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Variation in needle and cone characteristics and seed germination ability of *Abies bornmuelleriana* and *Abies equi-trojani* populations from Turkey

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Abstract: Turkish fir (*Abies bornmuelleriana* Mattf.) and Trojan fir (*Abies equi-trojani* (Aschers. et Sint. ex Boiss) Mattf.) are economically and ecologically important endemic species to Turkey. These species are also becoming increasingly popular in Europe and North America due to their suitable characteristics for use as Christmas trees coupled with their pest resistance. Provenance features, as well as needle and cone characteristics and seed germination ability, of three Turkish fir and two Trojan fir populations were studied. Provenance features (vigor score, crown score, and color) and mother tree characteristics (height class, diameter at breast height, and height) were very similar between species and among populations within species. Needles of Turkish fir were significantly ($P < 0.05$) longer and wider than those of Trojan fir. Turkish fir also had wider cones and a higher cone width/length ratio than Trojan fir. There were moderate, positive, and significant correlations between needle-cone characteristics and location variables (elevation, latitude, and longitude). Needle size tended to increase northwards, eastwards, and upwards along an altitudinal gradient. Cone width and the cone width/length ratio showed a weak trend of increasing northwards, while the cone width also showed a weak trend of increasing eastwards. Bract and cone length were not significantly correlated with any of the location variables. The overall mean cumulative germination percentage of Turkish fir seed (57%) was significantly higher ($P < 0.05$) than that of Trojan fir (36%). The natural genetic resources of both species should be conserved and managed sustainably to preserve the variation in their endemic locations because of their valuable benefits to Turkey and other countries.

Key words: Christmas trees, firs, genetic conservation, Trojan fir, Turkish fir

1. Introduction

Taxonomic classification of the genus *Abies* in the Mediterranean Basin has been difficult to define due to various reasons, such as hybridization among certain species, wide geographical range, diverse ecological habitats, and great genetic variability within and/or among species (Scaltsoyiannes et al., 1999; Aussenac, 2002; Linares, 2011; Kormutak et al., 2013). According to several authors (Liu, 1971; Farjon, 1990; Eckenwalder, 2009) firs in Turkey are divided into two main taxonomic species (Nordmann fir and Cilician fir) and three or four subspecies or hybrids. However, recent studies proposed four (Debreczy and Racz, 2011; Linares, 2011) or even five different species (Ata and Korgavus, 2012; Tayanç et al., 2012) in the country.

There are five native fir taxa in Turkey that have economic, ecologic, silvicultural, and recreational benefits; they are distributed mostly along the coastal regions of the country. *Abies nordmanniana* Stev.

(Caucasian or Nordmann fir) and *A. bornmuelleriana* Mattf. (Bornmüller's, Uludağ, or Turkish fir in Europe and North America) are mainly distributed along the Black Sea coast. *A. equi-trojani* (Aschers. et Sint. ex Boiss) Mattf. (Kazdağı or Trojan fir) and *A. × olcayana* Ata and Merev (Çataldağ fir) cover a small area in the Marmara Region (Bahadır and Emet, 2010; Ata and Korgavus, 2012). *A. cilicica* Carr. (Cilician or Taurus fir) is distributed in the Taurus Mountain ranges along the Mediterranean coast in southern Turkey. Cilician fir is represented by two geographically distinct subspecies: subsp. *cilicica* (central and eastern parts of the Taurus Mountains) and subsp. *isaurica* (western part of the Taurus Mountains). Bornmueller (Turkish), Kazdağı (Trojan), Çataldağ, and subsp. *isaurica* are endemic to Turkey (Bahadır and Emet, 2010; Tayanç et al., 2012).

Although Turkish fir and Trojan fir are often considered as subspecies of Nordmann fir, here we consider them separate because each has distinct morphological (Saribaş,

