parison of the data presented here with various weather factors may provide some suggestions in this direction.

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Pattern and Magnitude of Genetic Diversity in *Pinus nigra* ARNOLD Subspecies *pallasiana* Populations from Kazdağı: Implications for *in situ* Conservation

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Summary

To determine the genetic structure of black pine (*Pinus nigra* ARNOLD subspecies *pallasiana*), populations sampled from Kazdağı (Eybekli, Asar, Katrandağ, Kalkım, Gürgendağ, Kapıdağ, Mıhlıdere). Isozymes from 16 enzyme systems were investigated from haploid female megagametophytes by starch gel electrophoresis.

Twenty-nine loci were resolved from the 16 enzyme systems assayed. The results indicated that the mean number of alleles per locus (*A*) and polymorphisms (*P*) did not vary significantly in the populations studied. The mean number of alleles per locus (*A*) was around 1.67 (range, 1.65 to 1.69). Polymorphisms varied between 51.7% in Kapıdağ and 58.6% in Mıhlıdere populations. Observed heterozygosity (H_{obs}) was the highest (0.186) in Asar and the lowest (0.122) in Gürgendağ populations. The expected heterozygosities (H_{exp}) ranged between 0.283 (in Asar) and 0.248 (in Katrandağ). There were large differences between H_{obs} and H_{exp}. Ninety-four percent of the total observed genetic variation was within populations. NEI's genetic distances also showed that variation among populations is relatively low suggesting that no population differentiation has occurred. From the estimated average genetic distances between populations, it is evident that the genetic distances between population pairs were low, ranging from 0.01 to 0.04. Genetically, most similar population pairs were Eybekli-Asar and Kalkım-Gürgendağ, and the least similar ones were Eybekli-Katrandağ, Eybekli-Kapıdağ, Eybekli-Mıhlıdere, and Mıhlıdere-Gürgendağ.

Based on the genetic diversity measurements and genetic distance between populations, Asar (or Eybekli), Mıhlıdere and Gürgendağ populations seem to be forming genetically distinct groups. These populations were, therefore, recommended as potential Gene Manegement Zones (GMZ) to conserve the genetic resources of Anatolian black pine in the Kazdağı Region in Turkey.

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Introduction

Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) is one of the subspecies of European black pine, occurring naturally as a widespread mid-elevation species in Taurus, western Anatolian and northern Anatolian Mountains. The range in elevation varies from 250 m to 1550 m (*Figure 1A*). This subspecies covers a large area, more than 2 million hectares, in Turkey.

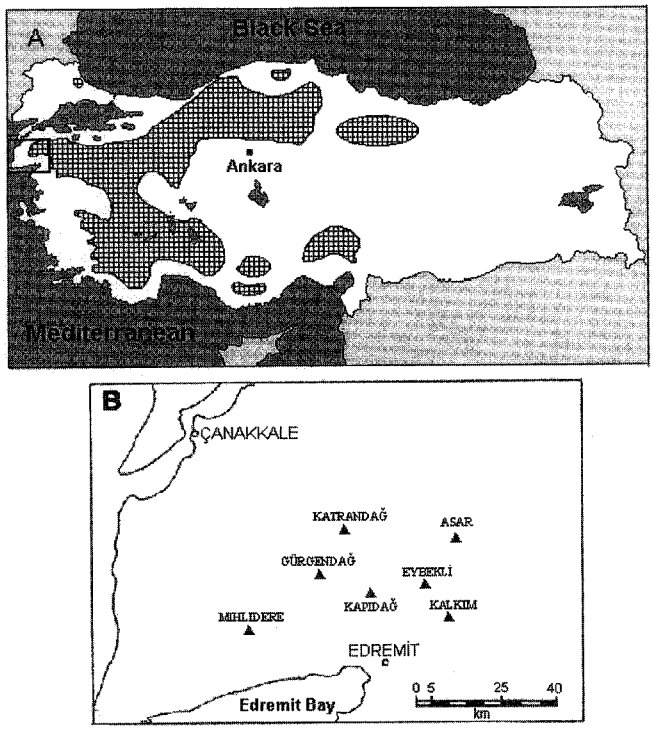

Figure 1. – The map showing the nautral distribution of *Pinus nigra* ARNOLD subsp. *pallasiana* in Turkey (A). Locations of the studied populations in the Kazdağı Region are indicated by ▲ (B).

Anatolian black pine is an important timber species and the first choice for afforestation of the high Anatolian steppes in Turkey (KAYA and TEMERIT, 1994). 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 years, large areas have been afforested using this subspecies. The subspecies is therefore of great importance in Turkish forestry (KOSKI and ANTOLA, 1993; KAYA and TEMERIT, 1994). The success of the plantations and the productivity of future forests established in high Anatolian steppes with Anatolian black pine will depend especially on how well the genetic resources of the subspecies are conserved, managed and used. Thus, the knowledge on genetic variation in a subspecies is necessary not only for identification of adapted seed sources of Anatolian black pine and establishment of plantations, but also for effective conservation of genetic resources of the species to ensure sustainable forestry manegement (MEFFE and GARROLL, 1994; KAYA *et al.*, 1997; LEDIG, 1998).

