Turk J Agric For
32 (2008) 137-145

Isozyme Variation in Four Natural Populations of *Cedrus libani* A.Rich. in Turkey

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Received: 10.10.2007

Abstract Genetic variation in 4 natural populations of *Cedrus libani* A.Rich. was determined using isoenzyme analyses. Isozymes from 12 enzyme systems extracted from haploid female gametophytes of the seeds were separated by horizontal starch gel electrophoresis. In the 12 enzyme systems, 16 loci and 37 alleles were observed. Average proportion of polymorphic loci for the populations ranged from 43.8% to 62.5%. The average number of alleles per locus per population was estimated as 2.0. Mean estimated expected-heterozygosity (He) of the populations was 0.168. The level of gene flow (Nm) was 12.7 per generation. A very high proportion of genetic variation was within populations (98.07%). Nei's genetic distance coefficient ranged from 0.003 to 0.008 among all possible population pairs. The mean value of Nei's genetic distance (0.005) confirmed the hypothesis that variation among the populations is low.

Key Words: Taurus cedar, genetic variation, geographical variation, starch gel electrophoresis

Türkiye'deki Dört Doğal Sedir (*Cedrus libani* A.Rich.) Populasyonunda İzoenzim Çeşitliliğinin Belirlenmesi

Özet: Bu çalışmada, izoenzim analizleri yoluyla *Cedrus libani* A.Rich.'in dört farklı populasyonunda genetik varyasyon belirlendi. Tohumun haploit dişi gametofitik dokusundan özütlenen 12 enzim sistemine ait izoenzimler, yatay nişasta jel elektroforezi tekniği kullanılarak ayrıldı. İncelenen 12 enzim sisteminde toplam 16 lokus ve 37 allel gözlendi. Populasyonlarda polimorfik lokusların oranı % 43.8 ile % 62.5 arasında değişmektedir. Her bir populasyonda her bir lokus için ortalama allel sayısı 2.0 olarak bulundu. Çalışılan sedir populasyonlarında genetik çeşitliliğin büyük oranda (% 98.07) populasyonlar içinde olduğu gözlendi. Populasyonların ortalama beklenen-heterozigotluk oranı (Hb) 0.168 olarak bulundu. Gen akışının düzeyi (Nm) her bir kuşakta 12.7 olarak hesaplandı. Nei'nin genetik mesafe katsayısı, bütün populasyon çiftleri dikkate alındığında 0.003 ile 0.008 arasında değişen değerler gösterdi. Nei'nin genetik mesafesinin ortalama değeri (0.005) de populasyonlar arasındaki farklılıkların düşük olduğu hipotezini doğrulamaktadır.

Anahtar Sözcükler: Toros sediri, genetik çeşitlilik, coğrafik çeşitlilik, nişasta jel elektroforezi

Introduction

Cedrus libani A.Rich. (Cedar of Lebanon or Taurus cedar) is a significant and salient tree species in historical, cultural, aesthetic, scientific, and economic terms. Its present distribution is restricted mainly to the Taurus Mountains in southern Turkey, where the most productive populations are found, especially in the Elmalı region near Antalya. Historical records indicate that extensive and magnificent forests of Lebanon cedar also grew in Syria and Lebanon, where only small populations remain today (Boydak, 1996, 2003; Khuri et al., 2000). Heavy human impact in the past likely caused genetic erosion, decrease in genetic variability, and eventually degradation of the gene pool of the species (Işık and Yıldırım, 1990; Rogers and Kaya, 2006; Fady et al., 2008). The elevational distribution of the species ranges between 800 and 2100 m in the Taurus Mountains. Marginal small populations can also be found at lower (500 m) (e.g., Babadağ-Fethiye) and higher (2400 m) (e.g., Bolkar Mountains) elevations in certain localities (Kantarcı, 1990; Boydak, 1996). In its distribution area, individuals and populations of *C. libani* exhibit distinct phenotypic characteristics (Işık and Yıldırım, 1990; Boydak, 1996).