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2008) and genetic characteristics (Kaya et al., 2008) from Nordmann fir and each other. Trojan fir differs from Turkish fir with its more rigid pointed needles and nonresinous buds (Debreczy and Racz, 2011). Provenance features (Ozel and Simsar, 2009; Özel and Ertekin, 2012), needle length and width (Şevik et al., 2013), cone and seed characteristics (Saribaş, 2008; Velioglu et al., 2012), and seed germination percentage (Karaşahin et al., 2000; Tilki, 2004; Ak et al., 2012) are significantly different among species and populations within species. The natural range of Trojan fir covers a total of 3591 ha. This species mostly (>90%) grows in mixed stands with black pine (*Pinus nigra* J.F. Arnold subsp. *pallasiana* Holmboe) and oriental beech (*Fagus orientalis* Lipsky) depending on the altitudinal zones (Simsar, 2007; Ozel and Simsar, 2009). The Turkish fir's natural range is nearly 200,000 ha. It grows from sea level to 2000 m above sea level. It grows in mixed and pure stands depending on region and altitudinal zones (Aussenac, 2002; Özel and Ertekin, 2012). Trojan fir grows faster than other native fir species both in pure and mixed stands (Ata, 1989; Ozel and Simsar, 2009). Both are keystone species in valuable forest ecosystems of Turkey and some fir species are under conservation programs, such as gene conservation forests, gene management zones, national parks, and nature conservation areas (Kaya and Raynal, 2001).

In Europe and North America, the sales of Christmas trees from short-rotation intensively managed plantations exceeds 80 million trees annually (Chastagner and Benson, 2000). Although many conifer species are used as Christmas trees, true firs (*Abies* spp.) have become increasingly popular. In Europe, Nordmann or Caucasian fir is the top-selling Christmas tree species, while in the United States, the noble (*A. procera* Rehd.) and Fraser (*A. fraseri* (Pursch) Poir) firs are popular. Firs are suited for use as Christmas trees due to their natural conical shape, pleasant color and aroma, and strong branches for holding ornaments (Frampton, 1998; Frampton and McKinley, 1999). Many fir species also have wonderful postharvest needle retention time, allowing them to be harvested and shipped weeks before their use in consumers' homes (Hinesley and Chastagner, 2004).

Although some native fir species of Europe and North America are well suited for use as Christmas trees, there are a number of diseases and insect pests, such as *Phytophthora* root rot and balsam wooly adelgid (*Adelges piceae* Ratzeburg), that can kill or seriously damage many of these species (Chastagner and Benson, 2000). A number of exotic fir species have been investigated to understand their adaptation to local conditions and susceptibility to disease and insect threats. Turkish and Trojan fir have resistance to some common threats that impact the production of Christmas trees and are well adapted to warm and dry climates. Experience also indicates that

Christmas tree consumers will buy Turkish fir, while the acceptance of Trojan fir is less well known (Frampton and Benson, 2012; Frampton et al., 2013). Therefore, the Collaborative Fir Germplasm Evaluation (CoFirGE) Project was organized in 2010 as a collaboration of university research and extension faculty and Christmas tree grower associations in five production regions of the United States (Connecticut, Michigan, North Carolina, Pennsylvania, and the Pacific Northwest) and Denmark. The initial objective of the CoFirGE Project was to obtain seeds of Turkish and Trojan fir. Seedlings have been propagated from this material and a series of field trials has been established to evaluate this germplasm for Christmas tree production in various regions of the United States and Denmark (Frampton, 2010).

In this study, cones from two Trojan and three Turkish fir populations were evaluated. The objectives were to describe: 1) differences among provenance features; 2) variations among needle, cone, and seed germination ability characteristics of species and populations within species; and 3) the relationship of needle and cone characteristics to the elevation, latitude, and longitude of mother trees.

2. Materials and methods

2.1. Sampled populations

Three Turkish fir and two Trojan fir populations representing a range of elevations were sampled during the first week of October (2–7 October 2010) (Table 1). All selected populations were seed stands except the Çanakkale-Çan population of Trojan fir, which is in a gene conservation area. Trojan fir trees were sampled from elevations of 280 m (Çan) to 1574 m (Kazdağı; Table 1), which nearly covered the entire altitudinal range (Simsar, 2007). Turkish fir populations were selected from the higher altitudes of the species (from 1024 m to 1690 m; Table 1). Twenty cone-bearing dominant to codominant mother trees showing good Christmas tree traits of form and growth were selected within each provenance. The selected trees were spaced at least 100 m from one another to reduce relatedness. From each tree about 60 mature cones that appeared healthy were collected in order to ensure enough seeds to establish future field trials. At the time of cone collection, the following measurements for each mother tree were made: latitude, longitude, elevation, vigor score (5 = very vigorous, 4 = above average, 3 = average, 2 = below average, 1 = poor), crown score (5 = very conical, 4 = above average, 3 = average, 2 = below average, 1 = flat-topped, no apical dominance), color (5 = dark green, 4 = green, 3 = medium green, 2 = light green, 1 = yellow green), height class (dominant or codominant), diameter at breast height (DBH), and height, presence, and frequency of subtending lateral branches (referred to as "tongues" by Christmas tree growers) (Table 1).

