



Population variation in drought resistance and its relationship with adaptive and physiological seedling traits in Turkish red pine (*Pinus brutia* Ten.)

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Abstract: Variation in drought resistance and its relationship with adaptive and physiological traits in forest trees are important in choosing suitable seed sources for reforestation and afforestation programs. A common garden experiment using 240 half-sib families originating from coastal and inland populations of Turkish red pine (*Pinus brutia*) in Turkey was set up with three replicates. The aims were to determine variation of drought damage, height growth, and phenology among populations and to investigate the relationship between drought damage and physiological traits (i.e. plant moisture stress and proline content). Three-year-old seedlings were subjected to drought treatment during the summer of 2000 and adaptive and physiological traits were measured. Except for bud burst, the majority of the variation resided between populations, leading to low heritability estimates for all traits. On average, inland populations were more resistant to drought and taller, with earlier bud burst and bud set times, than coastal populations. Proline content increased with higher drought damage, especially in cold-resistant and inland families. Inland populations are more drought-resistant than coastal populations. The results of the study demonstrate the possibility of selection for drought resistance for Turkish red pine at the population level.

Key words: *Pinus brutia*, Turkish red pine, drought resistance, phenology, proline

1. Introduction

The reports on potential impacts of climate change on agriculture and forestry in Europe (Maracchi et al., 2005) and Turkey (UIB, 2007; Kelleher et al., 2015) suggest that southern parts of the continent, especially in the Mediterranean coastal region, may be the most affected by changes in temperature and precipitation patterns. Furthermore, more frequent and intense drought events are expected to occur in parts of Turkey where Turkish red pine (*Pinus brutia* Ten.) is a key component of the Mediterranean forest ecosystem (Quezel, 1979; Kandemir et al., 2010). Other studies report that Turkish red pine is a highly drought-resistant tree species (Sevik and Cetin, 2015; Yigit et al., 2016). The natural distribution of Turkish red pine is confined to the eastern Mediterranean: the coastal regions of Anatolia, Lebanon, and Israel and the islands of Crete and Cyprus (Arbez, 1974; Kandemir et al., 2010). Turkish red pine is one of the most prevalent forest tree species in Turkey and accounts for 25.11% of the total forestland (22.3×10^6 ha) (OGM, 2015). The species is considered fast-growing and drought-tolerant with desirable wood characteristics. Thus, it has great potential

for industrial forestry not only in Turkey, but also in the Mediterranean basin and Australia (Dirik, 2000; Spencer, 2001). Turkish red pine is also widely used in reforestation and afforestation programs in Turkey. The species has been identified as a target species for intensive forestry and tree-breeding programs in Turkey (Koski and Antola, 1993; Kandemir, 2013).

Drought resistance is the capacity of a plant to withstand periods of insufficient soil water supply without damage (Turner, 1979; Larcher, 1995). Plants adopt either drought tolerance or drought avoidance strategies to overcome drought stress. Plants with drought avoidance acquire this characteristic through long-term developmental and morphological changes, while plants with drought tolerance achieve osmotic adjustment through immediate physiological and biochemical responses (McCue and Hanson, 1990; Blum, 2005). Drought resistance is determined mainly by hereditary properties that develop in the course of the evolution of a species (Penuelas et al., 2001) as well as in some plants. Deep root development may enhance this resistance (Newton et al., 1991). The level of resistance to soil drought can be evaluated through

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a variety of visual, physiological, and morphological parameters. Drought resistance can also be estimated chemically by determining the proline content (PC) of needles and measuring the osmotic potential in the cell sap (Sofu et al., 2004; Callister et al., 2006). Proline may be used in drought resistance assessment because of its role as a compatible osmolyte (Sofu et al., 2004), as a nitrogen-carbon source in the cell (Verbruggen et al., 1996), and in NADPH supply as the electron acceptor (Hare et al., 1999) during drought stress. In many woody plants, the relationships between osmotic adjustments and growth performances have been extensively studied. These studies suggest that proline accumulation occurs in drought-stressed plants (Newton et al., 1991; Sofu et al., 2004; Callister et al., 2006). Generally, plants accumulate high osmolytes during water stress in order to protect cell membranes from the adverse effects of this stress (Newton et al., 1991; De Diego et al., 2013).

To have a better assessment of drought resistance in perennial plants such as Turkish red pine, it is important that the quantity and pattern of genetic variation in adaptive traits such as drought stress responsive traits (e.g., growth and phenological traits) among populations be assessed. Furthermore, changes in physiological (osmotic pressure, water potential) and biochemical (PC) responses of populations should be evaluated to identify what kind of cellular-level changes occur during drought and how these traits are related to adaptive traits.

Given the importance of Turkish red pine in reforestation and afforestation programs as well as industrial plantations, information on its drought resistance mechanisms and its genetic control might be very useful, especially for the selection of appropriate seed sources. This type of information would be invaluable in future forestry activities in the Mediterranean basin with Turkish red pine, considering future climate change scenarios. Thus, in developing adaptive management strategies for Turkish red pine forests for the future, it is crucial to understand the degree and pattern in drought resistance of Turkish red pine seed sources. Furthermore, the ability to identify indicator traits that are easy to assess would be very useful in evaluating large numbers of genotypes for drought resistance.

