Genetic Structure of Black Pine (*Pinus nigra* ARNOLD subspecies *pallasiana*) Populations Sampled from the Bolkar Mountains

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(Received 11th March 1999)

Summary

In order to determine the magnitude and pattern of genetic diversity among Anatolian Black pine (*Pinus nigra* ARNOLD subspecies *pallasiana*) populations sampled in Bolkar Mountains and to recommend the potential populations which may be suitable for *in situ* conservation of genetic resources in this species, isoenzymes from 14 enzyme systems were investigated by starch gel electrophoresis. For this reason, open pollinated seed megagametophytes of half-sib families originated from the four populations (Çamlıyayla, Ulukışla, Cehennemdere and Gülekdere) were used.

24 loci were resolved for the 14 enzyme systems assayed. Polymorphism (*P*) varied between 41% in Çamlıyayla and 55% in Ulukışla. The mean number of alleles per locus (*A*) was around 1.6 and the expected heterozygosity (H_S) was about 21%. Moreover, only Ulukışla had a unique allele (second allele of GOT2) which gave the distinctive character to the population. Genetic diversity among populations relative to the total genetic diversity (G_{ST}) was averaged as 0.070, indicating that only 7% of the total genetic diversity was among populations. Furthermore, Nei's genetic distance values ranged from 0.007 to 0.032 among population, confirming that the diversity among populations was not very high. Based on the results of this study, it is recommended that Ulukışla and Gülekdere populations could be considered for *in situ* conservation of genetic resources of the species in the Bolkar Mountains.

Key words: Pinus nigra subsp. *pallasiana*, genetic diversity, genetic distance, *in situ* conservation, isoenzymes.

Introduction

European Black pine (*Pinus nigra* ARNOLD) 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 altitudinal distribution usually ranges from 250 meters to 1400 meters (VIDAKOVIC, 1991).

European Black pine has a very discontinuous distribution in its range. It is mainly a southern European species, extending from Spain to Turkey. Because of the pattern of its natural distribution, it is considered to be a very variable species in morphological, anatomical and physiological characteristics (SCALTSOYIANNES *et al.,* 1994). European Black pine should be regarded as a species subdivided into several subspecies and varieties. SCHWARZ (1938) divided the Black pine into six subspecies: subs. *pallasiana*, subs. *fenzlii*, subs. *dalmatica*, subs. *nigra*, subs. *laricio*, and subs. *salzmannii*. The Turkish variety of European Black pine, *Pinus nigra* subs. *pallasiana* (Anatolian black pine), is a widespread mid-elevation species in the Taurus, Western Anatolia and Northern Anatolian mountains (*Figure 1A*). The natural distribution covers more than 2 million hectares in Turkey. This subspecies is an important timber species and it is used for afforesting the high Anatolian steppes in Turkey (KAYA and TEMERIT, 1994).

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). The isozyme analysis is an important tool for describing population genetic structure, taxonomic status and mating systems of forest trees. Isoenzymes can also be used

