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Grouping of Scots Pine (*Pinus sylvestris* L.) Seed Stand Populations in Western Blacksea Region of Turkey by Seedling Morphological Distance

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Abstract: Nine populations of Scots pine, sampled from Western Blacksea Region, were grouped by seedling morphological distance and cluster analysis in this study. For that purpose, number of cotyledon, lengths of cotyledon, epycotyl and hypocotyl and height, root-collar diameter, number of branch, stem and root dry weights were measured on two-month old, and one-year old seedlings chosen randomly. The differences among the populations for the characteristics of one-year old seedlings were larger than two-months old seedlings. For instance, while epycotyl length varied between 11.9 and 15.3 mm (difference was 11%), stem dry weight varied between 164.3 and 256.6 mg (56%) among populations. Morphological distance among populations varied between 0.297 and 5.288. In addition, populations were gathered two main groups which are Ankara-Yenice, Kastamonu-Ballıdağ and Adapazari-Dokurcun and the others and three sub groups according to cluster analysis. Results of the study could be used in breeding and afforestation of the species such as preparation of gene maps, determination of breeding populations, gene conservation areas and seed sources.

Key words: *Pinus sylvestris*, breeding, seed sources, penrose, cluster analysis, seedling morphology

INTRODUCTION

Genetic variation is the fundamental component, which ensures survival and thus the stability of forest ecosystems as its quantity and quality determines the potential of population to adapt the changing in environmental condition. This is particularly important with changing populations and climatic condition and when the long-term stability of forest ecosystems is increasingly threatened by environmental stress. Thus, a genetic characterization of natural forest resources is the first step necessary for a better understanding of genetic resources for implementation of insitu and exsitu conservation activities^[1].

It is generally recognized that forest tree species, especially conifers, are characterized by a considerable variation, both across their native range and from tree within the stands. This potential reflects a mechanism of the adaptive strategy specific for these typically long-lived organism, which mostly respond with a wide genetic variation to environments heterogeneous in site and time^[2].

Scots pine (*Pinus sylvestris* L.) is the most widely distributed member of the family Pinaceae in the world.

Scots pine extends from 8°W in Spain to 141°E in Siberia and extends from 37°N in Turkey to 70°N in Norway, Sweden and Finland^[3]. Throughout much of its native range, Scots pine is an important commercial forest tree in Turkey, widely planted for industrial and conservation forestry systems such as soil erosion and living snow-fences. The southern boundary of its geographical distribution lies at around 38°34'N latitude. Scots pine occupies approximately 738 000 ha in Turkey, growing in the Blacksea coastal mountains on warm southern slopes, where the climate is humid. In the inner mountains, which are also parallel to the Black Sea coast, the tree species grows on humid northern slopes. On the eastern coast of the Black Sea, it is found at altitudes of 2000-2700 m. On the western region, it ranges from 1200 to 1600 m. In exceptional cases, it can be found from sea level (Trabzon-Çamburnu) to the alpine zone^[4]. Environmental conditions vary strongly within its distribution range. Scots pine is an important component of subalpine ecosystems of Blacksea region in Turkey because of its role in watershed protection, its function natural habitat for wild life, also, because of its esthetic values. It has, therefore, great importance especially in Blacksea forestry like oriental spruce.

Scots pine is one of the important forest tree species both Turkish Forestry and breeding program^[5]. Although it has twenty seed orchards in 111.3 ha and 36 seed stands in 4878 ha^[6], 91.0 % of seed demand is covered from seed stands for afforestation in the species^[7]. Seed stand is the most important seed source in the species at this stage. Determination of similarity/differences among populations/seed stands is an important stage of breeding programs. It should known to obtain maximum variability among genotypes and minimum loss of genetic diversity in current generation. It is also important for sustainable forestry and to transmit the current gene diversity to next generation by afforestation with suitable seed source (i.e. provenance trial). Besides, it can be used to determination of breeding populations and gene conservation areas and for establishment of seed orchards in the species. Morphological distance can play important role for the purposes.

Researchers have analyzed phenotypical and genetic traits of trees in *P. sylvestris* populations. Some of these studies were fragmentary; some were more intensive^[8,9]. However, the genetic variation of *P. sylvestris* populations in Turkey has not been studied so far, particularly using morphological and other analysis. In this study; some populations of Scots pine in Western Black Sea Region were grouped to contribute breeding, afforestation, artificial regeneration, gene conservation and nursery practice of the species.

MATERIALS AND METHODS

Seed collection and sowing: Open pollinated seed materials from nine different populations of *Pinus sylvestris*, collected from Western Black Sea Region, were provided by Forest Tree Seeds and Tree Breeding Research Directorate in Turkey. Locations of the populations are indicated in Table 1. While some seeds were sown at seed beds at 0.5 cm depth 10 cm x 1 cm spacing (250 seeds m⁻²) with five replications in a greenhouse (For cotyledon number and length, epycotyl and hypocotyl length). Seeds were sown to river sand, forest soil and peat in 1:1:2 proportion, respectively and covered by perlite, the other seeds were sown by using conventional methods at Forest Nursery in Taşköprü-Kastamonu (Altitude: 1160 m) with six replications (For seedling height, root-collar diameter, number of branches and stem-root dry weight). The seed density was 500 m⁻² in the nursery conditions.

