

*Full Length Research Paper*

# Variation in seedling morphology of Turkish fir (*Abies nordmanniana* subsp. *bornmulleriana* Mattf)

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**In this study, the genetic variation of some seedling characteristics of Turkish fir was investigated. A total of 303 trees were selected from 17 plots and 10 seedlings from each tree were used. Fifteen morphological characteristics were determined, including root collar diameter, seedling height, total needle, bud length and width, epicotyl length, needle length and width of 1 year seedlings and root collar diameter, shoot height and diameter, needle length and width, bud length and width of 2 years seedlings. Data were analyzed by using SPSS and SAS programs. The results show that there were significant differences among the populations in terms of morphological characteristics. In addition, 12.51% of the total variation is distributed among populations. The results of this research could be used for breeding studies in Turkish fir and *in-situ/ex-situ* conservation strategies, silvicultural purpose and breeding strategies.**

**Key words:** Genetic variation, *Abies nordmanniana* subsp. *bornmulleriana* Mattf, Turkish Fir.

## INTRODUCTION

The success of any sustainable reforestation program, among other things, depends on the quality of seeds (Hampton, 2002). Several factors affect the quality of seed and seedling production, example, insect infestation (El Atta, 1993; Dajoz, 2000), pollination failure and post-zygotic degeneration (Owens et al., 1990), infection by seed borne pathogens (Pritam and Singh, 1997), environmental conditions during seed and seedling development (Gutterman, 2000) as well as the genetic constitution (Bazzaz et al., 2000).

Genetic diversity is the richness of the hereditary information in the gene pool of one species (Şevik et al., 2010). Over a long term, the ability of a species to respond adaptively to environmental changes depends on the level of its genetic variability (Ayala and Kiger, 1984). As a process, genetic differentiation by natural selection to facilitate reproductive isolation involves the presupposition of the origin of geographic races, subspecies and species (Stebbins, 1999). A species without an appropriate amount of genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites. Therefore, the investigations regarding genetic structures of populations within a species may not only help the evolutionary process and mechanism but also provide information

useful for biological conservation (Schaal et al., 1991).

Up till now, in Turkey, studies about genetic diversity of the main forest trees have been concentrated on pine species. There are no enough studies on the other species.

Turkish fir (*Abies nordmanniana* subsp. *bornmulleriana*) has a special importance to Turkey due to its increasing economic value in the market and decorative characteristic in landscape architecture. For this reason, the species, endemic to Turkey, is mostly preferred to as Noel tree in the world. Turkish fir is distributed from Kizilirmak River to Mount Uludağ in Western Black Sea region, particularly in Ayancik, Ilgaz and Bolu Seben Mountains, Boyabat-Göktepe forests, Abant and Mount Uludağ. Stands of fir species occupy about 600.000 ha in Turkey (Anonymous, 2006).

The objective of this study was to investigate the genetic diversity between and within Turkish fir populations in Turkey, using 15 different morphological characters of species seedlings.

## MATERIALS AND METHODS

### Seed collection and sowing

Open pollinated seed materials were collected from seventeen



Figure 1. Locations of the populations.

different natural populations of Turkish fir in Western Black Sea region. Location and description of the studied populations are presented in Figure 1 and Table 1, while seeds were sown at seed beds of 0.5 cm depth in February. Seeds were sown in river sand (10%), forest soil (30%) and peat (60%), respectively and covered with perlite and snow. At the end of the vegetation period, morphological features were measured for 1 year old seedlings. After 1 year, morphological features were measured again for 2 years old seedlings.

#### Seedling morphological variables studied and data collection

In this study, root collar diameter (RCD1), seedling height (SH1), total needle (TN1), bud length (BL1), bud width (BW1), epicotyl length (EL1), needle length (NL1), needle width (NW1) of 1 year seedlings and root collar diameter (RCD2), shoot height (SH2), shoot diameter (SD2), needle length (NL2), needle width (NW2), bud length (BL2) and bud width (BW2) of 2 years seedlings were determined. All the length and width measurements were done by digital microcompas (0.01 mm) of 10 seedlings from 303 sample trees.

