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VARIATION OF SOME MORPHOLOGICAL AND ELECTROPHORETIC CHARACTERS OF 11 POPULATIONS OF SCOTS PINE IN TURKEY

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ABSTRACT

In this study, the genetic variation in populations of Scots pine (*Pinus sylvestris* L.), one of the species covering large areas in Turkey, was investigated. Open pollinated seeds were collected from 11 populations in natural distribution range. Six seed (length, width, ratio of length to width, weight/1000 seeds) and seedling (cotyledon number and hypocotyls height) characters and two enzyme systems (Leucine aminopeptidase, LAP; Gulutamate oxaloacetate transaminase, GOT) were investigated. Significant differences were detected among the populations for the morphologic characters. In addition, isozymes patterns of two enzyme systems revealed that Lap has two loci (one with 2 alleles and the other 3) while Got has three loci (two with 3 alleles and third one with 2 alleles). Polymorphic loci were 80% on the average. The mean number of alleles per loci was 2.02 and expected heterozygosity was 22%. The mean total genetic diversity was calculated 0.235; the mean gene diversity within populations was determined as 0.220 and the average between subpopulations diversity were found to be 0.014. Averaged genetic distance was 0.081. According to genetic distance, population number 2 was separated from the remaining populations.

Keywords: *Pinus sylvestris*, genetic diversity, isozymes, endosperm, seed, seedling

INTRODUCTION

Genetic variation is the fundamental component of adaptation and thus, of stability of the forest ecosystems. This is particularly important when the long-term stability of forest ecosystems is increasingly threatened by environmental stress and mismanagement. Thus, a genetic characterization of natural forest resources is an essential step for a better understanding of genetic resources for the implementation of *in-situ* and *ex-situ* conservation activities.

It is generally recognized that forest tree species, especially conifers, are characterized by a considerable variation, both across their native range and from tree to tree within stands. This potential reflects a mechanism of the adaptive strategy specific for these typically long-lived organisms, which mostly show a wide genetic variety to environments heterogeneous in site and time (Bergmann, 1975).

Electrophoresis techniques have come to be used routinely in the study of variations in enzyme systems, and they have been instrumental in determining the origin of populations of unknown ancestry. The allozyme variation expressed as the differences found in the allelic frequencies, is used to characterize the different populations under study.

Scots pine (*Pinus sylvestris* L.*)* is the most widely distributed member of the family Pinaceae in the world. The longitudinal range of Scots pine covers over 14 000 km and extends from 8^{o} W in Spain to 141^{o} E in Siberia. The latitudinal range of Scots pine covers over 3 700 km and extend from 37^{o} N in Turkey to 70^{o} N. The species covers a diverse array of physiographic regions. 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 in Turkey along Kayseri direction at 38° 34' N latitude. Scots pine occupies about 738 000 hectares in Turkey, growing in the Black Sea coastal mountains on warm southern slopes where the climate is humid. In the inner mountains, which are also parallel to the Black Sea coasts, 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) to Alpine zone. Within its distribution,

Scots pine can grow in absolutely different ecological conditions. Such a wide range of ecological conditions could favour the formation of variety of ecotypes.

Till recently, researchers analysed phenotypical and genetical traits of trees in *P. sylvestris* populations. Some of these studies were fragmentary and some of more intensively (Gullberg *et al*., 1985; Goncharenko *et al*., 1994). However, the genetic variation of *P. sylvestris* populations in Turkey has not been studied so far, particularly using morphological and electrophoretic analysis. This paper presents results on geographic variation in *P. sylvestris,* using conventional electrophoretic analysis and investigates its genetic structure, levels of variation and seed and seedling characters.

MATERIALS AND METHODS

The seed material from 11 different populations of *Pinus sylvestris* was provided by Forest Seeds and Tree Improvement Research Directorate. Bulked seeds for each population were collected from already established seed stands whose areas range from 7.5 ha. to 150 ha. As such, the number of trees per population is at least well over 100 different trees. The geographic distribution of Scots pine in the world (William and Elbert, 1966) and locations of studied populations in Turkey are indicated in Fig. 1 and Table 1.

Morphological characters: The seed length and width were studied using biometrical methods (compass and millimetres paper) on 50 randomly selected seeds from each population. An additional characteristic was derived from the ratio of length to width. In determining the seed weight, the seeds were taken randomly and divided into 4 replications (100 x 4 = 400). Seed samples were sown in trays containing soil. Seeds were considered germinated when the radicle extended at least 1 mm beyond the seed coat. Hypocotyls height was measured and number of cotyledons counted in 50 seedlings

per population.

