

## Local adaptation at a small geographic scale observed in *Juniperus excelsa* populations in southern Turkey

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*Juniperus excelsa* is one of the most common tree species and has a wide geographical and altitudinal distribution in Turkey. It is also resistant to drought and frost damages and can cope with poor soils. In this study, we explore whether there are any differences among eight *J. excelsa* populations from a narrow geographic region grown in a common garden test site in terms of growth and contents of photosynthetic pigments, proline and nutrients of their 10-year-old saplings. Phenotypic trait differentiation ( $Q_{ST}$ ) at all traits,  $F_{ST}$  at neutral SSRs among six of the populations and associations of traits with environmental conditions at provenance regions were also analysed to test for patterns of local adaptation. Sapling traits of eight *J. excelsa* populations of from Lakes District in Turkey at the test site showed that populations significantly differed for growth, photosynthetic pigments, proline and nutrient contents. The mean height and diameter of 10-year-old saplings were found as 94.5 cm and 41.6 mm, respectively. Eğirdir-Barla and Sütçüler-Tota populations showed the highest performance for the majority of traits at age 10 compared to all other populations. Nutrient contents in leaves were generally in the sufficiency range reported for plant growth. Considering photosynthetic pigments and proline, it could be concluded that the populations were not exposed to severe stress. Among the environmental variables, the best predictors of growth were annual mean minimum temperature and soil texture at the populations' origin, accounting for 49% of the variation in height and diameter, respectively. Also, higher phenotypic trait differentiation for most traits than genetic differentiation at neutral genetic markers suggests local adaptation at a small geographic scale. The present study revealed adaptive divergence between populations at a small geographic scale. However, environmental similarity between region of origin and test site was not a good indicator of growth-related traits. The results can be used in the early selection of provenances for *J. excelsa* for plantation establishment.

**Keywords:** *Juniperus Excelsa*, Chlorophyll, Proline, Phenotypic Trait Differentiation, Local Adaptation

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### Introduction

Understanding how tree species and populations will respond to climatic changes is crucial for the sustainability of forest management (Vizcaino-Palomar et al. 2019). This issue requires understanding the pattern and scale of local adaptation (Manzanedo et al. 2018), because genetic variation among populations of forest trees at traits highly fluctuates depending on the climate and environmental adaptation (Barbati et al. 2018). Common garden experiments are used to study genetic differentiation at phenotypic traits to detect patterns of local adaptation (Li et al. 2018, Vizcaino-Palomar et al. 2019).

For example, common garden tests play an important role in investigating adaptive responses to climate variation (Sáenz-Romero et al. 2019). Common gardens generally involve the cultivation of tree populations from different geographic regions with different environmental conditions in several common garden experiments established in different environments (Brancaion et al. 2015). In each common garden

experiment, saplings are planted using a randomized design (e.g., Randomized Complete Block Design) to minimize local environmental differences and separate between genetic and environmental effects on trait expression (Sewenet 2019). Common gardens were set up to identify the best populations for plantation programs under different environmental conditions (Sáenz-Romero et al. 2019).

Growth traits are strongly affected by the interaction between genotype and environmental resources such as water, soil fertility, etc. (Li et al. 2018). Therefore, it is advisable to conduct the measurements of growth traits in combination with the assessment of other traits in order to decrease the risks associated with sapling transfer (Isaac-Renton et al. 2018). Chlorophylls, carotenoids and proline play crucial roles for example in preventing various diseases associated with oxidative stress (Shah et al. 2017) and are good proxies for the general health of forests (Pakharkova et al. 2020). Macro- and trace elements such as sodium, magnesium, aluminium,

phosphorus, potassium, calcium, iron, copper and zinc are critical components of different biochemical processes in plants. For this reason, concentrations of these elements in leaves should be sustained within a certain range since their deficiency prevents growth and their excess can cause a toxic effect (Shtangeeva et al. 2017).

Junipers are keystone species in ecosystems of arid regions worldwide because they grow in many harsh environments and are often used for ecological restoration across arid and continental regions. In this regard, *J. excelsa* is one of the most common tree species and has a wide geographical and altitudinal distribution in Turkey (Yücedağ & Gailing 2013a). As it is resistant to drought and frost damages and can cope with poor soils (Yücedağ & Gailing 2013a), it grows at the boundaries of steppe in the interior parts of the mountains (Douaihy et al. 2011, Yücedağ & Gailing 2013a).

Up to now, there are studies conducted on genetic variation within and among populations of *J. excelsa* based on phenotypic traits measured in the nursery (Yücedağ & Gailing 2013b) and natural populations (Douaihy et al. 2012), isozymes (Hojjati et al. 2009), chloroplast DNA (Gulsoy et al. 2012) and nuclear microsatellite markers (Douaihy et al. 2011, Yücedağ & Gailing 2013a). However, there have been no previous studies on local adaptation to different micro-environments at a small geographic in a mountainous region. In the present study, we assessed the variability in growth and contents of photosynthetic pigments, proline and nutrients of neighbouring *J. excelsa* populations from distinct environments grown at a common garden test site. Specifically it will be tested: (1) whether there are substantial differences

in survival rates across eight *J. excelsa* populations at the test site; (2) whether there are significant differences in growth and contents of photosynthetic pigments, proline and nutrient among the eight populations at the test site; (3) whether there are correlations between the pairs of traits and of environmental variables at the regions of populations' origin with both growth and contents of photosynthetic pigments, proline and nutrient at the test site; (4) whether there is a pattern of local adaptation by comparing phenotypic trait differentiation ( $Q_{ST}$ ) in the current study and  $F_{ST}$  at neutral SSRs in an earlier study (Yücedağ & Gailing 2013a) among six of the populations at the same test site, as higher phenotypic differentiation than differentiation at neutral genetic markers could indicate divergent selection on the traits.

## Materials and methods

### Planting material and experimental site

One-year-old containerized saplings of eight *Juniperus excelsa* populations from the Lakes District of Turkey were planted at a common garden test site in the northwestern part of Davraz Mountain (37° 46' N, 30° 41' E; 1500 m a.s.l.) located about 25 km away from Isparta, Turkey, in early March 2009 (Fig. 1, Tab. 1). Parent trees were between 130 and 140 years old based on tree ring countings using a Pressler increment borer.

The test site was established on a northwestern slope. According to the results of the soil analysis conducted in the Soil Science and Ecology Institute, Çankiri Karatekin University, the soil at test site was sandy, stony and shallow, and was composed by low organic matter component, calcareous (1.8%), no salt (0.11%), too much

nitrogen (0.44%), much potassium (411 mg kg<sup>-1</sup>), much iron (18 mg kg<sup>-1</sup>), adequate phosphorus (9 mg kg<sup>-1</sup>), adequate magnesium (0.02%), adequate manganese (22.7 mg kg<sup>-1</sup>), adequate zinc (0.8 mg kg<sup>-1</sup>), adequate copper (1.6 mg kg<sup>-1</sup>) contents, and with slightly alkaline pH (7.5). The identification of adequacy in soil properties was made based on Sillanpää (1991). A soil cultivation with ripper was done at the test site. The annual average precipitation was 581 mm and the annual average temperature was 12 °C (Anonymous 2013).

### Experimental design

The layout of the experiment was a randomized complete block design with three replications. Sufficient homogeneity was achieved within the blocks extending from uphill to downhill, and environmental differences among populations were minimized. Spacing between saplings was 2 × 3 m. Each population was represented by 30 saplings (10 saplings per block) grown from seeds in Egirdir Forest Nursery (Yücedağ & Gailing 2013b). The total number of seedlings planted was 240.

