

ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT

***Acer velutinum* Bioss. (velvet maple) seedlings are more tolerant to water deficit than *Alnus subcordata* C.A. Mey. (Caucasian alder) seedlings**

DOI: 10.37427/botcro-2022-029

Mokarram Ravanbakhsh¹, Babak Babakhani¹, Mahmood Ghasemnezhad^{1,2}, Fariba Serpooshan¹, Mohamad Hassan Biglouie^{1,3}

¹ Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

² Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

³ Department of Water Engineering, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

Please cite this article as: Ravanbakhsh M., Babakhani B., Ghasemnezhad M., Serpooshan F., Biglouie M. H.: *Acer velutinum* Bioss. (velvet maple) seedlings are more tolerant to water deficit than *Alnus subcordata* C.A. Mey. (Caucasian alder) seedlings Acta Bot Croat, DOI: 10.37427/botcro-2022-029.

This is a PDF file of a manuscript that has been accepted for publication. The manuscript will undergo language and technical editing, formatting and author proofing before it is published in its final form.

***Acer velutinum* Bioss. (velvet maple) seedlings are more tolerant to water deficit than *Alnus subcordata* C.A. Mey. (Caucasian alder) seedlings**

Mokarram Ravanbakhsh¹, Babak Babakhani^{1*}, Mahmood Ghasemnezhad^{1,2}, Fariba Serpooshan¹, Mohamad Hassan Biglouie^{1,3}

¹ Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

² Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

³ Department of Water Engineering, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

*Correspondence: babakhani_babak@yahoo.com

Running title: RESPONSES TO WATER DEFICIT IN *ACER VELUTINUM* AND *ALNUS SUBCORDATA*

Abstract - Drought stress is a major environmental factor limiting plant growth. Selection of drought-tolerant plants is of critical importance in vegetation restoration and forestation programs. *Alnus subcordata* and *Acer velutinum* are two valuable, dominant, and endemic species in the Hyrcanian forests. There are fast-growing species and significant diffuse-porous hardwood in afforestation and reforestation. One-year old seedlings of both species were exposed to four water shortage treatments (100, 75, 50 and 25% of field capacity (FC) chosen as control, mild, moderate, and severe) for 12 weeks. Thereafter, their morphological characteristics such as height and basal area, total and organs biomass (root, stem, and leaf), leaf area (LA), specific leaf area (SLA), leaf area ratio (LAR), as well as physiological and biochemical characteristics such as relative water content (RWC), content of chlorophyll, free proline and malondialdehyde (MDA), and superoxide dismutase (SOD) and peroxidase (POD) activity were measured. The results showed that when exposed to reduced water availability, plants' height, basal diameter, total and organs biomass, LA, LAR, RWC and chlorophyll content decreased, but their proline concentration, MDA content, SOD, and POD activity increased in both species. The root to shoot ratio (R/S) and root mass ratio (RMR) increased at 50 and 25% FC treatments in *A. subcordata*, whereas no significant difference was found in *A. velutinum* under drought treatments. SLA increased significantly at 50% FC in *A. velutinum* and decreased in *A. subcordata* under drought treatments compared to control treatment. *A. velutinum* showed more proline content, RWC, POD, and lower increase in MDA content than *A. subcordata* under moderate treatment. Therefore, *A. velutinum* appears to possess a better mechanism to cope drought stress. The drought tolerance of *A. velutinum* may enhance its potential for climatic adaptations under drier conditions with the ongoing climatic change.

Keywords: *Alnus subcordata*, *Acer velutinum*, antioxidant enzymes, biomass, growth, water deficit

Introduction

The impacts of climate change on vegetation will appear as a combination of stress factors, including high temperatures, reduction of rainfall, and alterations in wildfire regimes. The principal aspect of global climate change, the frequency, and intensity of drought stress will increase in the future (Wu et al. 2017). Drought can damage afforestation and reforestation programs because seedlings are more prone to drought than mature trees. Drought-tolerant species should be considered to contribute to

sustainable forest ecosystems (Bhusal et al. 2020). Selection of drought-tolerance plants has a critical role in vegetation restoration and silvicultural strategies. (Khaleghi et al. 2019).

Drought affects various aspects of the plant; the roots are the first part to be affected in the face of drought. The chemical signals (abscisic acid) produced in the roots along with decreased leaf turgor and atmospheric vapor pressure can reduce stomatal conductance. The limitation associated with increased stomatal resistance (under mild to moderate water deficit), is known as a stomatal limitation. A limitation due to non-stomatal disturbance under severe drought stress (non-stomatal limitation) can be induced by the limited diffusion of CO₂ from the intercellular spaces to the chloroplasts or by metabolic factors such as a decrease in Rubisco activity, disturbances in the regeneration of ribulose diphosphate and reactive oxygen species (ROS) production from the excess excitation energy. Low growth can be due to suppress photosynthetic process that eventually reduces biomass (Du et al. 2010, Dulai et al. 2014). Chlorophyll content can directly influence photosynthetic potential and primary production. Reduction in chlorophyll content under water deficit has been regarded as a typical feature of oxidative stress (Liu et al. 2019). Photosynthetic pigments stabilization under stress conditions increases resistance to drought stress (Ge et al. 2014). Decreased chlorophyll content under water deficit was reported in tree species as *Fagus sylvatica* (Gallé and Feller, 2007), *Quercus variabilis* (Wu et al. 2013), *Alnus cremastogyne* (Tariq et al. 2018), and *Acer davidii* (Guo et al. 2019), while no-change in chlorophyll content was found in *Melia azedarach* (Dias et al. 2014).

Relative water content (RWC) is a key indicator of hydration degree and vital for optimal physiological functions and growth processes. RWC in woody and shrubby species reached 50 to 40% and seldom it was as low as 30 to 20% under severe water stress, which eventually causes leaf senescence (Wu et al. 2013). Relatively high RWC maintenance in water shortage is an indicator of drought tolerance (Ying et al. 2015, Toscano et al. 2016). *Quercus variabilis* seedlings could maintain sufficient RWC and slight growth at 40% field capacity (FC) (Wu et al. 2013). RWC of *Alnus cremastogyne* significantly decreased by 32.6 % under drought (Tariq et al. 2018). Decrease of RWC in response to moderate (50% FC) and severe (30% FC) drought treatment in *Maclura pomifera* has been reported (Khaleghi et al. 2019).

Resistance to biotic and abiotic stress in plants increases by accumulating significant amounts of free proline, soluble sugars (sucrose, glucose and fructose), and soluble proteins (maturation proteins). These compatible solutes are able to maintain the concentration of cell sap and prevent the loss of water in plasma (Mohammadkhani and Heidari 2008, Farooq et al. 2009, Guo et al. 2018). Proline functions not only as an osmolyte, but also as an antioxidant, thus helping ROS detoxification by membrane integrity protection and enzyme/protein stabilization (Ghaffari et al. 2019, Khaleghi et al. 2019).

The intercellular concentration of malondialdehyde (MDA), a breakdown product of lipid peroxidation, has been measured as an indicator of oxidative damage (Ge et al. 2014, Abid et al. 2018). To scavenge ROS, plants maintain an efficient antioxidant defense system including non-enzymatic antioxidants and antioxidant enzymes (Khaleghi et al. 2019). Peroxidase (POD) and superoxide dismutase (SOD) disintegrate ROS, and therefore, protect plants from drought stress (Geng et al. 2019). SOD catalyzes the conversion of superoxide radical (O₂^{•-}) to molecular oxygen (O₂) and hydrogen peroxide (H₂O₂). This H₂O₂ is detoxified to O₂ and H₂O through the activities of catalase (CAT) and POD as well as the ascorbate-glutathione (AsA-GSH) cycle (Wang et al. 2012, Abid et al. 2018).

