Genetic Variability of Seed Characteristics of *Abies* Populations from Turkey

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Abstract
Fir (*Abies*) species occupies 626.647 ha of forestland which constitutes 3 % of total forest areas in Turkey. There are five native taxa belonging to this genus growing in pure and mixed stands in the country. Three of these taxa(*Abies nordmanniana* subsp. *bornmülleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies cilicica* subsp. *isaurica*) are endemic and considered as low risk (LR) species. Four different morphological characters of seeds were studied from 9 origins (Pozantı-Ulukışla, Bolu-Kökez, Akyazı-Dokurcun, Kızılcahamam-Güvem, Torul-Örümcek, Şavşat-Veliköy, Şavşat-Yayla, Edremit-Gürgendağ, Can-Can) belonging to 4 taxa. 20 individuals were sampled from each individual for each population, and measurements were done for 20 seeds/individual. Seed characteristics of *A. cilicica* were significantly different than other three taxa. Weight of 1000 seeds was the heaviest in *A. cilicica* (143,70 gr) and the lightest in *A. nordmanniana* (6,6 gr). Average seed width, length and diameter of all populations were calculated as 6,54 cm, 11.97 cm and 3,34 cm, respectively. Seed weight were significantly correlated (P<0,01) with seed diameter and seed length. The dendrogram constructed by cluster analysis of populations were based on Squared Euclidean Distance and Nearest Neighbor Method. The dendrogram was divided into two main clusters. The first cluster contained only *A. cilicica* population and the second cluster contained other populations grouped according to taxa.
Keywords: *Abies*; Genetic variation; Seed characteristics

Introduction

Genus *Abies* belongs to the Pinaceae family of the Coniferae Class. This genus consists of about 49 species all native to the Northern Hemisphere. They are distributed in cool and temperate regions of the Northern and Central America, Asia, Europe and Northern Africa (Schopmeyer, 1989; Farjon, 2010). The taxonomy of the genus is very complicated due to the great variability of morphological traits (Bozkuş and Çoban, 2006; Yaltırık and Akkemik, 2010).

Firs are typical monoecious, evergreen trees. They have a spire-like or conic crown; however, it is flattened in older trees. Firs are distinguished by their needle-like leaves, attached to the twig by a base that resembles a small suction cup, and by erect, cylindrical cones. Firs typically have a single straight trunk with regularly spaced branch whorls which are produced annually.

Firs are quite resistant to wind and storms due to their tap root system. They require moist soil and temperate climate (Yaltırık and Akkemik, 2011).

Genus *Abies* has been divided into eight sections. All *Abies* species occurring in Turkey belong to "Section *Abies*". There are five *Abies* taxa naturally occurring in Turkey where three of them are endemic (Farjon, 2010). These are;

-*Abies cilicica*
  subsp. *cilicica*;
  subsp. *isaurica* (endemic);
-*Abies nordmanniana*
  subsp. *equi-trojani* (endemic);
  subsp. *nordmanniana*;
  subsp. *bornmülleriana* (endemic).

Abies cilicica Carr. (Cilician fir or Taurus fir) is naturally distributed in Southern Anatolia (Middle and East Taurus Mountains, Amanos Mountains). It occurs at elevations about 1000-1800 m at the northern slopes and 1300-1400 m at the southern slopes of the Taurus Mountains. There are two subspecies of *Abies cilicica* in Turkey; *Abies cilicica* subsp. *isaurica* Coode & Cullen and *A. cilicica* (Ant. &Kotschy) Carr. subsp. *cilicica*. The differences between those subspecies are young shoots and resinous buds. *A. cilicica* subsp. *cilicica* occurs at Mersin, Adana, Maraş and Hatay provinces of east Mediterranean region. *A. cilicica* subsp. *isaurica* has a natural distribution at western Mediterranean

provinces like Antalya, Muğla and Konya (Davis, 1965).