#### Statistical analysis

Data were standardized before the calculations and the

morphological distance among populations were estimated as:

$$Z_{i,k} = \frac{(X_{i,k} - \bar{X}_k)^2}{S_k}$$

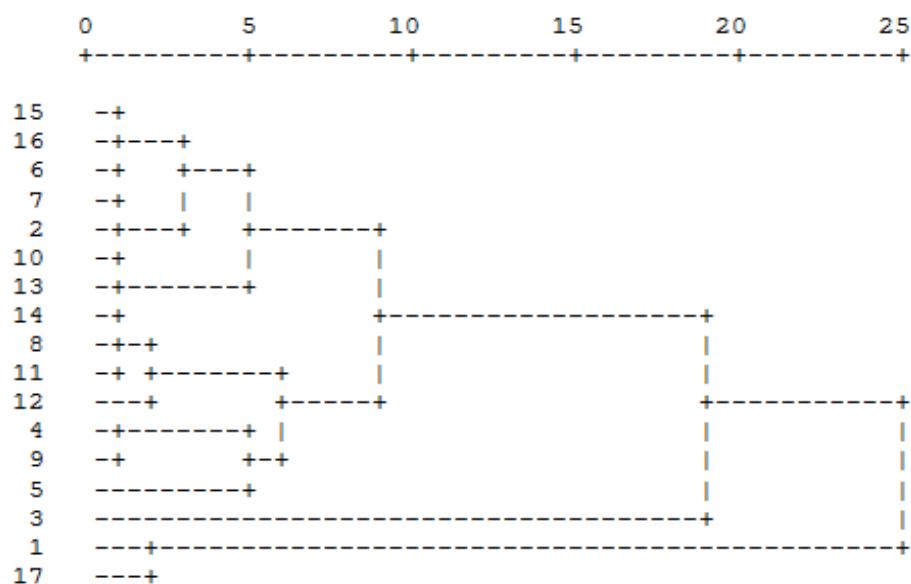
Where,  $Z_{i,k}$  is the standardized values of the  $k^{\text{th}}$  characteristics of the  $i^{\text{th}}$  population;  $X_{i,k}$  is the original average of the  $k^{\text{th}}$  characteristics of the  $i^{\text{th}}$  populations for the  $k^{\text{th}}$  characteristics and  $S_k$  is the standard deviation of the studied populations for the  $k^{\text{th}}$  characteristics (Yahyaoglu et al., 2001).

$$D_{ij} = \sum_{k=1}^p \frac{(\mu_{ki} - \mu_{kj})^2}{p \cdot V_k}$$

Where,  $D_{ij}$  is the morphological distance between the  $i^{\text{th}}$  population and the  $j^{\text{th}}$  populations;  $n$  is the number of studied characteristics;  $\mu_{ki}$  is the standardized values of the  $k^{\text{th}}$  of the  $i^{\text{th}}$  population;  $\mu_{kj}$  is the standardized values of the  $k^{\text{th}}$  characteristics of the  $j^{\text{th}}$  population;  $V_k$  is the variance of standardized averages of the  $k^{\text{th}}$  characteristics (Yahyaoglu et al., 2001). This was applied by standardized values in SPSS program (Şevik, 2010).

**Table 1.** Description of the studied populations.

Population number	Population name	City	Number of sample trees	Altitude (m)	Longitude (E)	Latitude (N)
1	Bafra1	Samsun	10	828	35°21'18"	41°34'01"
2	Bafra2	Samsun	10	1012	35°21'33"	41°33'28"
3	İskilip1	Amasya	20	1673	33°46'11"	41°22'36"
4	İskilip2	Amasya	20	1852	34°13'34"	40°49'01"
5	Türkeli	Sinop	13	1348	34°16'15"	41°44'58"
6	Ilgaz1	Kastamonu	20	1430	33°49'17"	41°09'27"
7	Ilgaz2	Kastamonu	20	1624	33°49'11"	41°08'60"
8	Ilgaz3	Kastamonu	20	1995	33°50'58"	41°07'47"
9	Ballıdağ1	Kastamonu	20	1056	33°29'02"	41°37'11"
10	Ballıdağ2	Kastamonu	20	1374	33°25'29"	41°34'12"
11	Ballıdağ3	Kastamonu	20	1640	33°22'37"	41°31'58"
12	Samatlar	Kastamonu	20	1497	33°15'32"	41°22'06"
13	Eflani	Karabük	20	1102	32°51'45"	41°29'02"
14	Aladağ	Bolu	10	968	31°37'15"	40°40'21"
15	Kıbrıscık2	Bolu	20	1499	32°00'42"	40°25'46"
16	Kıbrıscık1	Bolu	20	1791	32°02'22"	41°28'43"
17	Göynük	Bolu	20	1270	30°41'27"	40°30'08"

