase (PO) was extracted and determine in three fractions: the soluble (SPO), ionically-(IPO) and covalently-(CPO) bound fractions. SPO is involved in the stress response. IPO and CPO are supposed to be more related to the lignification and suberization of the cells (Abdolmaleki *et al.* 2007). Frozen samples were homogenized in 50 mM Tris-maleate buffer (pH 6.0) and centrifuged at 1000 × g for 10 min at 2 °C. The supernatant was re-centrifuged at 18000 × g for 20 min at 2 °C. This second supernatant was used to assay SPO. Pellets of the first and the second centrifugations were pooled, incubated with 0.2 M $CaCl_2$ for 2 h at room temperature and then centrifuged at 18000 × g for 20 min at 2 °C. The supernatant was used to measure the activity of IPO. The pellet was used directly for CPO assay. SPO activity was assayed in 60 mM K-phosphate buffer (pH 6.1) containing 28 mM guaiacol and 5 mM H_2O_2. The increase in the absorbance was recorded at 470 nm. For IPO and CPO assay the final reaction mixture (3ml) contained 41.6 µM syringaldazine, forty mM Tris-maleate buffer (pH 6.0) and 16 mM H_2O_2. Activity of IPO was expressed as the increase in absorbance at 530 nm per min per mg protein and of CPO was expressed as the increase in absorbance at 530 nm against cell wall dry weight (Ghanati *et al.* 2005).

Determination of amylase

Iodometric method was used to determine the endo-amylase activities (Douglas & Arshad 1996). The starch solution ($1.5 g L^{-1}$) was made by dissolving starch in 2 M NaOH solution at room temperature, followed by the addition of an equal volume of 2 M HCl. The resulting solution was then heated to a clear solution before its pH was adjusted to desired value by addition of 2 M NaOH. Final solutions were made by dilution with buffer at the desired pH. A solution ($126.9 mg L^{-1}$), containing the same volume of 0.1 M iodine and 5 g $100 mL^{-1}$ potassium iodide, was diluted with cold distilled water to obtain a cold iodine solution

(around 2 °C). Activity of Amylase were determined at 25 °C by allowing the enzyme solution in buffer (0.5 mL) to react with the starch solution (5 mL) for 2 min. The reaction was then stopped by adding 2.5 mL of cold iodine solution, which converts the non-hydrolyzed starch to starch-iodine blue complex. The resulting solution was afterwards placed in a thermostatic bath at 25 °C for 10 min and its absorbance was measured at 615 nm. The amount of the hydrolyzed starch (HS%) was determined as the absorbance value of the hydrolyzed starch, relative to a blank value of non-hydrolyzed starch solution, prepared without the addition of the enzyme (Douglas & Arshad 1996).

Determination of lignin

Cell walls of *Q. brantii* seedlings were isolated by sequential washing of filtered cells with 10 volumes of Na-Phosphate buffer (0.1 mM, pH 6.8), EtOH, $CHCl_3$-MeOH (2:1 v/v) and acetone followed by air-drying. Lignin content was measured via a modified acetyl bromide procedure (Iiyama & Wallis 1990). In brief, 6 mg of fine-powdered wall preparation was treated with a mixture (total of 2.5 ml) of 25% (w/w) AcBr in HOAc and 0.1 ml of 70% $HClO_4$ at 70 °C for 30 min with shaking at 10 min intervals. After cooling with ice, the digestion mixture was transferred to a 25 ml volumetric flask containing 5 ml of 2 M NaOH and 6 ml HOAc and made up to 25 ml. The lignin content was determined by measuring the absorbance at 280 nm using a specific absorption of 20.0 g^{-1} L cm^{-1} (Iiyama & Wallis 1990).

Nutrient measurements

Leaves of the seedlings were dried at 80 °C in oven for 24 h and then ground in a Wiley mill (20 mesh screens). Ca, an Mg and K concentration of the leaves were measured by digesting with perchloric acid and then analyzing with atomic absorption spectrophotometer (Shimadzu AA-670, Japan) and was expressed against leaf dry weight.

One pool of three leaves was analyzed for phosphorous concentration of whole plant colorimetrically (absorption 400 nm) using vanadat-molybdate (Kennedy 1984).

