Research Article

Mating system in natural populations of Taurus cedar (*Cedrus libani* A.Rich.)

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Abstract: The aim of this study was to estimate patterns of the mating system in 2 natural *Cedrus libani* A.Rich. populations in Elmalı, Antalya, with the aid of isozyme markers. Isozyme analysis was performed on both maternal and embryo tissues of the seeds collected from Avlankuzu and Çamkuyusu populations. Eleven loci encoding 8 enzyme systems were analyzed. The Avlankuzu and Çamkuyusu populations were a homogeneous and similar gene pool for the loci studied. Based on a multilocus estimator, the proportion of viable seeds originating from outcrossing (t_m) was 0.940 for the Avlankuzu population and 0.944 for the Çamkuyusu population. In other words, selfing in the Avlankuzu and Çamkuyusu populations was 6% and 5.6%, respectively. A high outcrossing rate, relatively high heterozygosity, and a lack of significant levels of inbreeding in the study populations are important components of the adaptive strategy of this species. Therefore these populations are not at risk for inbreeding depression is an important finding for the conservation of gene resources of this species.

Key words: *Cedrus libani*, inbreeding, isozyme, mating system

Doğal sedir (*Cedrus libani* A.Rich.) populasyonlarında eşleşme sisteminin belirlenmesi

Özet: Bu çalışmanın amacı, Elmalı-Antalya'da bulunan Toros sediri (*Cedrus libani* A.Rich.)'nin iki doğal populasyonunda eşleşme sistemi parametrelerini izoenzim analizleri yardımıyla tahmin etmektir. İzoenzim analizlerinde Avlankuzu ve Çamkuyusu populasyonundan toplanan tohumların megagametofit ve embriyo dokuları kullanılmıştır. Analizler, sekiz enzim sistemi tarafından kodlanan 11 lokusta yapılmıştır. Çalışılan lokuslar bakımından Avlankuzu ve Çamkuyusu populasyonlarının homojen olduğu ve benzer gen havuzlarına sahip oldukları belirlenmiştir. Çok-lokus tahmin yöntemine dayanarak yabancı döllenme sonucu oluşan yaşayabilir nitelikteki tohumların oranının (t_m) Avlankuzu populasyonunda 0.940, Çamkuyusu populasyonunda 0.944 olduğu bulunmuştur. Başka bir deyişle, kendileme sonucu oluşan tohumların oranının Avlankuzu ve Çamkuyusu populasyonlarında, sırasıyla % 6 ve % 5.6 olduğu belirlenmiştir. Yabancı döllenme oranının bu derece yüksek olması, heterozigotluk düzeyinin nispeten yüksekliği ve soy içi üremenin az olması sedir populasyonlarının adaptasyon stratejisinde önemli bileşenler olduğunu göstermiştir. Bu populasyonların soy-içi üreme çöküntüsü tehlikesi ile karşı karşıya olmaması türün gen kaynaklarının korunması açısından önemli bir bulgudur.

Anahtar sözcükler: *Cedrus libani*, eşleşme sistemi, izoenzim, soy-içi üreme

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Introduction

The distribution of Taurus cedar (*Cedrus libani* A.Rich.) is restricted mainly to the Taurus Mountains, excluding small and degraded populations in Lebanon and Syria (Boydak and Çalıkoğlu 2008). Natural cedar forests cover almost 110000 ha in Turkey, and there are widespread and productive forests of the species in Antalya, especially the Elmalı region. Historically, ecologically, economically and ethnobotanically, Taurus cedar is one of the most important forest trees. It has unique wood properties: it is decorative, soft and durable, and it has a unique smell and color. It is also easily processed by mechanical tools. Today in southern Turkey, a wood extract called katran is produced from *C. libani*. It is used to protect wooden structures against insects and fungi, to fight parasites and bacteria, and to heal wounds and cure various diseases in humans and domestic animals. However, forest fires and negative human activities such as heavy cutting and excessive grazing may have caused the destruction of cedar forests and reduction of genetic variation (Boydak 1996, 2003; Rogers and Kaya 2006; Fady et al. 2008; Kurt et al. 2008a).