Maintenance of genetic material in the wild on site (*in situ*) and maintenance of wild or domesticated material in gardens, orchards, seed collections, or laboratories (*ex-situ*) are essential aspects of managing the genetic diversity for preservation of species and for plantation forestry operations. The best solution for most species is to leave them *in situ* (LEDIG, 1988).

Gene Management Zones (GMZs) (KAYA *et al.*, 1997; LEDIG, 1988; KRUGMAN, 1984) are *in situ* gene conservation areas (naturally and semi-naturally protected areas) where the evolutionary processes are expected to take place in populations of target species. Target species may include endangered and economically important plant species as well as species with large potential genetic diversity and differentiation in a given site.

The GMZ concept was implemented, first time, in the context of the project named "*In situ* Conservation of Plant Genetic Diversity in Turkey", which was commenced with the collaboration of Turkish Ministry of Forestry, Ministry of Agriculture and Rural Affairs and Ministry of Environment in 1993. This project was supported by a special World Bank fund that is Global Environment Facility (GEF). The project aimed to protect genetic resources of important tree species as well as wild relatives of crop species in selected pilot sites. *Pinus nigra* subspecies *pallasiana* (Anatolian black pine) is one of the target species in selected pilot areas of the Bolkar and Kazdağı Mountains of Turkey. The Kazdağı Region was selected as one of the pilot areas because it is considered as a principle meeting place of Euro-Siberian and Mediterranean Phytogeographic Floristic elements of Turkey. Surveys indicated that the area is very rich in forest tree species as well as wild relatives of fruit trees. Anatolian Black Pine is the most widespread conifer species in the region. Thus, studying genetic diversity in Anatolian black pine populations in the Kazdağı Region would provide information on pattern of genetic variation in the area.

The genetic variation of forest tree species is commonly studied following the traditional approach of observing the offspring of a series of controlled cross or open-pollinated provenance or family trials for their performances in biologically important traits (adaptive traits) (LIBBY *et al.*, 1969; EL-KASSABY, 1991). Isoenzymes (isozymes; multiple molecular forms of enzymes as neutral markers) can also be used effectively to determine the magnitude and pattern of genetic variation in forest tree populations for quick decision making process in conservation of genetic resources *in situ* (MARKERT and MOLLER, 1959; WENDEL and WEEDEN, 1989; LEDIG, 1998).

Although the safest conservation strategy is to conserve virtually everything without any priorities, the high cost involved is often prohibitive. Information on the distribution of genetic variation can help to develop useful cost effective conservation strategies. In the Kazdağı Region, a conservation strategy which reduces the cost and increases the efficiency of *in situ* conservation site was selected. This involved potential Anatolian black pine GMZ sites in which selected target trees were evaluated. The implication of the results of this study for *in situ* conservation of genetic resources of the Anatolian black pine was discussed.

Material and Methods

Open-pollinated seeds of Anatolian black pine were collected from a total of 315 parent trees (half-sib families) from seven natural populations of Kazdağı, Turkey, in the fall of 1995 (*Table 1, Figure 1B*). In each population 45 trees were chosen with the following restrictions: (a) Parent trees had to be separated by at least 100 meters within each population to minimize the relatedness of families. (b) Elevational range of the parent trees had to be no greater than 300 meters within any one population. (c) Parent trees had to be approximately at the same age in a single stand. Then, at least 20 cones were collected from each mother tree. The elevation of sampled population ranged from 300 m in Asar to 1450 m in Kapıdağ population. Also, the aspects, stand age, density and stand types of the sampled Anatolian black pine populations sampled

Table 1. – Geographic description of the studied populations.

Population	Latitude(N)	Longitude(E)	Aspect	Altitude (m)	Sample Size	Average Stand Age	Stand Type	Stand Density [a]
Eybekli	39° 42'	27° 31'	West	1000	45	89	Mixed [b]	799
Asar	39° 49'	27° 08'	South	300	45	65	Pure	680
Katrandağ	39° 52'	27° 06'	West	950	45	72	Pure	975
Kalkım	39° 32'	27° 19'	West	750	45	50	Pure	601
Gürgendağ	39° 43'	26° 54'	Mixed	1280	45	61	Mixed [c]	531
Kapıdağ	39° 41'	26° 52'	North-East	1450	45	95	Pure	707
Mihlidere	39° 40'	26° 42'	North	680	45	86	Mixed [c]	481

[a]) Total number of trees per hectare.
[b]) Mixed with *Abies equi-trojani.*
[c]) Mixed with *Castanea sativa* and *Abies equi-trojani.*

in the Kazdağı showed variation. For example, the stand age was from 50 in Kalkım to 95 years in Kapıdağ population. The stand density was the lowest in Mıhlıdere and it was highest in the Katrandağ Population (*Table 1*).