Determination of the soluble carbohydrate accumulation content (glucose, rhamnose and mannose)

Frozen samples (100 mg fresh weight of leaf) were homogenized in 3 ml distilled water. The homogenates were filtered by filter paper. Sugar content of filtrates was determined colorimetrically according to phenol-sulfuric method (Debois *et al*. 1956). Glucose, rhamnose and mannose contents were determined at 490, 480 and 490 nm respectively. A standard curve was obtained at various concentrations (ranging from 0 to 30 $\mu g\ mL^{-1}$).

Statistical analysis

The data were analyzed as a factorial design with two factors of irrigation treatment (100, 75 and 50% of field capacity) and altitude classes (low, middle and high). The putative outliers were observed by box plot and homogeneity and normality of the data distribution were examined using Kolmogorov-Smirnov test. Data were subjected to the analysis of variance (GLM) for a randomized complete block design (Table1). The differences between means were measured by Tukey test at the 5% significant level. SPSS software was used for all data analyses.

RESULTS and DISCUSSION

The effects of water deficit were investigated on *Q. brantii* seedlings of different mother trees from different altitudes at four treatments during three growing months. The gradual exposing of oak seedlings to water shortage up to 25% FC within three months was not endurable for *Q. brantii*. The results also showed no significant differences concerning to the growth parameters and survival rates (%) between the seedlings of control (100% FC) and 75% FC, whereas the leaf and stem fresh weight were significantly lower at 75% FC compared to the control (Table 2), because leaf and stem expansion is more susceptible to drought (Ings *et al*., 2013). By increasing water deficit up to 50% FC, however, all growth parameters and

survival rates were decreased considerably in comparison with other treatments. Water deficit stress has been known to limit carbon fixation, growth and net primary production (Dawson 1993). The rate of root weight also decreased by drought at 50% FC (Table 2). In accordance with our results, Fort *et al.* (1997) reported that the growth of the root in *Q. robur* L. seedlings decreased under water deficit condition.

Table 1. The significance of different sources of variation for water regimes, interaction of water regimes × altitudes.

Source of variation	Water regimes	water deficit × altitude classes
Growth traits		
Fresh weight of shoot (g)	**	ns
Fresh weight of leaves (g)	**	ns
Fresh weight of root (g)	**	ns
Dry weight of shoot (g)	*	ns
Dry weight of leaf (g)	*	ns
Dry weight of root (g)	*	ns
Number of leaves	*	ns
Root/shoot	ns	ns
Biomass (g)	*	ns
Survival (%)	*	*
Antioxidant enzymes		ns
SOD (Δ Abs 560 mg^{-1} protein)	ns	ns
SPO (Δ Abs 470 mg^{-1} protein)	ns	ns
IPO (Δ Abs 470 mg^{-1} protein)	ns	ns
CPO (Δ Abs 470 g^{-1} DW)	ns	ns
Amylase (Abs 615 g^{-1} FW)	ns	ns
Lignin (µg mg^{-1} wall DW)	ns	ns
Lipid peroxidation (µM MDA g^{-1} FW)	ns	ns
Soluble carbohydrates		ns
Glucose (µg g^{-1} FW)	ns	*
Rhamnose (µg g^{-1} FW)	ns	*
Mannose (µg g^{-1} FW)	ns	*
Nutrition concentration		ns
Ca (mg gr^{-1})	ns	ns
Mg (mg g^{-1})	ns	ns
K (mg g^{-1})	ns	ns
P (mg gr^{-1})	*	ns

** $P \leq 0.01$, * $P \leq 0.05$, ns: not significant.

Table 2. Mean measured parameters of *Q. brantii* seedlings under different water regime treatments.