Based on climate modeling, the air temperature in Iran will raise by 2.7 °C up to 2050, which will increase the water needs of plants (Attarod et al. 2017). The Caspian forest climate has become warmer and the vegetation growth trend has been upwards of about one hundred meters in the last half-century (Taleshi et al. 2018). Reforestation by *Alnus subcordata* C.A. Mey. (Caucasian alder), and *Acer velutinum* Boiss. (persian or velvet maple) to increase production capacity reduced the pressure of wood exploitation on Hyrcanian forests (Abdolahi et al. 2017). *A. subcordata* and *A. velutinum* are the most valuable endemic species and indigenous to the Hyrcanian province in the Euro-Siberian region. Due to their importance, numerous studies have done on the quantitative and qualitative characteristics of species, mechanical properties of wood and nutrient elements (Naghdi et al. 2016, Naji et al. 2016, Tavankar et al. 2017, Ghorbani et al. 2018, Jourgholami et al. 2020).

According to few recent studies, nano priming technique increased drought tolerance of *A. subcordata* seeds (Rahimi et al. 2016). *A. subcordata* as an urban tree showed limited tolerance to water deficit by determination of midday leaf water potential (Ψ_L) and stomatal conductance (g_s) (Sjöman et al. 2021). However, their response to drought and the mechanism of these two species under artificial cultivation are still unclear and poorly understood. Therefore, the objectives of the present study were (i) to evaluate the effects of drought stress on *A. subcordata* and *A. velutinum* seedlings which are dominant species in Hyrcanian forest and have a high commercial value in wood industries, to discover their capacity to handle water deficit in the initial vegetative growth period by morphological, physiological and biochemical responses; and (ii) to determine these two species different adaptive responses to drought stress.

Materials and methods

Plant material and drought treatments

The experiment was carried out in a greenhouse at University of Guilan, Iran (37°15' N, 49°36' E). The average annual temperature was 15.9 °C and cumulative precipitation of 1329.1 mm (Allahyari et al. 2016). One-year-old *A. subcordata* C.A. Mey. and *A. velutinum* Boiss. seedlings were obtained from a local nursery called Pilambara (37°35' N, 49°05' E) in Resvanshahr, Guilan Province, Iran. The seedlings were transplanted to 9 L plastic pots filled with homogenized topsoil. The plants were grown in a naturally lit greenhouse (temperature range: 18–28 °C; relative humidity range 73–94%) under the semi-controlled environment (only sheltered from rainfall) from July 10 to October 10, 2019. The greenhouse was well ventilated by plastic side films rolled around it (Guo et al. 2013).

Drought treatments were performed three months after planting the seedlings (an acclimatization period, and when plants had reached fully expanded leaves) (Guo et al. 2013, Medeiros et al. 2013, Meng et al. 2013). A randomized complete design with two factors (two species and four watering regimes) was employed with three replications for four water shortage treatments (100, 75, 50 and 25% of field capacity performed as a control, mild, moderate, and severe, respectively). Using a scale with a capacity of 40 kg, transpiration water loss was measured gravimetrically by weighing all pot and re-watering by tap water every two days. The water added to each pot during the experimental period was 27, 18, 10.8 and 6.75 L for control, mild, moderate, and severe treatments respectively for seedling of *A. subcordata* and 22.5, 15, 9, and 6 L for seedling of *A. velutinum*. The evaluation was performed after three months at the end of experiment.

Growth parameters

Seedling height (cm) was measured from the soil surface to the terminal bud of the main stem using a measuring tape; also, the basal diameter (mm) was measured at the ground line by electronic calipers. Plants height, basal diameter and biomass (total dry mass) were recorded at the end of the experiments. Three seedlings were harvested randomly from each treatment. The leaves, stems, and roots were cut and dried in an oven at 65° C for 48 hours to calculate root, stem, and leaf biomass (the average weight of three samples per treatment). Biomass contribution including leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) was calculated by dividing the stem, leaf, and root biomass by the total biomass (root, stem, and leaf), respectively. Root: shoot ratio (R/S) was calculated using root biomass by total leaf and stem biomass in percentage. Leaf area (LA) was determined with a leaf scanner (model A3 Light box GCL Bubble Etch Tanks), and WinDIAS 3.2. software. Specific leaf area (SLA) was estimated by dividing the leaf area by leaf biomass, while leaf area ratio (LAR) was determined by dividing the total leaf area by every seedling total biomass (Wu et al. 2017.; Zhang et al. 2019).

Relative water content

Ten leaf discs with a diameter of 5 mm were cut from the interveinal parts of each plant and fresh weight (FW) was determined. After that, turgor weight (TW) was calculated by weighing discs when dipping them in water for 24 hours within the dark. Finally, leaf discs were oven-dried for 24 hours at 65° C to determine dry weight (DW). RWC was measured as follows: $RWC (\%) = (FW - DW) / (TW - DW) \times 100$ (Toscano et al. 2016).

Photosynthetic pigments content

For the extraction of photosynthetic pigments, 200 mg liquid nitrogen frozen tissue was ground by pestle and mortar and pigments were extracted by adding 10 mL of 80% cold acetone. The content of chlorophyll *a* (Chl *a*) and *b* (Chl *b*), total chlorophyll (Chl *a*+*b*) and carotenoids was measured spectrophotometrically at 663, 645 and 470 nm respectively by spectrophotometer (Ltd T80 + UV/VIS; PG Instruments, Leicestershire, UK) according to Lichtenthaler (1987). The chlorophylls and carotenoids concentration was expressed as $mg\ g^{-1}\ FW$ and were calculated as:

$$\begin{aligned} Chl_a &= [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V / 1000W \\ Chl_b &= [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V / 1000W \\ Chl\ a + b &= [(20.2(A_{645}) + 8.02(A_{663}) \times V)] / (1000 \times W) \\ Cartenoids &= \frac{1000 \times A_{470} - 2.27 \times Chl_a - 81.4Chl_b}{22} \times \frac{V}{1000W} \end{aligned}$$

where:

A – absorbance at specific wavelength

V – final volume of chlorophyll extract in 80% acetone

W – fresh weight of tissue extracted.

Free proline concentration

Free proline concentration was estimated by Bates et al. (1973). In this method, 0.5 g of frozen leaf samples were integrated with 10 mL of 3% (w/v) sulfosalicylic acid; 2 mL of an aliquot of the supernatant was mixed with 2 mL of acetic acid and 2 mL of ninhydrin acid incubated for 40 minutes at 100 °C. The reaction was stopped in an ice

bath and the reaction mixture was obtained with 4 mL of toluene and absorbance of the top layer was measured at 520 nm. Proline concentration was calculated by a standard curve, ranging from 0 to 400 µg/ml that was plotted with L-proline. Free proline concentration was calculated as:

$$\text{prolin } (\mu\text{mol/g FW}) = [(\mu\text{g prolin/ml}) \times (\text{ml toluene}/115)]/\text{g sample} /5$$

Malondialdehyde (MDA) content

The extent of lipid peroxidation was evaluated as malondialdehyde (MDA) content. 100 mg leaf tissue was blended in 2 mL 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12000 g for 15 min and then 0.5 mL the upper phase was mixed with 1.5 mL TCA 20% (w/v) containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was heated for 90 min at 90 °C and then rapidly cooled in an ice bath. Afterwards, the mixture was centrifuged at 10000 g for 5 min and the absorbance (A) of the supernatant was recorded at 532 and 600 nm. The MDA content was calculated by an extinction coefficient of 155 mM⁻¹ cm⁻¹ and exhibited as nmol/g (Chakhchar et al. 2015). MDA content was calculated as:

$$\text{MDA (nmol/g FW)} = (A_{532} - A_{600})/155 \times 1000$$

Enzyme activities

100 mg fresh leaves were ground in liquid nitrogen using a mortar and pestle, and the ground samples were homogenized with 1 mL 50 mM sodium phosphate buffer at neutral pH containing 2 mM α-dithiothreitol, 2 mM EDTA, 0.2% Triton X-100, 50 mM Tris-hydrochloric acid and 2% polyvinylpyrrolidone. The homogenate was centrifuged at 14000×g for 30 min at 4 °C and the supernatant was collected and stored at -80 °C for SOD and POD activity analysis (Yang and Miao 2010, Ghaffari et al. 2019). SOD activity (EC 1.15.1.1) was evaluated by inhibition ability of the photochemical reduction of nitroblue tetrazolium (NBT) reduction to formazan by O₂^{•-}. One unit of SOD was considered as the amount of enzyme required to causes 50% inhibition of NBT photochemical reduction which can be measured at 560 nm (Giannopolitis et al. 1977). Guaiacol peroxidase activity (POD) (EC 1.11.1.7) was assayed according to the guaiacol method (Plewa et al. 1991). POD catalyzes guaiacol to tetraguaiacol by H₂O₂. Absorbance was read at 465 nm for 2 min. The calculation were done through the following formulas:

$$\text{POD activity } \left(\frac{\mu\text{mol}}{\text{g FW min}} \right) = \frac{|A_{465}(t2) - A_{465}(t1)|}{t2 - t1} \times \frac{V_t}{E \times V_s}$$

Where:

A- absorbance at specific wavelength

V_t- total volume

V_s- enzyme volume

E- extinction coefficient

$$\text{SOD Activity } \left(\frac{\text{U}}{\text{g FW}} \right) = \frac{100 - \left[\frac{(\text{OD Control} - \text{OD Sample})}{\text{OD Control}} \times 100 \right]}{50}$$

Where:

OD Control- absorbance in the absence of SOD

OD Sample- absorbance in the presence of SOD.