Table 1. Range and means of geographic and tree characteristics of five Trojan and Turkish fir populations.

Populations	<i>Abies equi-trojani</i>		<i>Abies bornmuelleriana</i>		
	Çan	Kazdağı	Akyazı	Bolu	Karabük
Elevation, m (mean)	280–815 (596)	1469–1574 (1529)	1024–1448 (1248)	1134–1690 (1345)	1030–1472 (1307)
Latitude	39°54'3.7"N to 39°57'52.4"N	39°44'02.1"N to 39°45'59.1"N	40°36'0.5"N to 40°39'57.4"N	40°37'22.9"N to 40°39'59.4"N	41°02'03"N to 41°03'56.6"N
Longitude	27°05'0.3"E to 27°06'57.6"E	26°55'06.6"E to 26°56'59.2"E	30°50'00.9"E to 30°51'58.3"E	31°35'17.5"E to 31°38'57.3"E	32°28'0.08"E to 32°29'55.2"E
Vigor score* (mode)	2–4 (3)	2–3 (2)	1–3 (2)	1–3 (2 and 3)	1–5 (2)
Crown score ^o (mode)	1–5 (3)	1–3 (3)	1–3 (2)	2–3 (2)	1–5 (3)
Color* (mode)	2–4 (3)	1–3 (2)	1–3 (2)	1–3 (2)	1–4 (3)
Height class	Mostly codominant	Mostly codominant	Dominant–codominant	Dominant–codominant	Dominant–codominant
Trees with tongue branches	15 (75%)	20 (100%)	4 (20%)	6 (30%)	4 (20%)
DBH ⁺ , cm (mean)	21.6–47.1 (31.3)	18.1–51.9 (39.7)	17–50.5 (29.6)	22.5–53 (36.4)	21–42 (29)
Height, m (mean)	10.6–20.2 (14.8)	9.5–19.2 (14.9)	4.9–22.4 (13.1)	7.3–23.3 (15)	9.3–22.5 (16)

*5 = very vigorous, 4 = above average, 3 = average, 2 = below average, 1 = poor.

^o5 = very conical, 4 = above average, 3 = average, 2 = below average, 1 = flat-topped, no apical dominance.

*5 = dark green, 4 = green, 3 = medium green, 2 = light green, 1 = yellow green.

⁺Diameter at breast height.

2.2. Needle and cone measurements

Within each population, four sun-exposed branches from the upper third of the crown of each tree were randomly sampled during the cone collection process. There were a total of 80 branches (20 trees with 4 branches per tree) per population. During the cone collection, 4 cones were randomly selected from those collected from each tree. These cones, along with the 4 branches and current season needles (5 per branch) from each tree were labeled and photographed on lined paper with a ruler for reference. Subsequent to cone collection, the length and width of needles and cones were measured from pictures using the ImageJ program (Schneider et al., 2012).

The needle lengths were measured from base to apex, staying along the centerline. Immediately following each needle length measurement, the height of the paper line closest to the needle was measured to provide a standard for calculating the actual length of the needle. This was repeated for all 20 needles per tree. Once all of the lengths had been found, the widths of the needles, measured at the widest point, were assessed. The cone lengths were measured, as well as the widths at the widest point. For each cone, the lengths of the three longest bracts (the portion extending beyond the cone's scales) were also measured. As a reference, the width of the photographed branch tag was measured.

2.3. Germination tests

In Ankara, cones from each mother tree were placed into kraft boxes outside to continue ripening and were air-dried before seeds were manually extracted. The extracted seeds were fumigated before shipment to the United States (Seattle, WA). Germination tests were carried out at Washington State University, Research and Extension Center, Puyallup, WA. Prior to stratification, seeds were soaked in plain water for 24 h and surface-sterilized for 10 min in a 10% solution of bleach. Healthy seeds from each of the 100 seed lots (nearly 2000 for each population and a total of 9972 for five populations) were then divided into four repetitions of approximately 25 seeds each and placed in a petri plate 5 cm in diameter with 7.4 cm³ of moist vermiculite on 17 February 2011. The seeds were then stratified by placing the petri plates in a dark cooler maintained at 1 °C for 1 month (Bonner and Karrfalt, 2008).

After 1 month (17 March 2011), the petri plates were moved to a growth chamber set at temperatures of 30 °C with light for 8 h and 20 °C without light for 16 h. The number of germinated seeds in each plate was counted after 1, 2, 3, and 4 weeks (Bonner and Karrfalt, 2008). After the last count, the number of nongerminated seed in each plate was also counted to confirm the actual number of seeds that were in each plate. The cumulative percentage of

germination was then calculated for each seed lot over the 4 weeks that data were collected.