### Sampling strategy

Height, diameter and volume were determined for all the 30×8 saplings. For the plant nutrient, photosynthetic pigment and proline analyses, five saplings from each of the eight populations (at least one sapling from each block) were randomly sampled (a subset of 5×8 saplings). About 2 g leaf samples from each of the saplings were collected in August of 2018 to use as fresh material. They were immediately wrapped in aluminium foil and put on dry ice at the test site. Then, these samples were taken to the Department of Landscape Architecture, Çankiri Karatekin University without breaking the cold chain. Fresh leaf samples were kept at -20 °C until the day of analysis. Also, about 100 g leaf samples from each of the saplings were collected in late September of 2018 to use as dry material. After collecting, these samples were immediately dried at 70 °C in an oven for five days in the laboratory of Burdur Mehmet Akif Ersoy University and then dried samples were taken to the Department of Landscape Architecture, Çankiri Karatekin University.

### Plant analyses

The dried leaf samples were digested using the dry-ashing method in a muffle furnace at 500 °C for 6 hours, later dissolved in 10 N nitric acid (HNO<sub>3</sub>), and finally extracts were filtered and stored in plastic sample jars until the day of analysis of mineral nutrients (Miller 2004). The concentrations of P, K, Ca, Mg, Na, Cu, Mn, Fe and Zn were determined by ICP-OES (Optima® 2100 DV, Perkin-Elmer, Waltham, MA, USA). Nitrogen concentration was determined through the method developed by Kjeldahl (1883).

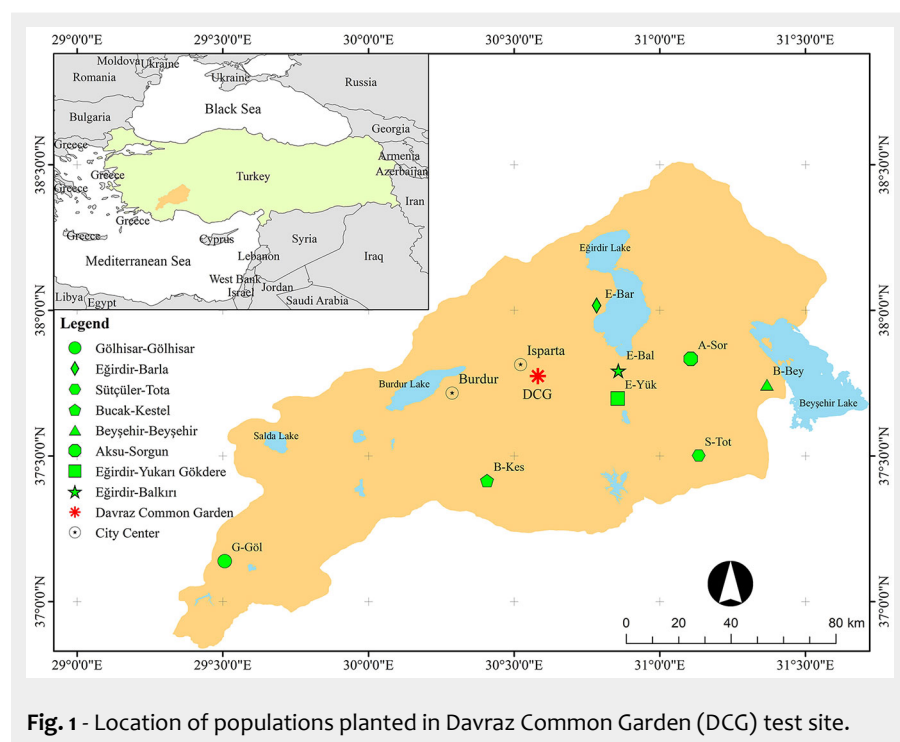


Fig. 1 - Location of populations planted in Davraz Common Garden (DCG) test site.

**Photosynthetic pigment analysis**

The fresh leaf samples (250 mg) were cut into small pieces and homogenized in 10 mL of acetone (90%, v/v). The extract was then filtered and the absorbance of the extract was measured at 645, 652, 663 and 470 nm using a spectrophotometer (UV/VIS-1201, Shimadzu Corp., Kyoto, Japan). The concentrations of chlorophylls (chl) and carotenoid (car) were calculated based on Lichtenthaler (1987).

**Proline analysis**

Free proline was extracted with 3% sulphosalicylic acid from fresh leaves (0.5 g) and determined according to the method of Bates et al. (1973). Extracts (2 mL) were maintained for 1 h in boiling water with the addition of 2 mL ninhydrin and 2 mL glacial acetic acid. Cold toluene (4 mL) was added afterwards and shaken with a vortex. Finally, absorbance was read at 520 nm. The proline concentration was determined from a standard curve using L-Proline and expressed in mmol kg<sup>-1</sup> FW.

**Growth analysis**

For the growth analysis, height (cm) and diameter (mm) of 30 saplings from each of the populations were measured in late September of 2018 using a tape measurer and a digital calliper, respectively. Furthermore, volumes of saplings were calculated using the equation developed for *J. excelsa* by Eler (1988) as follows (eqn. 1):

$$V = 0.035623846 \cdot d^{2.27029466} \cdot h^{0.595058217} \quad (1)$$

where V is the volume (cm<sup>3</sup>), d is the diameter and h the height of saplings.

**Environmental variables**

Soil texture for the region of origin of each of the eight populations was determined by using the feel method (FAO 2020). Accordingly, sandy, sandy loam and sand clay contained <10% (=1), 10-30% (=2) and 35-55% clay (=3), respectively.

Data of total annual precipitation (mm), annual mean temperature (°C), annual mean maximum and minimum temperatures (°C) in the eight meteorological stations (ms), that were the closest to the studied populations and common garden test site, were provided from Turkish State Meteorological Service. Then, total annual precipitation of populations (p) were interpolated using the formula of Schreiber (1904) as follows (eqn. 2):

$$P_p = P_{ms} + (h_p - h_{ms}) \cdot \frac{54}{100} \quad (2)$$

where  $P_p$  and  $P_{ms}$  are the total annual precipitations of populations and meteorological stations (mm),  $h_p$  and  $h_{ms}$  are altitudes of populations and meteorological stations (m), 54/100 is a constant showing 54 mm of precipitation increases at every 100 meters. Annual mean temperature, annual mean maximum and minimum temperatures of populations were interpreted using the lapse rate formula as follows (eqn. 3):

$$T_p = T_{ms} \pm (h_p - h_{ms}) \cdot 0.005 \quad (3)$$

where  $T_p$  and  $T_{ms}$  are the temperatures of populations and meteorological stations (°C), 0.005 is a constant (Kilinc et al. 2006).

Precipitation effectiveness index was calculated by taking the ratio of total annual precipitation to the annual mean maximum temperature, and then this index was used

to determine the climate type for each population (Erinç 1965).

Topographic wetness index was calculated as follows (eqn. 4):

$$TWI = \ln \left( \frac{\alpha_s}{\tan \beta} \right) \quad (4)$$

where  $\alpha_s$  is the specific catchment area (m<sup>2</sup>),  $\tan \beta$  is the local slope (Qin et al. 2011). Calculations were made using Arc-Map® v. 10.4.1 (ESRI, Redlands, CA, USA). Artificial digital elevation models with a series of resolutions finer than 25 m were used to evaluate TWI quantitatively.