Statistical analysis

A randomized complete design was employed with three replications ($n = 3$). First, the variables were analyzed using one-way ANOVA with water supply regimes as factors for each species, then The main effects of drought stress and species and their interactions were determined by two-way analysis of variances (ANOVA). When significant differences occurred among treatments, means were separated by Duncan's multiple range tests at $P \leq 0.05$. Pearson's correlation coefficients were used to calculate the bivariate relationships between some morphophysiological and biochemical traits.

Results

Growth parameters

The highest plant growth parameters (height, basal diameter, total and organs biomass and leaf area) were observed in the well-watered 100% FC treatment, while drought treatments significantly decreased plant height, basal diameter, total and organs biomass in both species ($P \leq 0.05$). Plant height decreased by 30.9, 26.6 and 16.9% when exposed to 25, 50 and 75% FC in *A. subcordata* respectively, and 23.3 and 17.8% in *A. velutinum* at 25 and 50% FC treatments, respectively in comparison with control treatment. Basal diameter decreased by 29.2, 32.7 and 13.8% at 25, 50 and 75% FC treatments in *A. subcordata* respectively, and 19.8% at 25% FC in *A. velutinum*, compared to control condition. Biomass traits showed a decreasing trend in both species under water treatment; namely, leaf biomass reduction was 79.1 and 80.8%, stem biomass was 40.5 and 75.8%, root biomass was 60.9 and 64.2%, and finally total biomass was 61.6 and 64.2% at 25% FC in *A. velutinum* and *A. subcordata* respectively compared to control condition (Tab. 1 and 2).

Drought stress significantly decreased leaf area in both species. Leaf area decreased 71.9 and 83.6% in *A. velutinum* and *A. subcordata*, respectively, when exposed to 25% FC. Specific leaf area (tended to increase with decreasing soil water contents and significantly increased for 70.9% when exposed to 50% FC in *A. velutinum*. In contrast, it decreased 19.3, 26.3 and 49.6% in *A. subcordata* at 75, 50 and 25% FC, respectively. Leaf area ratio significantly decreased for 41.85 and 67.7% at 25% FC in *A. velutinum* and *A. subcordata*, respectively (Tab. 1).

The biomass contribution was significantly affected by changing water availability. R/S increased by 45 and 53.3% in *A. subcordata* under moderate and severe treatments, while no significant difference among drought treatments was found in *A. velutinum*. RMR increased with reducing water availability in *A. subcordata*. The enhancement was 24.4% at 50% FC and 28.2% at 25% FC in comparison with control treatment, whereas no significant difference was observed in *A. velutinum*. Drought stress markedly decreased LMR by 45.9 and 44.1% when exposed to 25 and 50% FC in *A. velutinum* respectively, and 32.1 and 27.3% in *A. subcordata* in the 25 and 50% FC treatments, respectively in comparison with control treatment. SMR in *A. velutinum* significantly increased in all treatments in comparison with control treatment, while it showed a reduction tendency in *A. subcordata* (Tab. 3).

Relative water content and photosynthetic pigments content

RWC showed a significant decrease of 24.9 and 33.5% respectively at 50 and 25% FC in *A. subcordata*, whereas in *A. velutinum* only significant decrease was 27.3% at 25% FC compared with the well-watered seedlings (Tab. 4).

Chl *a* content was reduced for 24 and 28% at 50 and 25% FC in *A. velutinum*, respectively, and 21.9, 60.9 and 53.3% in *A. subcordata* in the 75, 50 and 25% FC treatments, respectively, compared to control condition. Chl *b* content decreased 20.4 and

53% in *A. velutinum* and 56.8 and 52% in *A. subcordata* at 50 and 25% FC respectively. Chl *a+b* decreased by 20, 60 and 53.3% when exposed to 75, 50 and 25% FC in *A. subcordata*, respectively, and 25.5 and 36.2% in *A. velutinum* in the 25 and 50% FC treatments respectively, in comparison with control treatment. The content of carotenoids significantly decreased under drought in *A. subcordata*, where reduction was 50 and 38.5% at 50 and 25% FC, whereas *A. velutinum* showed a tendency to increase in carotenoids under drought stress. (Tab. 4).

Biochemical responses

In the leaves of both species, increase in proline content was recorded upon stress treatments. Proline content in *A. velutinum* leaves increased 22.1 and 132.6% at 75 and 50% FC, respectively and 136.8% at 25% FC. In *A. subcordata* the increase was 34.9 and 62.2% at 75 and 50% FC, respectively and 169.8% at 25% FC in comparison with control treatment (Fig. 1A). The MDA content increased substantially as drought stress progressed in both species. In *A. subcordata* the increase was 93.7 and 133.8% at 75 and 50% FC, respectively and 142.7% at 25%, whereas in *A. velutinum* the increase was 60.5 and 65% at 50 and 25% FC (Fig. 1B).

In *A. velutinum*, SOD activity increased 12 and 8.9% at 50 and 25% FC, respectively. In *A. subcordata*, SOD activity was significantly increased by 36, 25 and 20.9% at 75, 50 and 25% FC, respectively (Fig. 1C). POD activity in *A. velutinum* increased by 113 and 327% at 75 and 50% FC, respectively and 40% at 25% FC, whereas of the values in *A. subcordata* were increased by 148 and 140% at 75 and 50% FC, respectively (Fig. 1D).

Correlation analysis

Correlation analysis indicated that there was a significant and positive correlation between SLA and Chl *a*, Chl *b* and Chl *a+b* in *A. subcordata*, but there was no significant correlation between SLA and Chl concentration in *A. velutinum*. Correlation analysis revealed that there was a significant and positive correlation between SOD and POD activities also, between proline and chl *a*, chl *a+b* in both species. According to correlation analysis there was no significant correlation between RWC and proline in *A. velutinum* but also, there was a negative correlation between RWC and proline in *A. subcordata*. Correlation analysis also revealed that there was a significant and positive correlation between carotenoids content and SOD activity in *A. velutinum* (Table. 5 and 6).

Discussion

Drought stress is the most adverse abiotic stress to plant growth. Permanent or temporary water shortage causes detrimental effects on plant growth and development (Tariq et al. 2018; Du et al. 2019). Height, total and organs biomass of both species significantly declined under moderate and severe treatments (50 and 25% FC) in comparison with control treatment. Basal diameter significantly decreased under moderate and severe treatments (50 and 25% FC) in *A. subcordata* and just reduced under severe treatments (25% FC) in *A. velutinum*. These results are in accordance with previous studies on *Salix paraqpleisia* and *Hippophae rhamnoides* (Fang et al. 2012) as well as *Prunus sargentii* and *Larix kaempferi* seedlings (Bhusal et al. 2020) which demonstrated that drought significantly reduced seedling growth and biomass.

We found that drought treatment significantly increased the R/S and RMR in *A. subcordata*. It was statistically ineffective in *A. velutinum*. The increase in R/S is the result of declining growth rate and biomass production and increase water uptake (Wu et al.

2008, Du et al. 2010). Many studies have shown that there is an increase in R/S ratio under water stress (Fang et al. 2012; Guo et al. 2019, Zhang et al. 2019). More biomass allocation to belowground and maintenance of higher R/S can be indicated as an important adaptive trait (Fang et al. 2012).