**Figure 2.** Dendrogram of 17 population of Turkish fir based on 15 morphological seedling traits.

### Evaluating quantitative genetic traits

Data analyses were conducted using the SAS program (SAS Institute Inc. 1987). All traits were subjected to analysis of variance (ANOVA), first for each individual test and then with the data sets combined over field sites in each population. Since error variances were consistent for each trait from site to site in each population, there was no adjustment necessary to combine the data over sites. Because of differences in statistical designs used in the seedlings, different random models were employed. The linear model for the combined data over the seedlings was:

$$y_{ij} = \mu + \alpha_i + \beta_{j(i)} + e_{ij} \quad (i=1, \dots, a, j=1, \dots, b, k=1, \dots, n)$$

Where,  $y_{ij}$  is the observation on the  $j$ th tree of the  $i$ th population;  $\mu$  is the overall mean;  $\alpha_i$  is the effect of the  $i$ th population;  $\beta_{j(i)}$  is the effect of the  $j$ th tree at the  $i$ th population and  $e_{ij}$  is the random error.

### RESULTS AND DISCUSSION

According to the results of cluster analysis (Figure 2), two

**Table 2.** Morphological distance among populations according to Penrose formula.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total
1	2.57	1.72	2.62	2.14	2.45	2.95	0.65	2.84	3.33	1.96	3.79	2.6	1.99	2.72	3.01	0.88	<b>38.22</b>
2		1.94	1.21	2.35	1.12	1.65	1.98	1.10	1.61	3.19	<b>4.43</b>	1.50	3.95	1.67	1.71	1.97	<b>33.95</b>
3			1.20	1.80	1.14	1.96	1.35	0.89	2.00	1.96	<b>4.13</b>	1.12	2.88	0.84	1.18	1.27	<b>27.38</b>
4				1.28	0.81	1.76	1.44	0.89	1.00	1.25	3.98	<b>0.36</b>	2.75	<b>0.54</b>	0.92	1.99	<b>24.00</b>
5					2.11	2.39	1.29	1.54	1.51	0.79	2.52	1.81	2.76	1.64	1.57	2.21	<b>29.71</b>
6						0.91	1.58	0.74	1.3	2.21	<b>4.46</b>	0.63	2.82	<b>0.47</b>	0.71	2.22	<b>25.68</b>
7							2.02	0.69	1.13	2.5	2.76	1.46	3.86	1.10	1.38	1.96	<b>30.48</b>
8								2.07	2.56	1.29	3.24	1.58	1.65	1.72	2.21	0.88	<b>27.51</b>
9									<b>0.55</b>	1.98	3.17	0.81	3.71	0.62	0.63	1.85	<b>24.08</b>
10										1.87	3.89	1.36	4.09	1.06	0.86	2.15	<b>30.27</b>
11											2.37	1.29	2.73	1.59	1.97	2.34	<b>31.29</b>
12												3.71	<b>4.16</b>	3.98	<b>4.88</b>	4.06	<b>59.53</b>
13													3.09	0.61	0.88	2.38	<b>25.19</b>
14														3.02	4.12	2.91	<b>50.49</b>
15															<b>0.42</b>	2.02	<b>24.02</b>
16																2.40	<b>28.85</b>
17																	<b>33.49</b>

distinct groups can be noticed: The first is pop1 (Bafra1) and pop17 (Göynük) and the others. The second group can distinguish between two groups, pop3 (Iskilip1) and the others. Accordingly, it can be said that there are three main groups. Morphological distance and grouping according to Penrose formula are shown in Table 2.

The highest 5 values were calculated between pop12 (Samatlar) and the other populations. A maximum of 5 values are between the pop12 and pop16 (4.88), pop6 (4.46), pop2 (4.43), pop14 (4.16) and pop3 (4.13). The lowest 5 values are 0.36 (pop4 and pop13), 0.42 (pop15 and pop16), 0.47 (pop6 and pop15), 0.54 (pop4 and pop15) and 0.55 (pop9 and pop10). Maximum total morphological distance values are shown by pop12 (59.53), pop14 (50.49) and pop1 (38.22), whereas minimum total morphological distance values are 24.00 (pop4), 24.02 (pop15) and 24.08 (pop9). Source of variation from SAS is shown in Figure 3. The results show that genetic variance among population were much lower than that within population. The ratio of genetic variation among the populations is 21% for RCD1, 23.2% for SH1, 16.2% for TN1, 5.5% for BW1, 8.2 for EL1, 12.7 for BL1, 19.8 for NL1, 24.6 for NW1, 7.6 for RCD2, 10.5 for SD2, 16.7 for SH2, 4.8 for NW2, 8.1 for BW2 and 4% for BL2. It ranged from 4 (BL2) to 24.6% (NW1).