Abies nordmanniana (Nordman fir), *A. bornmülleriana* (Uludağ fir) and *A. equi-trojani* (Kazdağı fir) have been differentiated by means of shoots (whether they are glabrous or not), twigs (resinous or not) and whether their needles are sharp pointed or not (Yaltırık and Efe, 1996; Anşin and Özkan, 1993). Although these three taxa have been accepted as distinct species in Turkey; *A. bornmülleriana* and *A. equi-trojani* are considered as synonyms in Farjon's latest review (2010). In addition, a distinct endemic taxon (Çataldag fir) was reported by Ata and Merev in1987. It is claimed that it occurs in 915 hectares in Çataldag where located between Susurluk (Balıkesir) and Mustafa Kemal Paşa (Bursa) provinces. Çataldag fir is also not accepted as a distinct taxon in Farjon's latest review (2010).

A.nordmanniana subsp. *nordmanniana* ((Stev.) Spach) has been found in the eastern Black Sea region and north-eastern Turkey (Yeşilırmak, Kelkit Valley, Koyulhisar and Şebinkarahisar provinces). It is distributed between 800-2300 m on the mountainous area (Anşin and Özkan, 1993). *A. nordmanniana* subsp. *bornmülleriana* has a fragmented distribution between Kızılırmak and Uludağ's western slopes (900-2000 m). *A. nordmanniana* subsp. *equi-trojani* (Asch. *Et* Sint.) occurs on a limited area at the Biga Peninsula on Ida Mountain between 800-1700 meters (Anşin, 1994; Asan, 1984).

Firs have a total of 626 647 hectares natural distribution in Turkey (Anonymous, 2010). They have significant ecological, economic and silvicultural functions in forestry.

All populations are genetically unique and peerless since they are adapted to their environments (Işık and Yıldırım, 1990). Therefore, while managing populations one should use population genetics data with biological information of the species (Namkoong, 1989). For the sustainable management of the populations, genetic variation data must be used. In order to determine the genetic variation of the populations, the best way is to compare the populations in several environments (Chmura and Rozkowski, 2002). Species with wide distributions have also several geographic variations and local races (Işık, 1981; Zobel and Talbert, 1984). Turkey's topographic components, climate and soil characteristics favor the divergence of local races. Therefore, wide distribution of our fir species might be a clue for their high genetic diversity.

Quantitative characters like seed's length, width and diameter are important for the selection (Ürgenç, 1982). These characters are controlled by several genes and their environment x genotype interaction is high (Işık, 1980; Falconer, 1989; Şimşek, 1993). Variations observed in quantitative characters are investigated by the application of population genetics and quantitative genetics rules (Işık, 1980; Falconer, 1981; 1989). Genetic variations can be determined by morphological or molecular markers (Suangtho, 1999; Yahyaoğlu and Genç, 2007).

Up to now, the studies on seed characteristics of fir taxa in Turkey involved only one taxon (Keskin and Şahin, 2000; Şevik, 2010; Turna et. al., 2010; Altun, 2011). Conversely, in this study seed morphological characteristics of 4 taxa distributed in our country were examined.

The aim of this study is to determine the genetic variations in seed characteristics in different fir taxa occurring in Turkey.

Materials and Methods

The objective of this study was to determine the seed characteristics of three *Abies* taxa which are widely distributed in Turkey. Therefore, particularly seed stands from those taxa were sampled (Şavşat-Yayla, Şavşat-Veliköy, Torul-Örümcek, Bolu-Kökez, Akyazı-Dokurcun, Edremit-Gürgendağı). In addition to those, Çan (Gene Conservation Forest) which is an extreme population for the Kazdağı fir; Kızılcahamam which is considered as an ecotype for Uludağ fir and Pozantı-Ulukışla population which is the most inland population in Central Anatolia have also been selected and sampled for this research. Detailed information of the studied populations is shown in Table 1.

Table 1.Detailed description of the studied populations.

