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Morphometric and electrophoretic analysis of 13 populations of Anatolian black pine in Turkey

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Abstract: *The genetic variation in populations of Anatolian black pine (Pinus nigra Arn. subsp. pallasiana (L.) Holmboe.), one of the species covering large areas in Turkey, was investigated. Open pollinated seeds were collected from 13 populations in a natural distribution range. Six characters of seeds (length, width, ratio of length to width, weight/1000 seeds) and seedling characters (cotyledon number and hypocotyls height) and two enzyme systems viz. leucine aminopeptidase (LAP) and glutamate oxaloacetate transaminase, (GOT) were investigated. Significant differences were detected among the populations for the morphological characters. In addition, isozyme patterns of two enzyme systems revealed that LAP has two loci (one with 2 alleles and the other with 3), while GOT has three loci (two with 3 alleles and the third one with 2 alleles). Polymorphic loci were 74% on the average. The mean number of alleles per loci was 1.94 and expected heterozygosity was 19%. The mean total genetic diversity was calculated as 0.203; the mean gene diversity within populations was determined as 0.188, and the average between subpopulations diversity was 0.016. The relative magnitude of genetic differentiation among subpopulations was measured as 0.074 indicating that only 7.4% of the total genetic diversity was there between populations. Average genetic distance was 0.093 according to Gregorius. Nei's genetic distance was 0.022.*

Key words: *Pinus nigra, 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.

The remarkable increase of computerized image analysis in recent years has brought about a great improvement in morphometric characterization a speeding up of the process of measurements, more accurate measurement and the number of data that as a result can be handled by the computer is much greater (Ortiz *et al*., 1990). 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 respond with 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. 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. Anatolian black pine (*Pinus nigra* Arn. subsp *pallasiana* (Lamb.) Holmbe.) is one of the major species for afforestation of arid and rocky terrains in the sub Mediterranean region. This species is a light demanding species and its attitudinal distribution usually ranges from 250 to 1550 m (Vidakovic, 1991; Tolun *et al*., 2000). It should be regarded as a species subdivided into several subspecies and varieties. The Turkish variety of European black pine, Anatolian black pine, is a widespread mid elevation species in the Taurus, Western Anatolia and Northern Anatolian Mountains (Fig. 1). The natural distribution covers more than 2 million ha in Turkey. This subspecies is an important commercial species and it is also used for afforesting the high Anatolian steppes in Turkey (Kaya and Temerit, 1994). Also such a wide range of ecological conditions could favour the formation of variety of ecotypes. Its natural distribution is greater than that of any other species in this region suggesting that there are genetic resources available for diverse habitats. In recent year, large areas have been afforested using this species.

This paper presents results on geographic variation in *Pinus nigra* subsp. *pallasiana,* using conventional electrophoretic analysis and investigates its genetic structure, levels of variation and seed and seedling characters.

Materials and Methods

The seed material from 13 different populations of *Pinus nigra* subsp. *pallasiana* was provided by forest tree seeds and tree improvement research directorate. The division manages 84 seed stands spread over 10,567 ha for the species

Table – 1: Description of the studied populations.

Popn. No .	Population names	Type*	Altitude (m)	Latitude (N)	Longitude (E)	Aspect
1	Corum	N.S	-	40° 58' 21"	34° 32' 48"	NE
2	Sinop - Boyabat	S.S	1210	41° 32' 14"	34° 31' 00"	N
3	Kastamonu - Tasköprü	S.S	1150	41° 33' 54"	34° 28' 46"	N
4	Bolu - Mengen	S.S	950	40° 57' 20"	32° 11' 02"	SE
5	Kütahya - Domanic	S.S	1400	39° 51' 30"	29° 29' 00"	NE
6	Kütahya -Tavsanlı	P.S	1100	39° 33' 12"	29° 27' 00"	SW
7	Canakkale - Yenice	S.S	270	39° 50' 00"	27° 08' 30"	E
8	İzmir - Bayındır	S.S	850	38° 19' 57"	27° 40' 52"	NW
9	Denizli	S.S	1250	37° 40' 50"	29° 04' 20"	E
10	Mugla - Yılanlı	S.S	1050	37° 11' 19"	28° 30' 57"	SE
11	Mersin - Erdemli	S.S	1500	36° 49' 22"	34° 13' 13"	NE
12	Adana - Pos	S.S	1200	37° 35' 30"	35° 18' 45"	N
13	K.Maras - Göksun	S.S	1800	38° 02' 30"	36° 22' 30"	S