Water regimes	**100%FC (control)**	**75%FC**	**50%FC**
Growth traits			
Fresh weight of shoot (g)	0.159^{a}	0.137^{b}	0.065^{c}
Fresh weight of leaves (g)	0.244^{a}	0.192^{b}	0.082^{c}
Fresh weight of root (g)	1.365^{a}	1.346^{a}	0.583^{b}
Dry weight of shoot (g)	0.093^{a}	0.078^{a}	0.036^{b}
Dry weight of leaf (g)	0.114^{a}	0.079^{ab}	0.038^{b}
Dry weight of root (g)	0.784^{a}	0.736^{a}	0.318^{b}
Number of leaves	3.65^{a}	3.59^{a}	1.47^{b}
Root/shoot	9.57^{a}	10.47^{a}	8.25^{a}
Biomass (g)	1.75^{a}	1.7^{a}	0.722^{b}
Survival rate (%)	70.4^{a}	60.9^{a}	24.6^{b}
Antioxidant enzymes			
SOD (Δ Abs 560 mg^{-1} protein)	0.0080^{a}	0.0079^{a}	0.0079^{a}
SPO (Δ Abs 470 mg^{-1} protein)	0.0014^{a}	0.0022^{a}	0.0019^{a}
IPO (Δ Abs 470 mg^{-1} protein)	0.0045^{a}	0.0065^{a}	0.0089^{a}
CPO (Δ Abs 470 g^{-1} DW)	13.84a	15.25a	15.72a
Amylase (Abs 615 g^{-1} FW)	26.85a	28.99^{a}	29.62^{a}
Lignin (µg mg^{-1} DW)	0.48^{a}	0.405^{a}	0.47^{a}
Lipid peroxidation (µM MDA g^{-1} FW)	0.0086^{a}	0.0095^{a}	0.0082^{a}
Soluble carbohydrae			
Glucose (µg g^{-1} FW)	27.25^{b}	30.46^{ab}	36.12^{a}
Rhamnose (µg g^{-1} FW)	24.02^{b}	27.45^{ab}	32.00^{a}
Mannose (µg g^{-1} FW)	24.74^{b}	27.8^{ab}	32.28^{a}
Nutrition concentration			
Ca (mg g^{-1})	7.43^{a}	6.65^{a}	6.62^{a}
Mg (mg g^{-1})	1.58^{a}	1.75^{a}	1.82^{a}
K (mg g^{-1})	6.65^{a}	6.84^{a}	6.65^{a}
P (mg g^{-1})	10.43^{b}	15.82^{b}	28.74^{a}

Different letters in each row indicate significant mean differences at $p \leq 0.05$ based on Tukey test.

Growth parameters of water shortage-treated seedlings were also impressed by different altitudes of the locations where seeds had been collected (Ma *et al.* 2014). The seedlings originated from the lower altitudes showed more biomass at 50% FC and root/shoot ratio at 75% FC than the seedlings originated from higher ones (Table 3).

Volaire & Thomas (1995) found that drought-resistant population had significantly more

root and aerial biomass than drought–susceptible population. Also, study on *Picea abies* populations from different altitudes showed that there was a correlation between root size and resistant to drought (Modrzynski & Eriksson 2002). This may be related to the fact that mother trees of these seeds belonged to the higher temperature (lower altitudes) in natural environment, so they responded to water deficit earlier and faster particularly compared to higher altitudes.

Water deficit did not affect the activities of SOD, SPO, IPO, CPO and amylase of *Q. branti* seedlings at different treatments after approximately 3 months experiment (Table 2). There was also no significant difference in MDA content of *Q. brantii* at different treatments, so the oxidation of membrane lipids was not different among the treatments. In accordance with our results, Pinheiro *et al.* (2004) did not find a general link between MDA and drought tolerance when four clones of *Coffea canephora* were subjected to long period drought. In contrast, by studding of two clones of *Coffea canephora* in rapid drought stress, Lima *et al.* (2002) proposed that drought tolerance might at least in part be associated with enhanced activity of antioxidant enzymes.

Smironff & Colombe (1998) also showed that MDA content increased in the early period of drought but then decreased. Therefore, the *Q. brantii* seedlings might gradually be adjusted to changing conditions, whereas in the other cases like coffee, the change is relatively abrupt and might exceed the metabolic capacity of plants to acclimate (schwanz *et al.* 1996). We also observed a gradual increase in glucose, rhamnose and mannose content at different water deficit treatments and the highest amount was at 50% FC (Tables 2).

Therefore, at this treatment level, the osmotic adjustment was possibly due to the accumulation of glucose, mannose and rhamnose (Slama *et al.* 2007). Hartmann *et al.* (2013) also found an elevated concentration of sugar in organs of *Picea abies* tree during drought period.

Also, interaction between different altitudes and water deficit was significant for soluble carbohydrate content (Table 1). So that, it was significantly higher in high altitude seedlings than in lower ones at 50% FC (Table 4).