	Taxon	Population	Latitude	Longitude	Altitude (m)	Aspect
1	*Abies nordmanniana* subsp. *nordmanniana*	Torul-Örümcek	40°35'45"	39 15 00	1700	N
2	*Abies nordmanniana* subsp. *nordmanniana*	Şavşat-Yayla	41°13'25"	42°27'20"	1800	S
3	*Abies nordmanniana* subsp. *nordmanniana*	Şavşat-Veliköy	41°19'00"	42°31'20"	1800	S
4	*Abies nordmanniana* subsp. *bornmülleriana*	Akyazı-Dokurcun	40°37'30"	30°51'00"	1300	SE, SW
5	*Abies nordmanniana* subsp. *bornmülleriana*	Bolu-Kökez	40°39'05"	31°36'56"	1300	NW
6	*Abies nordmanniana* subsp. *bornmülleriana*	Kızılcahamam-Güvem	40°42'00"	32°42'29"	1750	NE
7	*Abies nordmanniana* subsp. *equi-trojani*	Edremit-Gürgendağı	39°45'48"	26°57'50"	1300	S
8	*Abies nordmanniana* subsp. *equi-trojani*	Çan-Çan	39°56'00"	27°05'33"	750	E
9	*Abies cilicica*	Pozantı-Ulukışla	37°33'21"	34°29'23"	1560	N

Cones were collected in September - October 2010. 20 trees (families) were sampled from each population. While sampling, the following criteria has been applied to decrease the chance of relatedness between the families:

-the trees have to be separated at least 100 meters,

-the difference in elevation between the trees must be no more than 300 meters,

-the trees have to be at the same age.

Therefore, we tried to sample the best representatives of the gene pool (Işık, 1998). Medium-diameter trees have been selected while sampling. Collected cones were kept and labeled separately with the required information. Then, cones were kept at room temperature to dry them. The cones were blended every day so that they don't get any contamination or degradation due to humidity. The seeds were obtained in 10-15 days. Before the measurements, decayed and empty seeds were removed. All measurements have been made by the same technician in the Seed Quality Control Laboratory of our Institute. 20 healthy seeds were selected from each family for measurements. Therefore, 3600 (20 seeds x 20 families x 9 populations) seeds were used for measurements. Digital calipers were used for length, width and diameter measurements with 0.01 mm accuracy. Seeds were weighed with 0.001 g accuracy. 1000 seed weight were calculated by weighing randomly 100 seeds from 8 random seed family (ISTA, 1993).

The data obtained were used for the analysis of variance test by SPSS 16.0 (SPSS Inc., 2007). Duncan test was also applied to determine the differences between the groups. The dendrogram constructed by cluster analysis of populations were based on Squared Euclidean Distance and Nearest Neighbor Method of SPSS 16.0.

Results and Discussion

Effect of environmental factors on seed and cone characteristics of trees are very low and therefore, they are one of the preliminary characters representing genetic structure of a tree. These characters have been frequently used in genetic diversity studies. Variation of seed and cone characteristics in relation to various factors have been studied for important forest tree species of Turkey. These studies provide necessary information about nursery and regeneration practices for practitioners.

Table 2. Mean values of seed characteristics of populations

Taxa	Population	Seed width	Seed length	Seed diameter	Seed weight*
A. nordmanniana	Örümcek	6.80	12.42	3.33	68.80
A. nordmanniana	Yayla	6.57	11.90	3.13	68.35
A. nordmanniana	Veliköy	6.57	11.23	3.02	62.70
	Mean	**6.65**	**11.85**	**3.16**	**66.62**
A.nordmanniana subsp. *bornmülleriana*	Dokurcun	6.45	11.88	3.34	83.35
A.nordmanniana subsp. *bornmülleriana*	Kökez	6.46	11.87	3.41	86.30
A.nordmanniana subsp. *bornmülleriana*	Kızılcahamam	6.61	12.21	3.48	76.75
	Mean	**6.51**	**11.99**	**3.41**	**82.13**
A. nordmanniana subsp. *equi-trojani*	Gürgendağ	6.17	10.95	3.15	71.85
A. nordmanniana subsp. *equi-trojani*	Çan	6.24	10.88	3.30	71.70
	Mean	**6.21**	**10.92**	**3.23**	**71.78**
Abies cilicica	Ulukışla	7.22	13.76	3.88	149.70
	Mean	**7.22**	**13.76**	**3.88**	**149.70**

