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Genetic diversity of natural *Cyclamen alpinum* populations

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Abstract: The focus of this study was *Cyclamen alpinum* (formerly *C. trochopteranthum*). Habitat fragmentation, environmental degradation, and overharvesting of tubers have exerted pressure on native populations of this valuable ornamental species. Although the entire *Cyclamen* genus is in the Convention on International Trade in Endangered Species of Wild Fauna and Flora-Appendix II (CITES II), no species has yet been red-listed. Estimating the level and distribution of genetic variation in populations of rare and endemic species is important for conserving genetic diversity within a species in the context of well-developed conservation strategies. Currently, DNA markers are the most effective means used to infer genetic variation at the molecular level in conservation genetics. In this study, random amplified polymorphic DNA (RAPD) analysis was employed to assess the genetic diversity within and among 6 natural *C. alpinum* populations in the south and southwest of Turkey. A total of 190 loci were determined by using 15 polymorphic primers. Total genetic variation (H_T) was 0.27 ± 0.02. A high proportion of this variation, 0.16 ± 0.01 (59.26%), was due to within-population genetic variation (H_S). The genetic differentiation coefficient (G_{ST}) was 0.41, and the level of gene flow (Nm) within a generation among the 6 populations studied was 0.73. As a result of these findings, we propose in situ combined with ex situ conservation of all *C. alpinum* populations. In addition, our results support prior recommendations to add *C. alpinum* to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List under the critically endangered (CR) category.

Key words: *Cyclamen alpinum, Cyclamen trochopteranthum,* genetic diversity, RAPD

Introduction

There are more than 500 geophyte species growing naturally in Turkey, and the bulbs of most of these species are exported (1,2). In general, they are propagated by vegetative means (3). However, the horticulturally important genus *Cyclamen* L. is a tuberous perennial geophyte, and seeds are used in its propagation (4). *Cyclamen* species have a limited dispersal capacity due to their primary dependence on ants to transport their relatively few, large seeds (5,6). They show some form of summer dormancy in response to a lack of moisture sufficient to maintain normal physiological activity. The arrival of autumnal precipitation and cooler temperatures induce an important regrowth in this species. Aboveground parts of the plant die back, and drought is avoided by means of a summer-dormant underground storage organ, the tuber. Regrowth occurs from belowground perennating buds. Both the avoidance of summer drought and rapid regrowth in response to environmental cues are enabled by the storage of nutrients in tubers (6).

Cyclamen has been the focus of unusual taxonomic activity over the last 70 years. Nine

infrageneric classifications were proposed over that period for *Cyclamen*, a small genus with fewer than 30 species (7). Therefore, *Cyclamen alpinum* has a confusing taxonomic history. It was first described in the last part of the 19th century and was known as *Cyclamen alpinum* Sprenger until it was redescribed as *Cyclamen trochopteranthum* by Otto Schwarz in 1975. Today it is again known as *C. alpinum* (8). It is an ornamental species with attractive pink flowers and dark green leaves that are grey-green and cream-colored in the center. It is 1 of 10 *Cyclamen* species that grow naturally in Turkey. When it was described as *C. trochopteranthum*, it was accepted as 1 of 6 endemic species among the 10 *Cyclamen* species of Turkey (1). However, under the name of *C. alpinum*, its endemism is controversial. It grows in the south and southwestern parts of Turkey, especially in Antalya, Muğla, Denizli, Burdur, and Isparta. In general, it is found at elevations of 350-1500 m under *Pinus brutia* Ten. and *Liquidambar orientalis* Mill. trees or under *Laurus nobilis* L. and *Ceratonia siliqua* L. shrubs, in partially shaded moist habitats with soils rich in humus. However, some populations of this species can be found close to sea level in Dalyan and Marmaris, and there are populations growing at 1670 m on Sandras Mountain, as well (9).

