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# Some seed characteristics and genetic similarities of Western Black Sea Fir populations including an isolated population

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**Abstract:** West Blacksea fir (*Abies nordmanniana* (Stev.) Spach subsp. *equi-trojani*) is an endemic species of Turkey. Six populations, four of which are a seed stand and three gene conservation forests (Akyazi-Dokurcun, Aladag-Sarialan, Kizilcahamam-Guvem, Safranbolu-Safranbolu), a Nature Reserve Area (Istanbul-Beykoz) and a natural forest (Pinarbasi-Kurtgirmez) were selected to study. Cones were harvested from 118 trees in six populations. Some seed characters obtained from cones were measured and evaluated. The average seed width, length, and thickness were 6.63 mm, 12.06 mm, and 3.46 mm, respectively. The average 1000 seed weight was 82.80 g. Statistical analyses indicated significant differences at the population level in all characteristics except seed length. Regarding genetic similarity, populations were divided into two main groups: East and West. The isolated Istanbul-Beykoz population was similar to the West group showing artificial migration or cut-off from the West group. Due to including seed stand and gene conservation forest, the findings are expected to contribute to forest management, besides Christmas trees and landscape use.

**Keywords:** Seed width, seed thickness, seed length, 1000 seed weight, gene conservation

## Introduction

The forests of Turkey are very rich in terms of the number of species. In addition, a significant part of the natural species that make up forests is endemic. In addition to the importance that endemic species bring to the country's flora, a responsibility is required for forestry practices to be made for these species. Since two of the four fir taxa in Turkey are endemic, research studies to be carried out with fir taxa will supply knowledge and enable better implementations over firs thanks to knowledge. The ornamental use (Bilgili et al., 2012; Sakıcı et al., 2012), Christmas trees (Kurt et al., 2016; Nielsen et al., 2020), and consisting of approximately of raw material % 10 of total industrial wood production between 2000 and 2021 in Turkey (OGM, 2023) also increases research over firs

Firs, which are included in the *Coniferae* class of the *Pinaceae* family, are represented by 48-49 species in North and Central America, Asia, Europe, and North Africa (Farjon, 2010). Alizoti et al. (2011) stated that 10 fir taxa are distributed in the Mediterranean, two of which are in North Africa (Figure 1), including Turkey's fir *(Abies. cilicica* subsp. *cilicica and* subsp. *isaurica, Abies nordmanniana* subsp. *equi-trojani*, subs. *nordmanniana, and* subs. *bornmülleriana)* (Farjon, 2010). However, the fir species was revised (Akkemik 2020). In this revision, no change was made for *Abies cilicica*. *Abies nordmanniana* (Stev.) Spach subsp. *nordmanniana (*Eastern Black Sea Fir*)* and *Abies nordmanniana* (Stev.) Spach subsp.*equi-trojani* (Western Black Sea Fir), were renamed. With this revision, *Abies nordmanniana* subsp. *bornmülleriana* (Uludağ Fir) and *Abies nordmanniana* subsp. *equi-trojani* (Kazdag Fir) were combined into a subspecies (Western Black Sea Fir).