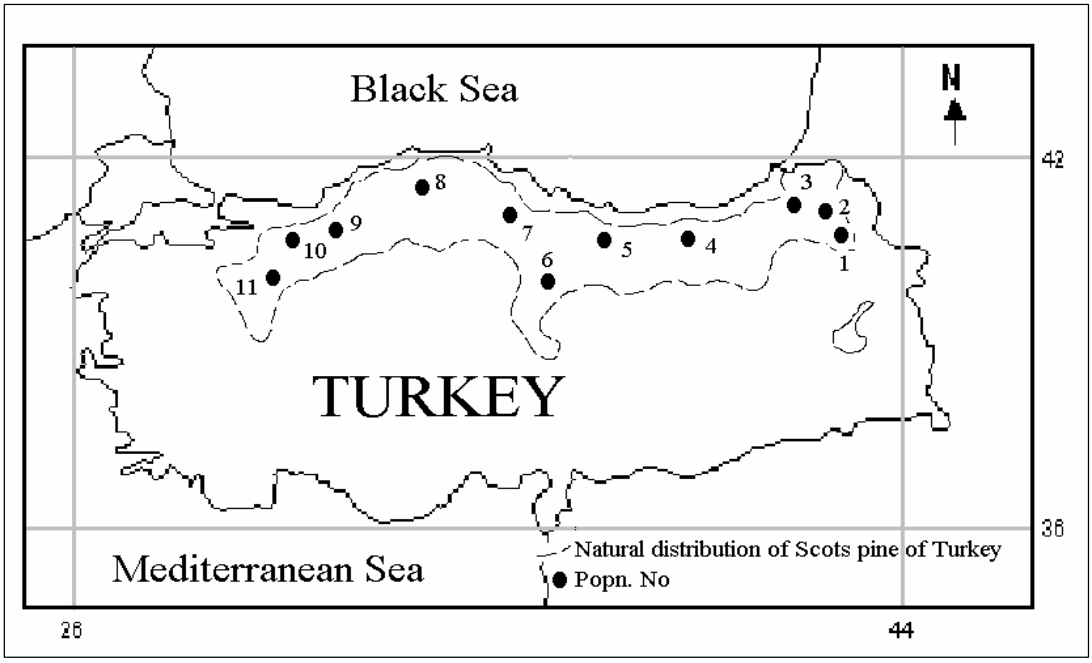


Figure 1. Geographical distribution of the Scots pine and location of study area in Turkey.

Table 1. Description of the studied populations

Popn. N^{o}	Population Names	Altitude (m)	Latitude (N)	Longitude (E)	Aspect
1	Sarıkamış- Kars	2350	40° 18' 00"	42° 37' 30"	NW
2	Şenkaya – Erzurum	2250	40° 45' 20"	42° 30' 30"	W
3	Yusufeli – Artvin	2050	40° 45' 53"	41° 35' 13"	S
4	Gümüşhane – K. Dere	900	40° 19' 48"	39° 16' 00"	N
5	Mesudiye – Arpaalan	1650	40° 22' 45"	37° 52' 30"	N-NE
6	A.Madeni – Yozgat	1750	39° 34' 30"	35° 50' 00"	W-N
7	Vezirköprü – Samsun	1300	41° 10' 51"	35° 02' 13"	E
8	Ilgaz - Yenice	1500	41° 02' 40"	33° 47' 36"	W
9	Bolu – Aladağ	1400	40° 38' 00"	31° 41' 30"	W-SW
10	Akyazı – Adapazarı	1450	40° 37' 30"	30° 50' 00"	S
11	Çatacık – Eskişehir	1550	39° 58' 20"	31° 07' 30"	E

Electrophoretic Study*:* Extracts for isozyme analysis were obtained individually from 100 endosperms per population in order to obtain reliable estimates of allelic frequencies. The procedures for the seed preparation, gel preparation, slicing and staining method using techniques for horizontal starch gel electrophoresis in conifer seeds as described by Cheliak and Pitel (1984). The leucine aminopeptidase (Lap-E.C.3.4.11.1) and glutamate oxaloacetate transaminase (Got-E.C.2.6.1.1) isozyme systems were studied. The extraction buffer consisted of 0.08 M Tris-HCl, 3% PVP with 100 ml distilled water. The electrode buffer contends 0.19 M boric acid, 0.026 M lithium hydroxide, pH 8.2 and the gel buffer was 0.05 M Tris-HCl, 0.01 M citric acid and 10% electrode buffer, at pH 8.2.