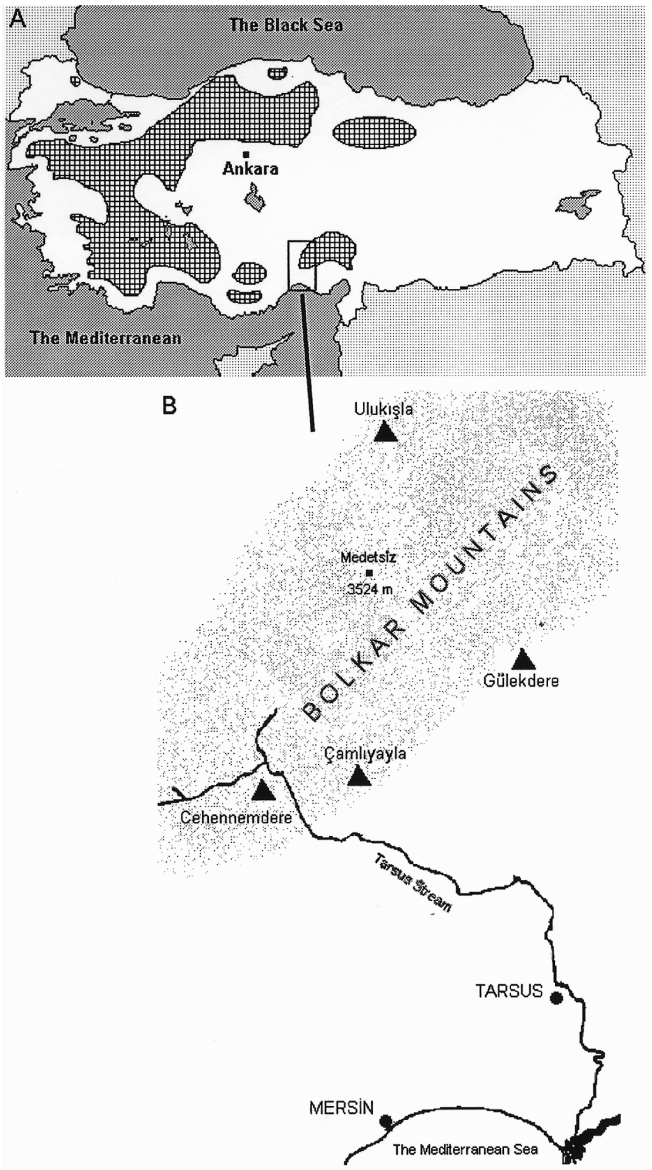

Figure 1. – The map showing natural distribution of *Pinus nigra* ARNOLD subspecies *pallasiana* in Turkey (*A*). Locations of studied populations are indicated by ▲ in the map (*B*).

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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).

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 such as; Kazdağı, Bolkar Mountains and Ceylanpınar State Farm. *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 as indicated in the National Plan for *In situ* Conservation of Plant Genetic Diversity in Turkey (KAYA *et al.,* 1997). Moreover, Anatolian Black Pine is the most widespread conifer species in the region. Thus, studying genetic diversity in Anatolian black pine populations in the Bolkar Mountain Region would provide information on pattern of genetic variation in the area.

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 Bolkar Mountains, a conservation strategy which reduces the cost and increase the efficiency of *in situ* conservation site was selected. This involved potential Anatolian black pine GMZ sites in which selected target trees were evaluated. To determine the potential GMZ sites concerning Anatolian black pine in the Bolkar Mountains in southern Turkey, effectively, open pollinated seeds from 190 half-sib families of four populations representing optimum and extreme habitats for the species in the region were collected. The genetic structure of four populations was determined using 14 isozyme systems and the implications for *in situ* conservation of genetic resources of the species were discussed.

Material and Methods

Open pollinated seeds of Anatolian black pine were collected from a total of 190 parent trees (half-sib families) of 4 natural populations of Bolkar Mountains, Turkey, in fall of 1995 (*Table 1, Figure 1B*). The seeds were stored at 4 °C for about one year. Conifer seeds do not loose their viability for about 10 years so there were no selection pressure of this storage on seed viability.

The seeds were germinated in germination boxes for 2 days to 4 days at room temperature in daylight. When the germinated radicles were 2 mm to 3 mm long, megagametophytes were separated from the embryos and crushed in extraction buffer (0.2 M phosphate). Megagametophytes from 8 seeds of each of 190 randomly selected mother trees (half-sib families) were analyzed by horizontal starch gel electrophoresis run at 4 °C for 4 hours to 5 hours at constant amperages. Gel and tray buffers used were as reported by Conkle et al.(1982). The 14 enzyme systems investigated were leucine aminopeptidase (LAP); phosphoglucoisomerase (PGI); phosphoglucomutase (PGM); glutamate oxaloacetate transaminase (GOT); glutamate dehydrogenase (GDH); mannose phosphate isomerase, (PMI); superoxide dismutase (SOD); aconitase (ACO); acid phosphatase (ACP); menadione reductase (MNR); shikimate dehydrogenase (SKDH); diaphorase (DIA); isocitrate dehydrogenase (IDH); and malate dehydrogenase (MDH).

Table 1. – Geographic description of the studied populations and number of seed trees (half-sib families) in each population.