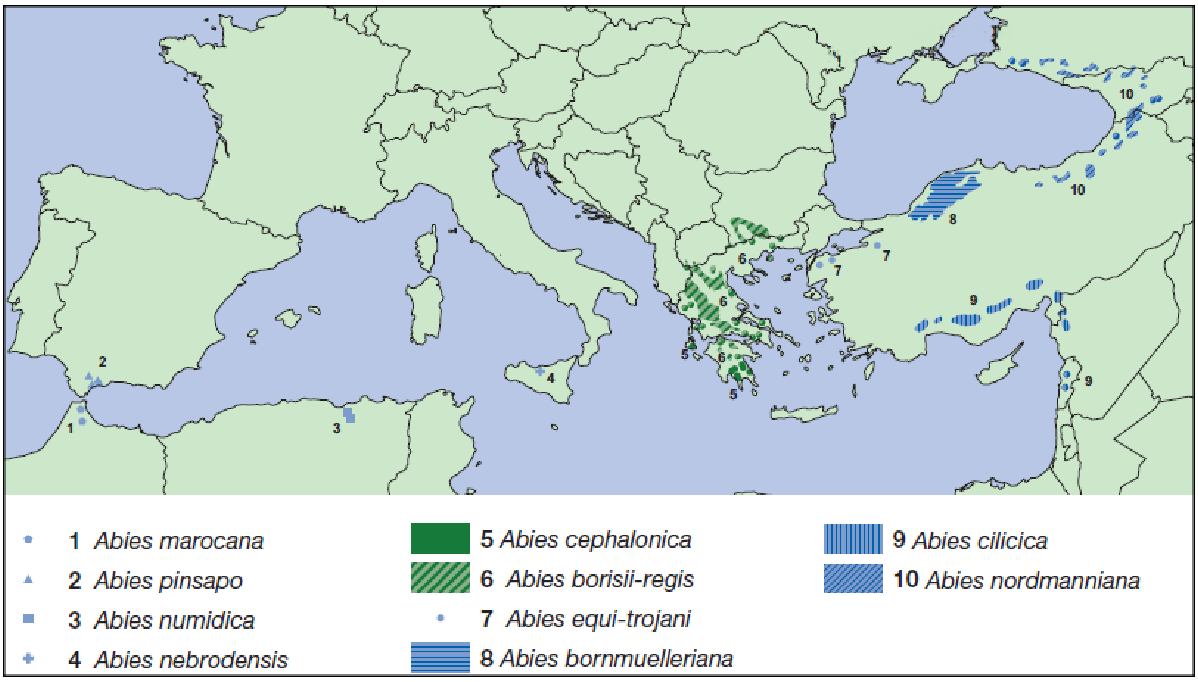


Figure 1. Distribution of firs' taxa in the Mediterranean Region (Alizoti et al., 2011)

Western Black Sea Fir (*Abies nordmanniana* (Stev.) Spach subsp. *equi-trojani*), which is endemic among the fir taxa, is an economically significant forest tree for Turkey that can grow up to 30-40 meters in height and up to 1.4 meters in diameter. It distributes between the Western Black Sea Basin and Kocaeli Basin, from Bafra, where the Kizilirmak reaches into the sea, to Uludağ, in the north of our country, at an altitude of approximately 1000-2000 meters (Figure 1). In addition, there is an isolated population of 46.5 hectares in Istanbul Beykoz at an altitude of 180 meters.

Genetic diversity in a species can be achieved by revealing the characteristics of populations in different habitats and comparing this information (Chmura and Rozkowski, 2002). Fir forests have unique genetic resources due to their evolutionary history and special adaptations. On the other hand, it is reported that its protection is necessary due to the threats arising from its endemism characteristics and scattered distribution. In this context, it is stated that the gene sources of Mediterranean firs are of particular importance (Alizoti et al., 2011; Caudullo and Tinner, 2016).
A seed contains an embryo formed by sexual reproduction and transforming into the next generation of plants. The seed occupies a critical position in the life history of the higher plant. The success with which the new individual is established -the time, the place, and the vigor of the young seedling—is primarily

determined by the physiological and biochemical features of the seed (Bewley et al., 2013). Researching seed characteristics is an effective way in population studies (Moreno-Saiz et al., 2003). Regarding 1000 seed weights, one of the seed characteristics, fast new methods were researched due to the importance of the commercialization process, directing storage, sowing, and germination (Felix et al., 2021). Seed source variations concerning cone, seed, and seedling characteristics were well-documented for several tree species (Isik, 1986; Dvorak et al., 1996; Rawah and Bakshi, 2011). In addition, it is claimed that seed production is a crucial stage in a plant's life history, and seed traits can directly affect plant fitness and persistence (Cochrane et al., 2015; Wu et al., 2018).

On the other hand, as phenotypic variation can benefit plants' adaptation to heterogeneous environments, variation in seed traits may increase this species' fitness and facilitate its persistence under future climate change (Wu et al., 2018). In this context, a description of the population's genetic structure and distribution of genetic variation among populations in this species is necessary for decisions regarding tree breeding and the conservation of plant genetic resources (Rawah and Bakshi, 2011). Moreover, further research that integrates ecology and emerging evolutionary genetic techniques to identify the distribution of seed traits within foundation species and the mechanisms driving them is emerged to guide the management and maintenance of  systems in the face of rapidly changing climates (Cochrane et al., 2015).