Gels were run at 4 ^{o} C and constant amperage (80 mA) but were not allowed to exceed 156 V. The loci and the alleles within each locus were numbered in decreasing order of anodal mobility. The locus that specifies the isozyme with the smallest anodic migration was labelled as 1, running to the next as 2, and so forth. At each locus, alleles of different isozymes were further designated into numbers such as 1, 2, and 3 in order of decreasing mobility from the anode.

Statistical Analysis*:* One-way analysis of variance (ANOVA) was used to determine the genetic variation or diversity among the 11 populations. The varieties were quantified with Duncan Multiple Range Test at $P<0.01$ significance level. These statistical analyses were performed by means of SPSS 11. for windows. Analyses of variance were performed for each character studied.

Allozyme frequency data were used to calculate, on a population basis, single locus measures of genetic diversity. Several different statistics were calculated, including proportion of polymorphic loci (*P*), mean number of alleles per locus (*A*), mean expected heterozygosity (*He*), gene diversity within populations (*Hs*), total gene diversity (*Ht*),

among populations (*Dst*), relative degree of genetic differentiation (*Gst*) and genetic distance (*do*) were estimated using GSED computer program (Gillet, 1994).

RESULTS

The mean values of the seed and seedling characters by populations are shown in Table 2. The differences were statistically significant when the average values for the parameters were used in the morphometric characterization of the seed and seedling, except the seed length. Popn. N^{o} 10 (Akyazı-Adapazarı) had the widest seeds and popn. N^{o} 8 (Ilgaz-Yenice) with the narrowest seeds. Again popn. N^{o} 10 had the highest, and popn. N^{o} 2 (Şenkaya-Erzurum) had the lowest cotyledon numbers; popn. N^{o} 7 had the longest and popn. N^{o} 11 had the shortest hypocotyls length; popn. N^{o} 9 had the height and popn. N^{o} 4 had the lowest weight of 1000 seeds. Even though the parameters in this case may be considered dependent to each other, the technique would appear to be useful in the characterization of different populations.

Five loci were identified from two enzyme systems analysed, Lap and Got. They showed at most two and three loci in zymograms of the gametophytes, respectively. Two zones of Lap activity were observed. The first zone (Lap-1) exhibited two variants (allele); the first variant is a double band and the second has single band. The second zone (Lap-2) exhibited three single-banded variants (1, 2, 3).

Allele frequencies for five loci in 11 populations of *P. sylvestris* were calculated by GSED programme (Gillet, 1994) and are shown in Table 3. According to the allele frequencies of Got and Lap enzyme, alleles frequencies were not quite similar in different populations. From the table, allele 1 of Got-1 was found in popn. N^{o} 3, 6 and 7 with low frequencies. Allele 5 of Got-2 was also present in popn. N^{o} 6, 7 and 8, but not in other populations. Generally, the frequency of allele 1, at Lap-1, and the frequency of allele

2, at Lap-2 were very high in all populations. Similarly, the frequency of allele 2, at Got-1 and the frequency of allele 3, at Got-2 were relatively high in all populations. In popn. N^{o} 2, however, the frequency of allele 2, at Got-2 was higher than the frequency of allele 3, at Got-2 (Table 3.).

Table 3. Estimated allele frequencies and expected heterozygosity in Scots pine.

