



Morphological and physiological changes in *Acer velutinum* Boiss seedlings under drought stress

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Abstract

Water is needed for numerous metabolic functions and its loss can affect all characters of plant life. *Acer velutinum* is a fast-growing species and significant diffuse-porous hardwood in afforestation and reforestation. In this study, the impacts of water deficit on growth, biomass, pigment contents, relative water content, osmolytes such as free proline, soluble protein, and soluble sugar, and also antioxidative enzymes including guaiacol peroxidase and catalase were studied to describe the protective mechanisms related to their drought resistance. One-year-old seedlings were exposed to four drought treatments (100, 75, 50, and 25% of the field capacity). The drought treatments had negative impacts on *A. velutinum* growth as exhibited by reduced plant height, basal diameter and biomass. Drought stress decreased significantly relative water content and chlorophyll content while increasing electrolyte leakage, osmotic substances, and catalase and peroxidase activities. Findings demonstrated that *A. velutinum* seedlings have the capacity to adapt to water deficiency and can be recommended for afforestation and reforestation.

Keywords: antioxidant enzymes, biomass, water deficit

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Introduction

Limitation to water access changes morphological, physiological, biochemical, and molecular characters in plants (Barros et al., 2020). Plants can avoid drought through undergoing morphological changes such as wide, deep, or dense roots (increasing water uptake), restricted leaf growth, and closed stomata to prevent water

loss. In addition, plants have developed physiological and biochemical mechanisms such as the assemblage of protective solutes and drought-resistance non-enzymatic and enzymes antioxidants as module of drought tolerance (Wuet al., 2013). Accumulation and mass production of reactive oxygen species (ROS) including singlet oxygen (¹O₂), superoxide radical (O⁻²), hydroxyl radical (*OH) or hydrogen peroxide (H₂O₂) in drought stress can cause oxidative damage (Guo, Yang, Wang, Yang, and Sun, 2010; Guo, Yu, Yang, Kong, and Zhang, 2018; Sarker and Oba, 2018). Destruction of the membrane

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integrity causes the release of essential solutes from the cell and organelles, and as a result increases electrolyte leakage (EL). Increment in the antioxidant system helps eliminate ROS, which reduces electrolyte leakage and lipid peroxidation (Ge et al., 2014; Kapoor et al., 2020). Plants reduce osmotic potential through assembling more compatible solutes (soluble sugars, proline, free amino acids, and soluble protein). The osmotic adjustment could also improve cell membrane stability and macromolecules stabilization, helping with ROS detoxification and ultimately improving plant resistance against drought (Abid et al., 2018; Khaleghi et al., 2019).

Acer velutinum Boiss (Persian or velvet maple tree) is the most valuable endemic species indigenous to the Hyrcanian forest, growing in a wide range of altitudes from 200–2000 m. It is a diffuse-porous hardwood with fast-growing ability in forest restoration (Farhadi et al., 2013; Naghdi et al., 2016; Naji et al., 2016; Picchio et al., 2019; Ramezani et al., 2008).

The aim of our study was to characterize the responses of velvet maple seedlings to four levels of drought stress treatments in the early growth phase. To this end, morphological, physiological, and biochemical changes in response to water shortage were assayed.

Materials and Methods

Materials and experiment treatments

The research was performed in a greenhouse in University of Guilan, Iran. One-year-old velvet maple seedlings were planted in plastic pots. A randomized complete design was applied with three replications for four irrigation regimes including 100% (control), 75% (mild), 50% (moderate), and 25% (severe) field capacity. Transpiration water loss was measured by weighing the pot and re-watering it every other day.

Growth parameters

Plant height and basal diameter were determined at 30-day intervals. Also, three seedlings were harvested randomly from each treatment. Plant materials were harvested and dried at 65 °C for 48 hours to calculate root, stem, and leaf biomass.