Some studies were carried out on seed and seedling characteristics of fir species (Turna et al., 2010; Velioğlu et al., 2012; Kurt et al., 2016; Yüksel and Dirik, 2021). However, unlike the studies mentioned, some seed sources (seed stand and gene conservation forest) and the Pinarbasi-Kurtgirmez population which represents one of the regions where the Western Black Sea Fir is widely distributed, were included in this research. Another dimension that makes this study quite different (unique) from the earlier studies is that the Istanbul-Beykoz population, which spreads outside the fir distribution area and is registered as a Nature Conservation Area (TKA), is also the subject of the study. The isolated or “island” population is in Istanbul Province, Beykoz District, and the only fir naturally distributed in the region, rare and under threat due to its characteristic of a forest ecosystem (Urker, 2021). Revealing the variation of populations, including an isolated population, using seed characteristics for Western Black Sea Fir was considered valuable for contributing to tree breeding and gene conservation.

This study aimed to reveal the seed characteristics of endemic Western Black Sea Fir populations, expose the relationships between the populations, including isolated population, and link their effects on geographical distributions using seed characteristics.  Besides managing the firs population, findings also have the potential to be used in landscape implementation and the growing of noel trees due to the properties of Western Black Sea fir.

## Material and Method

### Material

An isolated population, Istanbul-Beykoz, and five populations in natural distribution (Akyazi-Dokurcun, Aladag-Sarialan, Kizilcahamam-Guvem, Safranbolu-Safranbolu, and Pinarbasi-Kurtgirmez) including seed stands and gene conservation forests of Western Black Sea Fir were studied (Table 1, Figure 2).

Table 1. Sampled West Black Sea fir poulations

| Populations | Latitude | Longitude | Altitude | Aspect | Status |
|---|---|---|---|---|---|
| Istanbul-Beykoz | 41° 09' 26" | 29° 05' 54" | 180 | GD | TKA* |
| Akyazi-Dokurcun | 40° 37' 30" | 30° 51' 00" | 1300 | GD-GB | TM** |
| Aladag-Sarialan | 40° 39' 26' | 31° 46' 03" | 1650 | D | GKO*** |
| Kizilcahamam-Guvem | 40° 37' 08" | 32° 46' 31" | 1800 | D | GKO |
| Safranbolu-Safranbolu | 41° 20' 04" | 32° 36' 11" | 1700 | D | GKO |
| Pinarbasi-Kurtgirmez | 41° 34' 02" | 33° 12' 30" | 1150 | K | Natural forest |

*TKA, Nature Protection Area **TM: seed stand ***GKO, Gene Conservation Forest

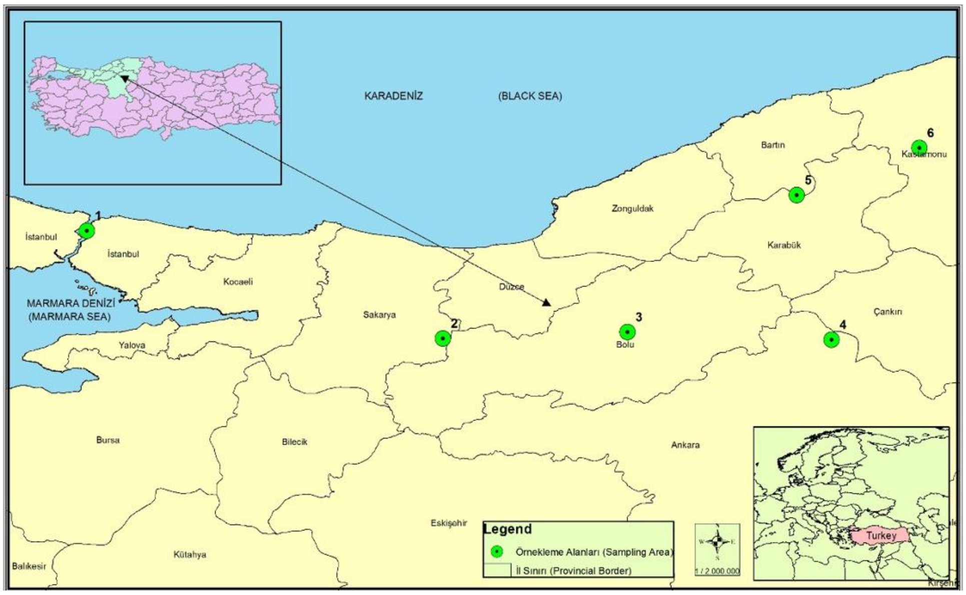


Figure 2. Location of sampled populations (1, Istanbul-Beykoz; 2, Akyazi-Dokurcun; 3, Aldag-Sarialan; 4, Kizilcahamam-Guvem; 5, Safranbolu-Safranbolu; 6, Pinarbasi-Kurtgirmez)