In the present study, drought decreased LA in both species under drought stress. SLA showed an increasing trend in *A. velutinum* under drought stress treatments. However, it decreased in all drought treatments in *A. subcordata*. Also, LAR significantly decreased under drought in both species. Decreased LA usually occurs due to inhibition of leaf development, loss of access to photosynthetic products to make new cells (Tariq et al. 2018). Some plant species adjust LA to prevent transpiration or a relative increase in root water uptake capacity (Guo et al. 2019). SLA and LAR increased under severe stress compared to the control in *Jatropha curcas* seedlings, which is considered a drought-tolerant plant (Díaz-lópez et al. 2012). In our study, *A. velutinum* significantly increased the SLA under moderate treatment (50% FC), which indicates that it probably has been able to cope with drought stress by increasing photosynthetic capacity and carbon assimilation (Wu et al. 2017, Barros et al. 2020). Correlation analysis indicated that there was a significant and positive correlation between SLA and Chl a, Chl b and a+b in *A. subcordata*, but there was no significant correlation between SLA and Chl concentration in *A. velutinum*.

We found that Chl a, Chl b, and Chl a+b content significantly decreased under drought stress in both species. *A. velutinum* had a higher chlorophyll content Chl a, Chl b, and Chl a+b than *A. subcordata* under moderate and severe treatment (50 and 25% FC). According Lei et al. (2006), the dry climate population of *Populus przewalskii* had higher chlorophyll content than the wet climate population under the drought treatment. Drought stress also significantly decreased chlorophyll content of *Juglans mandshurica*, *Juglans nigra* and *Juglans regia* seedlings (Liu et al. 2019). Our results also showed that the carotenoids content was not significantly increased by drought in *A. velutinum*, while it was significantly decreased under moderate and severe treatment (50 and 25% FC) in *A. subcordata*. Reduction of carotenoids suggested that drought stress caused noticeable oxidative stress by ROS accumulation (Lei et al. 2006). The slight increase in carotenoids content in *A. velutinum* could suppress photosynthetic apparatus damage by oxygen consumption in xanthophyll cycle or detoxification of ROS (Ashraf and Harris, 2013, Medeiros et al. 2013). Correlation analysis also revealed that there was a significant and positive correlation between carotenoids content and SOD activity in *A. velutinum*.

In our study, *A. velutinum* seedlings showed a decline in RWC just under severe treatment (25% FC), whereas *A. subcordata* showed a significant decrease in the moderate and severe treatments (50 and 25% FC, respectively). Díaz-López et al. (2012) indicated that *Jatropha curcas* can be considered a drought-resistant species as it has been able to sustain its RWC level under mild to severe stress drought treatments. Moreover, Ying et al. (2015) suggested that provenance Kunming (KM) had higher RWC than provenance Nanchang (NC) of *Camptotheca acuminata* under moderate and severe treatments (50 and 30% FC) and exhibited greater drought stress tolerance as expected given the natural habitat of this provenance. Proline content of both the species, investigated in this study, was significantly increased under drought treatments with respect to the well-watered plants although the higher increase was recorded in *A. velutinum* compared to *A. subcordata* under moderate treatment (50% FC), whereas the increment was significantly greater in the *A. subcordata* compared to *A. velutinum* under severe treatment (25% FC). According to correlation analysis, there was no significant correlation between RWC and proline content in *A. velutinum*, while negative correlation between RWC and proline was recorded in *A. subcordata*. Ashrafi et al. (2018) reported

a negative correlation between RWC and osmoprotectants in *Thymus vulgaris* and *T. kotschyanus*, and found that osmoprotectants accumulate by reduction of RWC to maintain plant water. Similarly, Bangar et al. (2019) found that proline content was negatively associated with RWC in *Vigna radiate*.

MDA is a product of poly-unsaturated fatty acids degeneration in phospholipids of cellular membrane, and is used as an index of oxidative stress magnitude under drought (Wang et al. 2012, Guo et al. 2018). MDA content increased along with the drought stress in both species, in this study. The significant increase of MDA content with progressive drought stress, suggests that drought stress caused oxidative damage. Our results, in according to Wu et al. (2013) in *Quercus variabilis* and Tariq et al. (2018) in *Alnus cremastogyne* subjected to drought stress, showed an increase of MDA content. In *A. velutinum*, the values increased under moderate and severe treatment (50 and 25% FC), while in *A. subcordata* MDA content was elevated upon all drought treatments. The increases in MDA content in *A. velutinum* were lower than those in *A. subcordata*. This indicated that drought led to more damage in the cellular membranes under stress treatments in *A. subcordata*. Similarly, Ying et al. (2015) found that drought stress significantly increased MDA content in *Camptotheca acuminata* provenance KM and NC and the increases in MDA content in provenance KM were lower than those in provenance NC. They suggested less production of ROS in provenance KM under water deficit has led to better membrane integrity.

The ability of antioxidant enzymes to eliminate ROS and reduce its harmful effects may be related to plant drought resistance (Anjum et al. 2011). High accumulation of ROS initiated and accelerated lipid peroxidation. POD plays an essential role in reducing the accumulation of H₂O₂, reducing MDA content and maintaining cell membrane integrity. Increased SOD and POD activity in stress treatments reflects an increase in ROS removal capacity and thus a reduction in membrane lipid damage (Ge et al. 2014, Guo et al. 2018). Toscano et al. (2016) suggested that *Eugenia uniflora* and *Photinia × fraseri* subjected to mild and moderate water stress showed increasing activities of antioxidant enzymes. We found that drought stress induced POD and SOD activity in both species under drought treatments in our study, although the highest activities were measured under mild and moderate treatments (75 and 50% FC) compared to the control. Our results are in good accordance with those published by Ge et al. (2014), who reported an increase of POD and SOD activities in *Phoebe bournei* subjected mild and moderate water stress and a decrease under severe drought. In addition, Ge et al. (2014) demonstrated that the increase in MDA content acts as a feedback mechanism to control the activities of antioxidant enzymes. In our study, *A. velutinum* showed higher POD activity and lower increment MDA compared to *A. subcordata* under moderate and severe treatment. Similarly, Wang et al. (2012) found that a stronger protective mechanism by drought-tolerant apple rootstock (*Malus prunifolia*) in comparison to sensitive-tolerant apple rootstock (*Malus hupehensis*) can be ascribed to lower MDA content, higher values for leaf RWC, and greater antioxidative defense system. Wu et al. (2013) has also shown that the MDA content at 60% FC treatment kept a lower increase compared with 40 and 20% FC treatments, indicating better protection against membranes lipid peroxidation, more efficient repairing mechanisms, including the antioxidative system, osmotic adjustment, and photosynthetic pigments in *Quercus variabilis* seedlings.

Conclusion

The present study concluded that although there were common responses in investigated parameters between two Hyrcanian endemic species i.e., *A. velutinum* and *A.*

subcordata, certain different responses were also recorded under drought stress. Our results demonstrated that drought stress significantly reduced growth, biomass and photosynthetic pigments content, but increased free proline content, POD and SOD activities in both species. *A. velutinum* showed slight reduction in seedlings height, basal diameter, biomass and had higher RWC and photosynthetic pigment than *A. subcordata*. *A. velutinum* also showed more efficient antioxidant systems with higher activities of POD, and a lower increase in MDA content under drought stress. Our results highlight that *A. velutinum* maintained stronger drought tolerance based on the measured parameters. According to these findings, it is recommended that *A. velutinum* plantation has more priority compared to *A. subcordata* in water deficit regions.