**Statistical analysis**

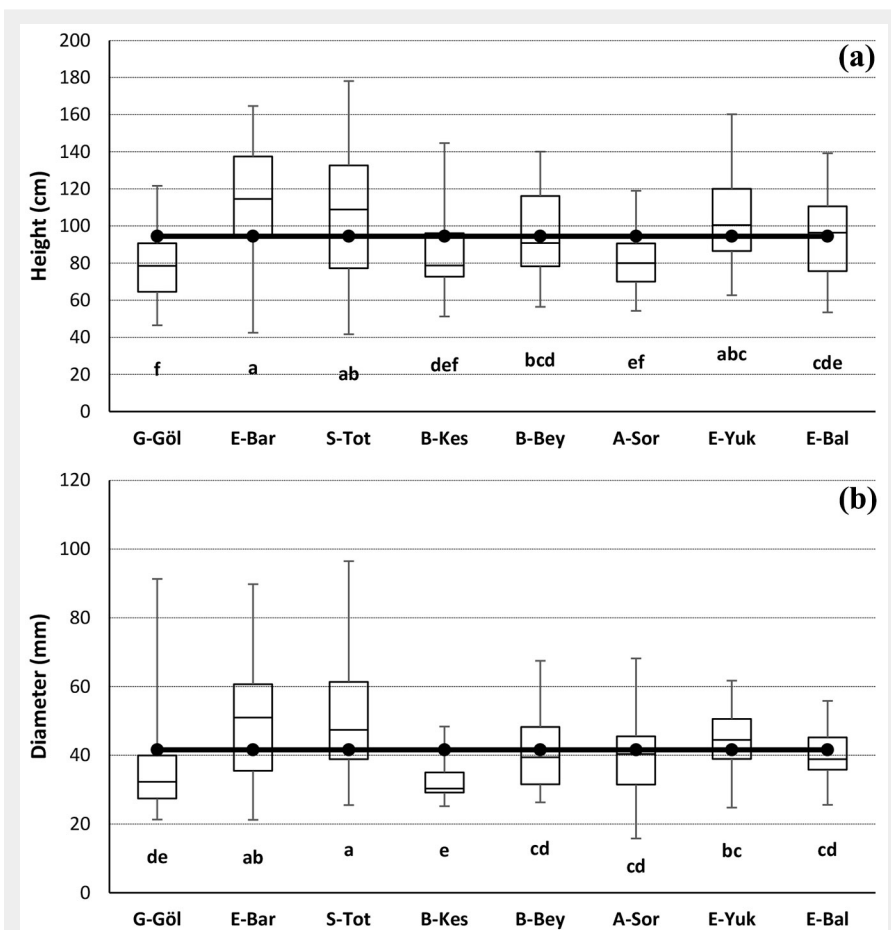
Normal distribution of the data in height, diameter and volume was tested through a Kolmogorov-Smirnov test. Parametric tests were performed as residuals of the model were homoscedastic. Mean, standard deviation, F ratio and probability (P-value) of all the traits were calculated and compared among populations through analysis of variance (ANOVA) and adjusted Duncan's multiple range tests ( $p < 0.05$ ). The means of height and diameter were compared between soil textures using a t-test. Quantitative trait differentiation (Spitze 1993) among populations was estimated for all traits as follows (eqn. 5):

$$Q_{ST} = \frac{\sigma_{G(\text{among})}^2}{\sigma_{G(\text{among})}^2 + 2\sigma_{G(\text{within})}^2} \quad (5)$$

where  $\sigma_{G(\text{among})}^2$  is the variance among populations and  $\sigma_{G(\text{within})}^2$  the variance within populations.  $Q_{ST}$  were calculated not only for the six populations for which  $F_{ST}$  values were available, but also for all eight populations.  $Q_{ST}$  describes the genetic differentiation at traits among provenances in the

**Tab. 1** - Geographic positions, environmental and climatic features of Davraz common garden and the eight *Juniperus excelsa* populations used in the present study (modified from Yücedag & Gailing 2013a). (TAP): total annual precipitation; (MAT): mean annual temperature; (MAT<sub>max</sub>): mean annual maximum temperature; (MAT<sub>min</sub>): mean annual minimum temperature; (PEI): precipitation effectiveness index; (TWI): topographic wetness index.

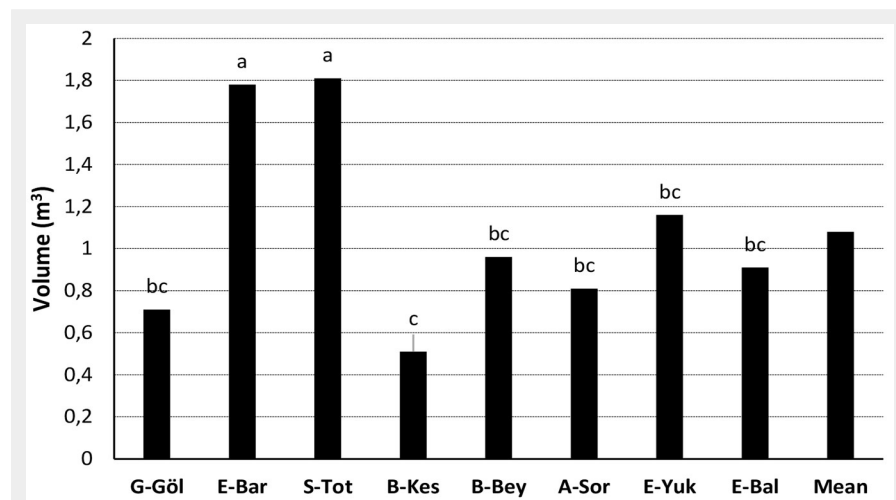
Population (test site)	Altitude (m a.s.l.)	Aspect	Coords	Soil Texture	TAP (mm)	MAT (°C)	MAT <sub>max</sub> (°C)	MAT <sub>min</sub> (°C)	PEI	Climate	TWI
Göhlhisar-Göhlhisar (G-Göl)	1413	SE	36° 57.115' N 29° 27.236' E	Sandy loam	510.7	11.2	24.2	-0.1	21.1	Semi-arid	6.6
Egirdir-Barla (E-Bar)	1027	SE	37° 58.162' N 30° 46.289' E	Sandy clay	542.2	13.8	23.8	4.5	22.8	Semi-arid	5.7
Sütçüler-Tota (S-Tot)	1546	NE	37° 34.678' N 31° 04.306' E	Sandy clay	1407.2	11.2	23.3	1.2	60.4	Much humid	7.7
Bucak-Kestel (B-Kes)	1197	SE	37° 24.863' N 30° 21.036' E	Sandy loam	745.8	13.2	25.7	1.9	29.0	Semi-humid	13.4
Beysehir-Beysehir (B-Bey)	1151	NE	37° 39.043' N 31° 42.058' E	Sandy clay	483.4	11.1	23.3	-1.3	20.8	Semi-arid	7.1
Aksu-Sorgun (A-Sor)	1416	NE	37° 50.488' N 31° 05.782' E	Sandy clay	964.5	11.0	23.2	-2.0	41.6	Humid	6.4
Egirdir-Yukari Gökdere (E-Yuk)	1150	SE	37° 40.558' N 30° 50.570' E	Sandy clay	918.9	11.8	22.3	1.4	41.2	Humid	8.5
Egirdir-Balkiri (E-Bal)	1493	E	37° 47.101' N 30° 48.981' E	Sandy loam	1104.1	10.0	20.5	-0.4	53.9	Humid	9.8
Davraz Common Garden (DCG)	1500	NW	37° 46.103' N 30° 41.285' E	Sandy	843.8	9.8	20.0	-0.6	42.2	Humid	8.6