The objectives of the study were to investigate the genetic variation for drought resistance in Turkish red pine and its relationship with growth and phenological traits and to identify changes in growth, physiological, and biochemical traits associated with drought resistance. To meet these objectives, 3-year-old seedlings of 240 half-sib families originating from six seed sources (three from coastal areas and three from inland areas) of Turkish red pine were evaluated at a nursery in Ankara.

2. Materials and methods

2.1. Plant material

Six populations of Turkish red pine were sampled from southwestern Turkey. Three of these populations were from inland regions while the other three were from coastal regions (Table 1). Open-pollinated seeds from half-sib families were collected from 40 randomly selected parent trees, which were at least 100 m apart from each other, for all six populations (240 families in total). Information about the sampled populations is given in Table 1. A detailed description of these populations and the sampling procedure of families has been provided elsewhere (Lise et al., 2007; Kandemir et al., 2010).

In the spring of 1998, seeds of the 240 families were sown as six-seedling row plots in three replications, where families were randomly allocated to plot locations in rows, on standard nursery beds (1.20 m in width and 15 m in length) at a nursery in Ankara, Turkey (elevation: 780 m; 39°57'N, 32°53'E; mean annual temperature: 11.7 °C; and mean annual precipitation: 378 mm). One row of buffer seedlings was also planted in tubes (15 cm × 30 cm) with peat, ground pine tree bark, granite soil, and perlite (4:3:2:1 ratio as volume) around the nursery beds. Spacing between seedlings within rows, as well as between rows, was 15 cm. The seedlings were irrigated twice a week from April to September in 1998 and 1999. There was no fertilizer application during the course of experiment.

2.2. Water stress induction

On 22 June 2000, water stress induction was initiated by covering experimental seedlings (in their third growing season at the time) with transparent plastic at 2 m height. After the last irrigation of the seedlings, predawn water potentials (PWP) of 20 randomly selected experimental seedlings were recorded between 0300 and 0400 hours on 7, 22, and 30 July as 3.88 ± 1.6 bar, 7.35 ± 2.56 bar, and 10.8 ± 6.04 bar (10 bar = 1 MPa), respectively. The effects of water stress were first visible on 31 July 2000, with the presence of yellowish needles in experimental seedlings when the water stress reached 10.8 bar. The average mean daily temperature in July 2000 and the maximum temperature on 30 July 2000 were 26.5 °C and 40.8 °C, respectively. This period, from 1 to 30 July, also coincided with the dates when water stress was applied. Drought conditions were the most severe in the month of July in 2000 in Ankara (Table 1).

Height growth and adaptive seedling traits such as phenology and cold resistance were evaluated in 1998, 1999, and 2000 and were published previously (Kandemir et al., 2010). The trait data on height growth in the year 2000 (the same as height growth after drought here), bud burst (BB), and bud set (BS) in the year 2000 from Kandemir et al. (2010) were included in the current study, since these traits are also important in evaluating the effect of drought

Table 1. Description of the six *Pinus brutia* populations (seed stands) included in this study.

Population*	Altitude (m)	Longitude (E)	Latitude (N)	Average over the last 30 years			
				Mean daily maximum temperature in June/July/August (°C)	Mean temperature in June/July/August (°C)	Annual average precipitation (mm)	Potential evapotranspiration in June/July/August (mm/m ²)
1- Alanya (C)	350	31°57'	36°36'	27.9/30.9/31.2	22.6/25.5/25.6	1103	157/178/182
2- Yaylaalan (C)	500	31°30'	36°57'	27.8/31.2/31.4	21.9/25.1/24.9	1050	159/185/169
3- Çalkaya (C)	50	30°50'	36°55'	30.2/33.7/33.8	24.6/27.5/24.2	1060	175/201/184
4- Fethiye (I)	800	29°28'	36°44'	28.1/31.9/32.1	21.2/24.4/24.3	993	154/182/166
5- Gölhisar (I)	1100	29°32'	37°40'	27.6/31.3/31.3	18.8/22.4/22.3	634	146/175/159
6- Çameli (I)	800	29°07'	37°06'	28.5/32.1/32.2	20.9/24.2/23.8	1222	162/189/173
Ankara nursery (study site)	780	32°53'	39°57'	25.5/29.1/29.2	18.8/22.0/21.9	378	148/186/165

*C: Coastal, I: inland.

stress on seedling growth in the same year (i.e. in 2000). Thus, the combined assessments of the growth, phenology, and drought resistance traits of 3-year-old seedlings in 2000 are reported here. The seedling height was measured from the cotyledon scar to the base of the terminal bud at the beginning (HTBD) and the end (HTAD) of the drought induction. BB was recorded in the spring of 2000 (12–18

April), as the presence or absence of new needles in the terminal bud. BS was recorded at the end of the growing season of 2000 (9–16 August), as the presence or absence of brown bud scales on the terminal bud. Drought damage (DRO) on each seedling was visually assessed on a scale from 0 (no damage) to 5 (all needles damaged) between 9 and 16 August 2000 (Table 2).

Table 2. Measured seedling characteristics and measurement methods.