References

- Abdolahi, A., Ali Arab, A. R., Parhizkar, P., Pourmalekshah, A. A. M. A., 2017: Effect of gap size and position within gaps on growth characters and survival of Chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey.), Cappadocian maple (*Acer cappadocicum* Gled.) and Caucasian alder (*Alnus subcordata* C. A. Mey.). Iranian Journal of Forest and Poplar Research 25, 275–285.
- Abid, M., Ali, S., Qi, L. K., Zahoor, R., Tian, Z., Jiang, D., Snider, J. L., Dai, T., 2018: Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Scientific Reports, 1–15. <https://doi.org/10.1038/s41598-018-21441-7>.
- Allahyari, M. S., Ghavami, S., Daghighi Masuleh, Z., Michailidis, A., Nastis, S. A., Masuleh, Z. D., Michailidis, A., Nastis, S. A., 2016: Understanding farmers' perceptions and adaptations to precipitation and temperature variability: Evidence from Northern Iran. Climate 4, 58. <https://doi.org/10.3390/cli4040058>.
- Anjum, S. A., Xie, X. yu, Wang, L. chang, Saleem, M. F., Man, C., Lei, W., 2011: Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research 6, 2026–2032 <https://doi.org/10.5897/AJAR10.027>.
- Ashraf, M., Harris, P. J. C., 2013: Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190 <https://doi.org/10.1007/s11099-013-0021-6>.
- Attarod, P., Kheirkhah, F., Sigaroodi, S. K., Sadeghi, S. M. M., Dolatshahi, A., Bayramzadeh, V., 2017: Trend analysis of meteorological parameters and reference evapotranspiration in the Caspian region. Iranian Journal of Forest 9, 171–185.
- Barros, V., Melo, A., Santos, M. G. M. M. G. M. M. G. M., Nogueira, L., Frosi, G., Santos, M. G., 2020: Different resource-use strategies of invasive and native woody species from a seasonally dry tropical forest under drought stress and recovery. Plant Physiology and Biochemistry 147, 181–190 <https://doi.org/10.1016/j.plaphy.2019.12.018>.
- Bhusal, N., Lee, M., Reum Han, A., Han, A., Kim, H. S., 2020: Responses to drought stress in *Prunus sargentii* and *Larix kaempferi* seedlings using morphological and physiological parameters. Forest Ecology and Management 465, 118099 <https://doi.org/10.1016/j.foreco.2020.118099>.

- Chakhchar, A., Wahbi, S., Lamaoui, M., Ferradous, A., Mousadik, A. El, Ibsouda-Koraichi, S., Filali-Maltouf, A., Cherkaoui, Modafar, E., El Modafar, C., 2015: Physiological and biochemical traits of drought tolerance in *Argania spinosa*. *Journal of Plant Interactions* 10, 252–261 <https://doi.org/10.1080/17429145.2015.1068386>.
- Dias, M. C., Azevedo, C., Costa, M., Pinto, G., Santos, C., 2014: *Melia azedarach* plants show tolerance properties to water shortage treatment: An ecophysiological study. *Plant Physiology and Biochemistry* 75, 123–127 <https://doi.org/10.1016/j.plaphy.2013.12.014>.
- Díaz-lópez, L., Gimeno, V., Simón, I., Martínez, V., Rodríguez-Ortega, W. M., García-sánchez, F., 2012: *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. *Agricultural Water Management* 105, 48–56 <https://doi.org/10.1016/j.agwat.2012.01.001>.
- Du, N., Guo, W., Zhang, X., Wang, R., 2010: Morphological and physiological responses of *vitex negundo* L. var. *heterophylla* (Franch.) Rehd. to drought stress. *Acta Physiologiae Plantarum* 32, 839–848 <https://doi.org/10.1007/s11738-010-0468-z>.
- Du, L., Liu, H., Guan, W., Li, J., Li, J., 2019: Drought affects the coordination of belowground and aboveground resource-related traits in *Solidago canadensis* in China. *Ecology and Evolution* 9, 9948–9960 <https://doi.org/10.1002/ece3.5536>.
- Dulai, S., Molnár, I., Szopkó, D., Darkó, É., Vojtkó, A., Sass-Gyarmati, A., Molnár-Láng, M., 2014: Wheat-Aegilops biuncialis amphiploids have efficient photosynthesis and biomass production during osmotic stress. *Journal of Plant Physiology* 171, 509–517 <https://doi.org/10.1016/j.jplph.2013.11.015>.
- Fang, J., Wu, F., Yang, W., Zhang, J., Cai, H., 2012: Effects of drought on the growth and resource use efficiency of two endemic species in an arid ecotone. *Acta Ecologica Sinica* 32, 195–201 <https://doi.org/10.1016/j.chnaes.2012.05.001>.
- Farooq, M., Wahid, A., Kobayashi, N., S.M.A. Fujita, D. B., Fujita, D., Basra, S. M. A., 2009: Plant drought stress: Effects, mechanisms and management. *Sustainable Agriculture* 29, 153–188 https://doi.org/10.1007/978-90-481-2666-8_12.
- Gallé, A., Feller, U., 2007: Changes of photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. *Physiologia Plantarum* 131, 412–421 <https://doi.org/10.1111/j.1399-3054.2007.00972.x>.
- Ge, Y., He, X., Wang, J., Jiang, B., Ye, R., Lin, X., 2014: Physiological and biochemical responses of *Phoebe bournei* seedlings to water stress and recovery. *Acta Physiologiae Plantarum* 36, 1241–1250 <https://doi.org/10.1007/s11738-014-1502-3>.
- Geng, D., LU, L., Yan, M. jia, Shen, X. xia, Jiang, L., Li, H., Wang, L., Yan, Y., Xu, J., Li, C., Yu, J. tao, Ma, F. wang, Guan, Q. mei., 2019: Physiological and transcriptomic analyses of roots from *Malus sieversii* under drought stress. *Journal of Integrative Agriculture* 18, 1280–1294 [https://doi.org/10.1016/S2095-3119\(19\)62571-2](https://doi.org/10.1016/S2095-3119(19)62571-2).
- Ghaffari, H., Reza, M., Muhammad, T., Cheema, M., Razmjoo, J., Tadayon, M. R., Nadeem, M., Cheema, M., Razmjoo, J., Reza, M., Muhammad, T., Cheema, M.,

- Razmjoo, J., 2019: Proline-mediated changes in antioxidant enzymatic activities and the physiology of sugar beet under drought stress. *Acta Physiologiae Plantarum* 41, 23 <https://doi.org/10.1007/s11738-019-2815-z>.
- Ghorbani, M., Sohrabi, H., Sadati, S. E., Babaei, F., 2018: Productivity and dynamics of pure and mixed-species plantations of *Populus deltoids* Bartr. ex Marsh and *Alnus subcordata* C. A. Mey. *Forest Ecology and Management* 409, 890–898 <https://doi.org/10.1016/j.foreco.2017.11.016>.
- Giannopolitis, C. N., Ries, S. K., Gutteridge, J. M. C., 1977: Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology* 59, 309–314 <https://doi.org/10.1080/09553008314551231>.
- Guo, X., Guo, W., Luo, Y., Tan, X., Du, N., Wang, R., 2013: Morphological and biomass characteristic acclimation of trident maple (*Acer buergerianum* Miq.) in response to light and water stress. *Acta Physiologiae Plantarum* 35, 1149–1159 <https://doi.org/10.1007/s11738-012-1154-0>.
- Guo, X., Luo, Y.-J. J., Xu, Z.-W. W., Li, M.-Y. Y., Guo, W. H., 2019: Response strategies of *Acer davidii* to varying light regimes under different water conditions. *Flora* 257, 151423 <https://doi.org/10.1016/j.flora.2019.151423>.
- Guo, Y. Y., Yu, H. Y., Yang, M. M., Kong, D. S., Zhang, Y. J., 2018: Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of *Lycium ruthenicum* Murr. seedling. *Russian Journal of Plant Physiology* 65, 244–250 <https://doi.org/10.1134/S1021443718020127>.
- Jourgholami, M., Fathi, K., Labelle, E. R., 2020: Effects of litter and straw mulch amendments on compacted soil properties and Caucasian alder (*Alnus subcordata*) growth. *New Forests* 51, 349–365 <https://doi.org/10.1007/s11056-019-09738-5>.
- Khaleghi, A., Naderi, R., Brunetti, C., Maserti, B. E., Salami, S. A., Babalar, M., 2019: Morphological, physiochemical and antioxidant responses of *Maclura pomifera* to drought stress. *Scientific Reports* 9, 1–12 <https://doi.org/10.1038/s41598-019-55889-y>.
- Lei, Y., Yin, C., Li, C., 2006: Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. *Physiologia Plantarum* 127, 182–191 <https://doi.org/10.1111/j.1399-3054.2006.00638.x>.
- Lichtenthaler, H. K., 1987: Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods in Enzymology* 148, 350–382 [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1).
- Liu, B., Liang, J., Tang, G., Wang, X., Liu, F., Zhao, D., 2019: Drought stress affects on growth, water use efficiency, gas exchange and chlorophyll fluorescence of *Juglans* rootstocks. *Scientia Horticulturae* 250, 230–235 <https://doi.org/10.1016/j.scienta.2019.02.056>.
- Medeiros, D. B., Silva, E. C. da, Nogueira, R. J. M. C., Teixeira, M. M., Buckeridge, M. S., 2013: Physiological limitations in two sugarcane varieties under water suppression and after recovering. *Theoretical and Experimental Plant Physiology* 25, 213–222 <https://doi.org/10.1590/s2197-00252013000300006>.