Population	Latitude(N)	Longitude(E)	Altitude(m)	Number of half-sib families sampled	Average Stand Age
Cehennemdere	37° 08'	34° 30'	1550	42	70
Ulukışla	37° 33'	34° 29'	1560	50	83
Çamlıyayla	37° 08'	34° 33'	1350	48	80
Gülekdere	37° 12'	34° 48'	1500	50	78

In order to determine the amount of genetic diversity in a standardized way, allelic richness (n_a), proportion of polymorphic loci (P) and heterozygosity (h) was estimated by the following equations, respectively:

$Mean(n_a) = \frac{\sum n_{a_i}}{r}$, where ($n_{a_i}$) is the number of alleles at the i^{th} locus and r is the number of loci.

$\hat{P} = \frac{n_p}{r}$, where n_p is the number of polymorphic loci in r number of loci (NEI, 1987).

The sample variance of proportion of polymorphic loci was estimated as;

$$V\left(\hat{P}\right) = \frac{\hat{P}\left(1 - \hat{P}\right)}{r} \text{ (Nei, 1987).}$$

Heterozygosity was estimated using the following equation;

$$\hat{h} = \frac{2N\left(1 - \sum \hat{x}_i^2\right)}{2N - 1} \text{ (Nei, 1987)}$$

For the variance of single locus estimates of ($\hat{h}$), the following equation was used (NEI, 1987);

$$V(\hat{h}) = 2\left[\sum \hat{x}_i^3 - \left(\sum \hat{x}_i^2\right)^2\right] / N$$

Estimations of heterozygosities: the observed heterozygosity of an individual in a subpopulation (H_I),

$$H_I = \frac{\sum_{j=1}^{s} \hat{h}_{oj}}{s},$$

where s is the number of subpopulations and ($\hat{h}_{o_j}$) is the observed heterozygosity in subpopulation j (NEI, 1987).

Expected heterozygosity of an individual in a subpopulation (H_S),

$$H_S = \frac{\sum_{j=1}^{s} \hat{h}_j}{s},$$

where ($\hat{h}_j$) is the expected heterozygosity in subpopulation j (NEI, 1987).

Expected heterozygosity of an individual in the total population (H_T)

$H_T = 1 - \sum_i x_{ia}^2$, where ($x_{ia}$) is the frequency of the i^{th} allele averaged over all subpopulations (NEI, 1987).

Finally, the average diversity between subpopulations (D_{ST})

$H_T = H_S + D_{ST}$

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 estimates of standard genetic distance (D), unbiased for sample size (NEI, 1978) for all pair-wise population comparisons were calculated to show the genetic relationships between populations.

$D' = \ln I'$ where I is the identity between two populations x and y.

All the estimations were carried on by the use of GeneStat computer program (LEWIS, 1993) and POPGENE software (YEH *et al.,* 1997).

Results

The genetic control of isozymes was postulated from the banding patterns observed in megagametophytes of the 190 mother trees. 14 enzyme systems were studied. However, in α-EST, G-6PDH and 6-PGD enzyme systems, clear banding patterns were not observable. For this reason, those enzymes were excluded from the study. In total, 24 zones of activity were resolved for the14 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 was monomorphic: GOT1, PMI1, SOD1, SOD2, GDH, ACO, DIA2, IDH1, IDH2, MDH1 and MDH3. Whereas, heterogeneity ($p < 0.01$) in allele frequencies among populations were detected at the remaining 13 loci (*Table 2*).

The estimated parameters which are used to describe the genetic diversity of the populations were given in *table 3*. The proportion of polymorphic loci (P) varied from 0.417 in Çamlıyayla to 0.542 in Ulukışla population of Anatolian black pine. When all four populations were considered, the polymorphism was 0.479 on the average (*Table 3*). The mean number of alleles per locus (A) had the lowest value in Çamlıyayla and Gülekdere populations (1.500) whereas, it was the highest in Ulukışla (1.625). Moreover, Ulukışla had a unique allele that could not be detected in the other populations. The mean number of alleles per locus was calculated as 1.552 on the average (*Table 3*). Among the populations, the heterozygosities calculated directly from genotypes (H_{obs}) varied from 0.216 in Cehennemdere to 0.278 in Çamlıyayla. Whereas, heterozygosities estimated (H_S) according to HARDY-WEINBERG expectations varied from 0.193 in Cehennemdere to 0.224 in Gülekdere populations of Anatolian black pine. When the expected and the observed heterozygosities were compared, the observed heterozygosities were found to be slightly higher than the expected values. The mean values calculated for observed heterozygosity (H_{obs}) and the expected heterozygosity (H_S) was 0.256 and 0.211, respectively. However, these differences do not seem to be significant considering the standard errors of the estimation (*Table 3*).