Analysis of isozyme variation is one of the fastest, most useful, and inexpensive methods for studying

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genetic variation at DNA level (Feret and Bergmann, 1976; Weber and Stettler, 1981; Buth and Murphy, 1999; Fallour et al., 2001). Various populations of *C. libani* have been studied by different researchers, using molecular markers such as isozyme, RAPD, and AFLP (Panetsos et al., 1992, 1994; Bariteau et al., 1999; Scaltsoyiannes, 1999; Piola et al., 1999; Fady et al., 2000, 2003; Bou dagher-Kharrat et al., 2007). There are also certain studies that focused only on the *C. libani* populations in Turkey. For example, Yahyaoğlu et al. (1997) studied 1 enzyme in 8 populations, and Gülbaba and Özkurt (2002) studied 13 enzymes in 7 populations. Kayıhan (2000) and Kayıhan et al. (2006), using DNA markers (RAPD), studied large number of populations representing the natural range of the species. None of these studies, however, covered *C. libani* populations in the Elmalı region, where the species exhibits the most magnificent and well-protected natural stands in its natural range. Detailed genetic variation studies employing additional enzyme systems need to be performed on these least-exploited populations in the Elmalı region. In this study, 2 populations (Avlankuzudağı and Çamkuyusu) from the Elmalı region and 2 populations (Mut and Pozantı) from south-central parts of the Taurus Mountains were sampled (Figure 1).

The aims of the study were (1) to obtain additional information on variation in allele and genotype frequencies of isozymes of *C. libani* in the Taurus Mountains, (2) to obtain information on intrapopulation variation, and (3) to investigate if any variation along altitudinal gradients appears in allozyme frequencies.

Materials and Methods

Plant Material

Megagametophytes from seeds of 4 *C. libani* populations were used in the study (Table 1). Two populations (Avlankuzu (A) and Çamkuyusu (C)) were located near Antalya, 1 population in Mut (M) near Mersin, and 1 in Pozantı (P) near Adana (Figure 1). The seeds from Mut and Pozantı populations were bulked, and obtained directly from the Forest Tree Seeds and Tree Breeding Research Directorate of the Ministry of Environment and Forestry, Ankara, Turkey. The general guidelines for bulked seed collection of the Ministry are that "several tons of cones from hundreds of trees within a given stand" are collected and thoroughly mixed during the seed extraction process. Therefore, the exact number of trees for the bulked seed sources is not known. The seeds from individual trees in the Avlankuzu and Çamkuyusu populations were collected under the guidance of the authors in October 2004. The cones were collected from at least 25 trees in each of these 2 populations, and labeled by mother tree, referred as "family" from now on. The distance between the mother trees in the populations was at least 30 m. Total range of seed collection area was about 300 ha in each population. Following the collection, the cones were stored in a cold

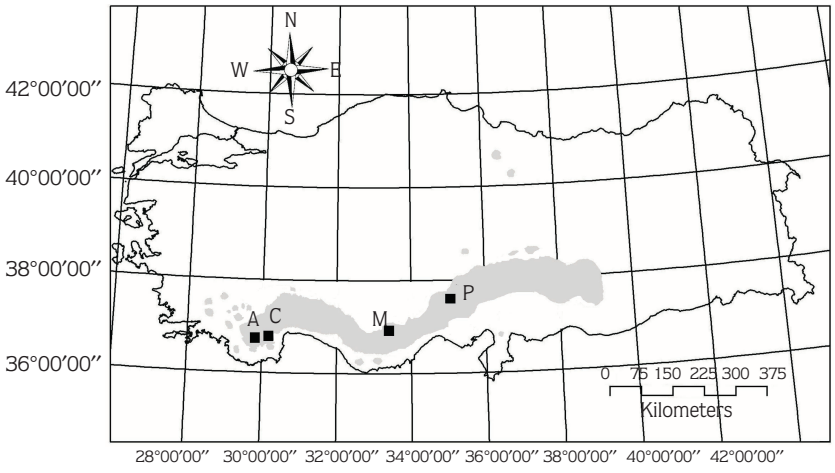

Figure 1. Locations (dark squares with letters A, C, M, P) of 4 *Cedrus libani* populations sampled in southern Turkey [shaded areas show the gross natural distribution range of *C. libani* in Turkey (data from Kantarcı, 1990; Boydak, 1996, 2003)].

Table 1. Locations of *Cedrus libani* populations analyzed in the study.