- Meng, G. T., Li, G. X., He, L. P., Chai, Y., Kong, J. J., Lei, Y.B., 2013: Combined Effects of CO₂ Enrichment and Drought Stress on Growth and Energetic Properties in the Seedlings of a Potential Bioenergy Crop *Jatropha curcas*. *Journal of Plant Growth Regulation* 32, 542–550 <https://doi.org/10.1007/s00344-013-9319-7>.
- Mohammadkhani, N., Heidari, R., 2008: Effects of drought stress on soluble proteins in two maize varieties. *Turkish Journal of Biology* 32, 23–30.
- Naghdi, R., Solgi, A., Labelle, E. R., Zenner, E. K., 2016: Influence of ground-based skidding on physical and chemical properties of forest soils and their effects on maple seedling growth. *European Journal of Forest Research* 135, 949–962 <https://doi.org/10.1007/s10342-016-0986-3>.
- Naji, H. R., Nia, M. F., Kiaei, M., Abdul-Hamid, H., Soltani, M., & Faghihi, A., 2016: Effect of intensive planting density on tree growth, wood density and fiber properties of maple (*Acer Velutinum* Boiss.). *IForest* 9, 325–329 <https://doi.org/10.3832/ifor1333-008>.
- Plewa, M. J., Smith, S. R., Wagner, E. D., 1991: Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 247, 57–64.
- Rahimi, D., Kartoolinejad, D., Nourmohammadi, K., Naghdi, R., 2016: Increasing drought resistance of *Alnus subcordata* C.A. Mey. seeds using a nano priming technique with multi-walled carbon nanotubes. *Journal of Forest Science* 62, 269–278 <https://doi.org/10.17221/15/2016-JFS>.
- Taleshi, H., JalaliS.GH., S.J., A., Hosseini, S. M., Naimi, B., 2018: Impacts of Climate Change on the Distribution of Oriental Beech (*Fagus orientalis* Lipsky) in the Hyrcanian Forests, Iran H. *Iranian Journal of Forest* 10, 251–266.
- Tariq, A., Pan, K., Olatunji, O. A., Graciano, C., Li, Z., Sun, F., Zhang, L., Wu, X., Chen, W., Song, D., Huang, D., Xue, T., Zhang, A., 2018: Phosphorous fertilization alleviates drought effects on *Alnus cremastogyne* by regulating its antioxidant and osmotic potential. *Scientific Reports* 8, 1–11 <https://doi.org/10.1038/s41598-018-24038-2>.
- Tavankar, F., Nikooy, M., Picchio, R., Bonyad, A., Venanzi, R., 2017: Effects of logging wounds on caucasian alder trees (*Alnus subcordata* C.A. Mey.) in Iranian caspian forests. *Croatian Journal of Forest Engineering* 38, 73–82.
- Toscano, S., Farieri, E., Ferrante, A., Romano, D., 2016: Physiological and biochemical responses in two ornamental shrubs to drought stress. *Frontiers in Plant Science* 7, 645 <https://doi.org/10.3389/fpls.2016.00645>.
- Wang, S., Liang, D., Li, C., Hao, Y., Ma, F., Shu, H., 2012: Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. *Plant Physiology and Biochemistry* 51, 81–89 <https://doi.org/10.1016/j.plaphy.2011.10.014>.
- Wu, F., Bao, W., Li, F., Wu, N., 2008: Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. *Environmental and Experimental Botany* 63, 248–255 <https://doi.org/10.1016/j.envexpbot.2007.11.002>.

- Wu, J., Li, J., Su, Y., He, Q., Wang, J., Qiu, Q., Ma, J., 2017: A morphophysiological analysis of the effects of drought and shade on *Catalpa bungei* plantlets. *Acta Physiologiae Plantarum* 39 <https://doi.org/10.1007/s11738-017-2380-2>.
- Wu, M., Zhang, W. H., Ma, C., Zhou, J. Y., 2013: Changes in morphological, physiological, and biochemical responses to different levels of drought stress in chinese cork oak (*Quercus variabilis* Bl.) seedlings. *Russian Journal of Plant Physiology* 60, 681–692 <https://doi.org/10.1134/S1021443713030151>.
- Yang, F., Miao, L.-F., 2010: Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. *Silva Fennica* 44, 23–37.
- Ying, Y. Q., Song, L. L., Jacobs, D. F., Mei, L., Liu, P., Jin, S. H., Wu, J. S., 2015: Physiological response to drought stress in *Camptotheca acuminata* seedlings from two provenances. *Frontiers in Plant Science* 6, 1–8 <https://doi.org/10.3389/fpls.2015.00361>.
- Zhang, Y., Yu, T., Ma, W., Tian, C., Sha, Z., Li, J., 2019: Morphological and physiological response of *Acer catalpifolium* Rehd. Seedlings to water and light stresses. *Global Ecology and Conservation* 19, e00660 <https://doi.org/10.1016/j.gecco.2019.e00660>.

Tab. 1. Effect of drought stress on height, basal diameter, leaf area (LA), special leaf area (SLA), and leaf area ratio (LAR) of *A. velutinum* and *A. subcordata* seedlings. Values are means of three replicates \pm standard deviation (SD). Different capital letters indicate significant ($p \leq 0.05$) differences between *A. velutinum* and *A. subcordata* subjected to the same treatment. Different lowercase letters indicate significant ($p \leq 0.05$) differences among different treatment subjected to the same species. Error bars are \pm SD ($n = 3$). Fs: Species effect, FD: drought effect, Fs \times FD: Species \times drought interaction effect. *, **, and ***: significant at $P \leq 0.05, 0.01, \text{ and } 0.001$, respectively.

	Field capacity (FC, %)	Plant height (cm)	Basal diameter (mm)	Leaf area (cm ²)	Special leaf area (cm ² /g)	Leaf area ratio (cm ² /g)
<i>Acer velutinum</i>	100	52.75 \pm 1.96 ^{Da}	14.51 \pm 0.37 ^{BCa}	123.40 \pm 11.21 ^{Ba}	117.23 \pm 5.22 ^{Eb}	30.51 \pm 1.53 ^{Ba}
	75	50.75 \pm 1.24 ^{Da}	14.06 \pm 0.52 ^{Ca}	78.55 \pm 3.41 ^{Cb}	131.84 \pm 10.34 ^{DEb}	24.49 \pm 0.85 ^{BCb}
	50	43.33 \pm 1.44 ^{Db}	13.35 \pm 0.59 ^{CDa}	60.80 \pm 4.30 ^{Cb}	200.35 \pm 14.53 ^{Ca}	29.00 \pm 0.21 ^{Ba}
	25	40.44 \pm 2.23 ^{Db}	11.63 \pm 0.38 ^{Db}	34.62 \pm 0.81 ^{Dc}	126.79 \pm 11.11 ^{DEb}	17.74 \pm 1.49 ^{Cc}
<i>Alnus subcordata</i>	100	132.67 \pm 7.97 ^{Aa}	18.69 \pm 0.80 ^{Aa}	153.66 \pm 12.50 ^{Aa}	356.28 \pm 22.99 ^{Aa}	57.95 \pm 3.74 ^{Aa}
	75	110.12 \pm 5.54 ^{Bb}	16.11 \pm 0.87 ^{Bb}	78.94 \pm 7.08 ^{Cb}	287.35 \pm 5.33 ^{Bab}	57.46 \pm 2.35 ^{Aa}
	50	97.33 \pm 6.32 ^{Cab}	12.58 \pm 0.62 ^{CDc}	37.36 \pm 3.90 ^{Dc}	262.53 \pm 30.17 ^{Bb}	31.78 \pm 7.17 ^{Bb}
	25	90.89 \pm 5.37 ^{Cc}	13.23 \pm 0.70 ^{CDc}	25.13 \pm 2.39 ^{Dc}	179.33 \pm 0.05 ^{CDc}	18.70 \pm 0.90 ^{Cb}
Fs		328.49 ^{***}	15.95 ^{***}	0.01 ^{ns}	98.39 ^{***}	53.38 ^{***}
FD		13.68 ^{***}	65.12 ^{***}	93.90 ^{***}	8.89 ^{**}	28.64 ^{***}
Fs \times FD		3.98 [*]	18.00 ^{**}	5.40 ^{**}	11.69 ^{***}	14.17 ^{***}