The altitudinal distribution of cedar species ranges from 1000 to 2500 m above sea level. In Lebanon and Turkey, *C. libani* occurs most abundantly at altitudes of 1000-2000 m, where it forms pure forests or mixed forests with *Abies cilicica*, *Pinus nigra*, and *Juniperus* species. Some populations are found on the upper slopes facing the Mediterranean side of the Taurus Mountains, but other populations occur on the north slopes facing the Central Anatolian side of the Taurus Mountains. *C. libani* forests are found on different kinds of parent rocks, such as calcareous or karstic. Therefore, *C. libani* individuals and populations showing different phenotypic characteristics can be seen in the distribution areas (Işık and Yıldırım 1999; Boydak and Çalıkoğlu 2008). There are some studies that explain the genetic basis of these phenotypic differences (Yahyaoğlu et al. 1997; Kayıhan 2000; Gülbaba and Özkurt 2002; Kurt et al. 2008b; Semaan and Dodd 2008). However, there is not enough information about the genetic structure, mating systems, and pollen contamination of *C. libani* populations grown on the Taurus Mountains in the Antalya region.

Human activities and climate change have caused habitat destruction and loss of species or loss of their genetic diversity (Booy et al. 2000; Heywood and Iriondo 2003). The mating system of a population provides an estimate of the distribution of genetic diversity among individuals and populations within a species. Therefore, mating systems play an important role in plant population genetics. Knowledge of the mating systems of forest trees is significant for managing breeding populations and developing efficient genetic conservation strategies (Adams and Birkes 1991). Seeds that have high genetic quality according to desired characters are necessary for successful afforestation studies. Consequently, for forestation purposes, the proportion of collected seeds produced by outcrossing or selfing should be known. Because self-fertilization causes low survival capacity in offspring if they even survive, this reproductive strategy leads to weak and unstable offspring. Under selfing, heterozygosity is lost among progeny. As a result, forests established from these seeds of poor quality have low genetic diversity.

The aims of this study were to estimate outcrossing and selfing rates in the study populations and determine the proportion of viable offspring produced by outcrossing and selfing in each population, to investigate the mating system pattern of populations, and to make suggestions for conservation strategies in *C. libani* populations.

Materials and methods

Plant material

In this study, 2 natural *C. libani* populations in the Taurus Mountains were used. The Avlankuzu (1100 m a.s.l.) and Çamkuyusu (1800 m a.s.l.) populations are located at 2 different altitudinal zones near Elmalı, Antalya (Table 1). The seeds from the Avlankuzu and Çamkuyusu populations were collected under the guidance of the authors in October 2008. The cones were collected from 30 randomly selected trees per population, with each tree separated by at least 50 m from other trees, and labeled separately according to the mother tree. The total seed collection range was about 300 ha for each population. After collection, cones were stored in a cold room at 5 °C for about 2 weeks. They were then soaked in tap water for 1 day,

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

Population name	Altitude (m)	Latitude	Longitude	Aspect*	Distance from sea (km)**
Avlankuzu	1100	36°34′48″	29°57′57″	NW	39
Çamkuyusu	1800	36°35′86″	30°01′55″	S	38

*Aspects: NW = northwest, S = south.
**As the crow flies.

whereby cone scales loosened, and the seeds were manually extracted. The seeds were air dried and stored at 4 °C until used in isozyme analysis.

Electrophoretic analysis

Megagametophytes and embryo tissues of 2 *C. libani* populations were used in the study. Germination of the seeds was enhanced with stratification at 4 °C for 15 days (Takos and Merou 2001). Horizontal starch gel electrophoresis was used to obtain the information necessary for estimating outcrossing rates: the multilocus genotype of each maternal parent and the multilocus genotype of the pollen gamete that fertilized the ovule of each seed progeny. For the analysis, seeds were germinated on moistened Whatman No. 3 filter paper in petri dishes at 24 °C. The seeds were dissected and the haploid megagametophyte and embryo tissues were homogenized separately in a grinding plate by adding 75 μL of 0.2 M phosphate buffer (pH 7.5) containing 0.1% Triton X-100, 1% BSA, 3% PVP-40, and 0.1% β-mercaptoethanol for all enzyme systems (Conkle et al. 1982). Homogenates were subjected to starch gel electrophoresis (12% starch) using 3 different buffer systems [morpholine citrate (pH 6.1), lithium-borate (pH 8.3), and tris-citrate (pH 8.0)] (Conkle et al. 1982; Cheliak and Pitel 1984). Gels were sliced and stained for each enzyme system according to the method of Conkle et al. (1982). *Pinus resinosa*, which is monomorphic for all of the enzyme systems studied, was used as a marker (Allendorf et al. 1982; Mosseler et al. 1991). In total, 8 enzyme systems, encoded by 11 loci, were assayed. The enzymes assayed were as follows: acid phosphatase (ACP; EC 3.1.3.2), glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), leucine aminopeptidase (LAP; EC 3.4.11.1), malate dehydrogenase (MDH; EC 1.1.1.37), menadione reductase (MNR; EC 1.6.99.2), 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), and phosphoglucose isomerase (PGI; EC 5.3.1.9). The loci (isozymes) and the alleles within each locus (allozymes) were numbered in decreasing order of anodal mobility.