LOCUS / ALLELE		Population N^{o}										
		1	2	3	4	5	6	7	8	9	10	11
LAP-1	1	1.00	1.00	1.00	1.00	1.00	0.96	0.96	0.96	0.96	0.96	0.99
	2	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.04	0.04	0.04	0.01
Expected het. (He)		0.00	0.00	0.00	0.00	0.00	0.08	0.08	0.08	0.08	0.08	0.02
LAP –2	1	0.00	0.20	0.07	0.00	0.12	0.04	0.09	0.04	0.20	0.11	0.07
	2	0.93	0.80	0.92	1.00	0.80	0.87	0.75	0.91	0.76	0.89	0.88
	3	0.07	0.00	0.01	0.00	0.08	0.09	0.16	0.05	0.04	0.00	0.05
Expected het. (He)		0.13	0.32	0.15	0.00	0.34	0.23	0.40	0.17	0.38	0.20	0.22
GOT-1	1	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	2	0.99	1.00	0.99	1.00	0.92	0.98	0.93	0.96	1.00	1.00	1.00
	3	0.01	0.00	0.00	0.00	0.08	0.01	0.06	0.04	0.00	0.00	0.00
Expected het. (He)		0.02	0.00	0.02	0.00	0.15	0.04	0.13	0.08	0.00	0.00	0.00
GOT-2	2	0.25	0.72	0.14	0.36	0.20	0.19	0.15	0.09	0.36	0.27	0.31
	3	0.75	0.28	0.86	0.64	0.80	0.73	0.79	0.88	0.64	0.73	0.69
	5	0.00	0.00	0.00	0.00	0.00	0.08	0.06	0.03	0.00	0.00	0.00
Expected het. (He)		0.38	0.40	0.24	0.46	0.32	0.43	0.35	0.22	0.46	0.38	0.43
GOT-3	2	0.35	0.35	0.40	0.40	0.27	0.21	0.33	0.23	0.27	0.33	0.20
	4	0.65	0.65	0.60	0.60	0.73	0.79	0.67	0.77	0.73	0.67	0.80
Expected het. (He)		0.46	0.46	0.48	0.48	0.39	0.33	0.44	0.39	0.39	0.44	0.32

The estimated genetic diversity parameters, which are expected heterozygosity (*He)*, mean number of alleles per locus (*A*), and number of alleles and proportion of polymorphic loci (*P*), are showed in Table 4.

Table 4. Genetic diversity parameters for studied populations.

Popn. N^o	Population Names	Number of alleles present	Mean number of alleles per locus (*A*)	Proportion of polymorphic loci (*P*)	Mean expected het. (*He*)
1	Sarıkamış – Kars	9	1.80	0.60	0.20
2	Şenkaya – Erzurum	8	1.60	0.60	0.24
3	Yusufeli – Artvin	10	2.00	0.60	0.18
4	Gümüşhane - K. Dere	7	1.40	0.40	0.19
5	Mesudiye - Arpaalan	10	2.00	0.80	0.24
6	A.Madeni – Yozgat	13	2.60	0.60	0.22
7	Vezirköprü – Samsun	13	2.60	0.80	0.28
8	Ilgaz –Yenice	12	2.40	0.60	0.19
9	Bolu – Aladağ	10	2.00	0.60	0.25
10	Akyazı – Adapazarı	9	1.80	0.60	0.22
11	Çatacık - Eskişehir	10	2.00	0.60	0.22
	Means	10.09	2.02	0.62	0.22

Expected heterozygosities *(He)* for populations range from 0.178 to 0.281, averaging 0.22. For the population whose (*He*) values are higher, their polymorphism and the number of alleles are also higher. Polymorphic loci (*P)* varied from 40% in popn. N^o 4 to 80% in popn. N^o 5 and 7. When all 11 populations were considered, the polymorphism was 62% on the average. Mean number of alleles per locus *(A)* was the lowest value in popn. N^o 4 (1.40), whereas it was the highest in popn. N^o 6 and 7 (2.60). The overall values in *P. sylvestris,* was found that *A* was approximately 2.02.

Genetic diversity and differentiation among populations within species could be examined further by analysing intra-and inter population components of genetic diversity. *Ht* was calculated as 0.235, *Hs* was determined as 0.220 and *Dst* was found to be 0.014. These mean that the great portion of genetic diversity was localized within populations (*Hs*=0.220) showing a low value for *Dst* (0.014). Moreover. *Gst* among populations was measured as 0.046 indicating that only 4.6% of the total genetic diversity was among populations (Table 5).

Table 5. Genetic diversity parameters estimated for 5 polymorphic loci.