*: N.S.: Native stand, S.S.: Seed stand, P.S.: Protected seed

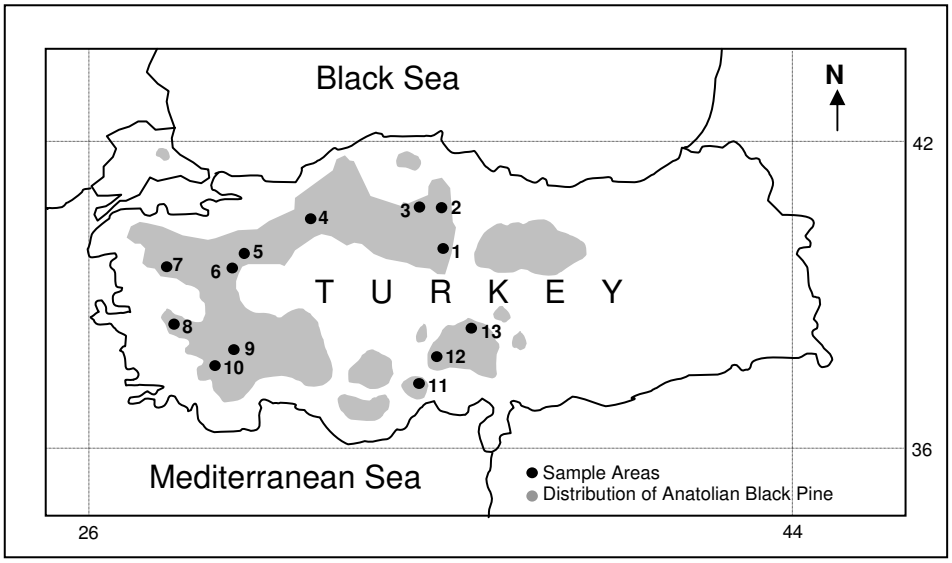

Fig. 1: Map showing the natural distributions of the Anatolian black pine and the location of study area in Turkey.

across the country. Bulk seeds were collected from 13 of these seed stands whose areas ranged from 6.5 to 100 ha. These seed stands are therefore not commercial seed lots. As such, the number of trees per population is at least well over 100 different trees. The natural distributions of *Pinus nigra* subsp. *pallasiana* in Turkey and location of studied populations 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 weight. 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.

Electrophoretic study: Extracts for isozyme analysis were obtained individually from 105 endosperm 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 Conkle *et al.* (1984) and Cheliak and Pitel (1984). The leucine aminopeptidase (LAP, EC 3.4.11.1) and glutamate oxaloacetate transaminase (GOT, EC 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 at pH 8.2. The electrode buffer consisted of 0.19 M boric acid, 0.026 M lithium hydroxide at 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.

Table – 2: Mean values and standard deviation of the morphometric analysis of seed and seedling parameters.