Population, abbreviation	Altitude (m)	Latitude N.	Longitude E.	Aspect*	Distance from the Mediterranean Sea (km)
Elmalı-Avlankuzu, A	1100	36º 34´ 48˝	29º57´57˝	NW	39
Elmalı-Çamkuyusu, C	1800	36º 35´ 86˝	30º01´55˝	S	38
Mut-Söğütözü, M	1650	36º 46´ 30˝	33º32´20˝	NW	51
Pozantı-Pozantı, P	1320	37º 30´ 32˝	34º57´38˝	W	73

* W = West, S = South, NW = Northwest

room at 5 ºC for about 2 weeks. They were then soaked in tap water for 24 h, whereby cone-scales were loosened, and then the seeds were extracted manually. The seeds were air dried and stored at 4 °C until they were used in isozyme analysis.

The Pozantı population was also included in the study by Panetsos et al. (1994) and by Scaltsoyiannes (1999). However, Avlankuzu, Çamkuyusu, and Mut populations were not sampled in any of the earlier studies on the species.

Electrophoretic Analysis

Electrophoresis was carried out on haploid megagametophytes of germinated seeds with a radicle 3 to 5 mm long. Germination of the seeds was enhanced with stratification at 4 °C for 15 days (Takos and Merou, 2001). Megagametophytes were dissected from germinated seeds and homogenized individually by adding 80 µl of 0.2 M phosphate buffer (pH 7.5) (Conkle et al., 1982). In electrophoretic analysis, on average 200 seeds were analyzed in bulked (Mut and Pozantı) populations. In the other 2 populations (Avlankuzu and Çamkuyusu), we analyzed 8 megagametophytes from each family. With a sample of 8 megagametophytes, the probability (P) of observing heterozygous maternal genotype at any single locus is > 0.992 [i.e. $P = (1/2)^{n-1} = (1/2)^7 = 0.992$] (Cheliak and Pitel, 1984). All the enzyme systems tested were resolved using horizontal starch gel (12% starch) electrophoresis (Conkle et al., 1982; Cheliak and Pitel, 1984). We used *Pinus resinosa*, which is monomorphic for all the enzyme systems studied, as a marker (Allendorf et al., 1982; Mosseler et al., 1991).

The homogenates were analyzed for the following enzyme systems: alcohol dehydrogenase (ADH; E.C. 1.1.1.1), acid phosphatase (ACP; E.C. 3.1.3.2), glucose 6-phosphate dehydrogenase (G6PDH; E.C. 1.1.1.49), glutamate dehydrogenase (GDH; E.C. 1.4.1.2), glutamate-oxaloacetate transaminase (GOT; E.C. 2.6.1.1), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42), leucine aminopeptidase (LAP; E.C. 3.4.11.1), malate dehydrogenase (MDH; E.C. 1.1.1.37), menadione reductase (MNR; E.C. 1.6.99.2), 6-phosphogluconate dehydrogenase (6PGD; E.C. 1.1.1.44), phosphoglucose isomerase (PGI; E.C. 5.3.1.9), and superoxide dismutase (SOD; E.C. 1.15.1.1). Gels were sliced and stained for each enzyme system according to Conkle et al. (1982) with slight modification as described in Kurt (2005). The loci (isozymes) and the alleles within each locus (allozymes) were numbered in decreasing order of anodal mobility. "Null" allozymes, which lacked staining activity, were specified by the letter "n".

Statistical Analysis

All calculations of parameters on intra- and interpopulation genetic diversity (mean number of alleles per locus, percentage of polymorphic loci, mean heterozygosity expected from Hardy-Weinberg proportions, estimation of genetic differentiation, and genetic distances) were done using Biosys-1, a computer program for the analysis of allelic variation in genetics (Swofford and Salender, 1981). Genetic differentiation and genetic distances were estimated according to Nei (1973, 1978). Cluster analysis, using the Unweighted Pair Group Method (UPGMA), was performed on the matrix of Nei's genetic distances (Sneath and Sokal, 1973).

Results

Twelve enzyme systems encoded by 16 loci were identified (Figure 2). Of the 16 loci, 4 (*Acp2*, *Idh*, *Pgi1*, and *Sod*) were monomorphic for all populations. We detected a total of 37 alleles for 16 loci in 4 populations.