## Method

After selecting 20 families (trees) from each population (18 families from the Istanbul-Beykoz population), twenty cones from each family were collected between September 15 and October 20. The total number of families collecting cones from six populations was 118. The trees from which the cones would be collected were considered at least 100 m away from each other (approximately twice the seed drop distance), the altitude difference between the trees was at most 300 m, and the tree ages were close to each other. After the collected cones were placed separately in labeled bags containing the necessary information for each tree, the mouths of the bags were opened under room conditions, and the bags were aerated. Cones were threshed (mixed) daily against mold and spoilage, and seeds were obtained on a family basis. Before starting the measurements, the seeds were blown, and the empty and rotten ones were removed.

A digital caliper was used for seed size measurements with 0.01 mm sensitivity. The dimensions of the seeds extracted from 20 cones collected from each tree and a total of 118 x 20=2360 cones were measured, and the weight of 1000 seeds was found for 118 trees. The length of the seeds for each tree was measured in millimeters, its width was measured in millimeters from its widest point and perpendicular to its long axis, and its thickness (mm) was measured from the thickest part of the seed. 1000 seed weight was calculated using ISTA (2017).

The collected data were analyzed in SPSS 16.0 statistical package program. If the differences between the groups were statistically significant in the analysis of variance, the Student Newman Keuls (SNK) test was applied to determine different groups. In addition, correlations between characters and characters with altitude, latitude, and longitude were estimated. Lastly, the similarity tree was created using the furthest neighborhood method after the Z transform to the characters.

The following statistical model used for variance analysis.

$$y_{ij} = \mu + P_i + e_{ij}$$

Where $y_{ij}$; is observation in $j^{th}$ *tree*, on the $i^{th}$ population, $\mu$; general mean, $P_i$ is $i^{th}$ population ($i$= 1, 2…, 6), $e_{ij}$ is experimental error.

**Results and Discussion**

The seed width of six Western Black Sea Fir populations ranged between 5.32-8.02 mm, and the average was 6.63 mm. In the same order, seed length 10.65-14.01 mm and 12.06 mm, seed thickness 2.93-4.05 mm and 3.46 mm, and 1000 seed weight 41.45-132.75 g and 82.80 g was found (Table 2). On the other hand, the coefficients of variation varied between 6% and 18%. Turna et al. (2010) reported 5.81 mm, 11.35 mm, and 3.88 mm for seed width, length, and thickness, respectively. Similarly, 6.51 mm, 11.99 mm, and 3.41 mm for Western Black Sea fir were found by Velioğlu et al. (2012). Our research findings were similar to Turna et al. (2010) and Velioğlu et al. (2012); however, the seed width and length were high, albeit slightly. Similarity can be seen in 1000 seed weights, 82,95, and 82.13 g, as found by Turna et al. (2010) and Velioğlu (2012).

Table 2. Basic statistics of seed traitss

| Characters | N | Ortalama±se* | Standard deviation | Coefficient of variation (%) | Min. | Max. |
|---|---|---|---|---|---|---|
| Seed width (mm) | 118 | 6,63±0,4 | 0,44 | 7 | 5,32 | 8,02 |
| Seed length (mm) | 118 | 12,06±0,07 | 0,72 | 6 | 10,65 | 14,01 |
| Seed thickness (mm) | 118 | 3,46±0,02 | 0,22 | 6 | 2,93 | 4,05 |
| 1000 seed weight (g) | 118 | 82,80±1,36 | 14,78 | 18 | 41,45 | 132,75 |

*se, standard error

The Istanbul-Beykoz populations at the lowest elevation were the highest 1000 seed weight (Figure 3). When the Istanbul-Beykoz population was excluded, the 1000 seed weight of the Pinarbasi-Kurtgirmez population at the lowest elevation (1150 m) was the lowest 1000 seed weight. Except for the Beykoz population, which is at a very low altitude compared to the distribution altitude of the species, an increase in 1000-seed weight was observed as the altitude increased. However, the 1000 seed weight in the Kızılcahamam-Guvem population (1800 m) with the highest elevation reversed this trend and approached the Akyazi-Dokurcun population at the lowest elevation. However, Istanbul-Beykoz, an isolated (island) population, has the highest 1000 seed weight; there was no trend with altitude and 1000 seed weight of Western Black Sea Firs.