A shift in carbohydrate formation from starch and sucrose to soluble glucose and fructose is a process which induces a biochemical adaptation to water deficit for osmotic adjustment (Epron & Dreyer 1996). In the present study, it occurred in the higher altitude seedlings earlier, because they are more susceptible to drought stress than the lower altitude ones.

According to the results, the foliage nutrient concentrations of Ca, Mg and K were not changed under drought treatments (Table 2), suggesting that either high availability of nutrients in pot experiment or equivalent reduction rate between growth and nutrient uptake can postpone the cation depletion of plants under water shortage (Chołuj *et al.* 2008). Haase & rose (1993) also obtained a similar result with Douglas-fir seedlings. So, they concluded that Ca and Mg are relatively immobile and can be uptaken only by increased soil water content. However, there was also a tendency toward the high P concentration in the seedling leaves grown under water stress treatment, such that the highest amount observed at 50% FC treatments. Baruch (1994) observed an increase in P concentration under drought treatment, because the uptake of phosphorus by root is less susceptible to drought conditions (Volaire & Thomas 1995). So, due to reducing in growth rate under water stress, phosphorus can accumulate in leaves. Nambiar & Fife (1987) also found that rapid replenishment of N and P into the needles coincided with the time of slow shoot growth under water deficit.

Table 3. Mean growth parameters and survival rate (%) of the *Q. brantii* seedlings from different altitudes.

Water regimes	**100% FC**			**75% FC**			**50% FC**		
Altitudes	Low	Middle	High	Low	Middle	High	Low	Middle	High
Fresh weight of shoot (g)	0.149^{a}	0.137^{a}	0.181^{a}	0.108^{b}	0.154^{a}	0.144^{a}	0.069^{a}	0.075^{a}	0.062^{a}
Fresh weight of leaves (g)	0.212^{a}	0.243^{a}	0.269^{a}	0.161^{a}	0.215^{a}	0.198^{a}	0.091^{a}	0.104^{a}	0.090^{a}
Fresh weight of root (g)	1.22^{a}	1.33^{a}	1.47^{a}	1.26^{a}	1.51^{a}	1.32^{a}	0.61^{ab}	0.76^{a}	0.46^{b}
Dry weight of shoot (g)	0.080^{a}	0.079^{a}	0.106^{a}	0.054^{b}	0.099^{a}	0.077^{a}	0.039^{a}	0.045^{a}	0.045^{a}
Dry weight of leaves (g)	0.130^{a}	0.140^{a}	0.083^{a}	0.069^{a}	0.087^{a}	0.081^{a}	0.030^{b}	0.050^{a}	0.038^{b}
Dry weight of root (g)	0.693^{a}	0.760^{a}	0.830^{a}	0.659^{a}	0.840^{a}	0.711^{a}	0.389^{a}	0.504^{a}	0.335^{a}
Number of leaf	3.36^{a}	3.76^{a}	3.75^{a}	3.22^{a}	3.83^{a}	3.67^{a}	1.48^{a}	2.35^{a}	1.38^{a}
Root/shoot	9.17^{a}	10.97^{a}	9.07^{a}	12.42^{a}	10.19^{ab}	9.41^{b}	8.50^{a}	9.74^{a}	7.59^{a}
Biomass (g)	1.58^{a}	1.71^{a}	1.92^{a}	1.55^{a}	1.88^{a}	1.66^{a}	0.86^{ab}	1.14^{a}	0.76^{b}
Survival rate (%)	68.46^{ab}	78.94^{a}	66.31^{b}	63.42^{ab}	71.86^{a}	57.21^{b}	31.09^{a}	24.80^{a}	22.44^{a}

Different letters in each row indicate significant mean differences at $p \leq 0.05$ based on Tukey test.

Table 4. Mean glucose, rhamnose and mannose of the *Q. brantii* seedlings under different water regimes and altitudes.