2.4. Statistical analysis

Data were analyzed with ANOVA using the general linear model procedure of SAS software (SAS Institute Inc., 2013) and the following model:

$$Y_{ij} = \mu + S_i + P_{j(i)} + e_{ij}$$

where Y_{ij} is an individual observation for one of the analyzed characters, μ is the overall mean, S_i is the fixed effect of species i , $P_{j(i)}$ is the fixed effect of provenance j within species i , and e_{ij} is the residual variation. Pearson correlation coefficients between all pairs of needle and cone characteristic values with the elevation, latitude, and longitude of the parent tree were explored with the CORR procedure of SAS software. Multiple comparisons of least squares means for cone and needle measurements as well as germination ability were assessed by Tukey–Kramer techniques. No data required transformation prior to analysis.

3. Results and discussion

3.1. Provenance features

Despite considerable variation in the trees sampled, the provenance modes and means were generally similar to each other, except that the means of the Trojan fir trees sampled from the Çan provenance were above average for all three of these traits. This difference can be attributed to the unique geographical, ecological, and/or bioclimatic characteristics of this population (Aussenac, 2002; Simsar, 2007; Linares, 2011).

The stand height class of sampled Trojan fir trees was mostly codominant, while sampled Turkish fir trees were codominant to dominant. Turkish fir is a dominant tree species in the higher altitudes of its forest, but codominant

and/or intermediate or even suppressed in the lower altitudes, where it is generally associated with oriental beech (Özel and Ertekin, 2012). Size characteristics (DBH and height) of Turkish fir populations were slightly smaller than those of Trojan fir populations. Trojan fir grows faster than other native fir species in pure stands and different forest tree species in mixed stands (Ata, 1989; Ozel and Simsar, 2009). One of the notable differences between species was the number and frequency of subtending lateral branches (tongues). Trojan fir (87.5%) populations had higher numbers and frequencies of tongues than Turkish fir (23.3%) populations (Table 1). Tongues are important for Christmas trees because they result in a fuller crown, a characteristic desired by most Christmas tree consumers (Frampton, 1998).

3.2. Needle and cone characteristics

Needles averaged 2.00 cm in length and 0.15 cm in width, while cones averaged 15.2 cm in length and 4.6 cm in overall width. The overall average cone width/length ratio was 0.31 cm and the average bract length (beyond the cone scale) was 0.86 cm. Differences between needle characteristics and cone width/length ratio of two species were small, but significant (Table 2). Turkish fir had longer and wider needles than Trojan fir (2.1 versus 1.8 cm and 0.16 versus 0.14 cm, respectively). Turkish fir also had wider cones and a higher cone width/length ratio than Trojan fir (4.69 versus 4.49 cm and 0.31 versus 0.29, respectively). Needle and cone characteristics of both species were found within the range of those previously reported (Liu, 1971; Farjon, 1990; Eckenwalder, 2009; Debreczy and Racz, 2011).

There were moderate, positive, and significant correlations between needle width and elevation, latitude, and longitude (Table 3). Needle length was also positively correlated with these location variables, but more weakly.

Table 2. Summary statistics of needle, cone, and seed germination ability characteristics of five Trojan and Turkish fir populations.

Populations	<i>Abies equi-trojani</i>			<i>Abies bornmuelleriana</i>			
	Çan	Kazdağı	Mean	Akyazı	Bolu	Karabük	Mean
Needle length, cm	1.73 ^{ba} (±0.08)	1.89 ^{ba} (±0.09)	1.81 ^b (±0.06)	2.21 ^a (±0.07)	2.19 ^a (±0.1)	1.98 ^{ba} (±0.11)	2.12 ^a (±0.06)
Needle width, cm	0.13 ^b	0.15 ^a	0.14 ^b	0.16 ^a	0.17 ^a	0.17 ^a	0.16 ^a
Cone length, cm	15.30 ^a (±0.42)	15.55 ^a (±0.3)	15.43 ^a (±0.25)	15.28 ^a (±0.69)	14.10 ^a (±0.28)	15.78 ^a (±0.66)	15.00 ^a (±0.34)
Cone width, cm	4.36 ^a (±0.1)	4.60 ^a (±0.07)	4.49 ^a (±0.06)	4.91 ^a (±0.17)	4.49 ^a (±0.07)	4.65 ^a (±0.32)	4.69 ^a (±0.11)
Width/length ratio of cone, cm	0.29 ^a (±0.01)	0.30 ^{ba} (±0.01)	0.29 ^b (±0)	0.33 ^a (±0.01)	0.32 ^{ba} (±0.01)	0.29 ^{ba} (±0.02)	0.31 ^a (±0.01)
Bract length, cm	0.84 ^{ba} (±0.04)	0.94 ^a (±0.03)	0.89 ^a (±0.03)	0.79 ^b (±0.04)	NA	0.85 ^{ba} (±0.05)	0.82 ^a (±0.03)
*Germination %, week 4	40 ^b	31 ^b	36 ^b	61 ^a	46 ^{ba}	62 ^a	57 ^a