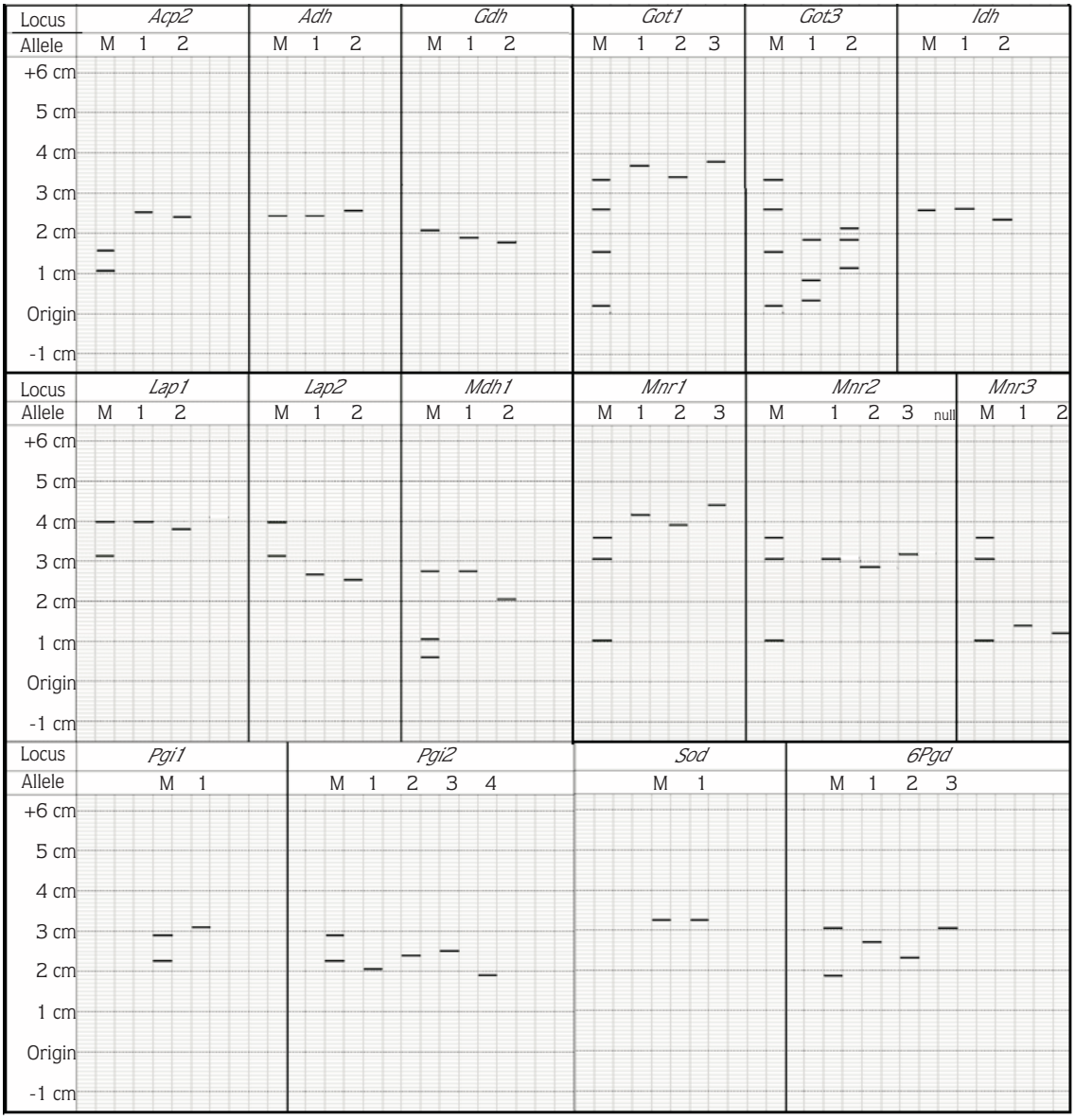

Figure 2. Zymogram of the enzyme systems in *Cedrus libani*. M: *Pinus resinosa* control or marker of migration.