According to the NEI's G-Statistics, the mean total genetic diversity (H_T) was calculated as 0.227, the mean expected heterozygosity in subpopulations (H_S) over all populations was determined as 0.211 and the average diversity between subpopulations (D_{ST}) was found to be 0.016. These mean that the greatest amount of genetic diversity was localized within populations ($H_S = 0.211$) showing a low value for D_{ST} (0.016). Moreover, the relative magnitude of genetic differentiation (G_{ST}) among subpopulations was measured as 0.070 indicating that

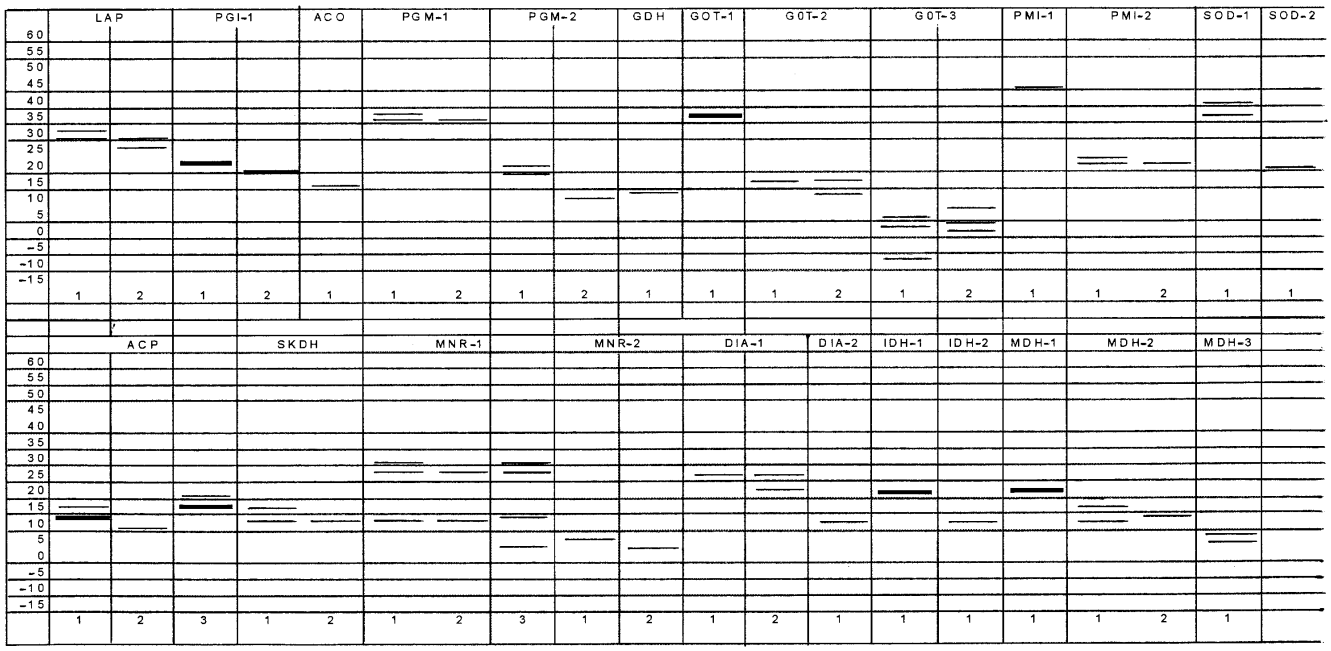

Figure 2. – Megagametophyte banding patterns and their allelic designations for 24 isoenzyme loci of Anatolian Black pine (numbers on vertical axis refer to migration distances from the origin in mm). Alleles with more than one band due to gene dublication. Alleles with darker bands due to overexpression of the related gene.

Table 2. – Allele frequencies in 24 isozyme loci studied in Anatolian black pine populations.