Popn. No**	Characters*					
	Seed			Seedling		
	Length (mm)	Width (mm)	Ratio of length to width	Weight/1000 seeds (g)	Cotyledon number	Hypocotyls height (mm)
1	5.69 ± 0.64 **abc**	3.20 ± 0.32 **bc**	1.78 ± 0.18 **ab**	18.64 ± 0.08 **b**	7.52 ± 0.81 **ab**	2.73 ± 0.60 **de**
2	5.79 ± 0.64 **bcd**	3.22 ± 0.29 **bc**	1.80 ± 0.19 **abc**	20.34 ± 0.11 **bcd**	8.42 ± 0.97 **d**	2.40 ± 0.65 **abcd**
3	6.59 ± 0.54 **f**	3.50 ± 0.36 **d**	1.89 ± 0.17 **bcd**	28.14 ± 0.11 **d**	8.04 ± 0.85 **bcd**	2.57 ± 0.64 **bcde**
4	6.14 ± 0.69 **de**	3.35 ± 0.27 **cd**	1.84 ± 0.20 **abcd**	23.19 ± 0.06 **ef**	7.86 ± 0.99 **bc**	2.22 ± 0.55 **ab**
5	5.78 ± 0.63 **bcd**	3.22 ± 0.39 **bc**	1.81± 0.21 **abc**	20.81 ± 0.08 **cd**	7.84 ± 0.96 **bc**	2.34 ± 0.63 **abc**
6	5.60 ± 0.60 **ab**	2.97 ± 0.33 **a**	1.90± 0.23 **bcd**	19.52 ± 0.10 **bc**	8.06 ± 0.77 **cd**	2.35 ± 0.62 **abc**
7	5.41 ± 0.53 **a**	3.13 ± 0.28 **ab**	1.74 ± 0.17 **a**	16.55 ± 0.08 **a**	7.06 ± 1.08 **a**	2.10 ± 0.67 **a**
8	5.99 ± 0.64 **cde**	3.27 ± 0.39 **bc**	1.85 ± 0.25 **abcd**	21.68 ± 0.10 **de**	7.86 ± 1.14 **bc**	2.47 ± 0.62 **bcd**
9	6.09 ± 0.65 **de**	3.24 ± 0.34 **bc**	1.89 ± 0.20 **bcd**	23.92 ± 0.12 **fg**	8.14 ± 0.81 **cd**	3.50 ± 0.60 **f**
10	6.35 ± 0.62 **ef**	3.27 ± 0.27 **bc**	1.95 ± 0.21 **d**	23.70 ± 0.10 **fg**	8.38 ± 0.73 **cd**	2.70 ± 0.58 **cde**
11	6.01 ± 0.58 **cde**	3.16 ± 0.36 **bc**	1.92 ± 0.22 **cd**	20.71 ± 0.11 **cd**	8.50 ± 0.93 **de**	2.69 ± 0.77 **cde**
12	6.26 ± 0.65 **ef**	3.33 ± 0.28 **cd**	1.89 ± 0.19 **bcd**	28.20 ± 0.10 **d**	9.22 ± 0.86 **ef**	2.91 ± 0.56 **e**
13	6.09 ± 0.78 **de**	3.24 ± 0.34 **bc**	1.87 ± 0.21 **bcd**	25.53 ± 0.09 **g**	8.94 ± 1.18 **f**	2.54 ± 0.55 **bcd**
Mean	5.98 ± 0.70	3.24 ± 0.35	1.86 ± 0.21	22.38 ± 0.35	8.14 ± 1.08	2.58 ± 0.70
F	13.16	7.12	4.41	52.65	18.33	16.23

*For each parameter, mean values with the same superscript letter are not significantly different at 1% level

** Bold letters show groupings among the populations resulting from Duncan multiply test at $p<0.01$

Gels were run at 4 ºC and constant amperage (80 mA) but were not allowed to exceed 155 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 variance analysis (ANOVA) was used to determine the genetic variation or diversity among the 13 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 allele frequencies, proportion of polymorphic loci *(P)*, and mean number of alleles per locus *(A)* using the GSED computer program (Gillet, 1994), and the mean expected heterozygosity *(He)*, gene diversity within populations *(Hs)*, total gene diversity *(Ht)*, gene diversity among populations *(Dst)*, relative degree of genetic differentiation (*Gst*) and genetic distance (*do*) were estimated according to Gregorius (1974) and Nei (1972).