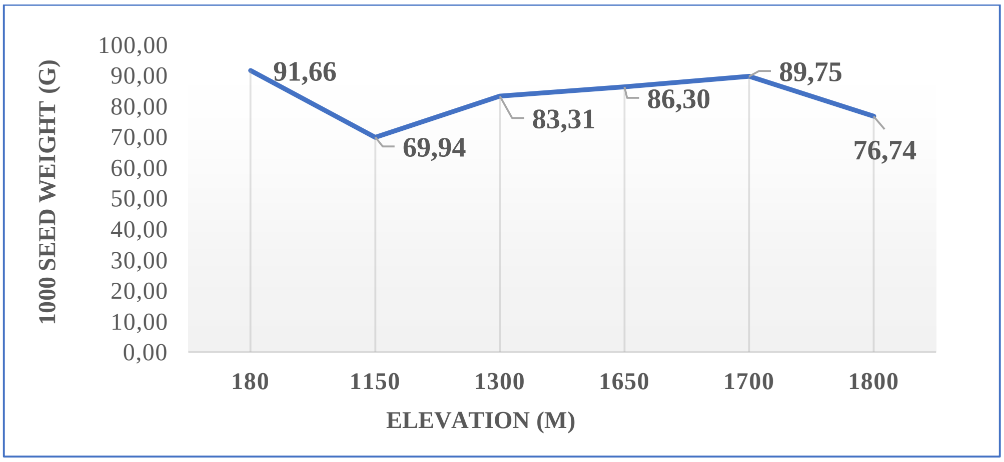


Figure 3. The changing 1000 seed weight with elevation

When the seed sizes were evaluated by the elevation (Figure 4), Pinarbasi-Kurtgirmez had the highest value in seed width, and Akyazi-Dokurcun and Istanbul-Beykoz populations had the lowest value. However, there is no apparent trend in seed width concerning elevation. A trend in seed thickness and length related to altitude was also observed.

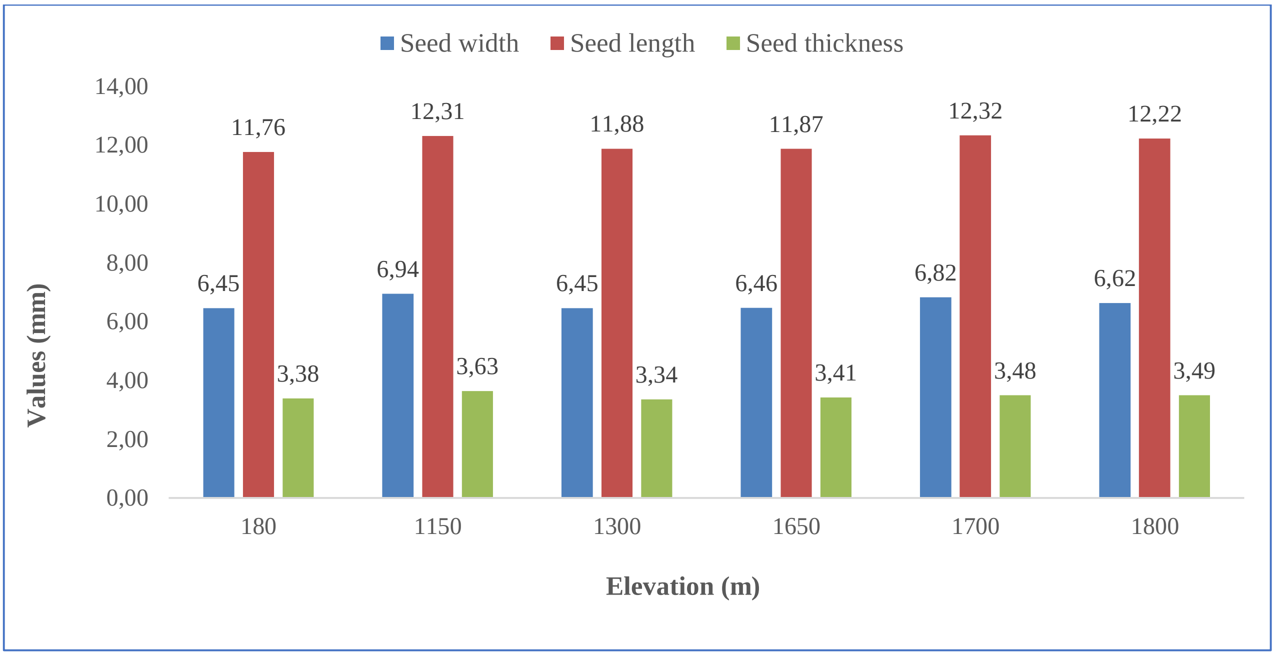


Figure 4. Altitude versus seed of width, length and thickness

In the analysis of variance at the population level for the four characters, statistically significant differences were found in seed width, seed thickness, and 1000 seed weight, while the difference was statistically insignificant for seed length (Table 3).