		Populations			
Locus	Allele	Çamlıyayla	Cehennemdere	Ulukışla	Gülekdere
LAP					
	1	0.573	0.929	0.870	0.792
	2	0.427	0.071	0.130	0.208
PGI1					
	1	0.600	0.893	0.710	0.542
	2	0.400	0.107	0.290	0.458
PGM1					
	1	0.583	0.524	0.702	0.424
	2	0.417	0.476	0.298	0.576
PGM2					
	1	1.000	0.786	0.964	0.300
	2	0.000	0.214	0.036	0.700
GOT1					
	1	1.000	1.000	1.000	1.000
GOT2					
	1	1.000	1.000	0.872	1.000
	2	0.000	0.000	0.128	0.000
GOT3					
	1	1.000	0.940	0.970	1.000
	2	0.000	0.060	0.030	0.000
PMI1					
	1	1.000	1.000	1.000	1.000
PMI2					
	1	0.500	0.488	0.470	0.625
	2	0.500	0.512	0.530	0.375
SOD1					
	1	1.000	1.000	1.000	1.000
SOD2					
	1	1.000	1.000	1.000	1.000
GDH					
	1	1.000	1.000	1.000	1.000
ACP					
	1	0.427	0.714	0.565	0.540
	2	0.479	0.274	0.402	0.460
	3	0.094	0.012	0.033	0.000
ACO					
	1	1.000	1.000	1.000	1.000
MNR1					
	1	0.208	0.107	0.160	0.220
	2	0.448	0.524	0.460	0.410
	3	0.344	0.369	0.380	0.370
MNR2					
	1	0.573	0.488	0.560	0.580
	2	0.427	0.512	0.440	0.420
SKDH					
	1	0.513	0.679	0.510	0.570
	2	0.488	0.321	0.490	0.430
DIA1					
	1	0.576	0.724	0.595	0.485
	2	0.424	0.276	0.405	0.515
DIA2					
	1	1.000	1.000	1.000	1.000
IDH1					
	1	1.000	1.000	1.000	1.000
IDH2					
	1	1.000	1.000	1.000	1.000
MDH1					
	1	1.000	1.000	1.000	1.000
MDH2					
	1	0.500	0.588	0.340	0.500
	2	0.500	0.412	0.660	0.500
MDH3					
	1	1.000	1.000	1.000	1.000

only 7% of the total genetic diversity was among populations (*Table 4*).

The estimates of standard genetic distance (*D*), unbiased for sample size (NEI, 1978) for all pair-wise population comparisons were estimated (*Table 5*). The genetic distance values among populations varied from 0.007 (between Çamlıyayla and Ulukışla) to 0.032 (between Ulukışla and Gülekdere). The distance values of all possible pairs of populations indicated that Ulukışla and Gülekdere populations are genetically the least similar ones while the Ulukışla and Çamlıyayla populations are the most similar ones (*Table 5*).

In order to see the differentiation of the four populations more clearly, the dendrogram was constructed by using NEI's genetic distances (1978) and the Unweighted Pair Group Method with Arithmetic means (UPGMA) (*Figure 3*). Two clearly separated branches were observable: one of them was with the strongly distant Gülekdere population and the other one was with Çamlıyayla, Cehennemdere and Ulukışla populations.

Discussions

The isozyme analysis provided important information about the genetic diversity in Anatolian Black pine. The gene frequencies, obtained from the analysis of our data, suggested that significant genetic differentiation existed among the populations.

In all of the populations, some of the loci yielded banding patterns consisting of double or more bands, such as, LAP, PGM1 and 2, GOT2 and 3, PMI2, SOD1, ACP, SKDH, MNR1, DIA1, and MDH2. Similar results have been reported in the previous isozyme studies dealing with *Pinus nigra* (NICOLIĆ and TUCIĆ, 1983; Doğan *et al.,* 1998). The reason for this multiple banding pattern may be explained by the probable duplication of the related genes.

The banding patterns observed in PGI, PGM, GOT, SOD, GDH, ACO and DIA systems were consistent with findings of other studies. However, in LAP, ACP, SKDH, IDH and MDH systems, some differences were observed in the banding patterns when compared to the results of the previous studies on *P. nigra* and other conifer species (PEI-SHOW and STOTZKY, 1973; CONKLE, 1979; ADAMS and JOLY, 1980a and b; MORAN *et al.,* 1980; EL-KASSABY, 1981; NICOLIĆ and TUCIĆ, 1983; MILLAR, 1985; GRUNWALD *et al.,* 1986; STRAUSS and CONKLE, 1986; SHIRAISHI, 1988a and b; MATHESON, 1989; THORMANN and STEPHAN, 1993; SCALTSOYIANNES *et al.,* 1994; AGUINAGALDE *et al.,* 1997; KIM *et al.,* 1997 and Doğan *et al.,* 1998). These differences might be due to the systems used or due to the differences in the genetic make up of the Anatolian black pine populations studied.