Results and Discussion

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 seeds and seedlings. Population (popn) No 3 (Kastamonu-Tasköprü) had the longest and widest the both seed length and width and popn. No 7 (Canakkale-Yenice) with the shortest seeds and popn. No 6 (Kütahya-Tavsanlı) had the narrowest seeds. Again popn. No 12 (Adana-Pos) had the highest cotyledon number and weight of 1000 seeds; popn. No 7 (Canakkale-Yenice) had the lowest cotyledon numbers and hypocotyls height and weight of 1000 seeds. Popn. No 7 generally had the minimal value of morphological characters. 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. Seed weight was the parameters that distinguish all populations. Seed length was the parameters that distinguish populations. The average values of this character showed 8 statistically different groups. The average values of seed width showed 5 statistically different groups. The average values of cotyledon number and hypocotyls height showed 9 and 10 statistically groups, respectively. According to the morphological characters, average values showed that differentiation among populations were significant at $p<0.01$ level. It can be concluded that morphological features are very sensitive to local environmental conditions. Similar results were also reported for European black pine populations by Aguinagalde and Bueno (1994), and Aguinagalde *et al*. (1997).

Allele frequencies for in 13 populations of *Pinus nigra* subsp. *pallasiana* were calculated by GSED program (Gillet, 1994) and are shown in Table 3. According to the allele frequencies of *LAP* and *GOT* enzyme, total 13 alleles at 5 loci

Table – 3: Estimated allele frequencies for LAP and GOT enzyme system in *Pinus nigra* subsp. *pallasiana.*

Popn. No.	Locus / Alleles												
	LAP – 1		LAP – 2			GOT - 1			GOT - 2			GOT – 3	
	Allele frequencies												
	1	2	1	2	3	1	2	3	2	3	5	2	4
1	1.00	0.00	0.05	0.86	0.10	0.00	1.00	0.00	0.42	0.58	0.00	0.08	0.92
2	1.00	0.00	0.12	0.73	0.15	0.03	0.97	0.00	0.49	0.51	0.00	0.05	0.95
3	0.88	0.12	0.07	0.76	0.17	0.00	1.00	0.00	0.41	0.59	0.00	0.04	0.96
4	0.97	0.03	0.16	0.79	0.05	0.02	0.98	0.00	0.42	0.58	0.00	0.16	0.84
5	0.87	0.13	0.02	0.92	0.06	0.00	1.00	0.00	0.41	0.59	0.00	0.07	0.93
6	1.00	0.00	0.06	0.88	0.07	0.00	1.00	0.00	0.32	0.68	0.00	0.05	0.95
7	0.89	0.11	0.09	0.81	0.10	0.00	0.99	0.01	0.71	0.29	0.00	0.14	0.86
8	1.00	0.00	0.02	0.95	0.03	0.00	1.00	0.00	0.69	0.31	0.00	0.11	0.90
9	1.00	0.00	0.03	0.81	0.16	0.00	1.00	0.00	0.43	0.57	0.00	0.11	0.89
10	1.00	0.00	0.03	0.88	0.10	0.00	1.00	0.00	0.22	0.73	0.05*	0.19	0.81
11	1.00	0.00	0.03	0.90	0.08	0.00	1.00	0.00	0.67	0.33	0.00	0.02	0.98
12	0.87	0.13	0.01	0.95	0.04	0.00	0.99	0.01	0.81	0.19	0.00	0.00	1.00
13	0.82	0.18	0.01	0.96	0.03	0.00	0.98	0.02	0.40	0.60	0.00	0.00	1.00
Mean	0.95	0.06	0.05	0.86	0.09	0.00	0.99	0.00	0.49	0.52	0.00	0.08	0.92

* Unique allele

were identified. They showed at most two and three loci in zymograms of the endosperms, respectively. Two zones of LAP activity were observed. The first zone (LAP-1) exhibited two variants (alleles); for a single band and the second zone (LAP-2) exhibited three single-banded variants (1, 2, 3). The enzyme system of GOT showed three zones in zymograms of the endosperms. Three zones of GOT activity were observed. The first zone (GOT-1) exhibited three variant (1. 2. 3) for a single band, the second zone (GOT-2) also exhibited three variant (2, 3, 5) for a single band and the third zone (GOT-3) exhibited two variant (2, 4) for a single band. 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. Nicolic and Tucic (1983), Scaltsoyiannes *et al*., (1994) found LAP A to segregate for two variants. These authors reported three and two variants at LAP-2, respectively. Generally, the frequency of 1 at LAP-1, and the frequency of 2 at LAP-2 were very high in all populations (Table 3). Three zones (GOT-1, GOT-2 and GOT-3) were detected on Got enzyme system. GOT-1 and GOT-2 had three variants and GOT-3 exhibited two variants, as reported previously for black pine by Scaltsoyiannes *et al*., (1994), Tolun *et al*., (2000) and in a number other conifers (Strauss and Conkle, 1986, Turna, 2003).