Seedling morphological variables studied and data collection: While Cotyledon Number (CN), Cotyledon Length (CL), Epycotyl Length (EL) and Hypocotyls

Length (HL) were measured from ninety seedlings, chosen randomly, which were two-month old and obtained from the greenhouse, Seedling Height (SH), Root-collar Diameter (RCD), Number of Branches (BN) and Stem Dry Weight (SDW) and Root Dry Weight (RDW) were measured from ninety seedlings, chosen randomly, which were one-year old and obtained from the nursery. The dry weights were determined after samples were oven-dried at 105°C for 24 h.

STATISTICAL ANALYSES

Collected data was standardized⁽¹⁾ before the calculations^[10,11], and the seedling morphological distance⁽²⁾ among populations were estimated as^[10-12]:

$$Z_{i,k} = \left[\frac{X_{i,k} - X_{aver}}{S_k} \right] \quad (1)$$

Where, $Z_{i,k}$ is standardized values of the k^{th} characteristics of the i^{th} population, $X_{i,k}$ is original average of the k^{th} characteristics of the i^{th} population, X_{aver} is original average of the studied populations for the k^{th} characteristics and S_k is the standard deviation of the studied populations for the k^{th} characteristics.

$$D_{i,j} = \sum_{k=1}^n \frac{[\mu_{k,i} - \mu_{k,j}]^2}{nV_k} \quad (2)$$

Where, $D_{i,j}$ is the morphological distance between the i^{th} population and the j^{th} populations, n is the number of studied characteristics, $\mu_{k,i}$ is the standardized values of the k^{th} characteristics of the i^{th} population, $\mu_{k,j}$ is the standardized values of the k^{th} characteristics of the j^{th} population, V_k is the variance of standardized averages of the k^{th} characteristics.

Morphological distances, obtained from Penrose's equation^[10-12], can play important role for the purposes. The different characteristics that have different units are pooled while calculations in morphological distances among populations. It is an important advantage to analysis of variance. When morphological distance, obtained the Penrose equation, approach to zero, the similarity among populations increases^[11].

Seedling morphology is a more recent approach, where very early developmental stages show appropriate stability. The advantage of using seedling morphology stems from their uniformity at the juvenile stages, before they are subjected to the diversity of factors that prevail in the case of mature plants^[13].

Hierarchical Cluster analysis was applied by standardized values in SPSS statistical package program^[14].

Table 1: Description of the studied populations

Population Code	Population name	Altitude (m)	Latitude (N)	Longitude (E)	Aspect
P1	Ankara-Yenice	1500	41°02'	33°47'	NW
P2	Ankara-Ulupinar	1550	40°53'	32°20'	S
P3	Ankara-Benliyayla ₁	1550	40°31'	32°08'	SE
P4	Adapazari-Dokurcun	1400	40°37'	30°50'	S
P5	Bolu-Aladağ	1400	40°38'	31°41'	W
P6	Kastamonu-Ballıdağ	1300	41°34'	33°19'	NW
P7	Kastamonu-Sarıçam	1250	41°22'	33°28'	Various
P8	Ankara-Eğriova	1550	41°26'	32°02'	Various
P9	Ankara-Benliyayla ₂	1550	40°31'	32°08'	N

Table 2: Averages of the studied seedling characteristics and pooled standard deviation.

Pop. Code	CN	CL (mm)	EL (mm)	HL (mm)	SH (mm)	RCD (mm)	BN	SDW (mg)	RDW (mg)
P1	6.4	23.3	11.9	15.0	32.7	1.4	1.3	164.3	147.6
P2	6.2	23.2	12.7	15.2	32.8	1.5	1.4	189.2	182.1
P3	6.6	24.6	13.4	15.9	39.3	1.6	1.6	212.9	172.3
P4	6.6	25.4	13.8	16.7	37.5	1.6	2.2	256.6	206.2
P5	6.8	24.5	12.8	14.6	38.4	1.6	1.8	217.7	178.1
P6	6.8	24.6	14.2	15.8	33.8	1.4	1.5	167.9	146.3
P7	6.6	22.4	12.1	15.1	34.8	1.5	1.5	225.1	186.8
P8	7.0	25.7	15.3	18.0	41.8	1.6	1.5	213.0	173.3
P9	6.8	25.3	15.0	16.8	39.2	1.5	1.7	206.3	167.5
Mean	6.6	24.3	13.5	15.9	36.7	1.5	1.6	205.9	173.4
St. dev.	0.24	1.13	1.21	1.09	3.28	0.08	0.27	28.8	18.7

Table 3: Morphological distances among populations

Pop. Code	P1	P2	P3	P4	P5	P6	P7	P8
P2	1.647	-						
P3	1.453	1.342	-					
P4	1.482	3.717	2.838	-				
P5	1.510	2.027	0.297	2.553	-			
P6	0.473	1.646	0.761	0.997	0.816	-		
P7	1.741	1.600	1.509	2.750	1.644	1.679	-	
P8	2.127	2.448	3.499	5.288	2.578	3.340	3.509	-
P9	1.251	0.786	0.990	2.816	0.802	1.305	0.810	2.439

RESULTS AND DISCUSSION

Morphological variables: As the differences among the populations for the characteristics of one-year old seedlings were larger than that of two-months old seedlings. For instance, while EL varied between 11.9 mm (P1) and 15.3 mm (P8), difference was 11% SDW varied between 164.3 mg (P1) and 256.6 mg (P4), 56% among populations (Table 2).