Relative water content

Ten leaf disks (5 mm) were cut and fresh weight (FW) was recorded. Afterwards, turgor weight (TW) was calculated by floating in distilled water for 24 h at 4 °C in darkness. The leaves were dried for 24 h at 65 °C to recorded dry weight (DW). Relative water content (RWC) was measured as follows (Barrs and Weatherley, 1962; Ying et al., 2015):

$$\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$$

Photosynthetic pigments

Leaf sample (200 mg) was ground and pigments were extracted using 10 mL 80% acetone. The absorbance was determined spectrophotometrically at 663, 645, and 470 nm according to Lichtenthaler (1987).

Soluble sugars, soluble proteins and free proline

Soluble sugar contents were analyzed by the phenol-sulphuric acid method. The leaves (0.2 g) were extracted with 95% ethanol. The supernatant was mixed with phenol (1%) and sulphuric acid (98%). The amalgam was placed in a boiling water bath for 10 min and absorbance was estimated at 625 nm. The concentration of soluble sugar was determined using glucose solution as the standard (Irigoyen et al., 1992).

Soluble proteins concentration was estimated using the Bradford G-250 reagent and absorbance was measured at 595 nm (Bradford, 1976).

Proline content was determined based on (Bates et al., 1973). Leaf samples (0.5 g) were homogenized with sulfosalicylic acid 3% (10 mL) in cold conditions; the supernatant (2 mL) was mixed with acetic acid (2 mL) and ninhydrin acid (2 mL) and incubated for 40 minutes at 100 °C. Toluene (4 mL) was added to cool the mixture. Absorbance of the extract was measured at 520 nm (Bates, 1973).

Electrolyte leakage

To determine electrolyte leakage, leaf segments (1 cm) were prepared and placed in vials containing 10 ml of distilled water, and electrolyte leakage was measured (EL1) after 24 hours of shaking at room temperature. The same vials were

autoclaved (120 °C for 20 min) before they were shaken for 12 hours to record electrical leakage (EL2). Electrolyte leakage was calculated using the following equation (Sarker and Oba, 2018):

$$EL\% = (EL1/EL2) \times 100$$

Enzyme activities

Guaiacol peroxidase activity (GPX) was assayed according to the guaiacol method. GPX catalyzes guaiacol to tetraguaiacol at 470 nm by H₂O₂. Absorbance were read for 2 min (Plewa et al., 1991).

Catalase activity (CAT) was measured using absorption method by following the decrease in absorbance at 240 nm as H₂O₂ was decomposed (Chakhchar et al., 2015).

Statistical Analysis

Data were submitted to one-way analysis of variance (ANOVA) to calculate the drought treatment effects. Tukey’s multiple test (P≤0.05 and P≤0.01) was used to test differences among treatment means. Statistical analyses were performed with the SPSS 16.0.

Results

A gradually height and basal diameter decrease with decreasing water supplies was observed. Significant differences in height occurred under moderate and severe stress. However, in basal diameter, only severe stress treatment showed a statistically significant difference with the control group (Fig. I).

Biomass production significantly reduced under drought stress conditions. Total biomass had a reduction of approximately 62% under severe drought. Root biomass declined by 55.3% and

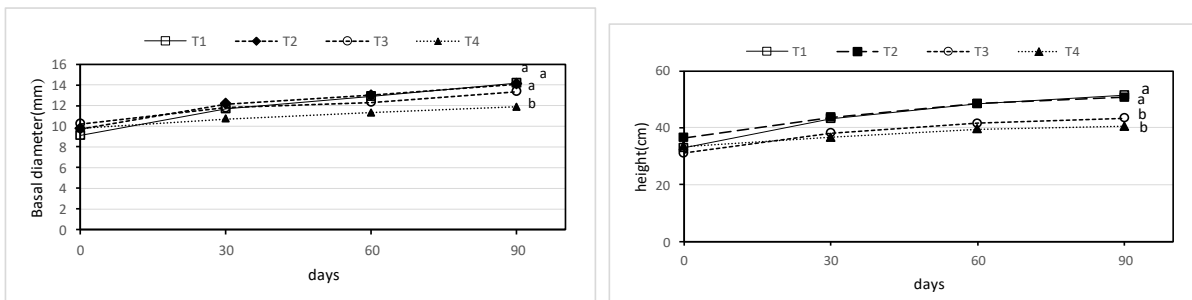


Fig. I. plant height (cm) and Basal diameter (mm) of *A. velutinum* under water treatments. T1 (100% FC), T2 (75% FC), T3 (50% FC), and T4 (25% FC). Data are means + SE of three replicates (n=3). The different Letters are significant differences (P ≤ 0.05) according to Tukey’s test.