Two enzyme systems, PMI and MNR, were studied for the first time in Anatolian Black pine so there were no available literature related to those enzyme systems. The gels stained for PMI and MNR had two zones of activity. Moreover, it was found that the first zone had only a single band and, PMI2 had two variants. MILLAR (1985) reported that PMI was yielding two monomorphic zones in many other conifer species. On the other hand, STRAUSS and CONKLE (1986), in their study with knobcone pine, determined two polymorphic zone in PMI and three loci for MNR. However, there are also reports indicating the presence of two zones of activities in MNR system (KIM *et al.,* 1997 and FALLOUR *et al.,* 1997).

In general, coniferous trees show very high genetic diversity when compared to other types of organisms (HAMRICK *et al.,* 1981). Although pines have somewhat lower genetic diversity relative to other conifers (SCHILLER *et al.,* 1986; MORAN *et al.,*

Table 3. – Parameters related to within population diversity of four Anatolian Black pine populations.

Populations	P	A	U	H_{obs}	H_S	H_{obs}/H_S
Çamlıyayla	0.417 ±0.103	1.500 ±0.135	0.000	0.278 ±0.077	0.217 ±0.054	1.281
Cehennemdere	0.500 ±0.104	1.583 ±0.133	0.000	0.216 ±0.064	0.193 ±0.046	1.119
Ulukışla	0.542 ±0.104	1.625 ±0.132	1.000	0.254 ±0.064	0.209 0.048±	1.215
Gülekdere	0.458 ±0.104	1.500 ±0.120	0.000	0.268 ±0.076	0.224 ±0.052	1.196
Means ±s.e.	*0.479 ±0.027*	*1.552 ±0.031*	*0.250 ±0.250*	*0.256 ±0.076*	*0.211 ±0.007*	*1.203*

P = proportion of polymorphic loci
A = mean number of alleles per locus
U = number of alleles unique to that population or private alleles
H_{obs} = observed heterozygosity
H_S = expected heterozygosity.

1988 and KIM *et al.,* 1997), *Pinus nigra* was one of the most diverse of the coniferous species (NICOLIĆ and TUCIĆ, 1983; SCALTSOYIANNES *et al.,* 1994 and AGUINAGALDE *et al.,* 1997). In the present study, *Pinus nigra* subsp. *pallasiana* exhibited slightly less genetic diversity relative to the species *Pinus nigra* considered as a whole, however, higher genetic diversity when compared to that of other conifers. Polymorphism varied between 41% and 55% while the frequency of the most common allele was 0.99 or more ($p < 0.01$). However, in their studies with *Pinus nigra,* NICOLIĆ and TUCIĆ, (1983) (66%) and *Scaltsoyiannes et al.,* (1994) (70%) determined considerably higher polymorphic values when compared to our result (48%). NICOLIĆ and TUCIĆ (1983) studied only a limited number of enzyme systems such as ACP, LAP and (-EST, and resolved for only 4 loci. On the other hand, SCALTSOYIANNES *et al.* (1994) studied 10 enzyme systems and determined 16 loci. These differences between the results were most probably due to the enzyme systems and the number of loci studied involved in estimation.

Table 4. – Genetic diversity parameters estimated for 13 polymorphic loci of the Anatolian Black pine populations studied.