*Weight of 1000 seeds

Results of variation analysis indicated that populations differ from each other at 95% confidence interval; therefore, Duncan Test was applied. Level of differentiation between populations was examined by comparing groups. Measurements of *Abies cilicica* subsp. *cilicica* seeds were the largest of all taxa (Table 2). According to results of Duncan Test, *Abies cilicica* subsp. *cilicica* were appeared in a different group in terms of all characters examined.

Mean seed width was the highest in Taurus fir population (7.22 mm) and the lowest in Çan (6.17mm) and Gürgendağı (6.24mm) populations of Kazdağı fir (Table 2). Mean seed width for Uludağ fir populations was 6.51 mm and Nordman fir populations was 6.65 mm. Keskin and Şahin (2000) reported the seed width of Taurus fir as 7.2 mm. This value is very close to seed width value determined in our study. Şevik (2010) found mean seed width of Uludağ fir as 5.81 which is slightly lower than the value found in this study. Also, the seed width (5.56 mm) of Nordman fir reported by Altun (2011) is lower than the value found in our study.

Table 3. Duncan Test results of seed width differentiation

Population	N	Subset				
		2	3	4	5	1
Gürgendağ	20	6.17				
Çan	20	6.24	6.24			
Dokurcun	20		6.45	6.45		
Kökez	20		6.46	6.46		
Yayla	20			6.57	6.57	
Veliköy	20			6.58	6.58	
Kızılcahamam	20			6.62	6.62	
Örümcek	20				6.80	
Ulukışla	20					7.22
Sig.		0.53	0.09	0.20	0.08	1

Populations were divided into 5 groups according to the results of Duncan test done for seed width (Table 3). First group contained only Taurus fir, second group contained Kazdağı fir populations, third group contained Kökez and Dokurcun

populations of Uludağ fir, fourth group contained Uludağ fir populations and Şavşat population of Nordman fir, fifth group contained Nordman fir and Kızılcahamam population of Uludağ fir.

As in the case of seed width, the highest value of seed length was (13.76 mm) reported for Taurus fir and the lowest values were reported for Çan (10.95 mm) and Gürgendağı (10.88 mm) populations of Kazdağı fir (Table 2). Estimated mean seed length for Uludağ fir and Nordman fir was 11.99 mm and 11.85 mm, respectively. Seed length for Taurus fir was reported as 14.4 mm by Keskin and Şahin (2000). This value is slightly higher than the value found in this study. Mean seed length of Uludağ fir (11.35 mm) determined by Şevik (2010) is parallel to the mean value found in our study. Similarly, the value (12.10 mm) found for Nordman fir (Altun, 2011) is parallel to our findings.

Table 4. Duncan Test results of seed length differentiation

Population	N	Subset			
		2	3	4	1
Çan	20	10.88			
Gürgendağ	20	10.96			
Veliköy	20	11.23			
Kökez	20		11.88		
Dokurcun	20		11.88		
Yayla	20		11.89		
Kızılcahamam	20		12.22	12.22	
Örümcek	20			12.41	
Ulukışla	20				13.76
Sig.		0.14	0.15	0.39	1

Populations were divided into 4 groups as a result of Duncan test conducted for seed length (Table 4). First group included only Taurus fir population, second group consisted of Kazdağı fir populations and Veliköy as a Nordman fir population. Third group consisted of Uludağ Fir population together with Yayla population of Nordman fir. Fourth group contained Kızılcahamam population of Uludağ fir and Örümcek population of Nordman fir.