±Standard error. *Cumulative germination percentage after 4 weeks. *Means with the same letter in the same row are not significantly different according to Tukey–Kramer least squares means. NA: not available.

Table 3. Correlation coefficients (upper) and Prob > |r| (lower) between morphological traits and location variables. **Bolded** r values are significantly different from 0 at the $P \leq 0.05$ level.

	Elevation	Latitude	Longitude
Needle length	0.25	0.26	0.31
(*n = 99)	0.0128	0.0084	0.0021
Needle width	0.55	0.51	0.56
(99)	<0.0001	<0.0001	<0.0001
Cone length	0.04	0.14	0.12
(92)	0.7358	0.1808	0.2482
Cone width	0.17	0.27	0.28
(92)	0.1054	0.0094	0.0075
Cone width/length ratio	0.19	0.17	0.21
(92)	0.0776	0.0953	0.0463
Bract length	0.21	-0.06	-0.05
(73)	0.0748	0.6085	0.6603

*Number of observations.

Thus, needle size generally increases from south to north, from west to east, and from lower to higher elevations. Cone width and cone width/length ratio showed a similar but weak trend for some location variables. Bract and cone lengths were not significantly correlated with any of the location variables (Table 3). It has been reported that needle oils (Ucar and Ucar, 2014) and seed characters (Turna et al., 2010) of Turkish fir exhibit notable variation depending on the geographic location. These correlations can be attributed to highly heterogeneous distribution area of species (even in a small area, like the Trojan fir range; Simsar, 2007), high altitudinal range (Aussenac, 2002), and different climatic conditions in the microhabitats of species (Kaya and Raynal, 2001; Bahadır and Emet, 2010).

3.3. Germination variation

The germination of the individual seed lots ranged from 0% to 86% after 4 weeks in the growth chamber. Across all populations, germination rates were greater than 30% for 82 of the 100 seed lots, yet nine Trojan fir seed lots had germination rates below 10%. A significant difference (at $P \leq 0.05$) between the two species' germination was found (Table 2). The lowest (31%) and the highest (62%) provenance cumulative germination percentages were found in the Kazdağı (Trojan) and Karabük (Turkish) populations, respectively (Table 2; Figure). Like in many other forest tree species (Escudero et al., 2002), seed germination percentages of *Abies* species are generally low because of several problems, such as collection of

immature cones, poor cone year, infestations of cones by insects, poor cone and/or seed postcollection handling practices, presence of resin vesicles within the seed coat, and physiological dormancy (Karaşahin et al., 2000; Tilki, 2004; Andersen et al., 2008; Ak et al., 2012).

According to cumulative percent germination results, Trojan fir populations germinated more poorly than Turkish fir populations (Table 2). Weekly germination percentages of Turkish fir populations were also better than those of Trojan fir populations. The germination percentage trend increased in the first and second week period and had nearly plateaued by the third week for both species and all populations within species (Figure). The average germination percentage of this study (36% for Trojan and 57% for Turkish fir; Table 2) was higher than previously published for Trojan fir (28.5%; Aslan, 1982) and Turkish fir (0% to 44%; Şevik et al., 2010). On the other hand, the average germination percentages of Trojan fir populations from the same regions (Çan and Edremit) in the same cone collection year (2010) were reported as 55% (Çan) and 78% (Edremit) (Ak et al., 2012). The average germination percentages of Akyazı and Bolu populations of Turkish fir from 2011 cone collection were reported to be 39% and 41%, respectively (Ak et al., 2012). These differences can likely be attributed to cone collection time (both in year and month), population variation (Karaşahin et al., 2000; Escudero et al., 2002), and geographic origin effect (Andersen et al., 2008).