Locus	*Hs**	*Ht**	*Dst**	*Gst**
LAP-1	0.044	0.045	0.001	0.022
LAP-2	0.231	0.243	0.012	0.049
GOT-1	0.040	0.041	0.001	0.024
GOT-2	0.370	0.422	0.052	0.123
GOT-3	0.417	0.423	0.006	0.014
Means	0.220	0.235	0.014	0.046

**Hs*: gene diversity within populations, *Ht*: total gene diversity,

**Dst*: among populations, *Gst*: relative degree of genetic differentiation

For all pair-wise population, comparisons were estimated in Table 6. Genetic distance (d_0) values among populations varied from 0.040 to 0.198. According to Nei (1972), the mean value of d_0 was 0.081. The data on the d_0 between populations revealed that genetic distance between popn. N^o 1 (Sarıkamış) and 10 (Akyazı) was the lowest, while between popn. N^o 2 (Şenkaya) and 8 (Ilgaz) was the highest (Table 6). In addition to, Table 6 clearly shows that popn. N^o 2 is distinctly different from the other populations as far as the genetic distance is concerned. This value indicated high average genetic distance among populations. Other populations are, however, genetically close to each other.

Table 6. Mean genetic distance by NEI's (1972) among the studied populations.

N^o	1*	2	3	4	5	6	7	8	9	10	11
2	0.136	-									
3	0.048	0.154	-								
4	0.044	0.122	0.062	-							
5	0.066	0.136	0.078	0.114	-						
6	0.066	0.178	0.090	0.110	0.064	-					
7	0.080	0.172	0.082	0.128	0.052	0.070	-				
8	0.078	0.198	0.068	0.122	0.068	0.050	0.076	-			
9	0.088	0.104	0.092	0.082	0.078	0.082	0.092	0.100	-		
10	0.040	0.120	0.058	0.062	0.068	0.062	0.070	0.066	0.056	-	
11	0.060	0.140	0.086	0.078	0.070	0.042	0.104	0.070	0.056	0.050	-

*: Population numbers as in Table 1

The cluster analysis based on Nei (1972) unbiased genetic distance (d_0) using the UPGMA algorithm reveals high levels of genetic separation between the populations at 95% significance level (Fig. 2). The phonetic dendrogram in Fig. 2 as well as data of Table 6 exhibits the extent of genetic relationships among the populations.

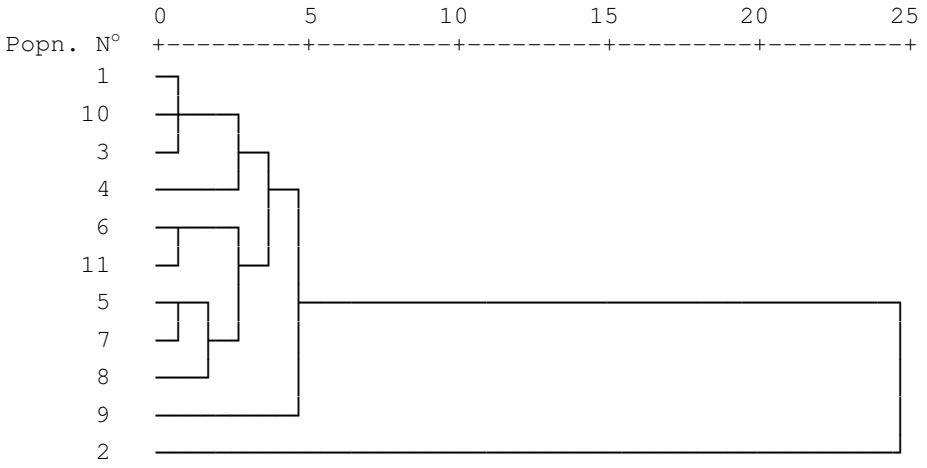


Figure 2. Dendrogram showing the clustering of the 11 natural populations of Scots pine based on NEI 's genetic distance coefficient.

DISCUSSION

In this study, five loci were identified from two enzyme systems, Lap (two loci) and Got (three loci). According to literature, visualisation of the products of electrophoresis indicated that isoenzymes of Lap occur on gels in two zones, which on the zymograms are designated as Lap-1 and Lap-2. Three zones of activity (Got-1, Got-2, Got-3) were detected on Got enzyme system. Got-1 and Got-2 had three variants and Got-3 exhibited two variants (Mejnartowicz, 1979; Rudin and Ekberg, 1978; Müller-Starck, 1982).