According to alleles frequencies of GOT and LAP enzyme, alleles frequencies were not quite similar in different populations. From the Table, allele 1 of LAP-1 was found in all populations with high frequencies, while allele 2 of LAP-2 was found in popn. No. 3, 4, 5, 7, 12 and 13 with very low frequencies. Allele 2 of LAP-2 was found in all populations with very high frequencies (%86). As well as LAP enzyme, in the GOT enzyme system, some alleles were high in some populations, while the others were low in some populations. For example the frequency of allele 2, at GOT-1 and the frequency of allele 4, GOT-3 was relatively high in all populations. Additionally, there is a unique allele (GOT 2, 5) in popn. No. 10 (Mugla-Yılanlı) that would indicate higher genetic richness than the other populations (Table 3). The estimated genetic diversity parameters, which are expected heterozygosity *(He)*, mean number of alleles per loci *(A)* and number of alleles and proportion of polymorphic loci *(P)*, are showed in Table 4.

Mean number of alleles per locus *(A)* was the lowest value in popn. No. 1, 6, 8, and 11 (1.80), whereas it was the highest in popn No. 4 and 7 (2.20). The overall values in *Pinus nigra* subsp *pallasiana*, was found that *(A)* was approximately 1.94. Scoltsoyiannes *et al.* (1994), and Tolun *et al.* (2000) reported the mean number of alleles as 2.0 and 1.6 respectively and expected heterozygosity as 20% and 21% respectively. These results in *Pinus nigra* were similar to our findings. Scots pines in Turkish populations were reported the similar results as 2.02 and 22% (Turna, 2003).

Proportion of polymorphic loci *(P)* varied from 40% in popn. No. 8, 11, 12 and 13 to 80% in popn. No. 5 and 7. When all 13 populations were considered, the polymorphism was 57% on average. This value for European black pine (*Pinus nigra* Arn.) was reported to be 99% (Aguinagalde *et al*., 1997), and 70% (Scaltsoyiannes *et al*., 1994), respectively. *P* for Anatolian black pine was determined as 47.9% (Tolun *et al*., 2000) and for Scots pine in Turkish populations was reported as 62% (Turna, 2003). These differences between the results were most probably due to the enzyme systems and the number of loci studied involved. It is known, however, that the expected genetic polymorphism of a species increases as it covers larger areas. The lower genetic polymorphism can be attributed to the smaller cover areas of the species in Turkey.

Table – 4: Genetic diversity parameters for studied populations.

Pop. No.	Number of alleles	Mean number of alleles per locus *(A)*	Proportion of polymorphic loci *(P)*	Mean expected het. *(He)*	Number of unique allele
1	9	1.80	0.60	0.18	0.00
2	10	2.00	0.80	0.22	0.00
3	10	2.00	0.80	0.23	0.00
4	11	2.20	1.00	0.24	0.00
5	10	2.00	0.80	0.20	0.00
6	9	1.80	0.60	0.15	0.00
7	11	2.20	1.00	0.24	0.00
8	9	1.80	0.60	0.14	0.00
9	9	1.80	0.60	0.20	0.00
10	10	2.00	0.60	0.19	1.00
11	9	1.80	0.60	0.14	0.00
12	10	2.00	0.80	0.13	0.00
13	10	2.00	0.80	0.18	0.00
Means	9.77	1.94	0.74	0.19	0.07

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

Locus	Hs*	Ht*	Dst*	Gst*
LAP-A	0.090	0.098	0.008	0.085
LAP-B	0.233	0.242	0.009	0.036
GOT-A	0.017	0.017	0.001	0.018
GOT-B	0.457	0.509	0.052	0.101
GOT-C	0.142	0.148	0.006	0.041
Means	0.188	0.203	0.015	0.074

*Hs= gene diversity within populations
Hat= total gene diversity
Dst= gene diversity among populations
Gst= relative degree of genetic diversity

Expected heterozygosis *(He)* for populations range from 0.13 to 0.24, averaging 0.19. For the population whose (*He*) values are higher, their polymorphism and the number of alleles are also higher.