To study the isozymes in Anatolian black pine, the procedures described in CONKLE *et al.* (1982) were used with some minor modifications. All the chemicals used in this study were purchased from Sigma Chemical Company, USA. Eight seeds of each of the 315 randomly selected mother trees (half-sib families) were analysed. When 8 seeds were used for determination of mother genotype the probability of error is less than $(1/2)^7 = 0.0078$ (MORRIS and SPIETH, 1978). The seeds were germinated on a germination boxes for 2 to 4 days at room temperature. When radicles reached 2 mm to 5 mm long, megagametophytes were separated from the embryos and placed in labelled grinding plates. Then, separated megagametophytes were crushed in 2 drops of extraction buffer at 4 °C to release enzymes from cell and organelle membranes. The extracts were then absorbed onto paper wicks (1.5 cm x 3.5 mm, WHATMAN chromatography paper, No.3 MM.). The 16 enzyme systems were eveluated by the use of four buffer systems. The enzyme systems studied were leucine aminopeptidase (LAP, EC.3.4.11.1); phosphoglocoisomerase (PGI, EC.5.3.1.9); phosphoglocomutase (PGM, EC.5.4.2.2); glutamate oxaloacetate transaminase (GOT, EC.2.6.1.1); glutamate dehydrogenase (GDH, EC.1.4.1.2); mannose phosphate isomerase, (PMI, EC.5.3.1.8); superoxide dismutase (SOD, EC.1.15.1.1); aconitase (ACO, EC.4.2.1.3); acid phosphatase (ACP, EC.3.1.3.2); alcohol dehydrogenase (ADH, EC.1.1.1.1); menadione reductase (MNR, EC.1.6.99.2); shikimate dehydrogenase (SKDH, EC.1.1.1.25); disphorase (DIA, EC.1.6.4.3); isocitrate dehydrogenase (IDH, EC. 1.1.1.42); malate dehydrogenase (MDH, EC.1.1.1.37); and 6-Phosphogluconate dehydrogenase (6-PGD, EC.1.1.1.44).

In order to determine the amount of genetic diversity in a standardised way; allelic richness *(A)*, proportion of polymorphic loci *(P)* and expected (H_{exp}) and observed (H_{obs}) heterozygosities were estimated for the populations (NEI, 1987). In addition, the observed heterozygosity of an individual in a population (H_I), the expected heterozygosity of an individual in a population (H_S), the expected heterozygosity of an individual in overall populations (H_T) and the average diversity between populations (D_{ST}) were estimated in accordance with the HARDY-WEINBERG expectations (NEI, 1987). The relative magnitude of genetic differentiation among subpopulations (G_{ST}) was obtained from D_{ST}/H_T (NEI, 1987). The WRIGHT's F-Statistics using the measure of heterozygosity defines three level of inbreeding such as F_{IT} = Fixation index within population, F_{IS} = Fixation index over the total populations and F_{ST} = Reduction in fixation index due differences among populations. These are essential in determining the presence of any deviation from the HARDY-WEINBERG expectations in populations and genetic differentiation of populations. The WRIGHT's F-Statistics were estimated for each locus and overall loci including all populations according to NEI (1987) using POGENE software (YEH *et al.*, 1987).

The estimates of NEI's (1972) standard genetic identity *(I)* and standard genetic distance *(D)*, unbiased for sample size (NEI, 1978) for all pair-wise population comparisons were calculated to show the genetic relationships between studied populations. All the estimations were carried out using BIOSYS-1 (SWOFFORD and SELANDER, 1989) and POPGENE software (YEH *et al.*, 1997).

Results

The genetic control of allozymes was postulated from the observed banding patterns. Twenty-nine zones of activity were resolved for the 16 enzyme systems assayed. The banding patterns and the distances of alleles from the origin are given in *figure 2*. The allele frequencies obtained are tabulated in *table 2*.

The following loci were monomorphic: LAP1, PGI1, GOT1, GOT2, PMI, SOD1, SOD2, GDH, ACP1, ACO, SKDH1, and MDH2. Heterogeneity ($p<0.01$) in allele frequencies among the populations was detected at the remaining 17 loci *(Table 2)*.

The mean number of alleles per locus varied from 1.65 in Eybekli, Kalkım and Kapıdağ populations to 1.69 in Gürgen-