Idh2 and *6Pgd3* were observed only in the Pozantı population, *Mnr2-2* and *Mnr2-3* only in the Mut-Söğütözü population, and *Adh-2* only in the Çamkuyusu population. In other words, these alleles are unique to the given populations. Four (*Adh-2*, *Idh-2*, *Mnr2-2*, and *Pgd-3*) out of the 37 alleles were rare (frequency ≤ 0.01) in all populations studied.

Genetic variability parameters are presented in Table 2. The overall mean percentage of polymorphic loci (P%) was 56.3%. The overall mean number of alleles per locus (A) was 2.0 (1.975) ± 0.2. The overall mean expected heterozygosity was calculated as 0.168 (ranging from 0.151 to 0.183).

A very high proportion (98.07%) of genetic variation was due to differences within populations. Level of gene flow among populations within a generation (N_m) was calculated to be, on average, 12.7.

Deviations from Hardy-Weinberg (H-W) equilibrium at each locus were determined in the Çamkuyusu (C) and Avlankuzu (A) populations, where seed collection and

Table 2. Genetic parameters for intra- and interpopulation variability in *Cedrus libani* populations in the study.

Population, abbreviation (elevation, m)	Mean sample size per locus	Percentage of polymorphic loci (P, %)	Mean number of alleles per locus (A)	Mean heterozygosity	
				Ho Observed	He Expected
Avlankuzu, A (1100)	28.0 (± 0.0)	62.5	1.9 (± 0.2)	0.187 (± 0.053)	0.165 (± 0.045)
Çamkuyusu, C (1800)	26.0 (± 0.0)	62.5	1.9 (± 0.2)	0.214 (± 0.062)	0.183 (± 0.048)
Mut, M (1650)	173.5 (± 8.5)	56.3	2.0 (± 0.2)	Not applicable*	0.151 (± 0.045)
Pozantı, P (1320)	183.6 (± 6.9)	43.8	2.1 (± 0.2)	Not applicable*	0.161 (± 0.051)
Mean		56.3	1.975 (± 0.2)	0.201 (± 0.055)	0.168 (± 0.046)

* Seeds from Mut and Pozantı are bulk seeds.

analyses were done at family level. According to the results, *Gdh* and *Pgi2* loci in Avlankuzu showed statistically significant deviations ($P \leq 0.05$ and 0.001, respectively). *Gdh*, *Mdh1*, and *Pgd* in Çamkuyusu also showed significant deviations ($P \leq 0.01$, 0.001 and 0.05, respectively). Nei's (1978) genetic distance coefficient (D_N) ranged from 0.003 to 0.008 among all possible population pairs (Table 3). The mean value of Nei's genetic distance was 0.05.

Table 3. Estimates of Nei's genetic distance (D_N) coefficient (Nei 1978) among the *Cedrus libani* populations in the study.

Population	Mut	Avlankuzu	Çamkuyusu
Pozantı	0.007	0.004	0.003
Mut	***	0.008	0.006
Avlankuzu		***	0.003

Discussion

Our observations on isozyme band patterns for each enzyme are discussed below.

ACP: We observed 4 zones of activity on the gel for ACP, but we were able to interpret only 1 (i.e. *Acp2*) because it was the only one that gave clear resolution. *Acp2* locus had 2 single-banded alleles. Gülbaba and Özkurt (2002) interpreted 2 loci in *C. libani*, and each locus had 2 single banded alleles. Fallour et al. (2001) observed 5 loci but identified only 2 polymorphic loci in *C. atlantica*.

ADH: We observed and identified 1 locus with 2 single-banded alleles. Fallour et al. (2001) were unable to resolve this enzyme because of its weak or nondetectable activity.

GDH: We observed and identified 1 polymorphic locus with 2 alleles. Gülbaba and Özkurt (2002) also found similar results.

GOT: We observed and identified 2 loci: *Got1* had 3 single-banded alleles, and *Got3* had 2 triple-banded alleles. We did not observe the *Got2* locus, which was stained in other conifers (Kara et al., 1997; Timerjanov, 1997; Konnert et al., 2001). Panetsos et al. (1992) found no polymorphism for this enzyme in any *Cedrus* species. Panetsos et al. (1994) identified 1 locus with 3 single-banded alleles in *C. libani* and *C. atlantica*. Yahyaoğlu et al. (1997) identified 4 alleles in the *Got1* locus and 2 alleles in the *Got3* locus in *C. libani*. Gülbaba and Özkurt (2002) identified 3 single banded alleles in the *Got1* locus and 2 triple-banded alleles in *Got3*.

