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Studies on tissue water relations and soluble sugars in Cilician Fir (*Abies cilicica*) seedlings during bud dormancy

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Abstract

Aim : The objective of this study was to determine seasonal changes in tissue water relations and total soluble sugars in Cilician fir (*Abies cilicica* subsp. *isaurica*) seedlings in relation to physiological processes, and their relationship during bud dormancy and its release.

Methodology : The water relations parameters, including osmotic potential at full turgor ($\Psi_{\pi 100}$), osmotic potential at the turgor loss point ($\Psi_{\pi TLP}$), symplastic water at saturated point per dry weight of the shoot (V_s/DW), dry weight fraction (DWF), relative water content at the turgor loss point (RWC_{ZT}), free water content at the turgor loss point (FWC_{ZT}) were obtained from the pressure-volume curves. Total soluble sugar content was determined by the phenol-sulfuric acid method, while dormancy intensity was estimated as number of days to reach the budbreak.

Results : The osmotic potential at full turgor and turgor loss point were lowest in January (-3.34 and -2.02 MPa, respectively) and highest in April (-2.27 and -1.26 MPa, respectively), and varied within season. The other water relation parameters did not show any change. The osmotic potential at full turgor and turgor loss point declined with the decrease of temperature during autumn and winter, and increased during dormancy release in spring. Total soluble sugars ranged between 103.8 and 224.0 mg g⁻¹ and it declined rapidly with shoot development in spring.

Interpretation : This study showed that osmotic adjustment is an important physiological adaptive mechanism to tolerance low temperatures or unfavorable conditions during winter in Cilician fir seedlings.

Introduction

Abies cilicica (Antoine and Kotschy) Carrière is one of the most important tree species of Turkey in aesthetic, economic and ecological perspectives. It can reach over than 40 m high and diameter of 1.5- 2.0 m in a suitable environment. *A. cilicica* occurs between 1150 and 2000 m on the north facing slopes, and 1450 and 1550 m on the south facing slopes on the Taurus Mountains, growing optimally at 1200–1800 m (Kaya and Raynal, 2001). There are two subspecies of *A. cilicica* in Turkey; *Abies cilicica* subsp. *isaurica* Coode and Cullen and *A. cilicica* (Ant. and Kotschy) Carr. subsp. *cilicica*. *A. cilicica* subsp. *isaurica* is an endemic species and grow in the area of western Mediterranean provinces like Antalya and Konya. The climate in the areas on which *A. cilicica* grows is completely different from the typical Mediterranean climate (which have above 15 °C of annual mean temperature) and it can be considered as a sub-class with severe winters and rather cool summers on the high mountain ranges. In the upper part of Taurus Mountains the mean value of temperature generally doesn't exceed 10 °C (Bozkus, 1988).

In such cold climate, plants are frequently exposed to sub-zero temperatures in autumn, winter, and spring seasons (Janmohammadi, 2012). Low temperature stress (cold or frost stress) is an environmental factor that limit the growing seasons and adversely affects the plant growth. To cope with extreme temperature and UV radiation conditions during winter, plants have evolved mechanisms (at cellular and metabolic levels) that allow timely acclimation to these adverse environmental conditions. Acclimation proceeds sequentially in response to short day length, low non-freezing temperatures, and then freezing temperatures (Welling *et al.*, 2004). This process is known as cold or frost acclimation. In woody plants, the processes of dormancy and cold acclimation overlaps (Wisniewski *et al.*, 2011). Dormancy induction is considered as a prerequisite to cold acclimation during the hardening period (Vitasse *et al.*, 2014). There are a series of physiological and biochemical responses with increasing freezing tolerance during cold acclimation, including decreased tissue water content and increased compatible compounds (Yang *et al.*, 2015). Tissue water content decreases during dormancy induction. At the cellular level, water activity is modulated by hydrophilic molecules, such as sugars and dehydrin proteins. These compounds accumulate during dormancy induction and cold acclimation, and change both the osmotic potential of water and its availability (Kalcsits *et al.*, 2009). Osmotic adjustment occurs when the osmotic potential of cells is lowered through active solute accumulation i.e., soluble sugars and proline (Scholz *et al.*, 2012). A higher soluble sugar content lowers the freezing point of the intracellular solution (Charrier and Améglio, 2011). Therefore, the observed increase in soluble carbohydrates in leaves and root in autumn and winter was associated with an increase of frost hardening (Oleksyn *et al.*, 2000).