**Fig. 2** - Boxplot diagrams of height (a) and diameter (b) of 10-years-old saplings from eight *J. excelsa* populations grown at the common garden test site ( $F_{\text{height}} = 8.429$ ,  $p < 0.001$ ;  $F_{\text{diameter}} = 8.357$ ,  $p < 0.001$ ). In both panels, different letters indicate significant differences ( $p < 0.05$ ) between the mean of the populations. Blue lines show the grand means of height (94.47 cm) and diameter (41.6 mm).

common garden test in which the environmental variance is minimized. Since we had no pedigree information we estimated broad-sense  $Q_{ST}$  which can include components of non-additive genetic components

(dominance, epistasis, maternal effects). However, genetic variance for complex traits has been reported to be additive in most cases (Hill et al. 2008). In addition, the correlations of both nutrient contents



**Fig. 3** - Mean volumes of 10-years-old saplings from eight *J. excelsa* populations grown at the common garden test site ( $F_{\text{volume}} = 8.229$ ,  $p < 0.001$ ). Different letters indicate significant differences ( $p < 0.05$ ) between the mean of the populations.

and environmental variables (total annual precipitation, annual mean temperature, annual mean maximum and minimum temperatures, precipitation effectiveness index, topographic wetness index and soil texture) at the populations' regions of origin with growth, photosynthetic pigments and proline were performed using Pearson's correlation coefficients. Differences among populations were visualized using a dendrogram from hierarchical cluster analyses based on growth, photosynthetic pigments, proline and nutrient contents. All statistical analyses were conducted using SPSS® v. 25 (IBM, Armonk, NY, USA).

## Results

We found very few saplings with damaged crown, but the survival rate of all populations was 100%.

The distribution of height and diameter for 10-year-old saplings of eight *J. excelsa* populations are presented in Fig. 2. The ANOVA showed significant differences among populations for height and diameter ( $p < 0.001$ ). E-Bar and G-Göl populations showed the highest (115.06 cm) and lowest (78.08 cm) performances in height, while S-Tot and B-Kes populations showed the best (50.8 mm) and worst (32.5 mm) performances in diameter. The mean height and diameter were 94.47 cm and 41.6 mm, respectively (Fig. 2). The differences (%) of the populations with the highest height and diameter from the grand mean of the populations in the current study were 22%. The differences between the populations with the highest (E-Bar and S-Tot) and lowest (G-Göl and B-Kes) heights and diameters were 47% and 56%, respectively. The highest mean volumes were 52.07 cm<sup>3</sup> and 51.13 cm<sup>3</sup> in S-Tot and E-Bar populations, respectively. Besides, B-Kes population had the lowest mean volume (14.82 cm<sup>3</sup> - Fig. 3). Overall, sapling materials from E-Bar, S-Tot and E-Yuk were above the general averages of height, diameter and volume compared to the other populations (Fig. 2, Fig. 3). The regions of these populations also had relatively high annual mean minimum temperatures as compared to the other populations, but the same soil texture (Tab. 1).

ANOVA revealed significant differences among populations for all photosynthetic pigments and proline ( $p < 0.05$ ). G-Göl population had the highest total chl (0.727 mg g<sup>-1</sup> FW), chl *a* (0.405 mg g<sup>-1</sup> FW) and chl *a+b* (0.567 mg g<sup>-1</sup> FW), while E-Bar population had the highest chl *b* (0.167 mg g<sup>-1</sup> FW) and car (0.192 mg g<sup>-1</sup> FW). S-Tot population had the highest proline concentration (0.523 mmol kg<sup>-1</sup> FW). The lowest total chl (0.394 mg g<sup>-1</sup> FW), chl *a* (0.182 mg g<sup>-1</sup> FW) and chl *a+b* (0.318 mg g<sup>-1</sup> FW) were found in E-Bal population. The lowest chl *b* (0.111 mg g<sup>-1</sup> FW) was determined in E-Yuk population. The lowest car (0.117 mg g<sup>-1</sup> FW) and proline (0.358 mmol kg<sup>-1</sup> FW) were found in B-Kes population. E-Bal population had significantly lower chl *a* than the other popu-

**Tab. 2** - Mean  $\pm$  standard deviation, range, F ratio and significance (p) for photosynthetic pigments and proline of *J. excelsa* 10-year-old saplings. (Total chl): total chlorophyll; (chl a, b and a+b): Chlorophyll a, b and a+b; (car): carotenoid. Different letters in the columns indicate significant differences ( $p < 0.05$ ) between population means.

Populations (Label)	Total chl (mg g <sup>-1</sup> FW)	chl a (mg g <sup>-1</sup> FW)	chl b (mg g <sup>-1</sup> FW)	chl a+b (mg g <sup>-1</sup> FW)	car (mg g <sup>-1</sup> FW)	Proline (mmol/kg FW)
Göhlhisar-Göhlhisar (G-Göl)	0.727 $\pm$ 0.12 <sup>a</sup>	0.405 $\pm$ 0.03 <sup>a</sup>	0.162 $\pm$ 0.03 <sup>ab</sup>	0.567 $\pm$ 0.05 <sup>a</sup>	0.184 $\pm$ 0.02 <sup>ab</sup>	0.475 $\pm$ 0.04 <sup>bc</sup>
Egirdir-Barla (E-Bar)	0.657 $\pm$ 0.07 <sup>ab</sup>	0.363 $\pm$ 0.04 <sup>ab</sup>	0.167 $\pm$ 0.02 <sup>a</sup>	0.529 $\pm$ 0.06 <sup>ab</sup>	0.192 $\pm$ 0.02 <sup>a</sup>	0.514 $\pm$ 0.03 <sup>ab</sup>
Sütçüler-Tota (S-Tot)	0.610 $\pm$ 0.08 <sup>bc</sup>	0.350 $\pm$ 0.04 <sup>b</sup>	0.150 $\pm$ 0.01 <sup>abc</sup>	0.500 $\pm$ 0.06 <sup>ab</sup>	0.136 $\pm$ 0.07 <sup>bc</sup>	0.523 $\pm$ 0.01 <sup>a</sup>
Bucak-Kestel (B-Kes)	0.568 $\pm$ 0.13 <sup>bc</sup>	0.328 $\pm$ 0.07 <sup>b</sup>	0.134 $\pm$ 0.02 <sup>bcd</sup>	0.462 $\pm$ 0.10 <sup>bc</sup>	0.117 $\pm$ 0.06 <sup>c</sup>	0.358 $\pm$ 0.01 <sup>d</sup>
Beyşehir-Beyşehir (B-Bey)	0.500 $\pm$ 0.06 <sup>cd</sup>	0.266 $\pm$ 0.04 <sup>c</sup>	0.130 $\pm$ 0.01 <sup>cd</sup>	0.396 $\pm$ 0.05 <sup>cd</sup>	0.142 $\pm$ 0.02 <sup>bc</sup>	0.517 $\pm$ 0.03 <sup>ab</sup>
Aksu-Sorgun (A-Sor)	0.449 $\pm$ 0.02 <sup>d</sup>	0.243 $\pm$ 0.01 <sup>c</sup>	0.117 $\pm$ 0.01 <sup>d</sup>	0.360 $\pm$ 0.02 <sup>de</sup>	0.140 $\pm$ 0.01 <sup>bc</sup>	0.439 $\pm$ 0.07 <sup>c</sup>
Egirdir-Yukari Gökdere (E-Yuk)	0.417 $\pm$ 0.09 <sup>d</sup>	0.239 $\pm$ 0.03 <sup>c</sup>	0.111 $\pm$ 0.01 <sup>d</sup>	0.349 $\pm$ 0.03 <sup>de</sup>	0.139 $\pm$ 0.02 <sup>bc</sup>	0.365 $\pm$ 0.01 <sup>d</sup>
Egirdir-Balkiri (E-Bal)	0.394 $\pm$ 0.03 <sup>d</sup>	0.182 $\pm$ 0.02 <sup>d</sup>	0.136 $\pm$ 0.04 <sup>bcd</sup>	0.318 $\pm$ 0.04 <sup>e</sup>	0.122 $\pm$ 0.02 <sup>c</sup>	0.377 $\pm$ 0.01 <sup>d</sup>
Mean	0.540 $\pm$ 0.14	0.297 $\pm$ 0.08	0.138 $\pm$ 0.03	0.435 $\pm$ 0.10	0.147 $\pm$ 0.04	0.446 $\pm$ 0.07
Range	0.280 - 0.860	0.160 - 0.440	0.090 - 0.200	0.290 - 0.630	0.020 - 0.210	0.340 - 0.560
F	10.883	19.277	4.375	14.295	2.820	22.010
p	<0.001	<0.001	0.002	<0.001	0.021	<0.001