Source of variations		**Glucose (μg g^{-1} FW)**	**Rhamnose (μg g^{-1} FW)**	**Mannose (μg g^{-1} FW)**
altitude classes	**Water regimes**			
Low	100% FC	29.5 (ABC)	25.4 (AB)	27.3 (ABC)
Middle	100% FC	26.1(BC)	22.9 (B)	24 (BC)
high	100% FC	27.3(BC)	24.2 (AB)	25 (BC)
Low	75% FC	26.3(BC)	24.7 (AB)	24.1 (BC)
Middle	75% FC	24.8 (C)	22.4 (B)	23 (C)
high	75% FC	36.7 (AB)	32.7(AB)	33.4 (AB)
Low	50% FC	39.7(A)	34 (A)	35.5 (A)
Middle	50% FC	29.5(ABC)	25.4 (AB)	27.3 (ABC)
high	50% FC	26.1 (BC)	22.9 (B)	24 (BC)

Different letters in each column indicate significant mean differences at $p \leq 0.05$ based on Tukey test.

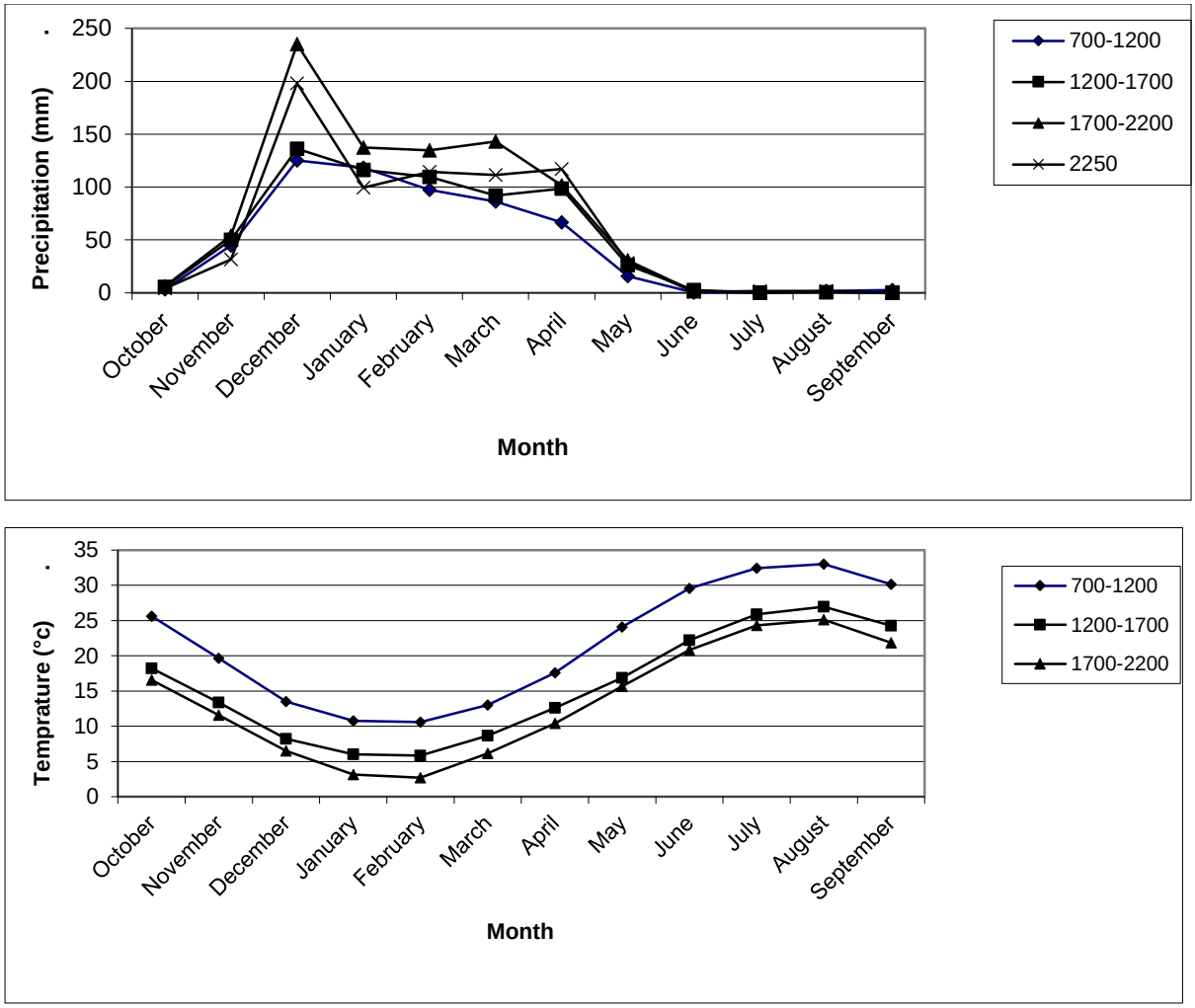

Fig. 1. Precipitation and temperature of different altitudes of Zagros Mountains forests in different months.