Fig. 1 : Minimum, maximum and mean air temperatures recorded during the study period under natural conditions in the nursery

The physiological mechanisms during cold acclimation induced by temperatures in *A. cilicica* subsp. *isaurica* are unknown. The changes that occur in soluble carbohydrate levels and water relations of seedlings under natural environmental conditions (e.g., extreme temperature and UV radiation conditions during winter) are not well understood and the information on physiological events involved during seasons is scarce. In view of the above, the objective of the present study was to determine the changes in tissue water relations and total soluble sugars in *A. cilicica* subsp. *isaurica* seedlings during winter and spring budburst. Further, relationship among these properties and to investigate the role of osmotic adjustment in response to environmental stresses under natural condition.

Materials and Methods

Study area and plant material : Cilician fir (*Abies cilicica* (Ant. and Kotschy) Carr. subsp. *isaurica* Coode and Cullen) seeds were collected from a seed stand (Bucak-Isparta/Turkey, 37°19' N, 30°37' E, 1200 m elevation) in October 2008, placed in polyethylene bags and refrigerated at 4±1 °C until sowing date. The study was conducted in Eğirdir Forest Nursery-Turkey. The nursery soil texture was sandy clay loam and its pH was 8.1. In the nursery area the mean of total annual precipitation was about 877 mm and the mean annual temperature was 13 °C. The seeds were sown on seedbeds on March 2009 and grown under natural conditions for three years. Irrigation (by sprinkling) and weed control were regularly applied during the three growing season. Moreover 18-20 gr m⁻² of ammonium sulfate fertilizer was applied June, 2009 and 2010. At the end of the third year (November, 2011), seedlings were lifted randomly from the nursery bed in order to determine morphological characteristics. The water relation parameters, total soluble sugar content and dormancy intensity of the seedlings were evaluated at monthly intervals from November 2011 to April 2012. Minimum, maximum and mean air temperatures were recorded in the nursery during the experiment period as shown in Fig.1.

Bud dormancy : Dormancy intensity of terminal buds of the seedlings was estimated as number of days to reach the

budbreak under controlled conditions. On each sample date, 18 seedlings (6 seedlings x 3 replication) were collected from the nursery beds and planted in plastic pots containing peat and perlite (3:1 v/v) and moved into a plant growth chamber. The plant growth chamber was maintained at 22-27 °C (night-day) and a photoperiod of 16 hrs. For the duration of the experiment, seedlings were watered regularly but not fertilized. Mean number of days to terminal budbreak was calculated following the procedure of Ritchie (1984).

Pressure-volume curves : Tissue water relations parameters were measured by the pressure-volume (P-V) method (Tyree and Hammel, 1972; Ritchie, 1984) with a pressure chamber (Plant Moisture Stress Instruments, Corvallis Oregon, USA) from November 2011 to April 2012. At each lifting time, shoots from each seedling were excised at the root collar level and immediately weighed. Shoot samples were kept at room temperature in distilled water for 24 hrs in dark for full saturation. Three turgid shoot samples were used and P-V curves were generated as described by Ritchie (1984). The parameters derived from these curves were osmotic potential at full turgor ($\Psi_{\pi 100}$), osmotic potential at the turgor loss point ($\Psi_{\pi TLP}$), symplastic water at saturated point per dry weight of the shoot (V_o/DW), dry weight fraction (DWF), relative water content at the turgor loss point (RWC_{ZT}), free water content at turgor loss point (FWC_{ZT}) (Tyree *et al.*, 1978; Ritchie, 1984; Doi *et al.*, 1986; Grossnickle, 1992).

Total soluble sugars : To determine the needle total sugar content, every month 30 seedlings were collected randomly from nursery from November 2011 to April 2012. Soluble sugar

analyses were performed on three replicates (needles of ten seedlings per replication). The needle samples were dried at 65 °C for 48 hrs and then ground to a fine powder in a coffee grinder. A 100 mg of ground needles, for each sample, was incubated with 10 ml of ethanol (80%) for 24 hrs, then centrifuged at 2500 rpm for 10 min and 0.05 ml of the supernatant was used for analysis. Total soluble sugar content ($mg\ g^{-1}$) was determined by phenol sulfuric acid method, using D-glucose as standard (Dubois *et al.*, 1956).