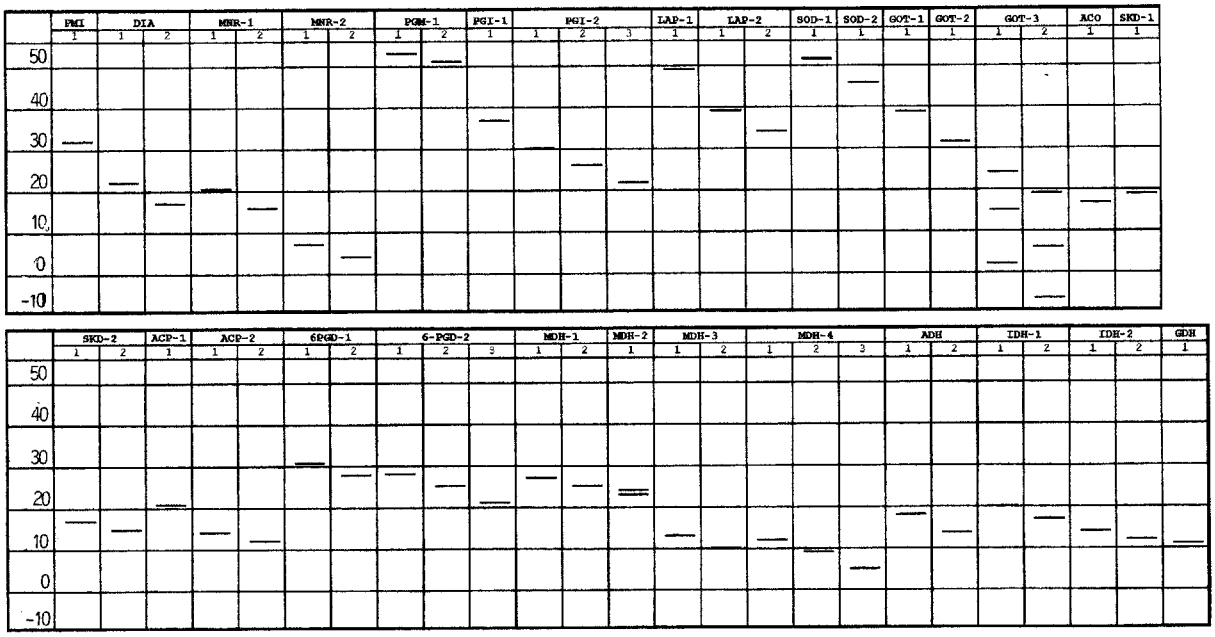

Figure 2. – Megagametophyte banding patterns and their allelic designations for 29 isoenzyme loci of Anatolian Black pine (numbers on vertical axis refer to migration distances from the origin in mm). (=) Alleles with more than one band due to gene duplication.

Table 2. – Allele frequencies for 29 isozyme loci studied in the Anatolian black pine populations in the Kazdağı Region in Turkey. (N is the sample size).

		Populations						
Locus	Allele	Eybekli	Asar	Katrandağ	Kalkım	Gürgendağ	Kapıdağ	Mıhlıdere
LAP1								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
LAP2								
(N)		45	45	45	37	45	45	45
	1	0.522	0.611	0.744	0.622	0.733	0.756	0.411
	2	0.478	0.389	0.256	0.378	0.267	0.244	0.589
PGI1								
(N)		45	45	45	42	43	45	45
	1	1	1	1	1	1	1	1
PGI2								
(N)		42	45	45	42	43	45	45
	1	0.119	0.156	0.167	0.179	0.360	0.578	0.344
	2	0.393	0.433	0.489	0.560	0.430	0.389	0.440
	3	0.488	0.411	0.344	0.262	0.209	0.033	0.211
PGM								
(N)		45	45	45	45	45	41	41
	1	0.878	0.778	0.889	1	0.956	0.976	0.744
	2	0.122	0.222	0.111	0	0.044	0.024	0.256
GOT1								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
GOT2								
(N)		45	45	41	45	45	45	45
	1	1	1	1	1	1	1	1
GOT3								
(N)		45	45	29	45	45	45	45
	1	0.389	0.356	0.603	0.300	0.456	0.300	0.256
	2	0.611	0.644	0.397	0.700	0.544	0.700	0.744
PMI								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
SOD1								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
SOD2								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
GDH								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1

		Populations						
Locus	Allele	Eybekli	Asar	Katrandağ	Kalkım	Gürgendağ	Kapıdağ	Mıhlıdere
ADH								
(N)		10	23	45	40	35	40	38
	1	1	0.804	0.756	0.538	0.714	0.663	0.553
	2	0	0.196	0.244	0.463	0.286	0.338	0.447
ACP1								
(N)		14	3	9	25	34	18	14
	1	1	1	1	1	1	1	1
ACP2								
(N)		45	45	45	45	45	45	45
	1	0.378	0.467	0.533	0.500	0.756	0.644	0.489
	2	0.622	0.533	0.467	0.500	0.244	0.356	0.511
ACO								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
MNR1								
(N)		45	45	45	43	45	45	45
	1	0.556	0.711	0.689	0.593	0.411	0.578	0.722
	2	0.444	0.289	0.311	0.407	0.589	0.422	0.278
MNR2								
(N)		45	45	45	43	45	45	45
	1	0.733	0.556	0.578	0.558	0.600	0.622	0.600
	2	0.267	0.444	0.422	0.442	0.400	0.378	0.400
SKDH1								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
SKDH2								
(N)		45	45	45	45	45	45	45
	1	0.567	0.511	0.578	0.700	0.622	0.500	0.711
	2	0.433	0.489	0.422	0.300	0.378	0.500	0.289
6PGD1								
(N)		45	44	45	45	45	45	45
	1	0.567	0.568	0.789	0.489	0.622	0.511	0.511
	2	0.433	0.432	0.211	0.511	0.378	0.489	0.489
6PGD2								
(N)		45	44	45	45	45	45	45
	1	0.444	0.273	0.167	0.389	0.256	0.156	0.244
	2	0.467	0.625	0.489	0.500	0.700	0.622	0.622
	3	0.089	0.102	0.344	0.111	0.044	0.222	0.133
DIA								
(N)		45	40	45	43	45	45	45
	1	0.533	0.650	0.611	0.837	0.844	0.722	0.467
	2	0.467	0.350	0.389	0.163	0.156	0.278	0.533
IDH1								
(N)		45	40	45	45	45	45	45
	1	0.644	0.613	0.744	0.678	0.722	0.700	0.933
	2	0.356	0.388	0.256	0.322	0.278	0.300	0.067
IDH2								
(N)		41	41	13	23	8	33	8
	1	0.500	0.557	1	0.500	0.500	1	0.875
	2	0.500	0.429	0	0.500	0.500	0	0.125
MDH1								
(N)		45	44	45	45	45	45	45
	1	0.478	0.557	0.644	0.556	0.544	0.611	0.611
	2	0.522	0.443	0.356	0.444	0.456	0.389	0.389
MDH2								
(N)		45	45	45	45	45	45	45
	1	1	1	1	1	1	1	1
MDH3								
(N)		45	44	45	45	45	45	45
	1	0.578	0.682	0.411	0.500	0.589	0.600	0.500
	2	0.422	0.318	0.589	0.500	0.411	0.400	0.500
MDH4								
(N)		45	44	45	45	45	45	45
	1	0.489	0.205	0.022	0.189	0.476	0.300	0.044
	2	0.356	0.455	0.714	0.733	0.478	0.456	0.744
	3	0.156	0.341	0.264	0.078	0.056	0.244	0.211