lations. B-Kes, E-Yuk and E-Bal populations were significantly different from other populations in terms of proline (Tab. 2).

According to the ANOVA, there were significant differences between populations for nutrient contents ( $p < 0.05$ ). The highest means of P (0.26%), K (73%), Mg (20%), Cu (9.15 mg kg<sup>-1</sup>) and Zn (21.29 mg kg<sup>-1</sup>) were found in S-Tot population. G-Göl population showed the highest means of N (1.35%) and Ca (1.62%). The highest means of Mn (42.12 mg kg<sup>-1</sup>) and Fe (76.75 mg kg<sup>-1</sup>) were determined in E-Bar and E-Bal populations, respectively. The lowest means of P (0.18%), K (62%) and Zn (13.46 mg kg<sup>-1</sup>) were found in G-Göl population. E-Yuk population revealed the lowest means of N (1.19%), Mg (0.12%) and Fe (51.19 mg kg<sup>-1</sup>). E-Bar population had the lowest means of Ca (1.34%) and Cu (6.14%). The lowest mean of Mn (28.85 mg kg<sup>-1</sup>) was found in B-Kes population. E-Yuk population had significantly lower Mg than the other populations. E-Bar

and B-Bey populations had significantly higher and lower Mn than the other populations (Tab. 3).

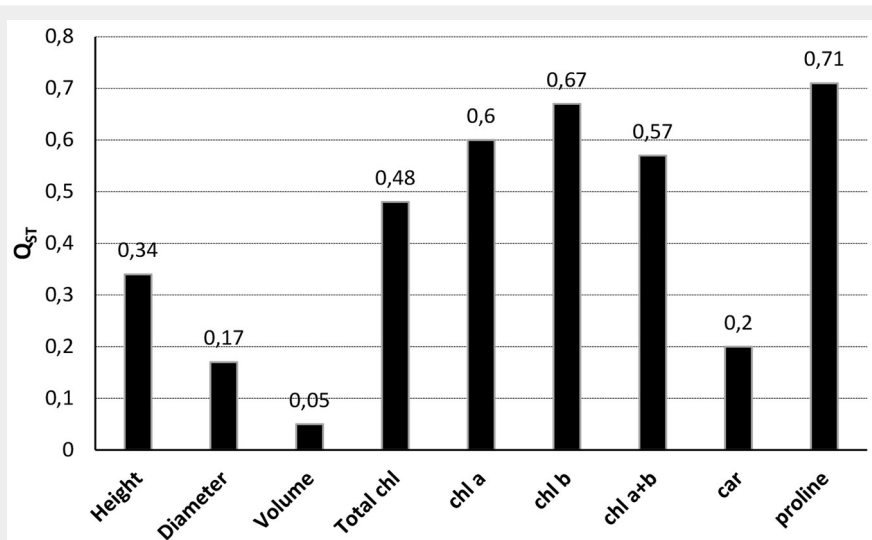
Proline ( $Q_{ST} = 0.71$ ), chl b ( $Q_{ST} = 0.67$ ), chl a ( $Q_{ST} = 0.60$ ) and chl a+b ( $Q_{ST} = 0.57$ ) had the highest  $Q_{ST}$  values among *J. excelsa* populations, while volume ( $Q_{ST} = 0.05$ ) and diameter ( $Q_{ST} = 0.17$ ) had the lowest  $Q_{ST}$  values (Fig. 4).

There were positive correlations between height and Zn, and between diameter and Fe. On the other hand, there were positive correlations of N with both total chl and chl b and of Mg with total chl, chl a and b, car and proline. There was no correlation between phenotypic and photosynthetic traits. While height was strongly correlated with diameter ( $R = 0.88$ ) and volume ( $R = 0.90$ ), diameter had a strong correlation with volume ( $R = 0.98$ ,  $p < 0.05$  – Tab. S1 in Supplementary material). However, there was no association between growth and distances of populations from the test site.

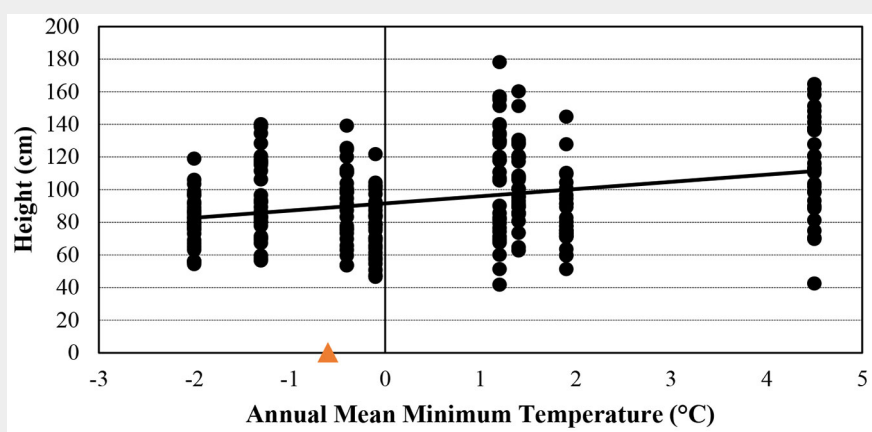
According to t-test results, saplings from populations with sandy clay had higher height and diameter than those from populations with sandy loam ( $t_{\text{height}} = 4.549$ ,  $p < 0.001$ ;  $t_{\text{diameter}} = 5.453$ ;  $p < 0.001$ ). Annual mean maximum temperature was positively correlated with total chl ( $R = 0.67$ ), chl a ( $R = 0.74$ ) and chl a+b ( $R = 0.67$ ,  $p < 0.05$  – Fig. S1 in Supplementary material) and annual mean minimum temperature was positively correlated with height ( $R = 0.70$ ,  $p < 0.05$  – Fig. 5). Finally, topographic wetness index was negatively correlated with car ( $R = -0.73$ ) and proline ( $R = -0.74$  – Fig. S2). *J. excelsa* saplings originating from regions with sandy clay and higher annual mean minimum temperature grew faster than those from regions with sandy loam and lower annual mean temperature. Populations with similar environmental conditions (low annual mean minimum temperature and sandy loam) as at the test site showed a comparatively low height and di-

**Tab. 3** - Mean  $\pm$  standard deviation, range, F ratio and significance (p) for the nutrient contents of *J. excelsa* 10-year-old saplings. (N): Nitrogen; (P): Phosphorous; (K): Potassium; (Ca): Calcium; (Mg): Magnesium; (Cu): Copper; (Mn): Manganese; (Fe): Iron; (Zn): Zinc. Different letters in the columns indicate significant differences ( $p < 0.05$ ) between the population means.