Table 3. ANOVA for seed characters for populations

| Sources of variation | Degree of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Seed width | Seed lengt | Seed thickness | 1000 seed weight |
| Population | 5 | 0,88*** | 1,24$^{ns}$ | 0,21*** | 1334,35*** |
| Error | 112 | 0,16 | 0,54 | 0,04 | 168,63 |

ns, non-significant; ***, p>0.001

Different groups were determined by using the SNK multiple comparison test for characters where differences between populations were significant (Table 4). Although the groups were intertwined, the Pinarbasi-Kurtgirmez population reached the highest values regarding seed sizes, while the same population had the lowest 1000 seeds weight. Seed characteristics can be decisive regarding species differentiation. In this context, Velioğlu et al. (2012) studied the seed characteristics of the fir species distributed in Turkey, showing that a species-based distinction could be made according to the seed characteristics. Kaliniewicz et al. (2019) also found that 11 fir species differed primarily in seed length and weight. In addition to species, for seed length, width, thickness, and 1000 seed weight, high statistical differences were revealed in 20 populations of *Pinus wallichiana* (Rawat and Bakshi, 2011), and Wu et al. (2018) also found significant differences in seed length, width, and mass in 11 populations of *Euptelea pleiospermum*. Therefore, it is claimed that populations can reflect their differences, including their geography, climate, and genetic structures, to seed characteristics (Morgenstern, 1996; Ducci et al., 2012; Dixit and Kolb, 2020; Dixit et al., 2020)

Tablo 4. SNK multiple comparison test for populations

| Populations | Seed width | Seed lengt | Seed thickness | 1000 seed weight |
|---|---|---|---|---|
| Pinarbasi-Kurtgirmez | **6,94 a** | 12,30 | **3,60 a** | 69,93 c |
| Safranbolu-Safranbolu | 6,82 ab | 12,32 | 3,48 ab | **89,75 a** |
| Kizilcahamam-Guvem | 6,62 bc | 12,22 | 3,49 ab | 76,74 bc |
| Aladag-Sarialan | 6,46 c | 11,87 | 3,41 b | 86,30 ab |
| Istanbul-Beykoz | 6,45 c | 11,75 | 3,38 b | **91,66 a** |
| Akyazi-Dokurcun | 6,45 c | 11,88 | 3,34 b | 83,31 ab |

Populations showed different attitudes related to each seed character. The Akyazi-Dokurcun population had low values in terms of seed size; however, it was in the middle with a 1000-seed weight. Istanbul-Beykoz and Safranbolu-Safranbolu populations reached the highest 1000-seed weight. The fact that these two populations are in a different order regarding seed size indicates no significant relationship between seed size and 1000-seed weight. There may be various reasons for the current study's high or low 1000 seed weight in populations. For example, Keskin and Şahin (2000) found that the 1000 seed weight in Taurus fir was 139.1 g in a poor seed year and 226.0 g in a mass seed year. Therefore, common garden tests can be better for supporting the seed characteristics of populations, although the findings show population differences in seed character.

On the other hand, seed weight is an essential life-history trait with effects on offspring phenotype and ultimately on fitness over germination, early growth, survival, abiotic stress tolerance, and biotic resistance for coniferous (Sorensen and Campbell, 1993; Wennstromet al.,2002; Parker et al., 2006; Blade and Vallejo, 2008; Zas and Sampedro, 2015; Chen et al., 2022). In this context, Beykoz and Safranbolu populations have more advantages in adaptation. These findings on seed weight supports to assigned as the natural reserve of Istanbul-Beykoz and the gene conservation forest of the Safranbolu-Safranbolu population.

The populations were subjected to the analysis of variance in terms of aspect, and there was no significant difference between the populations in terms of seed length. In contrast, a high level (p>0.001) statistical difference was observed between the populations in the other three characteristics (Table 5).