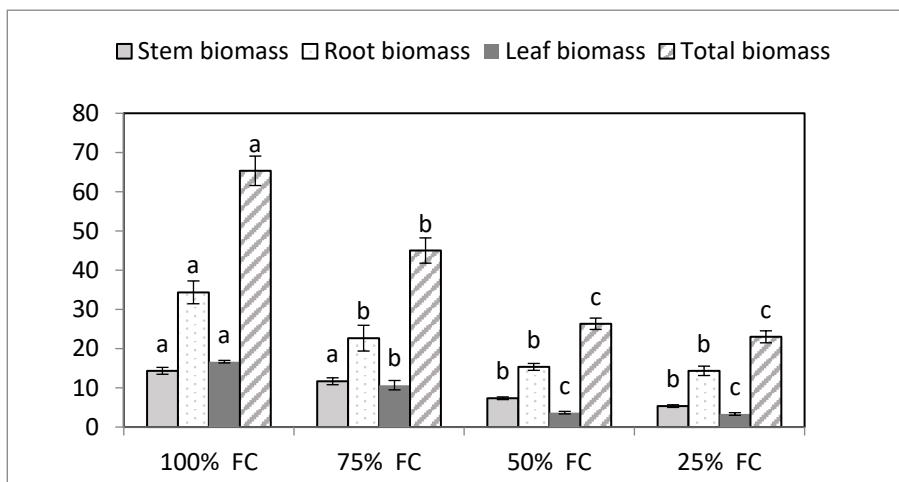


Fig. II. Comparisons of stem, root, leaf, and total biomass (g) of *A. velutinum* seedlings under different water treatments. Data are means + SE of three replicates (n=3). The different Letters are significant differences (P ≤ 0.05) according to Tukey’s test.

58.2% in plants exposed to 50% and 25% FC treatments. Significant differences were observed between control and severe drought in the leaves and stems by 80% and 62.7%, respectively (Fig. II).

Chlorophyll contents of the leaves significantly decreased with decreasing water supplies. A significant reduction in chlorophyll content was found at 50% and 25% FC. Carotenoids gradually increased with decreasing water supplies. However, no significant difference was observed among treatments (Fig. III).

Relative water content (RWC) of *A. velutinum* was significantly affected by drought. Severe drought stress had a significant effect on RWC, which declined by 27.3% in plants exposed to 25% FC compared with the control. *A. velutinum* seedlings exhibited continuous increase in the electrolyte leakage (EL) with increasing degree of drought. The increase in EL was 70.19%, 54.05, and 21.7% under 25, 50, and 75% FC treatment, respectively compared with the control (Fig. IV.)

Drought stress induced a notable increase in leaf proline contents of *A. velutinum* seedlings. The increase in proline was 131% and 136.4% under 25% and 50% FC treatment, respectively compared with 100% FC treatment.

Concentrations of soluble sugars (SS) increased under drought treatments. The increases in SS were 72.1%, 63.5% under 25% and 50% FC treatment, respectively compared with the control. Also, leaves showed significant increase in soluble protein. Increases in soluble protein (SP) contents were 27.7% and 23% under 25% and 50% FC treatments, respectively (Fig. V).

Drought stress was found to increase POD and CAT activities. Increases in POD activities were recorded by 40%, 327%, and 113.3% under 75, 50, and 25% FC treatments, respectively. CAT activity significantly increased compared to control plants with the maximum activities recorded under moderate and severe drought stress, respectively. Increased CAT activities were 41.3% and 30% under 50% and 25% FC treatments, respectively (Fig. VI.).