dağ and Asar populations. The average allelic richness for all populations was 1.67. Considering the standard errors of the estimations, it does not appear that in the Kazdağı populations of Anatolian black pine, there is a significant difference in average number of alleles per locus *(Table 3)*. Similarly, there was no significcant difference in the proportion of polymorphic loci (0.99 criterion) among the populations. The proportion of polymorphic loci varied from 55.2% (Kapıdağ, Katrandağ, and Kalkım) to 58.6% (Gürgendağ and Mıhlıdere) and the average proportion of polymorphic loci (using 0.99 criterlon) for all populations was 56.6% *(Table 3)*.

Observed heterozygosity was the highest (0.186) in Asar and the lowest (0.122) in Gürgendağ populations. On the other hand, the expected heterozygosities ranged from 0.283 (in Asar) and 0.248 (in Katrandağ). Considering the standard errors of these estimates, the differences in expected and observed heterozygosities between the populations are probably not significant. Mean heterozygosity for all populations was 0.151 for observed and 0.262 for expected. Again, there was a large difference between observed and expected heterozygosities *(Table 3)*.

In order to explore the pattern of genetic variation in each population and to further compare the populations, NEI's G-Statistics (NEI, 1973) was used. Depending on the locus, the expected heterozygosity of an individual in the total population (H_T) ranged from 0.198 in the PGM locus to 0.666 in the ACP-2 locus. Expected heterozygosity of an individual in a subpopulation (H_S) varied from 0.181 (PGM) and 0.614 (PGI-2). Mean total genetic diversity over all loci was calculated as 0.288 *(Table 4)*. The mean expected heterozygosity in subpopulations (H_S) was estimated as 0.269. This means that

Table 3. – Mean number of alleles per locus, proportion of polymorphic loci, mean observed and expected heterozygosities in the Australian black pine populations from the Kazdağı Region in Turkey.

Populations	Mean Number of Alleles (*A*)	Proportion of Polymorphic Loci (*P*)	Mean Heterozygosity (*H*)	
			Observed (H_{obs})	Expected (H_e)
Eybekli	1.65	0.553±0.09	0.138±0.032	0.270±0.048
Asar	1.69	0.582±0.09	0.186±0.041	0.283±0.046
Katrandağ	1.66	0.552±0.09	0.166±0.040	0.248±0.045
Kalkım	1.65	0.552±0.09	0.138±0.034	0.265±0.046
Gürgendağ	1.69	0.586±0.09	0.122±0.032	0.260±0.045
Kapıdağ	1.65	0.552±0.09	0.160±0.036	0.250±0.046
Mıhlıdere	1.69	0.586±0.09	0.148±0.035	0.259±0.044
Mean±s.e	*1.67*	*0.566±0.07*	*0.151±0.036*	*0.262±0.045*

greatest amount of genetic diversity occurs within populations. The average heterozygosity between populations (D_{ST}) was very low, that is 0.025, showing that populations are not significantly different from each other. As a result, genetic differentiation among sub-populations (G_{ST}) was also very low (0.060) *(Table 4)*.

F-Statistics is needed to detect any deviation from HARDY-WEINBERG expectations in gene frequencies *(Table 5)*. F_{IS} that is the fixation index within subpopulations, for this study ranged from –0.1583 (PGI-2) to 0.8857 (IDH-2); and the mean was about 0.4159. These results indicated that in some loci deviations from expectations were great. F_{IT} gives the total deviation of populations. Degrees were between –0.0764 (PGI-2) and 0.7534 (IDH-1), the mean was 0.4522. F_{ST} is equal to NEI's G_{ST} so it can be used for the same purpose. F_{ST} was 0.0603 meaning that 6% of total variation was between populations. 94% of total variation observed within populations *(Table 5)*.

NEI's (NEI, 1978) unbiased measures of genetic identity and genetic distance were used to determine relative degrees of relatedness among populations. Genetic identity and distance estimates between studied populations revealed that Eybekli-Asar, and Kalkım-Gürgendağ populations were the most genetically related population-pairs (genetic identity = 0.99). On the

Table 4. – Genetic diversity parameters estimated for 17 polymorphic loci of the Anatolian Black pine populations from the Kazdağı Region in Turkey.