Tablo 5. ANOVA for seed characters for aspects

| Sources of variation | Degree of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Seed width | Seed lengt | Seed thickness | 1000 seed weight |
| Aspect | 3 | 1,041*** | 1,51$^{ns}$ | 0,322*** | 1699,599*** |
| Error | 113 | 0,168 | 0,63 | 0,041 | 181,604 |

ns, non-significant; ***, p>0.001

SNK multiple comparison tests were performed for aspects using seed characteristics (Table 6). In this comparison, the sunny aspects (SE-SW, E, SE) seed sizes are smaller than the shadow aspects (N). In contrast, the sunny aspects (SE-SW, E, SE) 1000 seed weights were higher than the shadow aspect (N). However, while there are three populations in the sunny aspect of our study, there is one in the shadow aspect. On the other hand, Demir (2019) also reported that the seeds of the populations in the sunny aspect were heavier than in the shadow aspect in Juniperius excalsa M.Bieb. Therefore, the study showed Western Black Sea Firs produced heavier seeds in sunny aspects.

Tablo 6. SNK multiple comparison test for aspect of populations

| Aspect | Seed width | Seed Lengt | Seed thickness | 1000 seed weight |
|---|---|---|---|---|
| N | **6,94 a** | 12,30 | **3,63 a** | 69,93 b |
| SE-SW | 6,44 b | 11,87 | 3,34 b | **83,31 a** |
| E | 6,63 b | 12,13 | 3,46 b | **84,26 a** |
| SE | 6,45 b | 11,75 | 3,38 b | **91,65 a** |

N, North; SE, Southeast; SW, Southwest; E, East; SE, Southeast

The correlations between geographical factors (latitude, longitude, and altitude) with the four seed characters and among characters are given in Table 7. While there was a statistically significant and moderate correlation between seed width and seed length (0,58) and between seed width and seed thickness (0,61), and between seed length and seed thickness (0,57), other correlations were low despite sometimes being statistically significant. In this context, there were moderate and low correlations among characters and between characters and the geographic conditions of the population. Rawat and Bakshi (2011) also found low and statistically insignificant correlations between seed characters (seed length, width, thickness, and 1000 seed weight) and locations (altitude, latitude, and longitude). However, Kaliniewicz et al. (2019) found a similar correlation between characters like our study.

Tablo 7. Correlations with geographical factors (altitude, latitude and longtitute) and among characters

| Characters | Seed width | Seed Lengt | Seed thickness | 1000 seed weight |
|---|---|---|---|---|
| Seed width | | | | |
| Seed length | 0,58$^{**}$ | | | |
| Seed thickness | 0,61$^{**}$ | 0,57$^{**}$ | | |
| 1000 seed weight | 0,06$^{ns}$ | 0,25$^{**}$ | 0,18$^{*}$ | |
| Altitude | 0,11$^{ns}$ | 0,17$^{ns}$ | 0,09$^{ns}$ | -0,13$^{ns}$ |
| Latitude | 0,32$^{**}$ | 0,14$^{ns}$ | 0,26$^{**}$ | -0,03$^{ns}$ |
| Longtitude | 0,36$^{**}$ | 0,29$^{**}$ | 0,36$^{**}$ | -0,35$^{**}$ |

ns, non-significant; *, 0.05; **, 0.01,

The similarity tree (cluster), which was created by using the characteristics observed in the study (seed width, length, thickness, and 1000 seed weight), was first divided into two groups (Figure 5). Aladag-Sarialan, Akyazi-Dokurcun, and Istanbul-Beykoz populations were included in the first group, while the second group consisted of Safranbolu-Safranbolu, Kizilcaham-Guvem, and Pinarbasi-Kurtgirmez. While the first group populations are located very close to each other for similarity, the second group is separated into the cluster. When the cluster was associated with the geographical location, it was seen to be divided into the "western group" (Aladag-Sarialan, Akyazi-Dokurcun, and Istanbul-Beykoz), and the "eastern group" (Safranbolu-Safranbolu, Kizilcahamam-Guvem, and Pinarbasi-Kurtgirmez). The isolated population (Istanbul-Beykoz) was in the western group and showed similar seed characteristics with Aladag-Sarialan and Akyazi-Dokurcun. It is seen that the genetic distances of the western group were closer to each other and, therefore, more similar to each other than the eastern group. In addition, these results showed that the Istanbul-Beykoz population, which has an isolated population, may have been detached from the western group or could have been artificially transported. Due to the threats, endemism, and geographically scattered distribution, conserving Mediterranean firs and their genetic resources is a major challenge (Alizoti et al., 2011). In this context, our findings showed that seeds should not be transferred between the west and east group and used local seed sources. At the same time, our findings support before studies on gene conservation forests (Aladag-Sarialan, Kizilcahamam-Guvem, and Safranbolu-Safranbolu) and the natural reserve, Istanbul-Beykoz.