According to the results of the cluster analysis, Bafra1 and Göynük populations are very close to each other but very different from the others. This situation can be as a result of geographical conditions. These populations are

the eastern and the western populations according to geographical positions. Some populations are geographically and genetically close to each other such as, Kibriscik1 (pop15) and Kibriscik2 (pop16) populations. Some of them are geographically close to each other even though they are genetically different from each other, example, Bafra1 (pop1) and Bafra2 (pop2) populations. On the contrary, some populations are genetically close to each other even though they are geographically different from each other, for example, Bafra2 (pop2) and Ballıdağ2 (pop10) populations.

Morphological distance values range from 0.36 to 4.88 according to the results of Penrose formula. Similar to the results of cluster analysis, some populations are geographically and genetically close to each other, example, Kibriscik1 (pop15) and Kibriscik2 (pop16) populations. Some of them are geographically close to each other though genetically different from each other. For example, Ilgaz1 (pop6) and Kibriscik1 (pop16) populations and some populations are genetically close to each other even though they are geographically different from each other, for example, Bafra1 (pop1) and Göynük (pop17) populations.

These results could be used in preparation of gene map, seed transfer zones, determination of breeding populations, gene conservation areas, geographic variation and they result to provenance trials of the species in a short period. Preparation of forest gene maps and determination of seed transfer zones and geographical

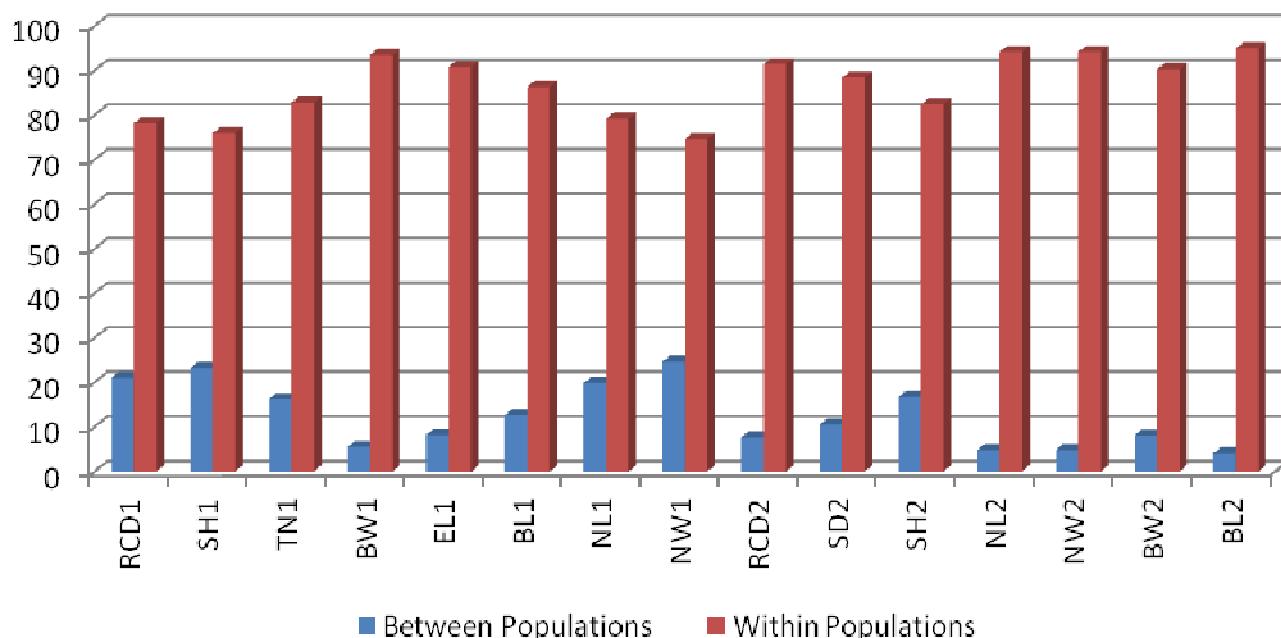


Figure 3. Source of variation (y-axis is in %).

variation by morphological distance were also suggested by Yahyaoglu et al. (2001).