Polymorphic loci in this study were 62% on the average. This value for European Scots pine was found as more than 90%, in Asian Scots pine was 99% (Goncharenko et al., 1994). *P* for European black pine was 99% (Aguinagalde et al. 1997), and 70%

(Scaltsoyiannes et al. 1994) respectively. This value for *P. brutia* was 70% (Kara et al. 1997). These differences between the results were most probably due to the enzyme systems and the number of loci studied. It is known, however, that the expected genetic polymorphism of a species increases as it covers larger areas. Similar studies indicate that genetic polymorphism of species such as *P. brutia* (Kara et al. 1997) and *P. nigra* (Tolun et al. 2000) in Turkey is lower when compared to that of European populations. The lower genetic polymorphism can be attributed to the smaller cover areas of the species in Turkey.

Mean number of alleles per locus *(A)* was approximately 2.02. This value was reported to be 2.47 in European Scots pine for central populations and was much lower values for the isolated populations (Goncharenko et al. 1994). Scaltsoyiannes et al. (1994), reported the mean number of alleles as 2.0. These results in *P. nigra* were similar to our findings on *P. sylvestris*. Average expected heterozygosities (*He*) value for this study was 0.22. According to the previous studies, *He* values were reported as 0.29 for *P.sylvestris* (Goncharenko et al. 1994), as 0.31 (Kinloch et al. 1986) and 0.26 for *P. brutia* (Kara et al. 1997).

The organisation of gene diversity in *P. sylvestris* is quite similar compared to that observed on other conifers. For example, *Gst* values reported for *P. nigra* were 6.0% (Scaltsoyiannes et al. 1994) and 5.3% for *P. brutia* (Kara et al. 1997). In this study, the relative degree of genetic differentiation *(Gst)* among populations was 0.046 indicating that only 4.6% of the total genetic diversity was among populations. The index of *Gst* varies for the Spanish populations from 0.014 to 0.100 with an average of 0.040, and for the eastern and northern European population from 0.019 to 0.041 with an average of 0.025 (Prus-Glowacki and Stephan, 1994). Also, *Gst* should be noted that in the

course of the previous analysis for Swedish, German, and Scotch populations of *P. sylvestris* subdivision was ranged from 1 % to 2.8% (Gullberg et al. 1985*,* Müller-Starck and Gregorius, 1986; Kinloch et al. 1986, Goncharenko et al. 1994*).*

Genetic distance values among population varied from 0.040 to 0.198 with an average of 0.081. According to previous studies, mean values of d_0 are 0.017 for *P. sylvestris* (Goncharenko et al. 1994) and 0.035 for *P. nigra* in European populations (Scaltsoyiannes et al. 1994). The reason for such a high d_0 value might be the result of large geographical distance among the populations and varying local ecological conditions. Furthermore, the high value would be a result of two enzymes used in this study. Therefore, genetic distance could be concluded that the genetic differences among natural populations of *P. sylvestris* in Turkey are very high compared to three other species indicated above.

Two main groups were separated with popn. N^o 2 and the others. High genetic distances were determined between the popn. N^o 2 and the others. Such high value of genetic distance may be attributed to both microenvironment and natural regeneration methods used over years. High values, obtained in terms of genetic variability between populations, may be explained by the fact that Scotch pine populations are scattered across wide ranges in Turkey. Since two enzyme systems were assayed using electrophoresis technique, the results should be interpreted cautiously.

The cluster analysis reveals high levels of genetic separations (Fig.2). When all populations are compared the cluster analysis, it is hard to see apparent relationship between the genetic distances of populations and their geographic locations. For examples, while popn. N^o 1 (Sarıkamış) and popn. N^o 2 (Şenkaya) are geographically close to each other (Fig. 1), their genetic distances are far a part from each other; they

are not in a same group (Fig. 2). On the other hand, popn. N^{o} 5, 7 and 8 are in a small group and they are geographically close to each other (Fig. 1).

Morphologic characters seem to be a useful tool for population differentiation and attributable to natural selection. Isozyme markers are genetic markers not always able to discriminate between very close populations. For the other populations, geographically further apart, the enzyme differentiation is so high that diagnostic loci might be detected and the genetic distance discriminates between them. Generally, our results show that sufficient genetic variation exists in *P. sylvestris* to explain its great ecological plasticity and evolutionary potential.

It can be concluded that morphological features are very sensitive to and modified easily by local environmental conditions. Similar results were also reported for European black pine populations by Aguinagalde and Bueno (1994). Furthermore, the gametophytic tissue of the seeds of conifers is an ideal material to evaluate the genotype of these trees; the fact that it is haploid material makes it very easy to identify the different isoenzymatic forms and the allele frequencies in those loci.

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