Variables	Description	Units
HTBD and HTAD	Seedling height before (22–29 June) and after (22–28 August) drought stress in 2000	mm
HTBA	Seedling height increment during the drought treatment period (between 22 June and 28 August) (= HTAD – HTBD)	mm
BS	Bud set in 2000 (9–16 August)	0 = no BS 1 = BS
BB	Bud burst in 2000 (12–18 April)	0 = no BB 1 = BB
DRO	Visual assessment of drought damage (9–16 August) in 2000	0 = no damage, 1 = up to 10% of the needles damaged, 2 = up to 40% of the needles damaged, 3 = up to 70% of the needles damaged, 4 = up to 90% of needle damaged, 5 = all needles damaged
PMS*	Predawn plant moisture stress measured using pressure chamber (31 July–8 August) in 2000	bar
PC*	Proline content	µmol g ⁻¹

*Measured on seedlings from 70 families. See objective 2.

2.2.1. Objective 1: Investigating genetic variation in drought resistance in Turkish red pine and its relationship with growth and phenological traits

All traits were subjected to analysis of variance (ANOVA). The linear model for ANOVA for the entire data set was:

$$Z_{ijkl} = \mu + B_k + G_l + P(G)_{j(l)} + F(P)_{i(j)} + e_{ijkl}, \quad (1)$$

where μ is the experimental mean, Z_{ijkl} is the mean performance of the i th family in the j th population in the l th group in the k th replication, B_k is the random effect of the k th replication, G_l is the fixed effect of the l th group (inland versus coastal), $P(G)_{j(l)}$ is the fixed effect of the j th population in group l , $F(P)_{i(j)}$ is the random effect of the i th family within population j , and e_{ijkl} is the experimental error.

When inland and coastal populations were combined (Eq. (1)), due to confounding effects, the genetic and phenotypic correlations could not be properly estimated. It is expected that coastal and inland populations of Turkish red pine will have different responses to drought (Kandemir et al., 2010). This may result in different patterns of correlation in inland and coastal populations. Therefore, a second linear model below was adopted and ANOVA was conducted for inland and coastal populations separately:

$$Z_{ijk} = \mu + B_k + P_j + F(P)_{i(j)} + e_{ijk}, \quad (2)$$

where μ is the experimental mean, Z_{ijk} is the mean performance of the i th family in the j th population in the k th replication, B_k is the random effect of the k th replication, P_j is the fixed effect of the j th population, $F(P)_{i(j)}$ is the random effect of the i th family within population j , and e_{ijk} is the experimental error.

For both all of the data (Eq. (1)) and inland versus coastal groups (Eq. (2)), ANOVA was conducted using PROC GLM (generalized linear models) of the SAS statistical package to test the significance of family differences as well as other main effects (groups, population, and families in Eq. (1), and populations and families in Eq. (2)) in the models (SAS Institute, 2006). Main effects comparisons were made with SAS using Duncan's multiple range test in PROC GLM. Variance components were then estimated using the restricted maximum likelihood (REML) method of the VARCOMP procedure. When an imbalance exists in data due to missing seedlings, REML estimates of variance components are considered more reliable than ANOVA estimates (White, 1996). All tests of significance were conducted at 0.05 and 0.01 probability levels.

Family heritabilities (h_f^2) were estimated (Kaya et al., 1989; Temel and Adams, 2000) as follows:

$$h_f^2 = \frac{0.25 \frac{\sigma_a^2}{\sigma_p^2}}{P}, \quad (3)$$

where $\frac{\sigma_a^2}{\sigma_p^2}$ is the additive genetic variation and $\frac{\sigma_p^2}{\sigma_f^2}$ is the phenotypic variance of family means. Because open-pollinated families came from parent trees in wild stands, it was assumed that individuals within open-pollinated families are more closely related than half-sibs (Campbell, 1979); thus, additive genetic variance ($\frac{\sigma_a^2}{\sigma_f^2}$) was estimated as $3 \frac{\sigma_f^2}{\sigma_p^2}$. The standard errors of family heritabilities were estimated according to Dickerson (1969).

Phenotypic and genetic correlations were estimated to investigate the relationships among the traits. Phenotypic correlation ($r_{p(x,y)}$) between traits x and y was estimated as in Kaya and Temerit (1994):

$$r_{p(x,y)} = \frac{MCP_{f(x,y)}}{MS_{f(x)} MS_{f(y)}}, \quad (4)$$

where $MCP_{f(x,y)}$ is the mean cross product between families within populations for traits x and y , and MS_f is the mean square between families within populations for respective traits x and y .

Genetic correlation ($r_{g(x,y)}$) between traits x and y was estimated as in Falconer and Mackay (1996):

$$r_{g(x,y)} = \frac{Cov_{f(x,y)}}{\sqrt{\frac{\sigma_a^2(x)}{2} \frac{\sigma_a^2(y)}{2}}}, \quad (5)$$

where $Cov_{f(x,y)}$ is the family covariance between traits x and y , estimated as $Cov_f(x,y) = \frac{f(x+y) - \frac{f(x)}{2} - \frac{f(y)}{2}}{2}$, and $\frac{\sigma_a^2}{2}$ is the respective additive genetic variances for traits x and y .