Tab. 2. Effect of drought stress on biomass in *A. velutinum* and *A. subcordata* seedlings. Values are means of three replicates \pm standard deviation (SD). Different capital letters indicate significant ($p \leq 0.05$) differences between *A. velutinum* and *A. subcordata* subjected to the same treatment. Different lowercase letters indicate significant ($p \leq 0.05$) differences among different treatment subjected to the same species. Error bars are \pm SD ($n = 3$). F_s: Species effect, F_D: drought effect, F_s×F_D: Species \times drought interaction effect. *, **, and ***: significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

	Field capacity (FC, %)	Root biomass (g)	Leaf biomass (g)	Stem biomass (g)	Total biomass (g)
<i>Acer velutinum</i>	100	33.33 \pm 3.38 ^{Ba}	16.00 \pm 0.58 ^{Ba}	12.33 \pm 1.20 ^{DEa}	61.67 \pm 3.76 ^{Ca}
	75	22.67 \pm 1.45 ^{CDb}	8.00 \pm 0.58 ^{Cb}	12.00 \pm 0.58 ^{DEa}	42.67 \pm 1.45 ^{DEb}
	50	13.00 \pm 0.58 ^{Ec}	3.67 \pm 0.33 ^{Dc}	8.33 \pm 0.33 ^{DEb}	25.00 \pm 0.58 ^{FGc}
	25	13.00 \pm 0.58 ^{Ec}	3.33 \pm 0.88 ^{Dc}	7.33 \pm 0.33 ^{Eb}	23.67 \pm 1.45 ^{Gc}
<i>Alnus subcordata</i>	100	46.66 \pm 2.90 ^{Aa}	20.33 \pm 0.66 ^{Aa}	58.00 \pm 4.16 ^{Aa}	125.00 \pm 4.00 ^{Aa}
	75	27.33 \pm 1.66 ^{Bb}	14.33 \pm 1.20 ^{Bb}	30.00 \pm 3.05 ^{Bb}	71.66 \pm 5.48 ^{Bb}
	50	21.33 \pm 2.02 ^{CDbc}	5.33 \pm 0.33 ^{Dc}	19.00 \pm 1.15 ^{Cc}	45.66 \pm 2.40 ^{Dc}
	25	16.66 \pm 2.18 ^{DEc}	3.90 \pm 0.92 ^{Dc}	14.00 \pm 0.57 ^{CDc}	34.56 \pm 3.47 ^{EFc}
F _s		26.21 ^{***}	44.43 ^{***}	211.64 ^{***}	185.72 ^{***}
F _D		60.19 ^{***}	195.72 ^{***}	61.62 ^{***}	162.89 ^{***}
F _s ×F _D		2.23 ^{ns}	7.25 ^{**}	39.88 ^{***}	25.16 ^{***}

Tab. 3. Effect of drought stress on biomass partitioning rate of *A. velutinum* and *A. subcordata* seedlings. Values are means of three replicates \pm standard deviation (SD). Different capital letters indicate significant ($p \leq 0.05$) differences between *A. velutinum* and *A. subcordata* subjected to the same treatment. Different lowercase letters indicate significant ($p \leq 0.05$) differences among different treatment subjected to the same species. Error bars are \pm SD ($n = 3$).Fs: Species effect, F_D: drought effect, F_S×F_D: Species \times drought interaction effect. *, **, and ***: significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

	Field capacity (%)	Root to shoot ratio (R/S)	Leaf mass ratio (LMR)	Stem mass ratio (SMR)	Root mass ratio (RMR)
<i>Acer velutinum</i>	100	1.18 \pm 0.12 ^{Aa}	26.19 \pm 2.12 ^{Aa}	19.96 \pm 1.33 ^{Ca}	53.85 \pm 2.60 ^{ABa}
	75	1.13 \pm 0.07 ^{ABa}	18.73 \pm 1.09 ^{BCb}	28.25 \pm 2.14 ^{Bb}	53.01 \pm 1.62 ^{ABa}
	50	1.09 \pm 0.08 ^{ABCa}	14.62 \pm 1.07 ^{CDEb}	33.36 \pm 1.50 ^{Bb}	52.01 \pm 2.00 ^{ABCa}
	25	1.22 \pm 0.05 ^{Aa}	14.16 \pm 1.48 ^{DEb}	30.78 \pm 1.92 ^{Bb}	55.05 \pm 1.04 ^{Aa}
<i>Alnus subcordata</i>	100	0.60 \pm 0.06 ^{Db}	16.26 \pm 0.01 ^{BCDa}	46.33 \pm 2.42 ^{Aa}	37.39 \pm 2.43 ^{Db}
	75	0.62 \pm 0.03 ^{Db}	19.97 \pm 0.44 ^{Ba}	41.75 \pm 1.60 ^{Aa}	38.26 \pm 1.31 ^{Db}
	50	0.87 \pm 0.08 ^{Ca}	11.82 \pm 1.40 ^{Eb}	41.64 \pm 2.25 ^{Aa}	46.53 \pm 2.25 ^{Ca}
	25	0.92 \pm 0.05 ^{BCa}	11.03 \pm 1.80 ^{Eb}	41.03 \pm 2.91 ^{Aa}	47.94 \pm 1.37 ^{BCa}
F _S		56.18 ***	14.74 ***	105.59 ***	65.76 ***
F _D		2.90 ns	20.74 ***	1.63 ns	4.58 *
F _S ×F _D		2.58 ns	5.90 **	8.20 **	4.09 *

Tab. 4. Effect of drought stress on photosynthetic pigments content, and RWC of *A. velutinum* and *A. subcordata* seedlings. Values are means of three replicates \pm standard deviation (SD). Different capital letters indicate significant ($p \leq 0.05$) differences between *A. velutinum* and *A. subcordata* subjected to the same treatment. Different lowercase letters indicate significant ($p \leq 0.05$) differences among different treatment subjected to the same species. Error bars are \pm SD ($n = 3$).FW: Fresh Weight, Fs: Species effect, F_D: drought effect, F_S×F_D: Species \times drought interaction effect. *, **, and ***: significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