Mean value of seed diameter was the highest in Taurus fir (3.88 mm) and the lowest in Yayla (3.13 mm) and Veliköy (3.02 mm) populations of Nordman fir (Table 2). Mean seed diameter of Uludağ fir populations (3.41 mm) were higher than that of Nordman fir and Kazdağı fir populations. Mean seed diameter of Nordman fir was 3.16 mm and Kazdağı fir was 3.23 mm. Keskin and Şahin (2000) reported seed diameter of Taurus fir as 4.3 mm which is slightly higher than the valuereported here. The mean seed diameter value (3.88 mm) found for Uludağ fir (Şevik, 2010) is also higher than that of this study. The mean seed diameter for Nordman fir (3.14) reported by Altun (2011) is very close to the value found in this study.

Duncan test performed for seed diameter yielded 4 groups (Table 5).

Table 5. Duncan Test results of seed diameter differentiation

Population	N	Subset			
		2	3	4	1
Veliköy	20	3.02			
Yayla	20	3.13			
Gürgendağ	20	3.15			
Çan	20		3.30		
Örümcek	20		3.33		
Dokurcun	20		3.34		
Kökez	20		3.41	3.41	
Kızılcahamam	20			3.48	
Ulukışla	20				3.88
Sig.		0.07	0.15	0.27	1

First group contained only Taurus fir, second group contained Gürgendağ population of Kazdağıfir together with Veliköy and Yayla populations of Nordman fir. Third group consisted of populations three taxa. Last group contained Kökez and Kızılcahamam populations of Uludağ fir.

The mean weight of 1000 seeds was the highest in Taurus fir (149.70 gr) and the lowest in Nordman fir (66.62 gr) (Table 2). The mean weight of 1000 seeds was 82.13 gr for Uludağ fir and 71.78 gr for Kazdağı fir. Seed weight of Taurus fir was determined as 138 gr in the study of Keskin and Şahin (2000). This value is lower than that of our result. The mean weight of 1000 seeds for the same taxon was reported as 120-144 gr by Saatçioğlu (1971). Results of both studies are in the range given by Saatçioğlu (1971). The mean seed weights of Uludağ fir were reported as 82.95 g (Şevik, 2010) and 83.39 gr (Beşkök, 1970) previously and close to the value found in our study. However, the mean weight of Uludağ fir seeds were given as 64.9 gr (Saatçioğlu, 1971), 63.19 g (Aslan, 1982) and 57.13 g (Erkuloğlu, 1993) in the other studies. Altun (2011) reported mean seed weight of Nordman fir as 43.9 g in poor seed year and 79.03 g in good seed year. In addition, weight of 1000 seeds for Nordman fir were estimated as 54.94 g (Saatçioğlu, 1971), 51.26 (Aslan, 1982) and 52.59 (Tolay, 1983) in other studies. These values are lower than ones calculated in our study. Saatçioğlu (1971) indicated that Taurus fir seeds were significantly heavier than other three taxa and the differentiation among other three taxa was not significant. Our findings also confirm this conclusion. In addition, Keskin and Şahin (2000) stated that relative presence of empty or rotten seeds in a lot affect weight of 1000 seeds.

Table 6. Duncan Test results of 1000 seed weight differentiation

Population	N	Subset					
		2	3	4	5	6	1
Veliköy	20	62.68					
Yayla	20	68.35	68.35				
Örümcek	20	68.81	68.81	68.81			
Çan	20		71.71	71.71			
Gürgendağ	20		71.83	71.83			
Kızılcahamam	20			76.74	76.74		
Dokurcun	20				83.31	83.31	
Kökez	20					86.30	
Ulukışla	20						148.96
Sig.		0.12	0.41	0.05	0.08	0.43	1

As a consequence of Duncan Test done for seed weight character, populations were divided into 6 groups (Table 6). First group contained Taurus fir, second group contained Nordman fir populations, third group contained Yayla and Örümcek populations of Nordman fir together with Kazdağı fir populations, fourth group contained Örümcek population of Nordman fir, Kazdağı fir populations and Kızılcahamam population of Uludağ fir, fifth group contained Kızılcahamam and Dokurcun populations of Uludağ fir, last group contained Dokurcun and Kökez populations of Uludağ fir.