2.2.2. Objective 2: Identifying the adaptive changes in morphological and physiological traits associated with drought resistance in Turkish red pine

After achieving the desired water stress of about 10 bar, predawn plant moisture stress (PMS) in the summer of 2000 and PC of seedlings belonging to 70 selected families were measured. In parallel to a previous study (Kandemir, 2002), a strong genetic correlation between cold and DRO ($r = 0.66$) was observed. Thus, selection of the 70 different families was based on their resistance to cold after the seedlings were naturally subjected to below-freezing temperatures in the winter of 1999. However, the cold damage to seedlings was mainly on needles and did not affect the survival of the seedlings in the studied populations. In some populations, such as the highly cold-sensitive coastal population Çalkaya, only a few families lacked cold damage. It was difficult to find sufficient numbers of families that were cold-resistant. Thus, only 19 cold-resistant and 51 cold-sensitive families with six seedling row plots in one of the replications were selected for PMS and PC measurements (Table 2).

2.3. Predawn water potential (PWP)

The PWP of the seedlings was measured using a pressure chamber (PMS Instruments Co., Corvallis, OR, USA). PWP measurements were conducted during predawn hours (0300 to 0500 hours) in 1 week, since the water potential of seedlings is expected to be stable during predawn hours. The drought treatment continued until the water potential difference between the control (i.e. well-irrigated seedlings) and water-stressed seedlings reached 10.8 bar (± 6.04) on 8 August 2000. For the measurement, a twig was sampled from each seedling and sealed in the chamber with the cut end exposed through a hole in the chamber cover. Chamber pressure was slowly increased until water in the twig was forced back to the cut surface and the amount of pressure (in bars) at that point was recorded.

2.4. Proline content

Since there are studies that report a strong correlation between the PC of pine seedlings and their water potential status, it was meaningful to determine the PC magnitude and its variation at the regional and population levels of Turkish red pine seedlings at the end of drought stress. The amount of proline accumulation was determined according to the method of Bates et al. (1973). For PC analysis, fresh leaf tissues were collected from the seedlings at the end of the water stress experiment, when PMS between control and water stress seedlings reached 10.8 bar, and they were stored at -80°C until they were used. The frozen tissues were thawed for 10 min and excess moisture was removed from needles by a filter paper. Sample leaves were then weighed (approximately 0.1 g), cut into smaller pieces, and ground in a mortar, with the addition of 1 mL of 3% sulfosalicylic acid. PC was measured by spectrophotometer (Shimadzu UV 160A) at 520 nm and calculated as $\mu\text{mol g}^{-1}$ weight against standard PC.

In order to investigate variation in PMS and PC among the selected families with respect to different population groupings (i.e. cold-resistant versus cold-sensitive or inland versus coastal), the following ANOVA model, which was carried out with family plot means, was employed:

$$Z_{ijk} = \mu + G_i + P(G)_{j(i)} + e_{ijk}, \quad (6)$$

where Z_{ijk} is the k th family in the i th group in the j th population, μ is the experimental mean, G is the i th group (cold-resistant versus cold-sensitive or inland versus coastal), $P(G)_{j(i)}$ is the j th population in the i th group, and e_{ijk} is the random error. The main effects in Eq. (6) were tested using PROC GLM in the SAS statistical package with Duncan's multiple range test. For the detection of the relationship of PMS and PC to other seedling traits, Pearson correlations were estimated among the traits by using the family means, which were obtained from the PROC COR of SAS with the Pearson correlations option.

3. Results

3.1. Objective 1: Genetic variation in drought resistance

Height growth of seedlings before drought (HTBD) varied significantly among populations within groups (coastal versus inland groups), as well as among families within populations. On the other hand, the height growth of seedlings after drought stress (HTAD) varied significantly only among populations. During the drought period, there was no variation in height growth among groups, among populations within groups, or among families within populations. However, DRO was different between coastal and inland populations, as well as among populations within coastal or inland groups (Table 3). Phenological traits (BB and BS) varied significantly among coastal and inland populations, as well as among families within populations (Table 3).

Table 3. Mean squares, variance components as percent of total variance (VC), family mean heritability estimates (h_f^2), and overall means (\pm standard errors) for the studied traits (see Table 2 for trait definitions). Analyses of variance were based on the entire data set.

Trait	Mean squares and variance components										
	Rep (df = 2)	Group (df = 1)	VC	Populations / group (df = 4)	VC	Families / populations (df = 234)	VC	Error (df = 444)	VC	h_f^2	Mean
HTBD	251741.0	10671.0 ^{ns}	0.9	30098.0 ^{**}	8.8	2563.4 [*]	3.9	2261.1	86.4	0.12 \pm 0.10	341.2 \pm 2.2
HTAD	346927.0	17335.0 ^{ns}	1.5	31909.0 ^{**}	6.1	3859.9 ^{ns}	4.0	3408.6	88.4	- ^a	363.2 \pm 2.8
HTBA	26249.0	275.9 ^{ns}	0	378.9 ^{ns}	0	513.8 ^{ns}	0	633.6	100.0	- ^a	22.05 \pm 1.2
BB	0.29	0.45 ^{ns}	2.02	0.40 ^{**}	3.45	0.09 ^{**}	17.53	0.05	77.0	0.40 \pm 0.09	0.27 \pm 0.01
BS	0.05	1.77 ^{ns}	6.28	2.89 ^{**}	20.96	0.09 [*]	5.76	0.07	67.0	0.20 \pm 0.10	0.66 \pm 0.01
DRO	100.5	19.3 ^{**}	8.4	10.9 ^{**}	9.2	0.9 ^{ns}	8.6	0.67	73.8	- ^a	2.4 \pm 0.04

^{ns} Not significant at $P < 0.05$; ^{*} significant at $P < 0.05$; ^{**} significant at $P < 0.01$; ^a not estimated due to lack of significant family variance.