Discussion

Plants' primary morphological response to drought stress is growth rate regulation, which occurs in the form of reduced shoot height, basal diameter, and biomass production and is known as adaptation or avoidance mechanisms (Lei et al., 2006; Wu et al., 2008). Drought-stress treatment significantly declined the height, biomass, and

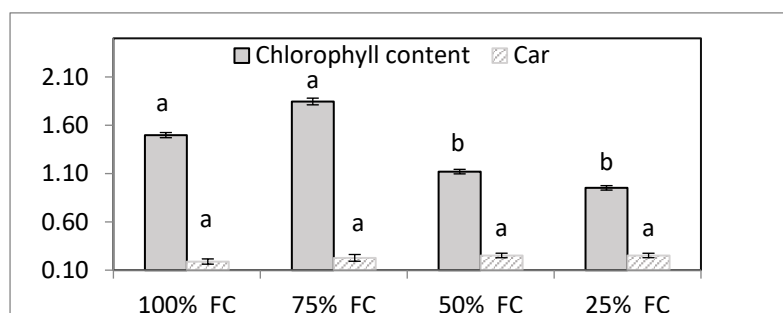


Fig. III. Chlorophyll content and carotenoids (Car) (mg/g fw) of *A. velutinum* under different water treatments; data are means + SE of three replicates (n=3). Different letters show significant differences ($P \leq 0.05$) according to Tukey's test.

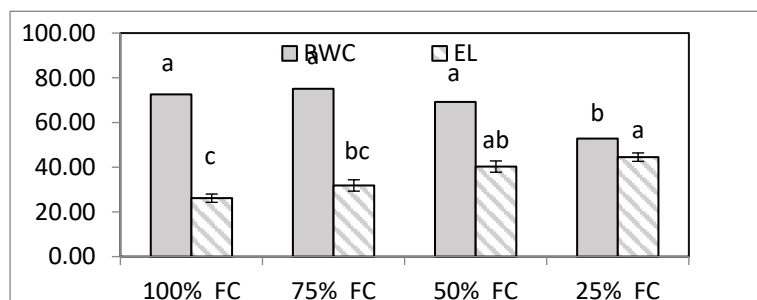


Fig. IV. Leaf relative water content (RWC) (%) and electrolyte leakage (EL) (%) of *A. velutinum* under different water treatments; data are means + SE of three replicates (n=3). Different letters show significant differences ($P \leq 0.05$) according to Tukey's test.

basal diameter of velvet maple seedlings from control to severe drought treatment that is consistent with studies on *Alnus cremastogyne* (Hu et al., 2012), *Maclura pomifera* (Khaleghi et al., 2019), *Acer catalpifolium* (Zhang et al., 2019), and *Juglans* (Liu et al., 2019).

The RWC basic index indicates the amount of water in cells and tissues, which is very important for proper physiological processes and growth (Ying et al., 2015). Therefore, it is a powerful and safe indicator for estimating the drought tolerance (Tariq et al., 2018). In our study, seedlings' RWC showed a noticeable decline just under severe water stress, suggesting relative ability of the species to maintain osmotic pressure and tolerance to drought stress.

Cell membranes undergo increased permeability and decreased stability under drought stress, so electrolyte leakage is evaluated as an indicator of membrane damage under water deficit (Gao et al., 2020; Guo et al., 2010). In this study, EL increased significantly in all treatments. The increase in EL

was reported in *Phoebe bournei* (Ge et al., 2014) and *Abies fabri* seedlings (Guo et al., 2010) under drought stress.

Decreased chlorophyll content occurs due to slow synthesis or rapid degradation due to increased chlorophyll activity and is a common symptom of oxidative stress (Medeiros et al., 2013). Chlorophyll contents significantly decreased with increasing water stress. However, carotenoid contents increased by drought, which was not significant. This may suggest an attempt to mitigate damages to the photosynthetic structure under adverse stress conditions. Carotenoids play a special role in the photosynthetic structure as they release excess energy in the form of heat, eliminate ROS, and prevent lipid peroxidation (Wu et al., 2013).