Table 7.Results of correlation analysis of studied seed characters

	Seed width	Seed length	Seed diameter	Seed weight
Seed width	1			
Seed length	0.684(**)	1		
Seed diameter	0.589(**)	0.684(**)	1	
Seed weight	0.503(**)	0.723(**)	0.744(**)	1

** Correlation is significant at the 0.01 level (2-tailed).

According to correlation analysis of 4 seed characters, each character is related at0.01significance level (Table 7). The highest correlation was found between seed weight and seed diameter with a value of 0.744. Correlation (0.723) between seed length and seed weight was also very high. The lowest correlation (0.503) was between seed width and seed weight. These results indicated that contributions of seed diameter and length to seed weight are significantly higher than the contribution of seed width. Seed width and diameter are equally correlated to seed length with a value of 0.684.

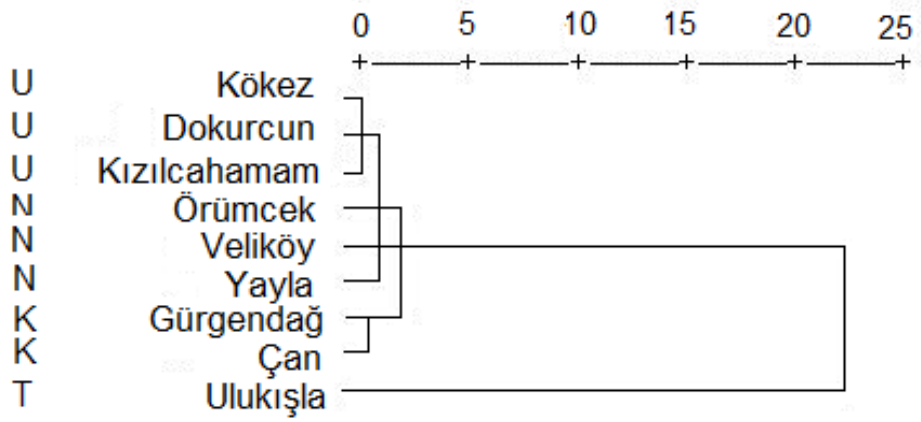

Figure 1.Dendogram constructed by cluster analysis. U: Uludağ fir, N: Nordman fir, K: Kazdağı fir, T:Taurus fir

Cluster analysis of populations in relation to seed characters yielded a dendrogram divided into two main clusters (Figure 1). The first main cluster contained only Taurus fir population and second main cluster divided into two sub clusters. The first sub cluster contained Kazdağı fir populations and the second sub cluster again divided into two groups each containing Nordman fir and Uludağ fir populations pooled separately. Anşin and Özkan (1997) noted that needle and cone morphology of Uludağ fir and Nordman fir samples were almost the same. The dendrogram revealed that, classification of fir taxa is in accordance with taxonomic classification of Yaltırık and Akkemik (2010). That is, Taurus fir is a separate species and other three taxa are subspecies of a distinct species.

Conclusions

Fir taxa distributed in Turkey had high genetic diversity in terms of studied seed characters. Populations differentiated from each other at 95% confidence level. Previous morphological (Velioğlu et al., 1999; Keskin and Şahin, 2000; Çiçek et al., 2005; Turna et al., 2009; Şevik 2010; Turna et al., 2010; Altun, 2011) and molecular studies (Şimşek, 1992; Gülbaba et al., 1996; Özer, 2002; Tayanç et al., 2012) also support our findings.

Fir taxa in Turkey possess endemic feature with systematic and ecological values as well as economic value. Improvement of *in-situ* and *ex-situ* conservation work by considering genetic studies will be beneficial for sustainable management of the species.

Acknowledgements

The authors thank Ünsal Altun, Hazin Cemal Gültekin, Muzaffer Topak and Nil Özbedel due to their support in cone collection. We also thank Ahmet Kaderoğlu for his effort in seed measurements. We also acknowledge the contribution of Dr. Murat Alan for assisting in statistical analysis.

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