G6PDH: We stained this enzyme in *Pinus resinosa* megagametophytes, but were not able to stain it in *C. libani* megagametophytes on the same gels. Scaltsoyiannes (1999) also was unable to resolve this enzyme in any *Cedrus* species.

IDH: We observed 1 monomorphic locus with 2 alleles. Panetsos et al. (1992) and Bariteau et al. (1999) identified 2 loci with 5 alleles in different *Cedrus* species, but some studies on *Cedrus* species showed that it had generally 1 monomorphic locus with 2 or 3 alleles at low frequency (Scaltsoyiannes, 1999; Fady et al., 2000; Fallour et al., 2001; Gülbaba and Özkurt, 2002).

LAP: We observed 2 zones of activity, *Lap1* and *Lap2*. Each locus had 2 alleles. Panetsos et al. (1992) identified 2 monomorphic loci: *Lap1* had 1 allele and *Lap2* had 2 alleles. Panetsos et al. (1994) and Bariteau et al. (1999) identified 2 loci: *Lap1* was monomorphic and *Lap2* was polymorphic and had 4 alleles. According to

Scaltsoyiannes (1999), *Lap1* was generally monomorphic with 3 alleles, and *Lap2* was polymorphic with 4 alleles.

MDH: We observed 4 zones of activity on gels for MDH, but we were able to interpret only 1 locus (*Mdh1*). This is because the 3 other zones of activity were either monomorphic or not clearly resolved. *Mdh1* was polymorphic and had 2 alleles. Panetsos et al. (1992) observed 2 loci and each locus had 2 alleles, but they identified both loci to be monomorphic with 1 allele for *C. libani*. Although Gülbaba and Özkurt (2002) identified 5 zones of activity, they interpreted 3 of the 5 loci. This enzyme was either monomorphic or not clearly resolved in other studies on *Cedrus* species (Panetsos et al., 1994; Bariteau et al., 1999; Scaltsoyiannes, 1999).

MNR: We observed 3 zones of activity: the *Mnr1* locus had 2 alleles, the *Mnr2* locus had 4 alleles (1 "null"), and the *Mnr3* locus had 2 alleles. Gülbaba and Özkurt (2002) also observed 3 loci for MNR, but they interpreted only 1 locus as *Mnr2* with 2 alleles (1 was "null"). They did not evaluate 2 other zones of activity because of their poor resolution. According to Scaltsoyiannes (1999), the *Mnr1* locus corresponds to the locus *Dia1* of diaphorase enzyme.

6PGD: We observed 1 locus with 3 alleles. Gülbaba and Özkurt (2002) observed 2 zones of activity. They observed that the *6Pgd1* locus had 1 allele and the *6Pgd2* locus 2 alleles. Although Panetsos et al. (1992) detected 1 locus with 3 alleles for 4 cedar species, *C. libani* had only 1 allele. Panetsos et al. (1994) detected 2 loci and they found the *6Pgd2* locus with 3 alleles. Bariteau et al. (1999) reported that PGD enzyme had 2 loci in *C. libani* and *C. atlantica*, and the *6Pgd2* locus had 4 alleles but there were only 3 of the 4 alleles in *C. libani*. According to Scaltsoyiannes (1999), 6PGD enzyme had 2 loci and the second locus had 6 alleles in 4 cedar species but Turkish populations of *C. libani* had only 3 alleles. Fady et al. (2000) identified in this enzyme 1 locus with 4 alleles. However, they observed 2 alleles in Turkish populations and 3 alleles in Lebanese populations.

PGI: We observed 2 zones of activity for PGI. The *Pgi1* locus had 1 allele and the *Pgi2* locus had 4 alleles. Gülbaba and Özkurt (2002) found similar results. Panetsos et al. (1992) observed that the *Pgi1* locus was either monomorphic or not clearly resolved. They observed the *Pgi2* locus with 6 triple-banded alleles. Panetsos et al. (1994) observed 2 loci but they found 4 single-banded alleles in the *Pgi2* locus. Bariteau et al. (1999) observed 6 single-banded alleles in the *Pgi2* locus but they found 4 alleles in Turkish populations of *C. libani*. Scaltsoyiannes (1999) observed 6 alleles in the *Pgi2* locus, which had 2 single-banded alleles and 4 triple-banded alleles.