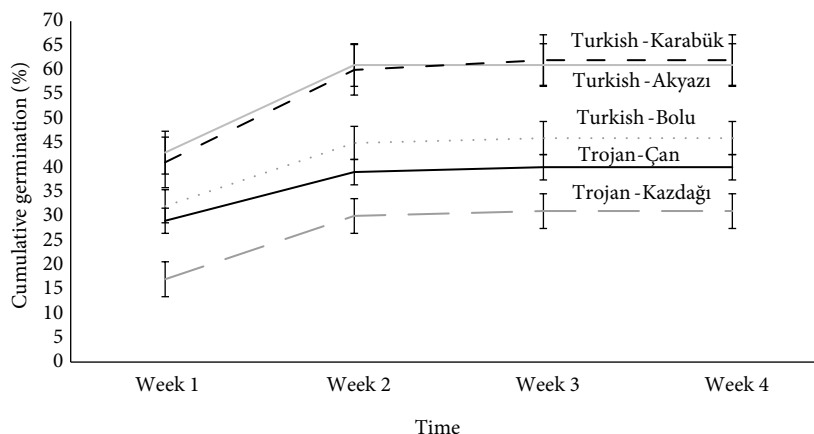


Figure. Cumulative seed germination percentages of Turkish and Trojan fir populations over the course of 4 weeks with standard errors.

Investigations of Turkish and Trojan fir germination are limited relative to the Nordmann fir, which is a closely related species and considered the main species of the Black Sea Region firs (subsp. *nordmanniana*, subsp. *bornmuelleriana*, and subsp. *equi-trojani*) according to some authors (Liu, 1971; Farjon, 1990; Eckenwalder, 2009). The germination percentages of stratified Nordmann fir seeds were reported from 8% to 40% and as less than 12% for unstratified seeds. It was also found that effects of stratification and temperature on germination were significant ($P < 0.05$) (Tilki, 2004). Karaşahin et al. (2000) collected cones weekly from 29 August to 10 October over the course of 5 years (from 1995 to 1999) to find the best cone harvesting time for Nordmann fir. In addition, cone harvest time is significantly different from year to year. There was also a significant relationship between germination percentage and total amount of time at more than 5 °C daily temperatures (must be more than 4000 growing degree-days) during the cone harvesting period. The germination percentage was higher than 50% when cones were collected in the first and/or following weeks of October (Karaşahin et al., 2000).

These results confirm this study's results for Turkish fir (Table 2; Figure). This study's populations were sampled during the first week of October and had a 57% mean cumulative germination percentage for Turkish fir (Table 2). Cone collection time is a dilemma for seed germination. If the cones are collected early, less germination occurs because of immature seeds. If the cone collection time is delayed too long, most of the cones may shatter and distribute their seeds. Therefore, the best cone collection time depends on many factors, such as species (Johnson et al., 2003), bioclimatic conditions (Ayari et al., 2011), and geographic variables (Karaşahin et al., 2000). Temperature and the moisture content of seed during storage also

significantly ($P < 0.001$) affects the germination of Nordmann fir (Karaşahin et al., 2001).

This study has described variation in needle, cone, and seed germination ability characteristics of Turkish and Trojan fir populations. These data are a small part of the CoFirGE Project. In the future, the CoFirGE Project will obtain detailed information across Christmas tree production regions in the United States and Denmark, resulting in a comprehensive performance database of Turkish and Trojan fir families. Fir trees will be identified with desirable Christmas tree characteristics and incorporated into regional applied tree improvement efforts to produce genetically improved planting stock alternatives that are pest-resistant and regionally adapted (Frampton, 2010). When field data are collected from progeny tests of these parent trees, morphological data of parents will be used to develop parent-offspring regressions. From that analysis, the degree of genetic control of these traits will also be estimated.

The natural fir forests of Turkey, especially the endemic Turkish and Trojan fir forests, are a treasured resource that has provided the country with bountiful aesthetic, ethic, ecologic, and economic benefits. Global climate change and various increasing anthropogenic activities will adversely affect fir forests in the future. The overall results of this study suggest that Turkish and Trojan fir populations have variations in needle and cone characteristics and seed germination ability. However, it is important to note that the sampling in this study covered only a small part of both species' natural range. Therefore, higher numbers of populations from both species, covering a wider range of distribution area and location gradients (altitude, latitude, and longitude), should be studied for further comparative morphologic and/or genetic variation analysis. Genetic resources of both species, like many other species, should

be conserved and managed carefully in Turkey due to their valuable benefits to Turkey and other countries.

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