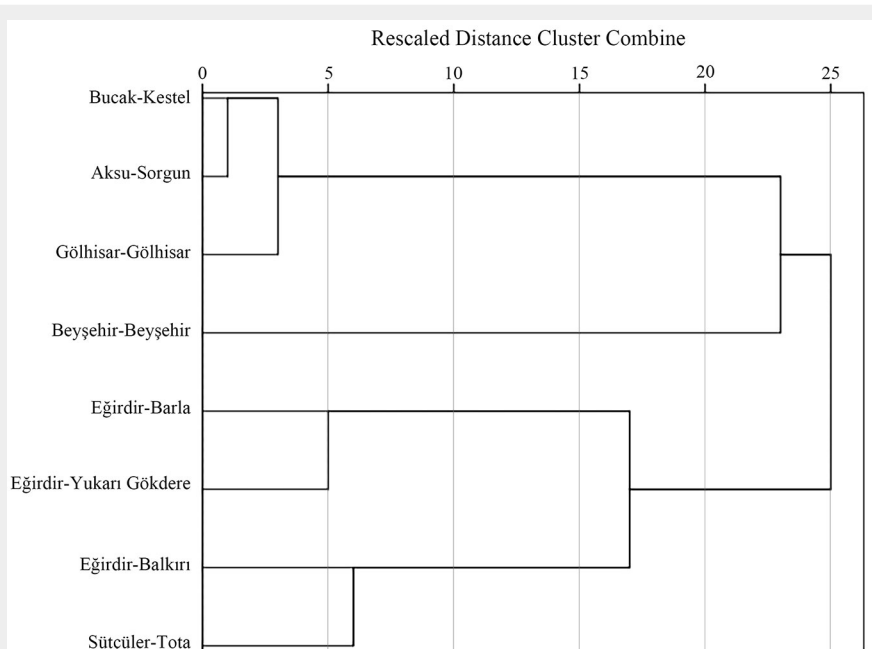
Pop	N (%)	P (%)	K (%)	Ca (%)	M (%)	Cu (mg kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
G-Göl	1.35 $\pm$ 0.14 <sup>a</sup>	0.18 $\pm$ 0.02 <sup>d</sup>	0.62 $\pm$ 0.07 <sup>b</sup>	1.62 $\pm$ 0.14 <sup>ab</sup>	0.17 $\pm$ 0.01 <sup>c</sup>	6.52 $\pm$ 0.76 <sup>bc</sup>	37.15 $\pm$ 1.68 <sup>b</sup>	54.79 $\pm$ 6.13 <sup>c</sup>	13.46 $\pm$ 0.89 <sup>f</sup>
E-Bar	1.33 $\pm$ 0.06 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>ab</sup>	0.69 $\pm$ 0.03 <sup>ab</sup>	1.34 $\pm$ 0.09 <sup>cd</sup>	0.18 $\pm$ 0.04 <sup>abc</sup>	6.14 $\pm$ 0.60 <sup>c</sup>	42.12 $\pm$ 2.92 <sup>a</sup>	58.00 $\pm$ 9.15 <sup>bc</sup>	20.95 $\pm$ 0.60 <sup>a</sup>
S-Tot	1.26 $\pm$ 0.05 <sup>ab</sup>	0.26 $\pm$ 0.03 <sup>a</sup>	0.73 $\pm$ 0.04 <sup>a</sup>	1.54 $\pm$ 0.23 <sup>abcd</sup>	0.20 $\pm$ 0.03 <sup>ab</sup>	9.15 $\pm$ 1.22 <sup>a</sup>	32.80 $\pm$ 3.89 <sup>bc</sup>	72.28 $\pm$ 10.92 <sup>a</sup>	21.29 $\pm$ 1.28 <sup>a</sup>
B-Kes	1.26 $\pm$ 0.10 <sup>ab</sup>	0.24 $\pm$ 0.01 <sup>ab</sup>	0.69 $\pm$ 0.03 <sup>a</sup>	1.43 $\pm$ 0.10 <sup>bcd</sup>	0.17 $\pm$ 0.02 <sup>bc</sup>	7.00 $\pm$ 0.45 <sup>bc</sup>	28.85 $\pm$ 2.28 <sup>c</sup>	65.85 $\pm$ 9.17 <sup>ab</sup>	17.49 $\pm$ 1.46 <sup>cd</sup>
B-Bey	1.27 $\pm$ 0.09 <sup>ab</sup>	0.20 $\pm$ 0.02 <sup>cd</sup>	0.74 $\pm$ 0.04 <sup>a</sup>	1.33 $\pm$ 0.05 <sup>d</sup>	0.21 $\pm$ 0.02 <sup>a</sup>	7.66 $\pm$ 0.63 <sup>b</sup>	21.27 $\pm$ 1.05 <sup>d</sup>	51.05 $\pm$ 5.09 <sup>c</sup>	14.98 $\pm$ 1.05 <sup>ef</sup>
A-Sor	1.32 $\pm$ 0.09 <sup>a</sup>	0.22 $\pm$ 0.01 <sup>bc</sup>	0.73 $\pm$ 0.01 <sup>a</sup>	1.57 $\pm$ 0.23 <sup>abc</sup>	0.17 $\pm$ 0.01 <sup>bc</sup>	7.37 $\pm$ 0.86 <sup>bc</sup>	36.98 $\pm$ 6.72 <sup>b</sup>	66.21 $\pm$ 5.68 <sup>ab</sup>	18.73 $\pm$ 0.62 <sup>bc</sup>
E-Yuk	1.19 $\pm$ 0.05 <sup>b</sup>	0.21 $\pm$ 0.02 <sup>c</sup>	0.66 $\pm$ 0.08 <sup>ab</sup>	1.44 $\pm$ 0.11 <sup>bcd</sup>	0.12 $\pm$ 0.01 <sup>d</sup>	6.15 $\pm$ 1.06 <sup>c</sup>	35.45 $\pm$ 2.02 <sup>b</sup>	51.19 $\pm$ 2.16 <sup>c</sup>	16.31 $\pm$ 2.12 <sup>de</sup>
E-Bal	1.34 $\pm$ 0.08 <sup>a</sup>	0.20 $\pm$ 0.02 <sup>cd</sup>	0.67 $\pm$ 0.08 <sup>ab</sup>	1.67 $\pm$ 0.19 <sup>a</sup>	0.17 $\pm$ 0.01 <sup>bc</sup>	6.25 $\pm$ 1.15 <sup>c</sup>	30.90 $\pm$ 0.28 <sup>c</sup>	76.75 $\pm$ 10.65 <sup>a</sup>	19.71 $\pm$ 0.66 <sup>ab</sup>
Mean	1.29 $\pm$ 0.09	0.22 $\pm$ 0.03	0.69 $\pm$ 0.06	1.49 $\pm$ 0.19	0.18 $\pm$ 0.03	7.03 $\pm$ 1.26	33.19 $\pm$ 6.67	62.01 $\pm$ 11.66	17.86 $\pm$ 2.88
Range	1.12 - 1.57	0.15 - 0.28	0.51 - 0.77	1.24 - 1.97	0.11 - 0.25	4.43 - 11.10	20.11 - 45.14	42.52 - 94.67	12.12 - 23.32
F	1.924	11.505	3.007	3.198	8.299	6.848	19.563	7.532	28.020
p	0.098	<0.001	0.015	0.011	<0.001	<0.001	<0.001	<0.001	<0.001



**Fig. 4** - Phenotypic trait differentiation ( $Q_{st}$ ) values among *J. excelsa* populations (except Eğirdir-Barla and Eğirdir-Yukarıgökdere populations). (Total chl): total chlorophyll; (chl a, b and a+b): chlorophyll a, b and a+b; (car): carotenoid.