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 0.203, *Hs* was determined as 0.188 and *Dst* was found to be 0.015. These mean that the great portion of genetic diversity was localized within populations (*Hs*=0.188) showing a low value for *Dst* (0.015). Moreover, *Gst* among populations was measured as 0.074 indicating that only 7.4% of the total genetic diversity was among populations (Table 5).

The organisation of gene diversity in *P. nigra* subsp. *pallasiana* 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), 46% for *P. sylvestris* (Turna, 2003) and 5.3% for *P. brutia* (Kara *et al*., 1997). 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).

Although conifer species differ in the manner by which they adapt to heterogeneous environments (Aguinagalde *et al*., 1997), high values of genetic diversity within Anatolian black pine populations have been attributed to adaptation mechanisms to the microenvironments (Kaya and Temerit, 1994).

For all pair wise population, comparisons were estimated in Table 6. Mean genetic distance below the diagonal by Nei (1972), above the diagonal by Gregorius (1974) values among the studied populations. Genetic distances *(d_0)* values among populations by Nei, (1972), varied from 0.000 (between popn. No. 1 and 9) to 0.093 (between popn. No. 10 and 12), and by Gregorius (1974), from 0.023 (between popn. No. 1 to 9) to 0.200 (between popn. No. 10 and 12). The data on the do between populations revealed that genetic distance between popn. No. 1 (Corum) and popn. No. 9 (Denizli) was the lowest, while between popn. No. 10 (Mugla-Yılanlı) and 12 (Adana-Pos) was the highest (Table 6). According to Nei (1972) and Gregorius (1974) the average genetic distances were 0.022 and 0.094 respectively. Both indicated high average genetic distance among populations. In addition to, Table 6 clearly shows that popn. No. 12 is distinctly different from the other population except 7, 8 and 11, as far as the genetic distance is concerned. According to genetic distance, it indicated high average genetic distance among some population and some populations are, however, genetically close to each other.

According to previous studies, mean values of d_0 are 0.035 for *P. nigra* in European populations (Scaltsoyiannes *et al*., 1994) and 0.081 for *P. sylvestris* in Turkish population (Turna, 2003). 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

Table – 6: Mean genetic distance (below the diagonal by Nei's, above the diagonal by Gregorius) among the studied populations.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	-	0.050	0.053	0.050	0.044	0.030	0.107	0.078	0.023	0.067	0.069	0.141	0.080
2	0.003	-	0.057	0.065	0.090	0.067	0.109	0.101	0.048	0.116	0.080	0.150	0.114
3	0.004	0.005	-	0.074	0.040	0.067	0.099	0.131	0.053	0.116	0.107	0.130	0.065
4	0.002	0.005	0.008	-	0.074	0.072	0.099	0.107	0.048	0.082	0.114	0.168	0.105
5	0.004	0.012	0.004	0.008	-	0.057	0.105	0.095	0.063	0.099	0.093	0.101	0.036
6	0.002	0.010	0.007	0.005	0.005	-	0.135	0.099	0.053	0.055	0.080	0.150	0.082
7	0.024	0.018	0.026	0.024	0.026	0.042	-	0.067	0.099	0.147	0.076	0.080	0.137
8	0.018	0.018	0.029	0.022	0.022	0.034	0.004	-	0.082	0.126	0.032	0.076	0.120
9	0.000	0.003	0.004	0.003	0.006	0.004	0.023	0.019	-	0.070	0.084	0.156	0.099
10	0.010	0.024	0.018	0.010	0.014	0.006	0.060	0.051	0.011	-	0.128	0.200	0.131
11	0.014	0.011	0.021	0.021	0.019	0.028	0.006	0.002	0.016	0.049	-	0.072	0.110
12	0.043	0.037	0.045	0.050	0.039	0.062	0.008	0.009	0.045	0.093	0.009	-	0.095
13	0.011	0.020	0.008	0.017	0.001	0.010	0.033	0.030	0.015	0.024	0.024	0.040	-

* Population numbers as in Table 1.