	Field capacity (%)	Chlorophyll <i>a</i> (mg/g FW)	Chlorophyll <i>b</i> (mg/g FW)	Total chlorophyll (mg/g FW)	Total Carotenoids (mg/g FW)	RWC (%)
<i>Acer velutinum</i>	100	1.00 \pm 0.05 ^{ABab}	0.49 \pm 0.13 ^{Aab}	1.49 \pm 0.07 ^{ABab}	0.18 \pm 0.02 ^{BCDa}	72.58 \pm 3.82 ^{Aa}
	75	1.30 \pm 0.20 ^{Aa}	0.53 \pm 0.03 ^{Aa}	1.84 \pm 0.23 ^{Aa}	0.22 \pm 0.03 ^{ABCa}	75.08 \pm 2.66 ^{Aa}
	50	0.76 \pm 0.07 ^{BCb}	0.39 \pm 0.09 ^{ABCab}	1.11 \pm 0.01 ^{Cbc}	0.25 \pm 0.02 ^{ABa}	69.20 \pm 3.82 ^{Aa}
	25	0.72 \pm 0.10 ^{BCDb}	0.23 \pm 0.03 ^{BCb}	0.95 \pm 0.12 ^{CDc}	0.25 \pm 0.02 ^{ABa}	52.77 \pm 1.36 ^{Bb}
<i>Alnus subcordata</i>	100	1.05 \pm 0.08 ^{ABa}	0.44 \pm 0.03 ^{Aa}	1.50 \pm 0.06 ^{Aa}	0.26 \pm 0.02 ^{Aa}	70.38 \pm 3.00 ^{Aa}
	75	0.82 \pm 0.12 ^{BCb}	0.40 \pm 0.06 ^{Aba}	1.23 \pm 0.11 ^{BCb}	0.21 \pm 0.00 ^{ABCb}	67.61 \pm 2.73 ^{Aa}
	50	0.41 \pm 0.00 ^{Dc}	0.19 \pm 0.01 ^{Cb}	0.60 \pm 0.02 ^{Dc}	0.13 \pm 0.0 ^{Dc}	52.83 \pm 3.08 ^{Bb}
	25	0.49 \pm 0.04 ^{CDc}	0.21 \pm 0.02 ^{BCb}	0.70 \pm 0.07 ^{Dc}	0.16 \pm 0.00 ^{CDc}	46.77 \pm 3.08 ^{Bb}
F _S		11.55 ^{**}	5.21 [*]	18.36 ^{**}	5.54 [*]	13.99 ^{**}
F _D		12.69 ^{***}	8.13 ^{**}	23.72 ^{***}	0.82 ^{ns}	23.10 ^{***}
F _S ×F _D		2.42 ^{ns}	0.81 ^{ns}	3.03 ^{ns}	7.69 ^{**}	1.94 ^{ns}

Tab. 5. Correlation analysis among some morphophysiological and biochemical traits in *Acer velutinum* under drought stress conditions. Each square indicates the Pearson's correlation coefficient of a pair of parameters. leaf area: LA (cm²), specific leaf area: SLA (cm²/g), relative water content: RWC (%), chlorophyll *a*: Chl *a*, chlorophyll *b*: Chl *b*, total chlorophyll: Chl *a+b*, and carotenoids: Car (mg/g FW), free proline: Pro (μmol/gFW), malondialdehyde: MDA (nmol/g FW), Peroxidase: POD (μmol /g FW min), superoxide dismutase: SOD (U/g FW). ** and * indicate a significant correlation between control and drought treatments at $p \leq 0.01$ and $p \leq 0.05$, respectively.

	LA	SLA	Car	SOD	POD	MDA	Pro	RWC	Chl <i>a</i>	Chl <i>b</i>	Chl
LA	1.000	-0.293	-0.476	-.695*	-0.306	-	-	.607*	0.466	0.426	0.524
SLA		1.000	0.300	.632*	.779**	0.323	0.427	0.047	-0.256	-0.196	-0.294
Car			1.000	.605*	0.365	0.343	0.415	-0.213	-0.096	-0.229	-0.181
SOD				1.000	.624*	.638*	.771**	-0.150	-0.224	-0.298	-0.314
POD					1.000	0.366	0.487	0.151	-0.332	-0.042	-0.293
MDA						1.000	.910**	-0.524	-.585*	-0.523	-.651*
Pro							1.000	-0.540	-.651*	-0.420	-.667*
RWC								1.000	.596*	.768**	.735**
Chl <i>a</i>									1.000	0.473	.939**
Chl <i>b</i>										1.000	.745**
Chl <i>a+b</i>											1

Tab. 6. Correlation analysis among some morphophysiological and biochemical traits in *Alnus subcordata* under drought. Each square indicates the Pearson's correlation coefficient of a pair of parameters. leaf area: LA (cm²), specific leaf area: SLA (cm²/g), relative water content: RWC (%), chlorophyll *a*: Chl *a*, chlorophyll *b*: Chl *b*, total chlorophyll: Chl *a+b*, and carotenoids: Car (mg/g_{FW}), free proline: Pro (μmol/g FW), malondialdehyde: MDA (nmol/g FW), Peroxidase: POD (μmol /g FW min), superoxide dismutase: SOD (U/g FW). ** and * indicate a significant correlation between control and drought treatments at $p \leq 0.01$ and $p \leq 0.05$, respectively.

	LA	SLA	Car	SOD	POD	MDA	Pro	RWC	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a+b</i>
LA	1.000	.836**	.873**	-0.566	-0.314	-.826**	-.757**	.775**	.869**	.758**	.889**
SLA		1.000	.648*	-0.300	0.094	-.797**	-.746**	.746**	.632*	.607*	.665*
Car			1.000	-0.559	-0.330	-.716**	-.626*	.799**	.944**	.698*	.925**
SOD				1.000	.705*	0.444	0.295	-0.250	-0.538	-0.210	-0.466
POD					1.000	0.281	-0.191	0.113	-0.309	-0.182	-0.288
MDA						1.000	0.543	-.702*	-.630*	-.697*	-.692*
Pro							1.000	-.812**	-.688*	-.655*	-.721**
RWC								1.000	.749**	.684*	.776**
Chl <i>a</i>									1.000	.725**	.974**
Chl <i>b</i>										1.000	.861**
Chl <i>a+b</i>											1.000

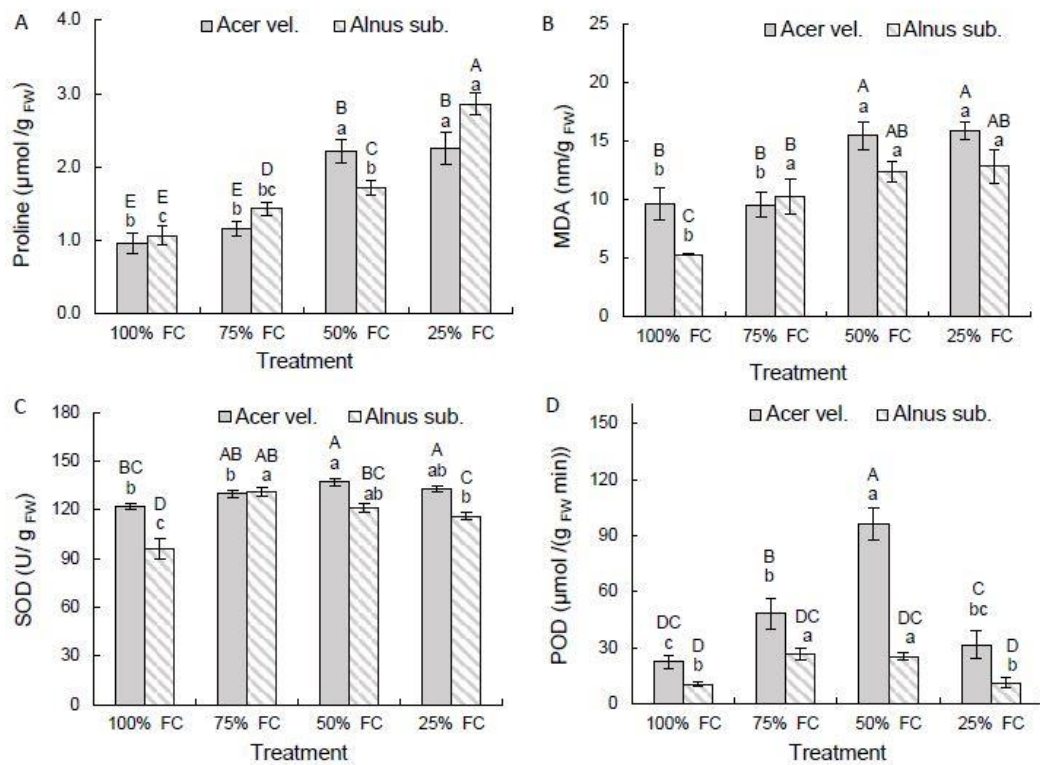


Fig. 1. Changes in proline (A), malondialdehyde (MDA) (B), superoxide dismutase (SOD) (C) and guaiacol peroxidase (POD) measured in leaves from *A. velutinum* and *A. subcordata* seedlings subjected to four drought treatments (100, 75, 50 and 25% of field capacity - FC). Values are means of three replicates \pm standard deviation (SD). Different capital letters indicate significant ($p \leq 0.05$) differences between *A. velutinum* and *A. subcordata* subjected to the same treatment. Different lowercase letters indicate significant ($p \leq 0.05$) differences among different treatment subjected to the same species.