Locus	H_S	H_T	D_{ST}	G_{ST}
6-PGD1	0.471	0.488	0.017	0.035
6-PGD2	0.546	0.571	0.025	0.044
ACP2	0.608	0.666	0.057	0.086
ADH	0.361	0.405	0.043	0.107
DIA	0.409	0.442	0.034	0.077
GOT3	0.446	0.471	0.025	0.053
IDH1	0.385	0.404	0.019	0.046
IDH2	0.316	0.415	0.099	0.239
LAP2	0.439	0.467	0.028	0.061
MDH1	0.485	0.490	0.005	0.011
MDH3	0.481	0.494	0.013	0.026
MDH4	0.522	0.583	0.061	0.105
MNR1	0.456	0.476	0.210	0.044
MNR2	0.468	0.476	0.008	0.018
PGI2	0.614	0.651	0.037	0.057
PGM	0.181	0.198	0.017	0.084
SKDH2	0.470	0.481	0.011	0.024
Means	***0.269± 0.043***	***0.288± 0.043***	***0.025 ± 0.0058***	***0.060 ± 0.010***

H_S: the expected heterozygosity in subpopulations
H_T: the total genetic diversity
D_{ST}: the average diversity between populations
G_{ST}: the relative magnitude of genetic differentiation

Table 5. – Summary of F-statistics for the 17-Polymorphic Loci.

Locus	F_{IS}	F_{IT}	F_{ST}
6PGD-1	0.3877	0.4105	0.0372
6PGD-2	0.3742	0.4021	0.0446
ACP-2	0.4669	0.4955	0.0536
ADH	0.0917	0.1887	0.1069
DIA	0.5626	0.5977	0.0803
GOT-3	0.2971	0.3331	0.0513
IDH-1	0.7414	0.7534	0.0464
IDH-2	0.8857	0.913	0.2390
LAP-2	0.5305	0.559	0.0609
MDH-1	0.3348	0.3419	0.0108
MDH-3	0.4308	0.4461	0.0268
MDH-4	0.5886	0.6312	0.1035
MNR-1	0.3896	0.4163	0.0437
MNR-2	0.5544	0.5603	0.0133
PGI-2	-0.1583	-0.0764	0.0707
PGM	0.7084	0.7327	0.0833
SKDH-2	0.3699	0.3857	0.0252
Mean	***0.4159***	***0.4522***	***0.0630***

F_{IS}: the fixation index within populations
F_{IT}: the fixation index over total populations
F_{ST}: the reduction in fixation index due to differences among populations.

other, the genetically least related population pairs were Eybekli-Kapıdağ, Eybekli-Mıhlıdere, Eybekli-Katrandağ and Gürgendağ-Mıhlıdere (genetic identity = 0.96) *(Table 6)*. To show the differentiation and grouping of studied populations graphically, a cluster analysis was performed using *Unweighted Pair Group Method* with Arithmetic Means (NEI, 1978). In cluster analysis, two large branches were observed *(Figure 3)*. The first branch covered two groups including Eybekli-Asar and Kalkım-Gürgendağ. The second branch also covered two groups, with Katrandağ-Kapıdağ forming one group, and Mıhlıdere the other group *(Figure 3)*. This grouping suggests that Eybekli and Mıhlıdere populations are not only the most geographically distant but also the most genetically different populations among the seven populations studied.

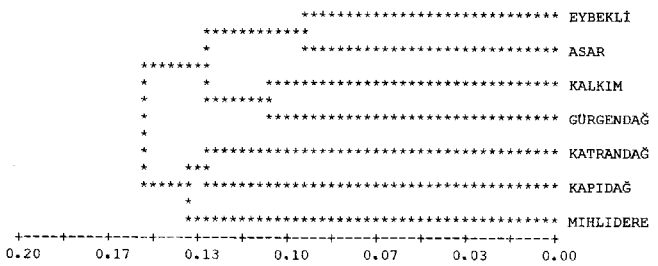

Figure 3. – Dendrogram constructed using NEI's (1978) Genetic Distance Statistics showing the clusters of Anatolian black pine populations from the Kazdağı Region.

Discussion

The present study provided considerable amount of information on the existing magnitude and pattern of genetic variation in Anatolian black pine populations in the Kazdağı Region.