Statistical analysis

To estimate the overall genetic variation of the populations, the following measures were calculated: mean sample size per locus, mean number of alleles per locus (A), percentage of polymorphic loci (Pp%) at the 0.95 criterion, and observed heterozygosity (H_o) and expected heterozygosity (H_e) for each population (Nei 1973). The BIOSYS (IBM PC version 1.7) computer program was used for all computations (Swofford and Salender 1981). In addition, F-statistics (F_{IS}, F_{IT}, and F_{ST}) were estimated for each locus across all populations using BIOSYS.

In order to determine mating system parameters, the following information should be estimated: genotypes of mother trees of each population, genotypic frequency of mother trees, allele frequency of the gene pool of each population, and inbreeding and outcrossing rates (Shaw and Allard 1982). Maximum likelihood estimates of mating system parameters based on the mixed mating model of Brown and Allard (1970) were made using the MLTR program (Ritland 2002). The multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), and individual multilocus outcrossing rate (t_{mi}) were calculated.

Results

In 2 populations of *C. libani*, 8 enzyme systems encoded by 11 loci were examined. Of the 11 isozyme loci assayed, 4 (*Acp*, *Idh*, *Lap-1*, and *Pgi-1*) proved to be monomorphic in the 2 populations. Estimates of allelic frequencies for each locus are presented in Table 2. In total, 22 alleles were observed at 11 loci for the parental trees of the 2 populations, and 29 alleles were observed at the same loci for embryos produced by parental trees sampled from the studied populations. The frequency of 11 alleles was higher than 50% in both the parental and pollen gene pools.

Table 2. Allele frequencies for 11 loci in 2 populations of *Cedrus libani*.

Populations		Avlankuzu		Çamkuyusu	
		Frequency		Frequency	
Locus	Allele	Parent	Pollen	Parent	Pollen
Acp	1	1.000	0.999	1.000	0.996
	2	0.000	0.001	0.000	0.004
Got-1	1	0.617	0.641	0.581	0.667
	2	0.383	0.341	0.419	0.328
	3	0.000	0.013	0.000	0.005
	4	0.000	0.005	0.000	0.000
Got-3	1	0.917	0.947	0.933	0.971
	2	0.083	0.053	0.067	0.029
Idh	1	1.000	0.993	1.000	0.996
	2	0.000	0.007	0.000	0.004
Lap-1	1	1.000	0.997	1.000	0.996
	2	0.000	0.003	0.000	0.004
Lap-2	1	0.967	0.986	1.000	0.986
	2	0.033*	0.014	0.000	0.014
Mdh-1	1	0.867	0.833	0.919	0.821
	2	0.133	0.163	0.081	0.179
	3	0.000	0.004	0.000	0.000
Mnr-2	1	0.933	0.941	1.000	0.820
	2	0.050*	0.050	0.000	0.078
	3	0.017*	0.009	0.000	0.102
6Pgd	1	0.732	0.721	0.726	0.691
	2	0.317	0.260	0.274	0.299
	3	0.000	0.020	0.000	0.011
Pgi-1	1	1.000	1.000	1.000	1.000
Pgi-2	1	0.583	0.455	0.500	0.517
	2	0.083	0.109	0.016	0.021
	3	0.317	0.375	0.484	0.393
	4	0.017*	0.055	0.000	0.064
	5	0.000	0.006	0.000	0.004

*Private or unique alleles.