Locus	H_S [a]	H_T [b]	D_{ST} [c]	G_{ST} [d]
LAP	0.297	0.343	0.045	0.132
PGI1	0.399	0.443	0.043	0.098
PGM1	0.478	0.500	0.022	0.044
PGM2	0.209	0.414	0.205	0.496
GOT2	0.056	0.064	0.008	0.119
GOT3	0.043	0.044	0.001	0.027
PMI2	0.497	0.502	0.005	0.009
ACP	0.508	0.527	0.019	0.036
MNR1	0.627	0.625	0.000	0.000
MNR2	0.498	0.496	0.000	0.000
SKDH	0.486	0.494	0.007	0.015
DIA1	0.472	0.487	0.014	0.030
MDH2	0.488	0.505	0.016	0.032
Means	*0.211± 0.049*	*0.227± 0.051*	*0.016*	*0.070*

[a]) the expected heterozygosity in subpopulations
[b]) the total genetic diversity
[c]) the average diversity between populations
[d]) the relative magnitude of genetic differentiation

Table 5. – NEI's Genetic Distances estimated between Anatolian black pine populations.

	Çamlıyayla	Cehennemdere	Ulukışla	Gülekdere
Çamlıyayla	–	–	–	–
Cehennemdere	0.018	–	–	–
Ulukışla	0.007	0.010	–	–
Gülekdere	0.030	0.026	0.032	–

It was found in the present study that the mean number of alleles per locus (A) was approximately 1.6, and the expected heterozygosity (H_S) was about 21%. SCALTSOYIANNES *et al.* (1994) reported the mean number of alleles as 2.0 and expected heterozygosity as 20%. These results in *Pinus nigra* were consistent with our findings. Moreover, out of the four populations only Ulukışla had a unique allele (second allele of GOT2), and this gave the distinctive character to that population which may be an important criteria in selection of *in situ* gene conservation sites as a GMZ for the Anatolian Black pine populations in the Bolkar Mountains.

Genetic diversity and differentiation among populations within subspecies could be examined further by analyzing intra- and interpopulation components of genetic diversity. Total genetic diversity (H_T) was averaged as 0.227. The greatest amount of genetic diversity was localized within populations ($H_S = 0.211$) showing a low value for differentiation of populations ($D_{ST} = 0.016$). 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). Genetic diversity among populations relative to the total genetic diversity (G_{ST}) was averaged as 0.070, indicating that only 7% of the total genetic diversity were among

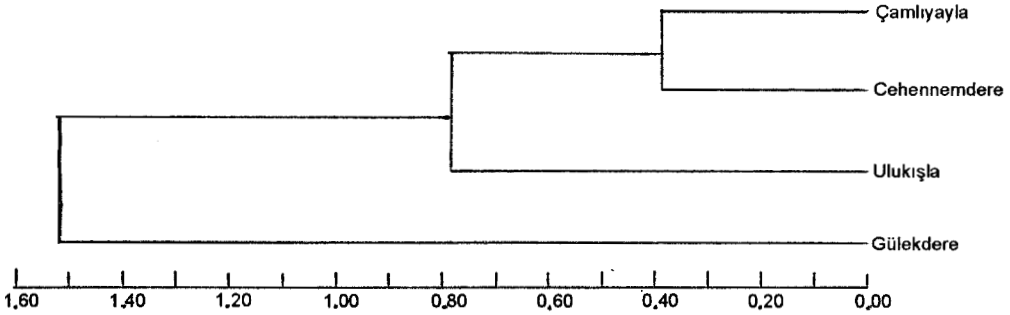

Figure 3. – UPGM dendrogram based on the Nei's (1987) genetic distances between populations showing the clustering pattern of four Anatolian black pine populations.

populations. Evidently, this suggests either that heterogeneity within populations was higher than heterogeneity among populations or that interpopulation genetic diversity was not so strong. The results of this study were consistent with the findings of the previous studies dealing with *P. nigra* (NICOLIĆ and TUCIĆ, 1983; SCALTSOYIANNES *et al.,* 1994) as well as other studies on conifers (EL-KASSABY, 1991; MÜLLER-STARCK *et al.,* 1992); clearly indicating that heterogeneity within populations was higher than heterogeneity among populations.

The data on the genetic distances between populations revealed that Gülekdere population was the most distant population and out of the four populations, Çamlıyayla and Cehennemdere were the most closely related ones. Furthermore, these two populations showed a genetic similarity to Ulukışla population. Gülekdere was geographically located further away from the other populations in the Bolkar Mountains. Ulukışla population, on the other hand, was located at the edge of the Bolkar Mountains so did not get much gene flow from Çamlıyayla or Cehennemdere population. Therefore, it is reasonable that these two populations Gülekdere and Ulukışla are genetically the most distant ones among the studies populations.