CONCLUSION

The current results showed that all growth parameters were decreased at 50% FC. Furthermore, the examined soluble carbohydrates and P concentration changed in the *Q. brantii* seedlings under water deficit treatments for osmotic adjustment. The results suggest the survival strategy of remaining seedlings in response to water deficit elevated by increased soluble carbohydrates and decreased growth.

As we initially hypothesized, the seedlings from higher altitudes showed a strong negative reaction to water deficit. So that, they had the lowest growth compared to the seedlings from lower altitudes, although the survival rate did not significantly decrease. The insignificant survival rate can be due to earlier increase in soluble carbohydrate contents of these seedlings at higher altitudes, while seedlings from the lower altitudes which their mother trees had grown under warmer climate condition, were more resistant to water deficit. The seedlings from lower altitude also had higher root to shoot ratio under water deficit treatments. These results suggest that the resistance to water deficit can transfer by inheritance of these parameters from acorns of mother trees.

These results can be different with the results in field condition because of altering of root system behavior in pot, compared to field condition. The results of this study can be employed in selecting seeds from mother trees which are more tolerant to water deficit in order to perform reforestation of *Q. branti* in Zagros destroyed forests.

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پاسخهای فیزیولوژیکی و بیوشیمیایی نهالهای بلوط ایرانی به تنش کمبود آب

ذوالفقاری ر.[1*]، اکبری‌نیا م.[2]

۱- گروه علوم جنگل، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران
۲- گروه علوم جنگل، دانشکده منابع طبیعی و علوم دریایی، دانشگاه تربیت مدرس، نور، ایران

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چکیده

کمبود آب یکی از مهمترین تنش‌های محیطی در مناطق مدیترانه ای محسوب می‌گردد. کیفیت نامطلوب نهال‌ها ممکن است دلیلی بر ناتوانی تجدید حیات بلوط باشد. برای تعیین بهترین مبداء بذر برودار، نهال‌های حاصل از بذور ۲۰ درخت مادری واقع در جنگل‌های رشته کوه زاگرس از ارتفاع ۷۰۰ تا ۲۲۰۰ متر بالاتر از سطح دریا بررسی گردیدند. نهالهای حاصل از ارتفاعات مختلف با ۲۵٪، ۵۰٪، ۷۵٪ و ۱۰۰٪ ظرفیت مزرعه‌ای از خرداد تا شهریور ۲۰۰۵ آبیاری شدند و سپس پارامترهای رویشی، فیزیولوژیکی و بیوشیمیایی آزمایش گردیدند. نتایج نشان داد که فعالیت پراکسیداز، سوپراکسیداز دیسموتاز و آمیلاز و نیز لیپید پراکسیداسیون غشایی و محتوای لیگنین تحت تنش کمبود آب تغییر نیافت. به هرحال، نرخ زنده مانی و رشد در تنش آبی کمتر از ۵۰٪ ظرفیت مزرعه ای کاهش یافت. نهالهای حاصل از درختان مادری ارتفاعات پایین نرخ رشد و زنده مانی بالاتری نسبت به ارتفاعات بالا در شرایط رژیم آبی کمتر از ۵۰ درصد ظرفیت مزرعه‌ای داشتند. هم‌چنین نهال‌های رشد یافته در شرایط رژیم آبی کمتر از ۵۰ درصد ظرفیت مزرعه‌ای دارای محتوای فسفر و قند محلول بالاتری بودند. در مجموع، پژوهش حاضر نشان داد که نهال‌های حاصل از ارتفاعات پایین‌تر، که درختان مادری آن‌ها شرایط رویشگاهی گرمتری را در طی فصل رشد تجربه می‌کنند، با بهره‌مندی از نرخ بالای ریشه به ساقه، محتوای فسفر و قند محلول، مقاومت بیشتری در برابر کاهش محتوای آب خاک نشان دادند.

*مؤلف مسئول