**Fig. 5** - Correlation between annual mean minimum temperature ( $^{\circ}\text{C}$ ) at populations' origin and height of *J. excelsa* 10-year-old saplings. The orange triangle indicates the mean annual minimum temperature at the common garden ( $-0.6^{\circ}\text{C}$ ).



**Fig. 6** - Dendrogram of hierarchical cluster analysis based on averages of all traits.

iameter growth (Fig. 5).

Total mean precipitation, annual mean temperature, precipitation effectiveness index and altitude had no correlation with growth, photosynthetic pigments and proline ( $p > 0.05$  – Tab. S2 in Supplementary material). No environmental variables were associated with leaf nutrient contents (Tab. S3).

A hierarchical cluster analysis based on averages of all traits was performed to quantify similarities among the populations. This analysis distinguished two main groups at the 25 rescaled distance units, comprising E-Yuk and E-Bar populations in the first group, and A-Sor, G-Göl, B-Kes, E-Bal and S-Tot populations in the second group. The southwestern populations G-Göl and B-Kes grouped together in the hierarchical cluster analysis confirming a weak phylogeographic pattern (Fig. 6). This hierarchical clustering corresponded with the results of Duncan tests relating to photosynthetic pigments, proline and nutrient contents (Tab. 2, Tab. 3).

## Discussion

### Survival and growth

Survival rate plays an important role in assessing the genetic variability among populations of different geographic origins when facing new environmental conditions (Apostol et al. 2020). In the present study, survival rate of all populations ten years after planting was determined as 100%. This value was higher than the findings of Ortel et al. (2020) stating that one-year-old containerized saplings of *J. excelsa* obtained from only one population (Eğirdir-Çamdağ) had a survival rate of more than 82% in the third year of a field experiment in Yalvaç, Isparta that was 150 km away from test site in the current study. These results confirmed that *J. excelsa* would be successful in the afforestation of dry and cold environments.

In the present study, significant variation in height and diameter was found among eight populations of *J. excelsa* growing in the Davraz Common Garden test site. E-Bar, S-Tot and E-Yuk populations were above the general averages of height, diameter and volume at age 10 compared to all the other populations. The test site had no similarity in altitude and environmental variables with the E-Bar population but showed a similarity in altitude and climate type with S-Tot population. The mean values of height and diameter of *J. excelsa* saplings at age 10 were 94.47 cm and 41.6 mm, respectively. Previous studies reported that the mean height of the species' saplings at age 8 in Burdur afforestation areas ranged from 80 to 151 cm (Cetin 2014), and the mean diameter at age 3 in Isparta-Yalvaç afforestation experiment was 8.86 mm (Ortel et al. 2020). The reason for the lower height and diameter in the present study may be related to environmental differences between sites (north exposure,

high altitude, shallow soil, steep slope at the test site).

E-Bal population (1493 m a.s.l.) can be considered as local population, as its origin was situated closer (about 10 km) to the test site (1500 m) as compared to the other populations, but surprisingly it showed lower growth compared to the mean of populations in terms of height and diameter at age 10. However, E-Bar population, located about 20 km from the test site, from lower altitude (1027 m a.s.l.) with higher annual mean temperature (13.8 °C), had the best performance at age 10 in terms of height, diameter and volume. On the contrary, the height and diameter of 10-year-old saplings from southwestern populations B-Kes and G-Göl (1197 and 1413 m a.s.l., respectively) were generally below the mean of populations at the test site. Boshier & Stewart (2005) emphasized that as the use of highly fertile nursery soil largely affects differences in growth among provenances as compared to traditional provenance trials, it is hard to conclude on the extent of local adaptation until several years after planting out in the field. In this sense, the results of the current study, 10 years after the establishment of the test site, may potentially already show some patterns of local adaptation.

Due to the complex topography, no pattern of isolation by distance was detected. For example, populations B-Bey and A-Sor, phenotypically similar to each other as reflected in the dendrogram, were geographically close to the other northern populations (S-Tot, E-Bal, E-Bar and E-Yuk), but differentiated at phenotypic traits. Indeed, these two populations are separated by a mountain range (Dedegöl mountain) from the other northern populations and also have lower annual mean minimum temperature. The dendrogram in the present study is similar to that of the earlier study based on allele frequency differences at nuclear microsatellites (Yücedag & Gailing 2013a). Similarly, Li et al. (2019) stressed that the barrier effects of mountains contributed to shape population differentiation of Chinese fir (*Cunninghamia konishii*) in Taiwan.

While there was no correlation of altitude of populations with diameter and height in the current study, both growth traits of 2 and 7-year-old saplings of *Picea abies* (L.) Karst. in a common garden at 150 m elevation in southern Poland decreased sharply for provenances from higher altitudes (Oleksyn et al. 1998). Similarly, Wilczynski & Kulej (2019) stated that the mean height of 4-year-old saplings of *Abies grandis* (D. Don) Lindl. from North America in a provenance trial in Krynica (Polish Carpathian Mts., 700 m a.s.l.) decreased for provenances from higher altitudes. Adaptation to a combination of environmental factors including temperature (see below) is likely related to the observed differences in growth.

The present study revealed that *J. excelsa*

saplings originating from regions with sandy clay and higher annual mean minimum temperature grew faster than those from regions with sandy loam and lower annual mean temperature than at the test site. As the temperature in the region of populations' origin was higher and thus the vegetation period was longer, saplings from these regions were likely adapted to higher temperatures and a longer growing period might explain their faster growth. Similarly, earlier studies reported that 13-year-old saplings of *Fraxinus americana*, *Betula alleghaniensis*, *Prunus serotina*, *Abies balsamea*, *Larix laricina*, *Picea glauca*, *Pinus banksiana* and *P. strobus* (Carter 1996) and 3-year-old saplings of *Pseudotsuga menziesii* (Leites et al. 2012) from regions with higher annual mean minimum temperature grew better than those from lower annual mean minimum temperature across test sites. Additionally, Chakraborty et al. (2015) reported that in 24-year-old trees of *P. menziesii* there was a weak positive correlation between annual mean minimum temperature in the regions of origin of populations and growth.

However, populations from regions with similar environmental conditions (e.g., annual mean minimum temperature and soil texture) as at the test site showed comparatively low height and diameter growth. Likewise, no pattern of isolation by adaptation was detectable by plotting growth differences against environmental differences between the populations' origins. Thus, it can be concluded from the present study that environmental similarity may not be a good indicator of growth-related traits and stress symptoms (proline and chlorophyll content).