According to the results of SAS, the variation among the populations is averaged at 12.51%. This result is in accordance with other studies. Yücedağ et al. (2010) stated that the variation among populations was less than 30% for 75 days seedlings of Crimean juniper. Shea (1990) reported that the variation among the populations is small (1.3%) but significant in *Abies Lasiocarpa*. Sorensen and Franklin (1977) reported that year effect including interactions with places and trees in places made up an estimated 45% of the variance in seed weight and 25% of the variance in cotyledon number. Among population, genetic variance was much lower than within population variance, ranging from 6.6 to 6.8% for drought resistance traits to 7.8–14.0% for bud-break dates and a maximum of 10.0 to 17.9% for height growth traits of *Abies alba*. Therefore, genetic variance was predominantly within population (Sagnard et al., 2002).

The average genetic distance for all pair-wise comparisons between the ten populations of *A. alba* in Italy was 0.014 (Parducci and Szmidt, 1997). 7.3% of the total genetic variation was due to the differences among populations for gymnosperms (Hamrick et al., 1992) and 10% for eight *Abies* species (Shea and Furnier, 2002). 13.3% of the total diversity is distributed among populations in *A. alba* (Vendramin et al., 1999). Great variation was observed in the heterozygosity among the populations studied and ranged from 0.010 (*Abies pinsapo*) to 0.328 (*Abies cephalonica*). The inter population genetic diversity was about 26% of the total genetic diversity. The average coefficient of gene

differentiation ( $G_{st}$ ) was 0.255, which means that approximately 26% of the total diversity of the Mediterranean firs exist among the populations. In particular, the geographical Area III (Turkey) has scored the highest value of  $G_{st}$  (25.8%), (Scaltsioyianne et al., 1999). The proportion of genetic diversity among the populations of *Abies sachalinensis* is 1.5% (El-Kassaby, 1992), populations of *Abies mariesii* is 2.6% (Suyama et al., 1992) and populations of *A. cephalonica* is 4.8% (Fady and Conkle, 1993).

Conte (2004) reported that most of the genetic variation resides within subsets (84%) by ANOVA done for *Abies nebrodensis*. More than 10% of the total genetic diversity was due to differences among populations of *A. nebrodensis* (Vicario et al., 1995).

Total percentage of genetic variation present in the population shown by interplot or among subpopulation differences is 0.35% of *Abies fraseri*. Thus, more than 99% of the genetic variation is due to within plot (that is tree to tree) variation (Diebel and Feret, 1991). Most of the genetic diversity lies within populations of *A. cephalonica* (Fady and Conkle, 1993). Less than 10% of the total observed variation appeared among populations of *A. cephalonica* (Hamrick, 1989) and the variation among the populations is 11% in *A. alba* (Vicario et al., 1995). Vendramin et al. (1999) reported that 13.3% of the total diversity is distributed among populations in *A. alba*. On the average, the genetic diversity among populations of *Abies* species has been found to be 6.3% (Hamrick et al., 1992).

The high within-population genetic diversity and low among-population differentiation observed in conifers

have been attributed to common lifehistory traits, such as longevity and extensive gene flow (Hamrick et al., 1992; Streiff et al., 1998). The biogeographic history of a species should also contribute significantly to current patterns of genetic variation (Planter et al., 2000). Despite the comparatively low levels of allozyme variation and the small genetic distances between populations, geographical differentiation among silver fir populations at different spatial scales could be demonstrated with markers (Konnert and Bergmann, 1995). Ecological and geographical differentiations are important factors that influence the breeding and sampling strategies of tree crops. It is also essential to consider the relationship between population structure in natural and domesticated populations (Chalmers et al., 1992; Şevik, 2010). Results of this study could be taken into consideration in silvicultural purpose (afforestation and artificial regeneration) and breeding strategies (that is determination of breeding populations, gene conservation areas, seed transfer zones, seed sources and geographic variation, resulting to provenance trial; establishment of seed orchard) of this species. Until now a few studies have been conducted about Turkish fir (Kaya et al., 2008; Şimşek, 1991; Veliöğlu et al., 1999; Kaya and Raynal, 2000; Nielsen and Chastagner, 2005). But there is no comprehensive study disclosing the spatial distribution of Turkish fir and providing background information for future studies. In the near future, the studies done with the morphological characters of genetical variations should also be analyzed with DNA markers and isosymes analysis.

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