The bulk of the variations observed in HTBD (86.4%), HTAD (88.4%), and DRO (73.8%) were a consequence of within-plot variation. The proportion of variation due to populations ranged from 0% to 20.9%, while it varied from 0% to 17.53% for families within populations (Table 3). The family component of variance accounted for 5.76% and 17.53% of the total variation in BS and BB, respectively. Estimated family heritability was high for BB (0.40) while family heritabilities for BS (0.20) and HTBD (0.12) were low. The family heritabilities for DRO, HTAD, and HTBA could not be estimated due to a lack of significant family variance (Table 3).

Visible DRO was more severe in coastal (mean DRO = 2.57) than inland (mean DRO = 2.24) populations. Among the coastal populations, DRO was highest in the Çalkaya population (3.07). Çalkaya had the lowest average height growth in 2000 before and after drought stress. On average, families from coastal populations had earlier BB (0.24 in coastal versus 0.29 in inland) and later BS (0.61 in coastal versus 0.71 in inland) dates than families from inland populations (Table 4). This difference was mainly due to the Çalkaya population, since a greater portion of the families had early BB (30%), but a relatively small number of families had late BS (39% of families). Nevertheless, growth response and contribution of growth during the stress period did not change among the coastal and inland populations (6.3% versus 6.7% HTBA, Table 4). However, there were significant differences in HTBD and HTAD among populations within inland as well as within coastal regions (Tables 3 and 4). DRO of families varied significantly in inland and coastal populations. In addition,

populations within the coastal and inland regions varied significantly in their DRO values. Within coastal regions, the Çalkaya population suffered greatly from DRO, while the inland population of Çameli was the most damaged (Tables 3 and 4). Although populations did not differ significantly with respect to HTBA, it appears that a greater number of families from the Fethiye population continued to grow during the stress period than families from other populations. On average, during the stress period, families from the Fethiye population attained a height growth rate of 7.3% (highest among all populations, Table 4).

In general, the families that were affected by drought stress were the ones with early BB (genetic correlation = 0.29) and late BS (genetic correlation = -1.00 (Table 5A)). This pattern held for BB as well as BS when genetic correlations were estimated for the coastal and inland populations separately. The families with early BS suffered less DRO (genetic correlation = -0.92 for inland and -1.00 for coastal populations, Table 5B). Genetic correlations between DRO and HTBD (-0.84) and DRO and HTAD (-0.86) were strongly negative (Table 5A). This pattern was observed only in inland populations (Table 5B). It appears that seedlings originating from inland high-elevation areas were taller and experienced less damage from drought than those originating from coastal low-elevation populations (Table 5).

3.2. Objective 2: Identifying adaptive changes in morphological and physiological traits associated with drought resistance in Turkish red pine

Analyses of variance were conducted to compare PMS and PC by grouping families as cold-sensitive or cold-resistant

Table 4. Population means (\pm standard deviations) for seedling height before (HTBD) and after (HTAD) the drought period and visually assessed DRO in *Pinus brutia*. See Table 2 for measurement methods of the traits. Duncan's multiple range test results indicated the population means within coastal or inland groups followed by different letters were statistically significant at $P < 0.05$.

Populations	HTBD (cm)	HTAD (cm)	HTBA (as % of HTBD)	BB	BS	DRO
Coastal						
Alanya	353.3 \pm 4.5 a	375.7 \pm 5.4 a	22.4 (6.3) a	0.24 \pm 0.02 a	0.73 \pm 0.03 a	2.31 \pm 0.09 a
Yaylaalan	348.6 \pm 4.4 a	369.5 \pm 5.3 a	20.9 (6.0) a	0.20 \pm 0.02 a	0.70 \pm 0.02 a	2.33 \pm 0.09 a
Çalkaya	312.6 \pm 4.8 b	333.4 \pm 6.2 b	20.8 (6.6) a	0.30 \pm 0.02 b	0.39 \pm 0.037 b	3.07 \pm 0.09 b
Mean	338.2 \pm 3.1	359.5 \pm 3.7	21.4 (6.3)	0.24 \pm 0.01	0.61 \pm 0.02	2.57 \pm 0.06
Inland						
Fethiye	345.1 \pm 5.1 a	370.5 \pm 6.0 a	25.4 (7.3) a	0.35 \pm 0.026 a	0.58 \pm 0.03 a	2.22 \pm 0.08 c
Göhlisar	340.8 \pm 4.7 a	362.8 \pm 6.0 b	22.0 (6.4) a	0.29 \pm 0.025 a	0.77 \pm 0.02 b	2.25 \pm 0.09 c
Çameli	352.2 \pm 5.2 b	374.8 \pm 6.1 a	22.6 (6.4) a	0.22 \pm 0.022 b	0.77 \pm 0.02 b	2.26 \pm 0.09 c
Mean	346.0 \pm 2.9	369.4 \pm 3.5	23.3 (6.7)	0.29 \pm 0.01	0.71 \pm 0.01	2.24 \pm 0.05