#### Phytochemical analyses

In *J. excelsa*, photosynthetic pigments and proline of leaf varied significantly among populations for 10 year-old seedlings. The mean values of chl *a* (0.297 mg g<sup>-1</sup> FW) and *b* (0.138 mg g<sup>-1</sup> FW) in the present study were higher than those of chl *a* (0.231 mg g<sup>-1</sup> FW) and *b* (0.79 mg g<sup>-1</sup> FW) found by Keles & Everest (2008) for *J. excelsa* naturally growing at 1500 m a.s.l. between Mersin and Anamur in the Middle Taurus Mountains, southern Turkey. Considering the results of photosynthetic pigments and proline as a whole, it can be concluded that populations were not exposed to severe stress factors at the test site.

In the current study, all nutrient contents of leaves varied among the populations of *J. excelsa*. Gülser et al. (2013) also showed that there were significant differences in N, P, Ca, Fe, Mn, Zn, and Cu contents of their leaves (but not in K and Mg) among nine natural populations of *J. excelsa* in Gevas county in eastern Turkey. Turner et al. (1977) reported that Mn was significantly different among eight provenances of *Pseudotsuga menziesii* (Mirb.) Franco in a test site, but no difference for the other

nutrients (P, Al, Ca, Mg, K and Na). The nutrient concentration in the leaves of a species mostly relies on the inherent properties of the species and provenances rather than the quantity of nutrients in the soil in which they grow (Jayasankar et al. 2003). The concentrations of N, P, Ca, Mn and Zn in *J. excelsa* leaves obtained from the present study were in accordance with those reported by Gülser et al. (2013) in *J. excelsa*, but the concentrations of K, Mg, Cu and Fe were lower.

In the present study, Zn and Fe in leaves were positively correlated with height and diameter of saplings, respectively. Accordingly, E-Bar and S-Tot populations with the highest diameters also had the highest Zn concentration in their leaves because these nutrients are effective in the photosynthesis process (Zvezdanovic & Markovic 2009). Accordingly, height growth was strongly and positively correlated with Zn in the needles of *Pinus sylvestris* L. (Semerci et al. 2017). Although Rout & Sahoo (2015) indicated that Fe and chlorophyll concentrations are often well correlated in green plants, such a correlation was not found in the present study. N and Mg are associated with chlorophyll biosynthesis, and a deficiency of these elements is responsible for a significant decrease in chlorophyll content in the leaves of *Acacia nilotica* ssp. *indica* (Krishan & Toky 1995). Similarly, the present study showed a positive correlation of chlorophyll content with N and Mg.

Total chl, chl *a* and chl *a+b* as an indicator of plant health was positively associated with annual mean maximum temperature at the populations' origin. This result may result from the adaptations to drought stress of populations from warmer regions. However, Klein et al. (2012) in *Pinus halepensis* stated that leaf chlorophyll concentration at the test site had no correlation with climatic conditions at the regions of provenances. These contrasting results may be explained by cardinal temperatures for each plant that control its growth and development (Fowells & Means 1990). Proline content in the leaves of *J. excelsa* saplings from regions with high topographic wetness index was lower than for those with low topographic wetness index at the test site in the current study. Increased concentration of osmolytes such as proline prevents negative effects of dehydration as an adaptation to drought stress. Accordingly, plants for drier regions (low topographic wetness index) have a higher proline content as adaptation to water deprived environments (Ashraf et al. 2018). Furthermore, both photosynthetic pigments and nutrient contents had no correlation with altitude of populations in this study. In contrast, Keles & Everest (2008) revealed that the contents of chl *a* and *b* decreased in leaves of natural *J. excelsa* populations from high altitude. Decrease in the contents of chlorophyll may be associated with reductions in photosynthesis at

high altitudes as compared with lower altitudes.

#### Phenotypic differentiation vs. differentiation at neutral markers

Phenotypic differentiation ( $Q_{ST}$ ) among *J. excelsa* populations for growth, photosynthetic pigments and proline ranged from 5% (volume) to 71% (proline). Proline, chl *a*, chl *b* and chl *a+b* showed a very high  $Q_{ST}$  value, potentially indicative of local adaptation. However,  $Q_{ST}$  is possibly overestimated for photosynthetic pigments and proline since the number of samples per population is low due to the comparatively high costs for the phytochemical analyses. Also, the  $Q_{ST}$  values estimated in the present study were considerably higher for most traits than the mean genetic differentiation ( $F_{ST} = 0.03$ ) among *J. excelsa* populations observed at four randomly selected nuclear microsatellite loci (Yücedağ & Gailing 2013a) suggesting local adaptation across a small geographic scale. However,  $Q_{ST}$  values for height and diameter growth were comparatively low (8% and 11%, respectively). High variation within populations (Fig. 2) indicates a phenotypic plastic response and potentially high genetic variation for growth related traits within populations. Common garden experiments at different sites are needed to evaluate phenotypic plasticity in response to different environments.

#### Conclusions

The present study is the first to provide variability in growth, photosynthetic pigments, proline and nutrient contents of different *J. excelsa* populations at a common garden test site. Sapling traits of eight *J. excelsa* populations from the Lakes District in Turkey at the test site showed that the origin of populations significantly influenced growth, photosynthetic pigments, proline and nutrient contents. E-Bar and S-Tot populations showed the highest performance for the majority of traits at age 10 compared to all other populations. Thus, they are likely the most suitable provenances to these harsh environmental conditions (low annual mean temperature, shallow soil depth, water deprivation, north exposure). Nutrient contents in leaves were generally in the sufficiency range reported for plant growth.  $Q_{ST}$  values in the present study were considerably higher than the mean genetic differentiation among *J. excelsa* populations observed at four nuclear microsatellite loci, suggesting different local adaptations of populations at a small geographic scale. However, populations from regions with similar environmental conditions as at the test site did not show a faster growth. Thus, it can be concluded from the present study that environmental similarity may not be a good indicator of growth-related traits.

The results of the present study can be useful in the early selection of provenances

for *J. excelsa* for plantation efforts. Further studies should be done in order to assess traits related to drought tolerance such as water use efficiency, photosynthesis rate and stomatal conductance of saplings in the same test site.

#### List of abbreviations

The following abbreviations have been used throughout the paper:

- $Q_{ST}$ : Phenotypic trait differentiation
- $F_{ST}$ : Genetic differentiation
- Total chl: total chlorophyll
- chl *a*: chlorophyll *a*
- chl *b*: chlorophyll *b*
- chl *a+b*: Chlorophyll *a+b*
- car: carotenoid
- TWI: topographic wetness index
- N: Nitrogen
- P: Phosphorous
- K: Potassium
- Ca: Calcium
- Mg: Magnesium
- Cu: Copper
- Mn: Manganese
- Fe: Iron
- Zn: Zinc

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## Supplementary Material

**Fig. S1** - Correlation between annual mean maximum temperature (°C) at populations' origin and total chlorophyll (chl), chl a and chl a+b.

**Tab. S1** - Correlation coefficients between the pairs of all the traits.

**Tab. S2** - Correlation coefficients for the associations of the environmental variables at the region of populations with growth, photosynthetic pigments and proline.

**Tab. S3** - Correlation coefficients for the associations of environmental variables with leaf nutrient contents.

**Link:** [Yucedag\\_3769@suppl001.pdf](mailto:Yucedag_3769@suppl001.pdf)