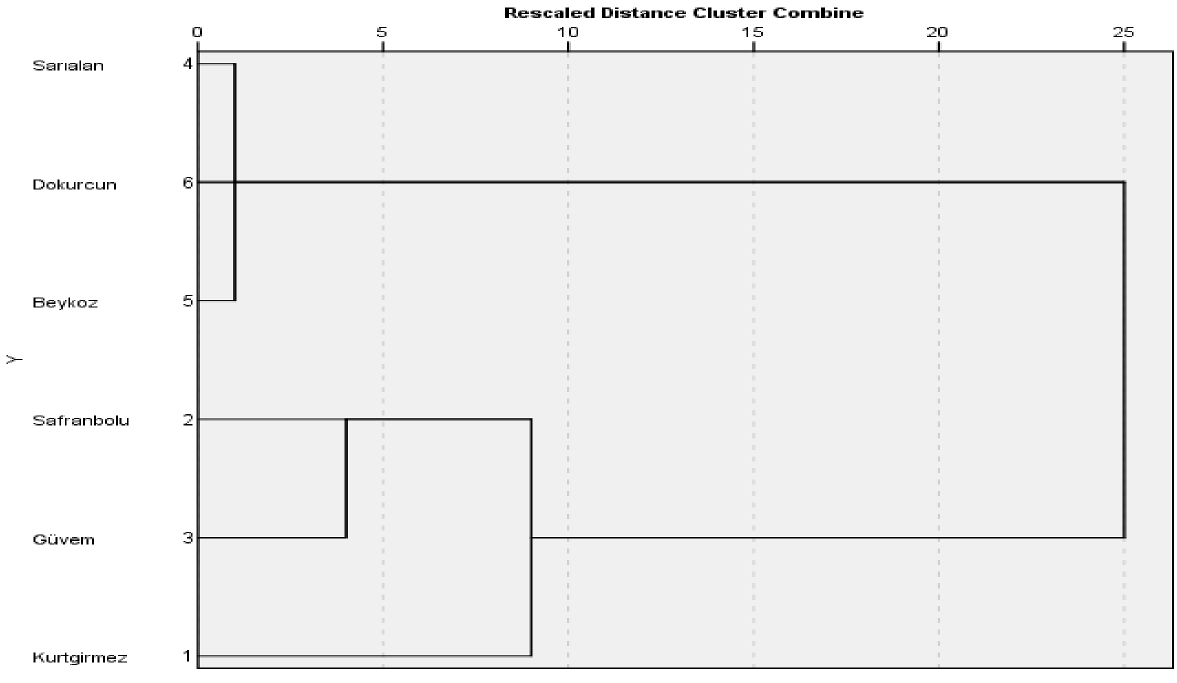


Figure 5. Similarity tree (cluster) between populations using all characters

## Conclusion

Populations of Western Black Sea Fir, which is an endemic species, showed highly significant differences in the studied seed characteristics except for seed length. While the populations in the sunny aspects reached lower values in seed size, 1000 seed weights were higher. Although the isolated population reached the highest 1000 seed weight, it was included in the lower populations in terms of seed size (seed width and seed thickness). There was no significant trend in seed characteristics regarding altitude, latitude, and longitude, and the correlations between altitude and seed characteristics were also low.

The studied populations were divided into two main groups in terms of genetic distance. Naming "East" and "West" groups is possible considering the distribution areas of these groups. The genetic distances of the populations in the west group were closer (similar) compared to the east group. In this context, the Istanbul-Beykoz population, which has an isolated population, was also cut off from the West group and showed that artificial migration was possible.

Considering that the research was based on a one-year observation, the study on six Western Black Sea Fir populations showed evidence that no seed transfer should be made between the west and east group or vice versa. In this context, the closest seed resources should be used in regeneration and forestation studies. Moreover, supporting seed characteristic findings with the common garden tests can be better in long-term.

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