Table 5. Genetic and phenotypic correlations (\pm standard error) between DRO and other studied traits: A) across all populations, B) within inland and coastal populations. See Table 2 for trait definitions.**A) Across all populations**

Traits	DRO	
	Genetic correlation	Phenotypic correlation
HTBD	-0.84 ± 0.10	-0.46 ± 0.07
HTAD	-0.86 ± 0.11	-0.48 ± 0.07
BB	0.29 ± 0.12	0.10 ± 0.08
BS	-1.00 ± 0.35	-0.31 ± 0.09

B) Within inland and coastal populations

Traits	DRO			
	Inland		Coastal	
	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation
HTBD	-1.00 ± 0.53	-0.41 ± 0.13	- ^a	-0.32 ± 0.13
HTAD	-1.00 ± 0.50	-0.44 ± 0.12	- ^a	-1.00 ± 0.11
BB	0.48 ± 0.39	0.03 ± 0.13	0.59 ± 0.29	0.19 ± 0.12
BS	-0.92 ± 0.51	-0.26 ± 0.14	-1.00 ± 0.51	-0.37 ± 0.12

^a Due to a lack of significant family variance components, genetic correlations could not be estimated.

and as coastal or inland. PC was significantly different between the cold-resistant and cold-sensitive population groups, while there was no difference within groups (Table 6A). When the grouping was based on population location (i.e. coastal versus inland), only PMS was significantly different between groups, but not among populations within groups. PC was significantly different among populations within groups, while it did not vary between coastal and inland populations (Table 6B).

Among the studied populations, PMS ranged from 6.5 to 12.45 bar and it was the highest in the Alanya population. Although the lowest PMS values were recorded for the Yaylaalan population, there were only three families included from this population in the analysis. PC ranged from 15.8 to 36.5 $\mu\text{mol g}^{-1}$. The highest values were observed in Gölhisar populations (Table 7A). PMS and PC values exhibited a similar pattern in cold-resistant families from cold-resistant groups, as well as in families from inland populations. Cold-resistant families and inland families both had lower PMS and higher PC values than cold-sensitive families and coastal families (Table 7B). Cold-resistant families or families from inland populations maintained lower water stress and high PC (Table 7B).

The relationships between physiological traits (PMS and PC), growth, and phenological traits exhibited similar patterns when families were grouped according to cold damage (cold-sensitive and cold-resistant). Among the physiological traits, PC had a significant but moderate negative relationship with growth for cold resistance ($r = -0.38$). In cold-sensitive and coastal families, only PMS had a negative significant relationship with growth traits (ranging from $r = -0.37$ to -0.40 , Tables 8A and 8B).

The only significant relationship between BS and physiological traits was observed in cold-sensitive families for PC ($r = -0.33$). BB was positively correlated with physiological traits, but it was only significant for PMS and PC ($r = 0.56$ and 0.35 , respectively) in cold-resistant families and for PMS ($r = 0.55$) in families originating from inland populations. The relationship between DRO and physiological traits was almost the same for all groups, but it was slightly higher when grouping was based on population location, especially in inland regions. There were significant correlations between DRO and physiological traits ($r = 0.77$) (Tables 8A and 8B).

Table 6. A) Mean squares for PMS and PC traits. Analyses of variance were based on 70 families (grouped as cold-resistant and cold-sensitive). B) Mean squares for PMS and PC traits. Analyses of variance were based on 70 families (grouped as inland or coastal populations).

A) Cold-resistant versus cold-sensitive

	Group (df = 1)	Population (group) (df = 4)	Error (df = 64)
PMS	30.20 ^{ns}	27.40 ^{ns}	15.40
PC	0.22 ^{**}	0.04 ^{ns}	0.03

B) Inland versus coastal

	Group (df = 1)	Population (group) (df = 4)	Error (df = 65)
PMS	102.20 ^{**}	17.40 ^{ns}	16.10
PC	0.03 ^{ns}	0.10 ^{**}	0.03

^{ns}:Not significant at $P < 0.05$, ^{**} significant at $P < 0.01$.

Table 7. A) Population means for PMS and PC values (\pm standard errors), B) group means for PMS and PC values (\pm standard errors) with respect to cold-resistant versus cold-sensitive families and coastal versus inland populations (see Table 2 for trait definitions). Duncan's multiple range test results indicated that the population means (A) and group means with respect to cold sensitivity and distance from the Mediterranean coast (B) for the studied traits followed by different letters are statistically significant at $P < 0.05$.