SOD: We observed 1 monomorphic locus with 1 allele. We could not find any report in the literature where this enzyme system has been described for cedar species. Kara (1996) found 2 monomorphic loci in Turkish red pine (*Pinus brutia* Ten.). Fallour et al. (1997) were unable to resolve this enzyme in *Pinus pinea* L.

The overall mean percentage of polymorphic loci (P%) was 56.3% (Table 2). Hamrick et al. (1992) found that the mean percentage of polymorphic loci for conifer species was 53.4%. Estimates of percentage of polymorphic loci by Gülbaba and Özkurt (2002) ranged from 47.6% to 66.7% and by Scaltsoyiannes (1999) from 33.8% and 83.3% in various *C. libani* populations from Turkey. Kayıhan (2000), using DNA markers, found the percentage of polymorphic loci to range from 30.0% to 42.52% in 14 different *C. libani* seed stands, all from Turkey. Renau-Morata et al. (2005) calculated the percentage of polymorphic loci to range from 38.6% to 62.1% with an overall mean of 51.0% in *C. atlantica* with RAPD analysis. The percentage of polymorphic loci of cedar populations in this study was estimated to range between 43.8 and 62.5.

The overall mean number of alleles per locus (A) was 1.975 ± 0.2 (Table 2). Hamrick et al. (1992) calculated the average A for conifer species to be 1.8. Other studies on *Cedrus* species (Panetsos et al., 1994; Bariteau et al., 1999; Scaltsoyiannes, 1999; Gülbaba and Özkurt, 2002; Fady et al., 2008) reported that the mean number of alleles per locus was between 1.50 and 3.00. Although the mean number of alleles per locus is the highest (2.1) in the Pozantı population, the polymorphism level was the lowest (43.8%) (Table 2). This means that although there are more monomorphic loci than polymorphic ones in this population, the number of alleles per polymorphic locus was higher.

Hamrick et al. (1992) found mean expected heterozygosity (He) to be 0.151 for conifer species. This value was between 0.000 and 0.339 in earlier studies on *Cedrus* species (Panetsos et al., 1992; 1994; Bariteau et

al., 1999; Scaltsoyiannes, 1999; Bou dagher-Kharrat et al., 2001; 2007; Gülbaba and Özkurt, 2002; Renau-Morata et al., 2005). Mean expected heterozygosity calculated in this study (i.e. 0.168) is compatible with other studies on conifer and/or *Cedrus* species.

A very high proportion (98.07%) of genetic variation was due to differences within populations. In addition, the level of gene flow among populations within a generation (N_m) was calculated to be, on average, 12.7. This means that gene exchange among *C. libani* populations studied in the Taurus Mountains is relatively high compared to those in other studies (N_m = 0.4140 in Kayıhan, 2000; and N_m = 2.71 in Gülbaba and Özkurt, 2002). A relatively high level of gene flow is expected in high elevation species such as *C. libani*, which grow in landscapes where other tree species are generally rare. Thus, free exposure to alpine winds and absence or rareness of associating tree species may contribute to effective pollen distribution within relatively long distances. Most forest tree species can disseminate their seeds within a limited distance of about 100 m diameter. If the distribution of a species over an area is continuous, then each population appears to consist of overlapping subpopulations. Furthermore, because forest tree species are long-lived, each subpopulation includes individuals that are adapted to specific environmental conditions in their local habitats through repeated influences of different selection pressures. Thus, while genetic differentiation among populations tends to decrease due to gene flow, intrapopulation genetic variation increases due to selection pressures specific to different microhabitats. Therefore, in species having such features, different races or subraces may occur in short distances along the environmental gradients (Bradshaw, 1972; Libby, 1973). As a result, populations of long-lived, widely distributed plant species could have typically high intrapopulation genetic variation (Brown, 1979; Giannini et al., 1991; Gülbaba and Özkurt, 2002). Therefore, the very high proportion of genetic variation within population (i.e. 98.07%) in *C. libani* could be explained on the basis of diversity of local environmental forces.