Data analysis : The effect of sampling time on days to terminal budbreak, water relation parameters, and total soluble sugar content were analyzed by one-way ANOVA using the SPSS 17.0. Mean comparison was carried by Duncan's test. Before statistical analysis, proportional values were subjected to transformation of arcsin $\sqrt{P}$. Relationship between studied traits were examined by correlation. Differences were considered statistically significant at $P < 0.05$ (Kalipsiz, 1981).

Results and Discussion

The changes in water relations parameters derived from P-V curves from shoots of Cilician fir seedlings (root collar diameter: 3.23 ± 0.30 mm, seedling height: 14.70 ± 0.61 cm, shoot dry weight: 1.91 ± 0.26 g, root dry weight: 0.94 ± 0.15 g, shoot/root ratio: 2.1 ± 0.13) from November 2011 to April 2012 are shown in Fig. 2. While $\Psi_{\pi TLP}$ varied between - 2.27 MPa and - 3.34 MPa during the study, $\Psi_{\pi 100}$ varied between - 1.26 MPa and - 2.02 MPa. $\Psi_{\pi TLP}$ decreased slowly from November (-2.93 MPa) reaching the lowest value in January (-3.34 MPa), and then reached highest level in April (-2.27 MPa). The highest values were observed in April for $\Psi_{\pi 100}$ too (Fig. 2a). Minimum values of $\Psi_{\pi TLP}$ were

Fig. 2 : Seasonal changes (mean $\pm$ SE) of three-year-old Cilician fir seedlings: (a) osmotic potential at full turgor (100py) and at the turgor loss point (TLppy), (b) relative water content at the turgor loss point (RWC_{ZT}) and free water content at the turgor loss point (FWC_{ZT}), (c) symplastic water at saturated point per dry weight of the shoot (V_o/DW), and (d) dry weight fraction (DWF) under natural conditions in the nursery. Data analyzed by ANOVA, Different letters show significant difference at 0.05 level between lifting dates

Fig. 3 : Seasonal changes (mean $\pm$ SE) of three-year-old Cilician fir seedlings: (a) days to terminal budbreak (DBB) and (b) total soluble sugar content under natural conditions in nursery. Data analyzed by ANOVA, Different letters show significant difference at 0.05 level between lifting dates

observed in midwinter, when mean air temperature was about 0 °C (Fig.1), while $\Psi_{\pi TLP}$ decreased with decreasing mean air temperature ($r^2 = 0.78$, $P < 0.05$). No statistical difference among the sampling dates were found analyzing the other parameters were studied. The difference among the sampling dates were not found to be significant with respect to RWC_{ZT} , FWC_{ZT} , Vo/DW and DWF , values (Fig. 2b, 2c, 2d).

$\Psi_{\pi TLP}$ and $\Psi_{\pi 100}$ measured in Cilician fir between November and April showed a seasonal change, but other water relations parameters did not show any seasonal change. $\Psi_{\pi TLP}$ decreased as temperature decreased during autumn and winter; especially it reached lowest values in January, then, both and also temperatures started to increase again and reached high values in April. This seasonal trend is an expected status and it was similarly detected in *Pinus nigra* subsp. *pallasiana* (Deligoz, 2011) species too. It was reported that seedlings would be resistant to environmental stresses such as frost and drought when $\Psi_{\pi TLP}$ is lowest while sugar and nutrient contents would be high (Grossnickle, 1992). It is known that many species decrease their osmotic potentials for adaptation to cold weather during freezing temperatures and for turgor maintenance or symplastic volume during drought (Abrams and Kubiske, 1994). Although no seasonal change was detected in Vo/DW values, it was detected that Vo/DW received low values in January and February. When $\Psi_{\pi TLP}$ reached the minimum value (in January), the minimum temperatures in January and February were -10 °C and -13 °C, respectively, with mean temperature as 0 °C (Fig. 1). An increase in water viscosity, a decrease in root water permeability and extracellular freezing might occur in winter at low temperatures. The decreasing trend of osmotic potential not only resulted in depression of freezing point of symplastic water, but also in maintaining the turgor against dehydration (Harayama *et al.*,

2006). Consequently, it was put forward that Cilician fir seedlings showed an osmotic adjustment against minimum temperatures.