A)

Populations (number of families)						
Traits	Alanya (7)	Yaylaalan (3)	Çalkaya (32)	Fethiye (9)	Göhlhisar (12)	Çameli (7)
PMS (bar)	12.45 \pm 1.3 a	6.50 \pm 1.4 b	11.26 \pm 0.5 a	9.05 \pm 1.05 c	8.44 \pm 1.70 c	7.78 \pm 1.60 c
PC ($\mu\text{mol g}^{-1}$)	20.1 \pm 6.6 a	23.13 \pm 6.7 a	26.4 \pm 2.1 a	15.8 \pm 2.0 b	36.5 \pm 4.14 c	29.56 \pm 6.92 c

B)

Traits	Grouping of families depending on cold sensitivity		Grouping of six populations depending on their locations, coast versus inland	
	Cold-resistant families (n = 19)	Cold-sensitive families (n = 51)	Inland families (n = 28)	Coastal families (n = 42)
PMS (bar)	8.20 \pm 0.95 a	10.74 \pm 0.50 a	8.47 \pm 0.88 a	11.10 \pm 0.52 b
PC ($\mu\text{mol g}^{-1}$)	33.94 \pm 3.4 a	23.49 \pm 1.75 b	28.10 \pm 2.99 a	25.14 \pm 2.00 a

4. Discussion

Since drought stress application occurred during a large part of the seedlings' vegetation period, there were no significant differences in HTBA between coastal and inland groups, among populations within groups, or among families within populations during this period. Air temperature and potential evapotranspiration in the summer of 2000 exceeded the last 30 years' average, resulting in very severe drought conditions, which probably contributed to the similar height growth in all seedlings

during this period. Similar results were reported in a study by Sánchez-Gómez et al. (2010) on *Pinus pinaster*: water availability is not a likely cause for observing a regional variation in the studied seedlings' traits.

On average, populations from the coastal regions were more prone to DRO than those from inland sources. While temperatures are higher in places where coastal populations are located, those places receive higher amounts of rainfall than the populations located inland. The combination of high air temperatures and lack of

Table 8. Pearson correlations among physiological (PMS and PC) and adaptive traits in cold-resistant and cold-sensitive individuals (A), and in inland and coastal families (B). See Table 2 for trait definitions.

A)

Traits	Cold-resistant families		Cold-sensitive families	
	PMS	PC	PMS	PC
HTBD	-0.17	-0.43 **	-0.40 *	-0.25
HTAD	-0.10	-0.38 *	-0.37 *	-0.21
BS	0.15	0.03	-0.31	-0.33 *
BB	0.56 **	0.35 *	0.14	0.12
DRO	0.14	0.66 **	0.34	0.44 **
PMS	-	0.28	-	0.25
PC	0.28	-	0.25	-

B)

Traits	Inland families		Coastal families	
	PMS	PC	PMS	PC
HTBD	-0.13	-0.44 *	-0.39 *	-0.22
HTAD	0.05	-0.52 **	-0.37 *	-0.19
BS	0.03	0.20	-0.24	-0.29
BB	0.55 **	0.28	0.05	0.11
DRO	0.14	0.77 **	0.29	0.38 *
PMS	-	0.20	-	0.19
PC	0.20	-	0.19	-

* Significant at $P < 0.05$, ** significant at $P < 0.01$.

sufficient water supply during the study at the nursery affected individuals coming from coastal populations more than those from inland populations. The magnitude and the patterns of genetic variation in adaptive traits (growth, phenology, and DRO) suggest that forestry practices such as regeneration, reforestation, afforestation, and industrial plantations could greatly benefit from properly matching sites with seed sources.

The most drought-sensitive population, Çalkaya, located at the lowest elevation (50 m), is the population that typically receives the highest mean annual precipitation among the studied populations. Being located at a low elevation probably provides better soil conditions, along with enough year-long water supply. Thus, resistance to drought has not evolved in this population. However, the Fethiye population was the most drought-resistant population, along with the other inland populations (Göhlisar and Çameli). These populations are located

further inland and at higher elevations than Çalkaya, Yaylaalan, and Alanya. Şevik and Ertürk (2015) reported that under the applied -8 bar water stress test, low-elevation populations (e.g., Isparta-Bucak - 350 m and Mersin-Silifke - 100 m) were the provenances least affected by water stress among the 14 tested provenances with elevation ranges from sea level to 950 m. Similar results were obtained for *Pinus pinaster* (Fernández et al., 1999), Turkish red pine (Dirik, 2000), *Pinus nigra* (Topacoglu et al., 2016), *Pinus halepensis* (Calamassi et al., 2001), and *Pinus sylvestris* (Cregg and Zhang, 2001) where seed sources from inland and xeric environments were more drought-resistant than mesic seed sources.

By looking at the geographical and precipitation data, one would expect the Göhlisar population to be the most drought-resistant population. The reason for this seemingly contradictory result may be the fact that this population is located very close to large inland water

bodies (the Lakes Region of Turkey). Relatively high humidity (with the lowest potential evapotranspiration; see Table 1), a high water table, and high air moisture due to the lakes possibly resulted in lower selection pressure via drought over generations. Thus, although the mean annual precipitation is the lowest for this population, it is not the most drought-resistant one among the studied populations.