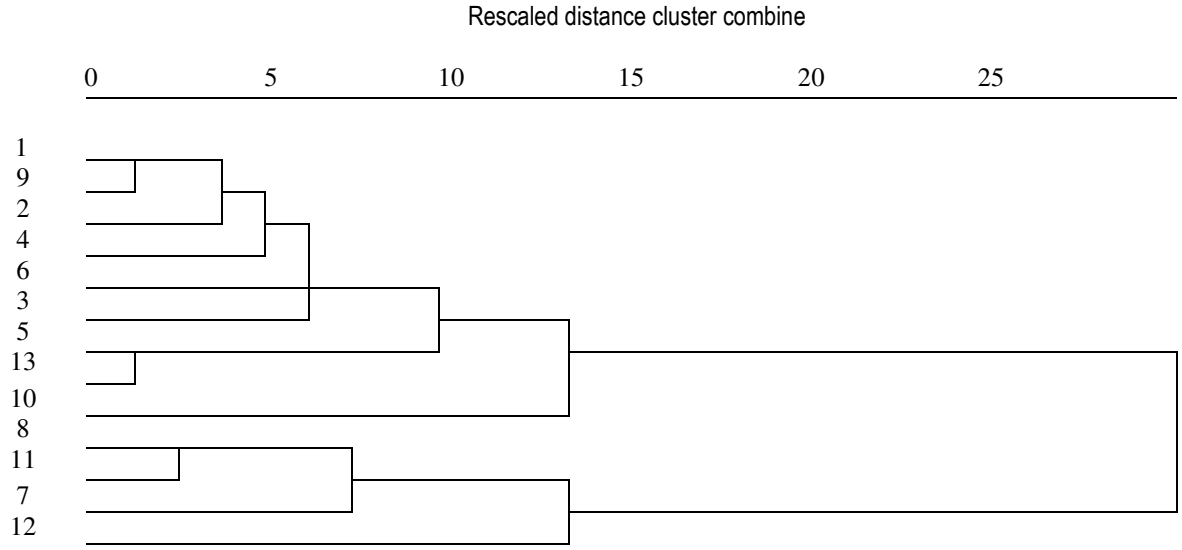

Fig. 2: Dendrogram showing the clustering of the 13 natural populations of Anatolian black pine based on Nei's genetic distance coefficient.

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. nigra* in Turkey are very high compared to three other species indicated above.

The cluster analysis based on Nei's (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). Since calculations based on both Nei's and Gregorius provide similar results, here we used only Nei's calculations. The phonetic dendrogram in Fig. 2 as well as data of Table 6 exhibit the extent of genetic relationships among the populations.

According to phonetic dendrogram in Fig. 2, two main groups were separated with population numbers of 7, 8, 11, 12 and the other populations. Genetic distances between populations were lower in population numbers 1 and 9, 5 and 13 and 8 and 11. High genetic distances were determined between the popn No. 12 (Adana-Pos) 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 Anatolian black pine populations are scattered across wide ranges in Turkey.

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 example, while popn. No. 13 (K.Maras-Göksun) and popn. No. 12 (Adana-Pos) 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). In general, coniferous trees show very high genetic diversity when compared to other types of organisms. Although pines have somewhat lower genetic diversity relative to other conifers (Kim *et al.,* 1997), *Pinus nigra* is one of the most diverse of the coniferous species (Nicolic and Tucic, 1983; Scaltsoyiannes *et al.,* 1994).

Morphological 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. nigra* subsp *pallasiana* 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 gametophytes 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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