The general phenotypes of Anatolian black pine's isozymes were consistent with those found in related species for number of loci for given enzymes and banding patterns. For example, the banding patterns for the PGM, PGI, LAP-2, PMI, 6PGD, SOD, SKDH enzyme systems were consistent with the patterns observed in *Pinus nigra* and other conifer species (GURIES and LEDIG, 1978; ADAMS and JOLY, 1980a; YEH and EL-KASSABY, 1980; EL-KASSABY, 1981; NICOLIĆ and TUCIĆ, 1983; KING and DANCIK, 1983; FINESCHI, 1984; CHELISK and PITEL, 1985; MILLAR, 1985; STRAUSS and CONKLE, 1986; ADAMS *et al.*, 1990; SCALTSOYIANNES *et al.*, 1994; DOĞAN *et al.*, 1998). Similarly, the banding patterns observed for the MNR, PMI, ACO, ADH, MDH, GDH, SOD, DIA enzyme systems were similar to the results from studies carried out in other conifer species (GURIES and LEDIG, 1978; ADAMS and JOLY, 1980a and b NEALE and ADAMS, 1981; MILLAR, 1985; STRAUSS and CONKLE, 1986; SUYAMA *et al.*, 1992; PARKER and HAMRICK, 1996; FALLOUR *et al.*, 1997; KIM *et al.*, 1997). However, the banding patterns determined for LAP-1 and GOT were consistent with only the results of DOĞAN *et al.* (1998), but these patterns were inconsistent with the banding patterns reported by Scaltsoyiannes *et al.* (1994), NICOLIĆ and TUCIĆ, (1983) who reported two and three LAP-1 variants for black pine, and BONNET-MASIMBERT and BIKAY-BIKAY (1978) who found four GOT zones in black pine. Although the banding patterns for the IDH and ACP enzyme systems were not similar to the results of DOĞAN *et al.* (1998) and SCALTSOYIANNES *et al.* (1994), similar results have been reported for other conifer species (MILLAR, 1985; STRAUSS and CONKLE, 1986). These differences observed in this study may be due to the systems used, the sample sizes in the populations or the differences in the genetic make up of the Anatolian black pine populations from the Kazdağı Region.

Generally, conifers are considered as one of the most genetically variable groups of species. Forest trees, particularly conifer species are among the most genetically variable organisms (HAMRICK and GODT, 1989). Long-lived woody plant species maintain, on average, higher levels of allozyme variation within their populations than other plant species and this is attributed to geographic range, and uniqueness of life-history features (HAMRICK *et al.*, 1992; EL-KASSABY, 1991).

The results of this study supported the existence of high genetic diversity within the seven populations of Anatolian black pine from the Kazdağı Region. The mean number of alleles per locus did not vary significantly among the studied populations. Similarly, the percent of polymorphic loci did not vary significantly among the seven populations studied. The finding that no significant differences were observed between populations for allelic richness and the proportion of polymorphic locus can be explained by the fact that these populations are located within the gene flow distance of each other. The amount of gene flow between populations would vary depending on prevailing wind direction during pollen shed, timing of pollen dispersal and the position of the population in the species range in the Kazdağı Region. NICOLIĆ and TUCIĆ (1983) and SCALTSOYIANNES *et al.* (1994) however, reported relatively higher polymorphism values than ours, that is 66.1% and 70% polymorphism, respectively. This discrepancy could be explained by the differences in the enzyme systems used in their studies and ours. Since the percent of polymorphic loci is dependent on the enzyme systems studied, it is not a reliable measure for genetic diversity. In fact, SCALTSOYIANNES *et al.* (1994) studied 10 enzyme systems while NICOLIĆ and TUCIĆ (1983) studied only four enzyme systems, compared to 16 enzyme systems used in this study.

The estimated average observed and expected heterozygosities were found to be similar to findings of NICOLIĆ and TUCIĆ (1983), which was 27.2%. The differences in expected and

Table 6. – Estimated NEI's genetic identities (above) and distances (below) between Anatolian black pine populations from the Kazdağı Region in Turkey.

	Eybekli	Asar	Katrandağ	Kalkım	Gürgendağ	Kapıdağ	Mıhlıdere
Eybekli		0.99	0.96	0.97	0.98	0.96	0.96
Asar	0.01		0.98	0.98	0.98	0.98	0.98
Katrandağ	0.04	0.02		0.97	0.97	0.98	0.98
Kalkım	0.03	0.02	0.03		0.99	0.98	0.98
Gürgendağ	0.02	0.02	0.03	0.01		0.98	0.96
Kapıdağ	0.04	0.02	0.02	0.03	0.02		0.98
Mıhlıdere	0.04	0.02	0.02	0.02	0.04	0.02	

observed heterozygosities between populations are not significant. However, the differences between expected and observed heterozygosities among populations were found to be considerable. This suggest that there must be forces operating to lower the observed heterozygosity especially in Gürgendağ which is located in a mixed forest of *Abies equi-trojani,* and some other deciduous tree species. The Gürgendağ population may therefore not be getting as much background pollen flow as the Katrandağ and Asar populations that are pure stands and open to prevailing winds in the area.

Genetic differentiation among sub-populations was low with G_{ST} = 0.060. This implies that 6% of total genetic variation is between populations and 94% of total genetic variation is within populations. These results are close to findings of BONNET-MASIMBERT and BIKAY-BIKAY (1978), NICOLIĆ and TUCIĆ (1983), and SCALTSOVIANNNES *et al.* (1994) for European black pine. The results of present and previous studies therefore indicated that Anatolian black pine exhibits a pattern of genetic diversity characterised by rather high intrapopulation variation and a moderate degree of inter-population genetic diversity, which results in a high total genetic variation. Also, the genetic diversity of Anatolian black pine (94%) is quite comparable to that observed for other conifers (GURIES and LEDIG, 1982; STRAUSS and CONKLE, 1986; KARA, 1996; PARKER and HAMRICK, 1996).