Kaya et al. (1995) found that those seedlings with continuous growth during the drought period originated from coastal and cold-sensitive populations. In the present study, seedlings did not vary significantly in height with respect to coastal versus inland grouping during drought, though inland populations had slightly higher increments. The reason for this might be the establishment of height growth differences between these groups before the drought experiment started. Early drought stress could have been triggered by the prolonged high temperatures during the summer of 2000. Thus, seedlings from both coastal and inland sources that experienced early drought stress avoided water stress by establishing long root systems before the proper drought stress was applied. This reasoning is supported by the strong negative genetic correlation between height growth (HTAD and HTBD) and DRO, which means that the taller seedlings before and after the drought period were the ones that suffered the least from drought stress because of their better established root system. Although it was reported by Cregg and Zhang (2001) that *Pinus sylvestris* populations varied in summer drought tolerance and slower-growing seed sources were more drought-tolerant than faster-growing populations, the findings in the present study can be explained by the fact that early bud-setting populations completed their growth before or during the drought period. Therefore, they were less affected by DRO (Kaya et al., 1995; Işık et al., 2001; Isik et al., 2002). Supporting the above statement, earlier BB and late BS in both coastal and inland populations were associated with higher DRO in the experimental seedlings.

In the presence of climate change scenarios in Turkey, such as increased temperatures coupled with changes in precipitation patterns (Önder et al., 2009; Yilmaz and Tolunay, 2012), an expansion of Turkish red pine's natural distribution from south to north is expected. The Inner Anatolian steppe, including the study site, will probably become a potential site for future Turkish red pine plantations. Given the strong genetic correlation between cold sensitivity (Kandemir et al., 2010) and DRO, physiological differences among cold-sensitive and cold-resistant families with respect to physiological traits related to drought resistance are of interest. PC was significantly different between cold-sensitive and cold-resistant families, but not PMS. When the selected families were grouped according to coastal or inland populations,

PMS was significantly different between groups (inland versus coastal) and PC was significantly different among populations within groups. This indicates that cold sensitivity may drive PC, while PMS is likely governed by source location. Mean values for PMS and PC were similar between the two grouping types; that is, PMS and PC were higher in cold-resistant and inland families. This similarity is not surprising because families originating from the inland sources or coming from cold-resistant groups were less damaged by the induced drought stress. This may be due to maintenance of lower water stress along with high PC. On the other hand, families from cold-sensitive and coastal sources were relatively more damaged by drought stress with the existence of higher water stress, and thus higher PC.

Phenotypic correlations between physiological traits (PMS and PC) and other traits revealed a similar pattern in both groupings (coastal versus inland or cold-resistant versus cold-sensitive). There was a significant negative correlation between physiological traits (PC), and growth traits (HTBD and HTAD) in cold-resistant and inland families. In cold-sensitive and coastal families, only PMS had a significant and negative correlation with height growth. The families with high growth before and after the stress period accumulated less proline, while increased DRO was positively correlated with increased proline accumulation. This was more prevalent in inland populations than in coastal populations. Decreased water potential affects water movement into growing regions and cell elongation rate (Bradford and Hsiao, 1982) as well as plant morphology and cellular metabolism. At lower water potentials, photosynthesis is inhibited (Kaiser, 1987). Decreasing metabolic activities can perhaps explain the negative correlation between plant moisture stress and height growth. The stronger negative correlation between PMS and growth traits in cold-sensitive or coastal families and during 2000 suggests that seedlings with low proline (drought-sensitive families) had less growth because they responded rapidly to induced water stress.

Higher PC values are associated with higher DRO. This relationship, on the other hand, was much stronger in cold-resistant and inland families than in cold-sensitive and coastal families. In both cold-resistant and cold-sensitive families, seedlings with more height growth in the summer of 2000 (HTBD and HTAD) had low PC, suggesting that families with more height growth before and after water stress may have maintained high water potential (low PMS) for continuation of growth. Tolerance to drought in some crop species is increased by osmotic adjustment. Osmotic adjustment is defined as active solute accumulation, decreasing the cell osmotic potential as water potential falls (Morgan, 1984). Generally, proline is known to accumulate in water-stressed plants (Rhodes,

1987; Wohlfahrt et al., 1998; Sofo et al., 2004; Anjum et al., 2011). In this study, high PC was also strongly correlated with visible DRO, regardless of cold-resistant versus cold-sensitive or inland versus coastal groupings. However, these correlations were stronger in inland and cold-resistant families than in coastal or cold-sensitive ones. High PC in cold-resistant and inland families and strong correlations with visual needle damage suggest that water-stressed seedlings may accumulate high proline to cope with drought. The coastal and cold-sensitive families seemed less responsive to water stress and maintained high water potential. According to Bokhari and Trent (1985), there is a counter effect between water potential and PC. Increased PC in water-stressed plants was accompanied by higher water potential. In this study, although it was not significant, a positive correlation between PMS and proline amount in both cold-resistant and cold-sensitive families, as well as inland and coastal families, indicated that increasing plant moisture stress was related with increasing proline amount.

In a 16-year study by Williston (1972), 57% of the first-year mortalities in pine plantations were due to drought. Therefore, early testing of the adaptability of genotypes

is important. Considering the possible climate change scenarios in Turkey (Kandemir et al., 2010), information on the adaptive characteristics (especially drought-related) of seed sources of Turkish red pine will be vitally important for future plantations. However, the obtained information about growth characteristics, physiological traits, and DRO should be further investigated with long-term field tests, such as provenance and progeny tests, conducted with a large number of populations in reciprocal field experiments.

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