Population of Ankara-Eğriova (P8) showed the highest performance for CN, CL, EL, HL, SH and RCD, while population of Adapazari-Dokurcun (P4) showed the highest performance for BN, SDW and RDW. Performances of Kastamonu populations (P6 and P7) were similar for the studied characteristics except of dry weights (Table 2). This result showed that studied characteristics were important factor on determination of similarity of populations.

Morphological distance and grouping : Populations of Ankara-Benliyayla₁ and Bolu-Aladağ were the most similar ($D_{P3, P5} = 0.297$), populations of Adapazari-Dokurcun and Ankara-Eğriova were the most different ($D_{P4, P8} = 5.288$) to each other (Table 3). Such a high D

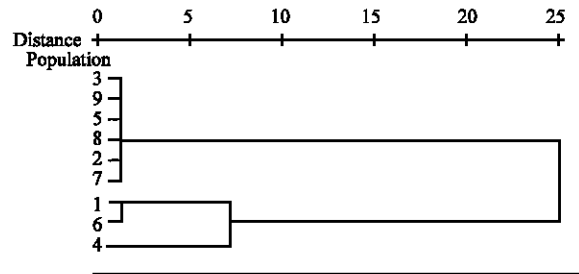


Fig. 1: Dendrogram of hierarchical cluster analysis

value might be the result from varying local ecological conditions. Although populations of Ankara-Benliyayla (P3 and P9, $D_{P3, P9} = 0.990$) were from the same district (Table 1), Ankara-Benliyayla₁ population was to most similar to Bolu-Aladağ population ($D_{P3, P5} = 0.297$, Table 3). Turna^[15] reported that it is hard to see apparent relations and their geographic locations. For example, while Sarıkamış and Şenkaya are geographically close to each other but their genetic distance is far apart from each other. This result showed that seeds from Bolu-Aladağ population could be used as a seed source instead of Ankara-Benliyayla₁ for the similar afforestation area, when enough seeds were not collected from Ankara-Benliyayla₁

population. So, similarity between Ankara-Benliyayla₁ and Bolu-Aladağ populations ($D_{P3, P5} = 0.297$) was more than three times to similarity between Ankara-Benliyayla₁ and Ankara-Benliyayla₂ ($D_{P3, P9} = 0.990$, Table 3). It was also valid for Kastamonu populations (P6 and P7, Table 3).

It could be explained that large genetic diversity and geographic variation among populations even they were from the same district. The similar results were found by Yahyaoglu *et al.*^[16] on *Cedrus libani* A rich. Classifications of genotypes were studied detail by different distances (i.e. Manhattan distance, Eucladian distance) by Crossa and Franco^[17].

Similarity among populations were shown in Fig. 1 by Hierarchical Cluster Analysis^[14]. According to results of the cluster analysis, Ankara-Yenice (P1), Kastamonu-Ballıdağ (P6) and Adapazari-Dokurcun (P4) populations were in the main same group and the other populations were in another main same group. When seen sub-group it was evident that Adapazari-Dokurcun (P4) population was very different than the others (Fig. 1). It could be because of its longitude (Table 1). Results of the cluster analysis (Fig. 1) were well accordance with morphological distances (Table 3). For instance, morphological distances of Ankara-Ulupinar (P2), Ankara-Eğriova (P8) and Ankara-Benliyayla₂ (P9) populations to Adapazari-Dokurcun (P4) were the highest ($D_{P2, P4} = 3.717$, $D_{P8, P4} = 5.288$, $D_{P9, P4} = 2.439$ and Table 3) than the others. It can be suggested that all populations, especially Ankara-Eğriova (P8) and Adapazari-Dokurcun (P4) populations, be considered for a gene conservation program. Also, future studies are necessary to provide deeper insights in to the subject.

These results could be used in preparation of gene map, seed transfer zones, determination of breeding populations, gene conservation areas, geographic variation and resulting of provenance trials of the species in short period. Preparation of forest gene maps and determination of seed transfer zones and geographic variation by morphological distance were also suggested by Yahyaoglu *et al.*^[16].

It may be concluded from the present study that studied characteristics were the important factors on morphological distance among populations and grouping of populations. There was a large genetic and geographic variation among the populations even they were at the adjacent district. Morphological distance among populations could be taken into consideration in silvicultural purposes (afforestation, artificial regeneration) and breeding strategies (i.e. determination of breeding populations, gene conservation areas, seed transfer zones, seed source and geographic variation, resulting of provenance trial; establishment of seed orchards) of this species.

Generally, our results show that large genetic diversity exists in *P. sylvestris* to explain its great ecological plasticity and evolutionary.

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