Genetic variability parameters are presented in Table 3. The overall mean Pp% was 50%, and the overall mean H_o and mean H_e were 0.171 and 0.168, respectively. Differences in allele frequencies between the Avlankuzu and Çamkuyusu populations were statistically nonsignificant at the 11 loci assayed. In the Avlankuzu population, 4 unique (private) alleles were observed (*Lap2-2*, *Mnr2-2*, *Mnr2-3*, and *Pgi2-5*). All genetic variability parameters of the Avlankuzu population gene pool, including unique alleles, were higher than those of the Çamkuyusu population gene pool (Table 3).

Table 4 also presents the results of the F-statistics, i.e. the genetic diversity coefficients, F_{IS}, F_{IT}, and F_{ST}, which were calculated according to the methods of Wright (1978). The mean within locality inbreeding (F_{IS}) over all loci between the 2 populations was −0.089 (ranging from −0.194 to 0.054); this suggests a relative deficiency in homozygotes in the loci of the following enzyme systems: *Got-1* (−0.194), *Mdh-1* (−0.101), and *Pgi-2* (−0.116). The mean apparent value of F (F_{IT}) was −0.099 (ranging from −0.195 to 0.052). The F_{ST} value was calculated as 0.010. These results show that most of the genetic diversity in this species lies within populations and only a little lies between populations. In addition, Wright's estimates of gene flow (Nm) between populations, based on the F_{ST} value, was 24.75.

The multilocus outcrossing rate (t_m) estimates for the Avlankuzu and Çamkuyusu populations were

Table 3. Genetic parameters for intra- and interpopulation variability in *Cedrus libani* populations in the study.

Population (altitude, m)	N	Pp%	A	Mean heterozygosity	
				H_o	H_e
Avlankuzu (1100)	30.0 (±0.0)	54.5	1.9 (±0.3)	0.206 (±0.074)	0.188 (±0.064)
Çamkuyusu (1800)	30.8 (±0.0)	45.5	1.5 (±0.2)	0.159 (±0.069)	0.150 (±0.065)
Mean	59.5 (±1.3)	45.5	1.9 (±0.3)	0.171 (±0.065)	0.168 (±0.063)

N = mean sample size per locus; Pp% = percentage of polymorphic loci; A = mean number of alleles per locus; H_o = mean observed heterozygosity; H_e = mean expected heterozygosity.

Table 4. Wright's F-statistics for polymorphic loci in the 2 populations of *Cedrus libani*.

Locus	F_{IT}	F_{IS}	F_{ST}
Got-1	−0.195	−0.194	0.001
Got-3	−0.082	−0.081	0.001
Lap-2	−0.034	−0.017	0.017
Mdh-1	−0.124	−0.101	0.021
Mnr-2	−0.057	−0.027	0.028
6Pgd	0.052	0.054	0.002
Pgi-2	−0.136	−0.116	0.018
Mean	−0.099	−0.089	0.010

0.940 and 0.944, respectively (Table 5). The high t_m value indicates that these 2 *C. libani* populations mate predominantly by outcrossing (approximately 95%). The single-locus inbreeding coefficients of maternal parents in the Avlankuzu and Çamkuyusu populations were 0.047 and 0.018, respectively.

Discussion

According to the results of this study, there is no significant difference between allele frequencies in the parental gene pools of the 2 populations. This shows that the gene pools of these populations are homogeneous. Populations that are close to each other and are found in the same geographical region might have similar gene pools due to similar environmental and climatic conditions. The gene pools of the Avlankuzu and Çamkuyusu populations might also have been similar due to a continuous distribution of *C. libani* populations in the study area and a high level of gene flow. The frequencies of alleles found only in the pollen gene pool and not in the parental gene pool varied between 0.001 and 0.02. The source of these rare alleles may be the parental population itself. These alleles may also be rare in the parent population and, because of this, infrequently chosen.

The results related to genetic variation parameters obtained in the study showed the parallelism found by Kurt et al. (2008b) in the same populations. The Pp% was 54.5% in the Avlankuzu and 45.5% in the Çamkuyusu population (Table 3). Estimates of Pp% by Gülbaba and Özkurt (2002) ranged from 47.6% to 66.7% and by Scaltsoyiannes (1999) from 33.8% to 83.3% in various *C. libani* populations from Turkey. Using DNA markers, Kayıhan (2000) found Pp% to range from 30.0% to 42.52% in 14 different *C. libani*

seed stands from Turkey. Renau-Morata et al. (2005) calculated Pp% from 38.6% to 62.1% with an overall mean of 51.0% in *C. atlantica* with RAPD analysis. Estimates of Pp% in this study were consistent with those of other studies.