Studies of genetic diversity guide the choice of populations for conservation (LEDIG, 1998). Knowledge of the distribution of genetic diversity among populations is critical in the formulation of management strategies (HAMRICK *et al.,* 1991). In the present study, using the isozyme markers, the genetic diversity within and among the four selected Anatolian black pine populations were determined. This data was further used as a guide in the recommendation of suitable populations. For the Bolkar Mountains, in order to be able to capture most of the genetic diversity existing in Anatolian Black pine populations in the area, at least two populations of the species should be considered for *in situ* conservation, as Gene Management Zones. Having high polymorphism, mean number of alleles per locus, the only unique allele and highest genetic distance when compared to the other populations, Ulukışla can be considered as the first choice for *in situ* reserves. On the other hand, Gülekdere, being the most distant population to Ulukışla and having the highest heterozygosity can be considered as the second choice for the in situ conservation of genetic resources of the species. However, for the final decision on Gene Management Zone selection in the Bolkar Mountains other factors such as, presence of more than one target species and degree of biological diversity in the site and suitability of the area for *in situ* preservation should be considered.

Acknowledgments

This study has been supported by the Ministry of Forestry as a part of "*In situ* Conservation of Plant Genetic Diversity" project which was funded by the World Bank (Project #: GEF-28632-TU).

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Levels and Partitioning of Genetic Diversity of *Camellia japonica* (Theaceae) in Korea and Japan

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(Received 23rd September 1999)

Abstract

We analyzed allozymes from 620 trees to estimate the levels of allozyme diversity within and genetic differentiation among populations of *Camellia japonica* in Korea and Japan. Both at the population and species levels, *C. japonica* (mean expected heterozygosity, H_e = 0.169 and 0.190) maintains high levels of genetic diversity. Although Korean populations of the species are located in edge of its distribution, they (0.209) harbor higher levels of genetic diversity than that found within populations in Japan (0.145). The mean G_{ST} value among 16 populations (0.091) of *C. japonica* was similar to those among six Korean (0.055) and 10 Japanese populations (0.086). The results indicate that a low degree of allozyme differentiation between populations in Japan and Korea, though the land connection between the southern Korean peninsula and southern Japanese archipelagos no longer existed after the middle Pleistocene. There was no significant correlation between genetic distance and geographic distance, indicating that isolation by distance may not be a primary factor for shaping genetic structure among populations. Instead, genetic diversity and structure in populations of *C. japonica* may result from the balance between occasional gene flow and genetic drift.

Key words: Allozyme, *Camellia japonica,* east Asia, gene glow, genetic differentiation.

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Introduction

The coastal forests of northeast Asia possess a unique suite of broad-leaved evergreen woody species such as *Camellia japonica* L., *Castanopsis* spp., *Eurya* spp., *Ficus* spp., *Ligustrum* spp., *Litsea* spp., *Neolitsea* spp., *Persea* spp., *Quercus* spp., etc. Since these species are important members of coastal forest vegetation in Japan and Korea (NUMATA, 1974), population studies such as population dynamics (e.g., YAMAMOTO, 1992; SATO *et al.,* 1994; TANOUCHI *et al.,* 1994), pollination ecology (e.g., YUMOTO, 1987), allozyme variation in local populations (e.g., WENDEL and PARKS, 1985; CHUNG and KANG, 1994, 1996), and population genetic structure (e.g., CHUNG and EPPERSON, 2000) have been conducted.

During the Ice Age (the glacial Würm), the Sea of Japan (East Sea) and Yellow Sea were about 100 m lower than at present and a land connection existed between Korea and Japan (KIM and HONG, 1991). However, little is known about the degree of genetic differentiation among plant populations extending over Korea and the Islands of Japan (e.g., M. G. CHUNG and M. Y. CHUNG, 2000a). The distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity of a plant species (HAMRICK *et al.,* 1991).

Camellia japonica is widely distributed in Japan (Honshu, Shikoku, and Kyushu) and the southern Korean peninsula. *Camellia japonica* usually occurs in old-growth forests on several islands near the southern coast of the regions and coexists *Eurya japonica, Persea thunbergii, Neolitsea sericea,* and *Cinnamonum insularimontanum,* etc. *Camellia japonica*