The seasonal change in osmotic potential or other tissue water relations were attributed to phenological events including flowering, shoot elongation and leaf development (Abrams and Kubiske, 1994). It was also found that in other conifer species osmotic potential increased in relation to budbreak and shoot elongation in spring (Grossnickle *et al.*, 1996; Deligoz, 2011). Similarly, in the present study, it was found that $\Psi_{\pi TLP}$ and $\Psi_{\pi 100}$ increased in April. The reason could be increase in temperature and the beginning of growing season. In addition, the osmotic potential of cells is lowered due to accumulation of active solute i.e., soluble sugars, proline (Scholz *et al.*, 2012). In spring, the osmotic potential might increase due to decrease in solute concentration, which accumulated during acclimation in autumn. Because in early spring, the soluble sugar concentration decreased, the carbohydrate reserves were probably used during initial spring growth.

The timing of induction and release from dormancy are synchronized with local climates and are highly heritable (Yordanov *et al.*, 2014). In late summer, shortening photoperiods triggered plant to begin the bud dormancy process that culminates in late fall. This condition is known as deep dormancy and can be released by exposure to a period of low temperatures. The release from dormancy requires a period of chilling and temperatures in the range of about 3 to 5 °C are most efficient (Ritchie *et al.*, 2010). In this study, dormancy intensity was determined as DBB. The time required for terminal budbreak progressively decreased from November to April ($P < 0.001$) (Fig. 3a). Dormancy intensity peaked at November, and then it gradually decreased towards late March. This showed that

seedlings received sufficient chilling by late November to break dormancy. Likewise in this period, the mean temperatures were less than 5 °C. Full dormancy release of seedlings occurred by late March. In April, mean temperature was above 10°C and development of new shoots started. In this period, stress resistance of seedlings was also reduced as a consequence of a decreased dormancy intensity and of an increased $\Psi_{\pi TLP}$ because, the major increase in stress resistance occurs during dormancy and stress resistance falls with renewed development and growth (Burr, 1990).

Total soluble sugar content varied during the seasons between 103.8 and 224.0 mg g⁻¹ but it was maintaining the same high level from November 2011 to March 2012, and only in April 2012 it decreased reaching the lowest value (103.8 mg g⁻¹) (Fig. 3b). It's known that the osmotic potential varies with changes in solute concentrations. In the present study, a significant relation was found between total soluble sugar content and $\Psi_{\pi TLP}$ ($r = -0.67$; $P < 0.01$), $\Psi_{\pi 100}$ ($r = -0.51$; $P < 0.05$). While $\Psi_{\pi TLP}$ decreased from November to January, the total soluble sugar content remained high. While $\Psi_{\pi TLP}$ increased until April, the total soluble sugar content decreased. Schaberg *et al.* (2000) reported that sugar concentration in *Picea rubens* Sarg. seedlings was maximum in winter and reached minimum value in spring. The reserve carbohydrates are important for metabolic activity during cold acclimation and for development and maintenance of cold tolerance (Wong *et al.*, 2003). It is known that carbohydrates such as soluble sugars accumulate as a response to low temperature stress (Jiang *et al.*, 2011). Chohan and Raina (2011) reported that total soluble sugars increased under chilling stress, thus maintaining the osmotic pressure of cells. Although total soluble sugar content was similar from November to March, it was characterized by high values in winter. During this period, average temperature remained under 5 °C (Fig. 1). A sudden decrease was observed in total soluble sugar content with increase in spring temperature and bud dormancy release (in April). The fact that total soluble sugar content reached minimum value in April can be explained by the fact that carbohydrate was consumed as energy source for budbreak and shoot growth during this period.

Thus, the present study focus on seasonal changes in water relations, total soluble sugars and dormancy intensity in *A. cilicica* subsp. *isaurica* three-years-old seedlings. $\Psi_{\pi TLP}$ showed seasonal changes and declined with decreasing temperatures during winter. The significant decreases observed in osmotic potentials might help seedlings to maintain turgor and tolerate freezing temperatures during winter. While total soluble sugars peaked in winter, and declined with increasing temperatures, depending at the start of shoot development in spring. This study showed that osmotic adjustment is an important physiological adaptive mechanism of tolerance against unfavorable conditions during winter in Cilician fir.

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