The overall mean number of alleles per locus (A) was 1.7 (Table 3). Hamrick et al. (1992) calculated an average A for conifer species of 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 estimates of A were between 1.50 and 3.00. Our study is compatible with other studies on conifer and/or *Cedrus* species.

Hamrick et al. (1992) found H_e 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; Gülbaba and Özkurt 2002; Renau-Morata et al. 2005; Dagher-Kharrat et al. 2007). The overall H_e was 0.169 in our study, and this is similar to other studies on conifer and/or *Cedrus* species.

Many factors such as selection, genetic drift, gene flow, and mating pattern determine the genetic structure of a population and the partitioning of genetic variation among populations in nature. In this study, divergence between populations (F_{ST}) was low (0.010), and variation within populations ($1-F_{ST}$) represented 90% of the total variance. Semaan and Dodd (2008) also reported that genetic differentiation was low (0.07) among remnant natural populations of *C. libani* of Lebanon. Low levels of genetic differentiation are attributed to effective gene flow. Indeed, Nm based on the F_{ST} value indicated that the partitioning of genetic variation in these populations could have been strongly influenced by

Table 5. Mating system parameters of *Cedrus libani* populations in the study.

Populations	Outcrossing rates			
	t_m [a]	t_s [b]	$t_m - t_s$	t_{mi} [c]
Avlankuzu	0.940 (±0.032)	0.880 (±0.041)	0.060	0.949 (±0.028)
Çamkuyusu	0.944 (±0.032)	0.863 (±0.046)	0.081	0.978 (±0.044)

a: multilocus outcrossing rates; b: single-locus outcrossing rates; c: individual-tree multilocus outcrossing rates.

gene flow. Effective gene flow, typical of most wind-pollinated conifers, would tend to decrease genetic heterogeneity among populations.

The estimated t_m values indicate a high level of outcrossing in all crops, as measured at the viable embryo stage. High outcrossing rates in different forest trees have been reported in many studies (Shaw and Allard 1982; Ritland and El-Kassaby 1985; El-Kassaby and Ritland 1986; El-Kassaby et al. 1989; Kaya et al. 2006; O'Connell et al. 2006; El-Kassaby et al. 2007). The estimated proportions of outcrossed seed in the Avlankuzu and Çamkuyusu populations were very high (0.940 and 0.944). This is consistent with estimates for other conifer species (Omi and Adams 1985; Ritland and El-Kassaby 1985; El-Kassaby et al. 1989; Harju and Muona 1989; Ladislav et al. 1993) and lies within the range reported for several other pines (El-Kassaby et al. 1987, 1989; Krutovskii et al. 1995; Burczyk 1996; Burczyk et al. 1997). A high outcrossing rate means a low proportion of viable selfed offspring. The level of selfing (s) in the Avlankuzu and Çamkuyusu populations was 0.06 and 0.054, respectively. However, the actual level of self-fertilization may be much greater because most selfings result in empty (nonviable) seeds (Neale and Adams 1985).

Individual-tree multilocus estimates (t_{mi}) ranged from 0.48 to 1.13 for the Avlankuzu population and from 0.26 to 1.45 for the Çamkuyusu population (Table 5). Neale and Adams (1985) reported t_{mi} values from 0.90 to 1.10 in Douglas-fir. Sorensen (1973)

reported t_{mi} values from 0.72 to 1.00+ in Douglas-fir. The variety in outcrossing rates between individual trees depends on density, age-class distribution of trees, and species mixture (Neale and Adams 1985).

The mating systems of plant species vary due to genetic or environmental factors such as population density, family structure, and tree heights (Clegg 1980; El-Kassaby and Jaquish 1996; O'Connell et al. 2004). The high outcrossing rate, relatively high heterozygosity, and lack of significant levels of inbreeding in the populations are important components of the adaptive strategy of this species. Mediterranean growth zones of *Cedrus* species have experienced degradation throughout the history of human civilization in the area (Seaman and Dodd 2008). The present distribution of *C. libani* is restricted mainly to the Taurus Mountains in southern Turkey, where the most productive populations are found, especially in the Elmalı region near Antalya, which includes the Avlankuzu and Çamkuyusu populations. However, the results of this study indicate that these populations are not at risk of inbreeding depression and genetic drift. This is an important finding for the conservation of gene resources of this species.

Acknowledgments

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