Although the pattern and magnitude of genetic diversity is important, determination of deviations from HARDY-WEINBERG expectations is also important. Because, these deviations indicate that there may be some forces operating on the heterozygosities. In order to estimate the deviations from expectations, WRIGHT's F-Statistics were used. Deviations were observed for all of the loci. For all loci considered, the fixation index within populations (F_{IS}) was 0.4159, meaning that within the populations homozygotes were 41.59% higher than expected. In addition, mean total deviation of populations (F_{IT}) was 0.4522, indicating that 44% excess homozygotes were observed than expected. Both F_{IS} and F_{IT} values reveal that excess of homozygotes genotypes in almost all loci with the exception of PGI2. This could be explained by the considerable amount of mating between relatives (or the gene flow from genetically like genotypes from neighbouring populations). The area of Kazdağı region where the study populations sampled is not physically isolated and relatively small (about 75 000 ha). Furthermore, the Anatolian black pine forests in the Kazdağı Region were intensively and selectively harvested and then regenerated naturally using seed tree- silvicultural system. This area is also very close to many ancient civilizations. Perhaps, due to these anthropogenic factors, Anatolian black pine populations in the Kazdağı Region may have experienced with past bottlenecks. It may also be stated that a strong selection for homozygote genotypes Anatolian black pine populations in environmentally and ecologically homogenous Kazdağı Region might be operating. There are also studies reporting the excess of homozygotes in some studied conifer populations (GRUNDWALD *et al.,* 1986; MILLAR and MARSHALL, 1991; FALLOUR *et al.,* 1997).

In fact, both G_{ST} and F_{ST} statistics indicated that the genetic differentiation is low (about 0.06) among sampled seven populations and comparable to other related studies (GURIES and LEDIG, 1982; STRAUSS and CONKLE, 1986; KARA, 1996; PARKER and HAMRICK, 1996; TOLUN *et al.*, 1999).

The estimated average genetic distances between populations indicated that the genetic distances between population pairs were low, ranging from 0.01 to 0.04. Genetically, most similar population pairs were Eybekli-Asar, Kalkım-Gürgendağ while the least similar ones were Eybekli-Katrandağ, Eybekli-Kapıdağ, Eybekli-Mıhlıdere, and Mıhlıdere-Gürgendağ. Cluster analysis (dendrogram) based on NEI's genetic distances also showed that Mıhlıdere and Eybekli populations were located in separate clusters, differentiating from the remaining populations. The Mıhlıdere population was the most distantly clustered one compared to other populations. These findings are not surprising since Mıhlıdere population is located in extreme habitats, at the western edge of the species range in the Kazdağı region. On the other hand, Eybekli and Asar populations, which are in the same cluster, are located at the eastern edge in the Kazdağı Region. Considerable amount of gene flow between these populations probably occurs considering the topography of the area, prevailing wind direction and physical distance between these populations.

Protection of all Anatolian black pine populations in the Kazdağı Region may not be feasible. However, for conservation, it may be sufficient to know which populations have the richest genetic diversity. The reason for this is because when faced with choices among alternative sites, conservationists generally prefer to save genetically diverse populations (LEDIG, 1998). Considering the lack of unique alleles, low differentiation of populations, and small genetic distance (or large genetic similarities) between populations, it is difficult to suggest any particular population (s) for effective *in situ* conservation of the genetic diversity of Anatolian black pine populations in Kazdağı as a GMZ. But the results of the another study dealing with the adaptive seedling traits and using the same Anatolian black pine populations and families revealed that Eybekli and Mıhlıdere populations were clearly discriminated when the canonical discrinminant function analysis was applied (VELIOĞLU *et al.*, 1999). In the same study, Gürgendağ population was discriminated as between Eybekli and Mıhlıdere populations. Asar (or Eybekli), Mıhlıdere and Gürgendağ populations are the representatives of the cluster groups formed in cluster analysis using genetic distances. Based on the results of adaptive seedling traits and neutral marker (present isozyme study) studies, Asar (or as an alternate population Eybekli) and Mıhlıdere populations should be seriously considered for the potential GMZs of the Anatolian black pine in the Kazdağı Region. Because Asar is an extreme population for black pine due to its altitude (300 meters); and it has the highest heterozygosity and percent polymorphic loci. The Gürgendağ population has a lower heterozygosity, but it represents the second group in the dendrogram. The Mıhlıdere population on the other hand has a higher percentage of polymorphic loci, and is one of the extreme population of Anatolian black pine in the Kazdağı region with respect to habitat (growing for example in dry, low elevation areas).

The genetic diversity of a species is not the only criterion that must be considered when selecting an area as a potential GMZ site for the species. Other criteria such as, accessibility of the site, ease of protection, presence of other target species, amount of biological diversity etc., are all-important factors which should also be considered. Although, information on the status of biological diversity in Anatolian black pine population sites is lacking in the Kazdağı region, there are unpublished results suggesting that the Eybekli, Mıhlıdere and Gürgendağ population sites are also rich in plant biodiversity, not only tree species, but also other plant species (Personal Communication, NIHAL ÖZEL, Aegean Forest Research Institute, June 1998). Thus, The final decision on designing of GMZs for the Anatolian black pine in the Kazdağı Region will however be made after consideration of the other target species, suitability of the sites for *in situ* conservation of genetic diversity and long-term protection of land as *in situ* reserve.

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