Plants produce osmoprotectants e.g., proline, soluble sugars, and soluble proteins under drought stress. The primary physiological response of plants to water deficit is stomatal closure and osmotic adjustment, which is done to

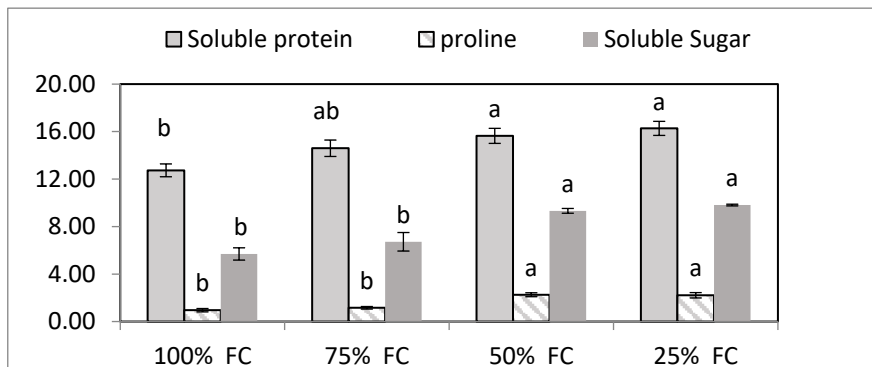


Fig. V. Free proline (μmol/g fw), soluble sugar (mg/g fw), and soluble protein (mg/g fw) content of *A. velutinum* under different water treatments; data are means + SE of three replicates (n=3). Different letters are significant differences (P ≤ 0.05) according to Tukey's test.

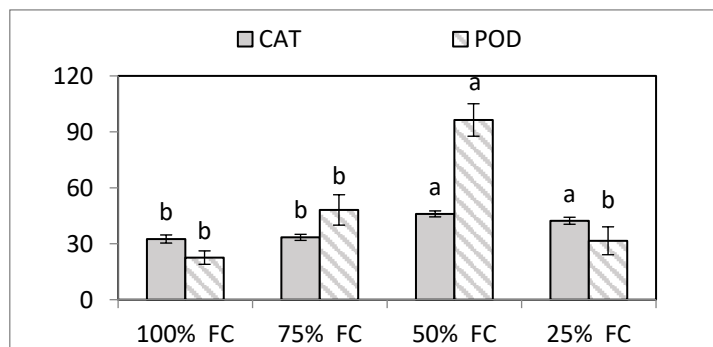


Fig. VI. Effect of drought stress on CAT (u/g fw), and POD (u/g fw) activities in leaves of *A. velutinum*; data are means + SE of three replicates (n=3). Different letters show significant differences (P ≤ 0.05) according to Tukey's test.

maintain the moisture, as well as absorb the water, and ultimately maintain the biochemical and physiological activities (Bargali and Tewari, 2004; Gao et al., 2020; Yang and Miao, 2010). In this study, the three osmotic regulators showed a significant increase in moderate and severe stress treatment. Soluble protein along with soluble sugar and proline increased under drought stress to strengthen the water transport and absorption capacity (Hu et al., 2012). The increase in the soluble protein content under water deficit can be attributed to the synthesis of special proteins and reprogramming to new environment, as well as cell protection (Guo et al., 2018; Yang et al., 2010).

SOD, CAT and POD are important antioxidant enzymes that can substantially decrease the accumulation and damage of ROS, thus being able to regulate lipid peroxidation levels. The antioxidant enzyme reactions are associated with drought resistance (Toscano et al., 2016; Ying et al., 2015). We found that drought stress induces POD and CAT under drought treatments. Quantities of antioxidant enzymatic activities are associated with the plants' potential to combat unfavorable environment (Guo et al., 2018). Observation of variations in the two enzymes showed that POD had a more significant increase than CAT. In this study, the highest level of antioxidant enzyme was observed under

moderate drought condition. Toscano et al. (2016) found increases in antioxidant enzyme activities of several adapted plants under medium drought stress.

Conclusion

Plants adapt to drought stress through variation in some parameter to manage water supply. *A. velutinum* used an avoidance mechanism consisting of the reduction in biomass and growth as well as tolerance strategy such as increasing osmotic substance (free proline, soluble sugar, and soluble protein) and CAT and POD activities. We only considered the physiological responses of seedlings to short-term drought under greenhouse condition. It is necessary to examine *velvet maple* adaptation under long-term drought condition in both field and greenhouse environments for the effectiveness of afforestation and reforestation projects.

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