Significant deviations from Hardy-Weinberg (H-W) equilibrium at each locus in Çamkuyusu (C) and Avlankuzu (A) populations indicate that populations at different altitudes might have experienced different levels of selection pressure.

Nei's (1978) genetic distance coefficient (D_N) ranged from 0.003 to 0.008 among all possible population pairs (Table 3), the mean value being 0.005. This indicates that variation among the populations is low. Genetic distance coefficient estimates in *Cedrus* species ranged from 0.000 to 1.529 (Panetsos et al., 1992, 1994; Yahyaoğlu et al., 1997; Scaltsoyiannes, 1999; Fady et al., 2000; Kayıhan, 2000; Gülbaba and Özkurt, 2002; Renau-Morata et al., 2005). The phenogram, based on UPGMA clustering, was constructed using D_N values (Figure 3). Çamkuyusu and Avlankuzu populations, which are geographically close to each other, were genetically more similar. The Pozantı (P) population, although geographically closer to Mut than the others, appears to be clustered together with C and A populations. This support the hypothesis that they all derived from the same refugia in the western Taurus ranges (Fady et al., 2008). Although the Mut population is located between the A, C, and P populations (Figure 1) and probably derived from the same refugia, it might have somewhat differentiated due to habitat fragmentation, isolation, and anthropogenic effects in the region. It is also likely that evolutionary forces, mainly natural selection, might have operated in the same direction in A, C, and P populations. These hypotheses can be tested by additional and more intensive sampling of populations in the region as well as by use of more powerful markers.

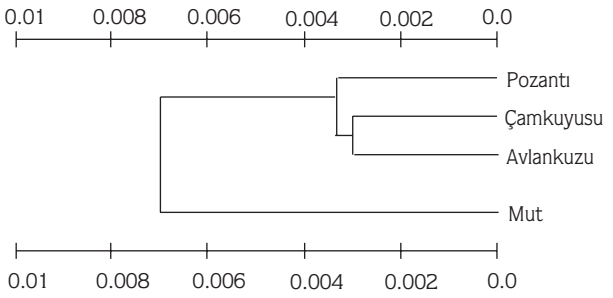

Figure 3. Phenogram constructed by using Nei's genetic distance values (Nei, 1978) for 4 *Cedrus libani* populations in Turkey.

Results from isozyme analyses indicate that *C. libani* exhibits a high intrapopulation variation while it has a low genetic variation among populations. Assuming that this trend is reflected in the adaptive features of the species, then it can be postulated that *C. libani* is a suitable species for tree improvement and afforestation programs. Accordingly, the emphasis should be given to

intrapopulation selection for genetic improvement. Selection of few large populations having high genetic variation rather than several small populations appears to be appropriate for gene conservation purposes for the species. In this way, most of the desired genetic variation for conservation can be captured in a large population having high intrapopulation genetic variation, as is the case in the Elmalı region. Furthermore, such a population is preferable as a seed source or in establishing seed orchards because of its higher adaptability to a relatively wide range of microhabitats as displayed in its distribution range in the Elmalı region. There have been several attempts by the Ministry of Environment and Forestry to establish seed transfer zones on *C. libani* as well as other forest tree species in Turkey (Orman Ağaçları ve Tohumları Islah Araştırma Müdürlüğü, 2004). It is important to note that such studies should be verified by genetic variation studies on traits of molecular and adaptational nature in related species.

Acknowledgments

This study was supported by Akdeniz University Scientific Research Projects Unit (Project No: 2004.02.0121.019). We collected seeds from Çamkuyusu and Avlankuzu populations on an individual tree basis through logistic support provided by Yusuf CENGİZ and M. Süleyman KAÇAR (Southwest Anatolia Forest Research Institute, Antalya). Dr. Hikmet ÖZTÜRK (Turkish Ministry of Environment and Forestry, Ankara) provided the bulked seed material from Mut and Pozantı populations. Dr. Valerie HIPKINS (Pacific Southwest Research Station, National Forest Genetics Laboratory, CA, USA) helped us in the interpretation of MDH gels. Dr. Bruno FADY (INRA-URFM, Avignon, France) kindly commented on the earlier version of the manuscript. Two anonymous reviewers provided valuable comments to further improve the manuscript. We gratefully acknowledge the contributions of all these persons and institutions.

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