Because of the tremendous impact of humans on their environment over the past 50 years (e.g., increase in agricultural and residential areas, tourism activities, and the construction of roads and dams), the natural growing areas of many plant species, including *C. alpinum*, have been destroyed. Intense pasturing, collection of tubers for medicinal purposes, and exportation of the plants are additional human-created pressures on *C. alpinum* populations. Therefore, conservation is important for the continued existence and evolution of this species.

A primary goal of conservation genetics is to estimate the level and distribution of genetic variation in the populations of rare and endemic species and then create a plan to preserve genetic diversity within the species. Currently, DNA markers are the most effective means by which conservation genetics infers variation at the molecular level. They are also effective tools that enable clarification of taxonomic synonyms and detection of the origin of species and cultivars. These markers have been used to study genetic diversity in various plant species (10). However, DNA marker systems are complex and there are basic difficulties involved in using most of them, such as restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs) (11). Although AFLPs and SSRs are generally preferred due to their informativeness, random amplified polymorphic DNAs (RAPDs) are still widely used; they are simple to perform, have low costs, and do not require DNA sequence information (12). Additionally, the RAPD system provides a more arbitrary sample of the genome and can generate essentially unlimited numbers of loci for use in genetic analysis (13).

In this study, RAPD analysis was employed to assess the genetic variability within and among 6 *C. alpinum* populations located in the southern and southwestern regions of Turkey at different elevations. Although there are several studies related to its taxonomy (7), morphology, ecology, and cytology (14) as well as in vitro and in vivo seed germination (4), this is the first report characterizing the populations of this valuable yet taxonomically confusing and controversial ornamental species using a DNA marker system.

Materials and methods

Plant material

Four *C. alpinum* populations at elevations of 20, 30, 40, and 50 m from Dalyan-Muğla, 1 population at 560 m from Çukurköy-Denizli, and 1 population at 1100 m from Elmalı-Antalya were sampled and used in the study (Figure 1). Populations sampled were from rarely found *C. alpinum* populations in Turkey, and they were located in a region that is very important for tourism and agriculture. For this reason, some of the populations screened in this study could only be minimally sampled. For example, Pop-4 and Pop-6 were represented by only 3 and 6 individuals, respectively (Figure 1). For the same reason, in the Çukurköy-Denizli and Elmalı-Antalya populations we obtained pieces of tuber tissues from individual plants collected for another purpose and used them

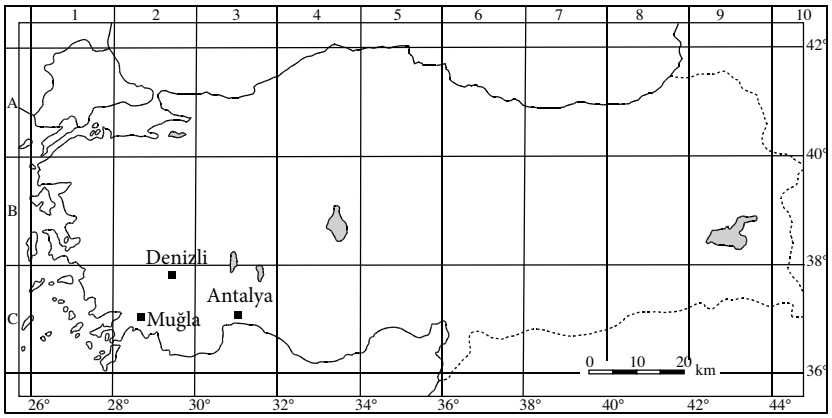

Figure 1. Locations of the 6 *C. alpinum* populations.
Pop-1: Dalyan-Muğla (20 m), Pop-2: Dalyan-Muğla (30 m), Pop-3: Dalyan-Muğla (50 m), Pop-4: Dalyan-Muğla (40 m), Pop-5: Elmalı-Antalya (1100 m), Pop-6: Çukurköy-Denizli (560 m)

in the analysis, while leaves from individual plants were used to analyze the remaining 4 populations from Dalyan-Muğla.

Individual plants in each population were collected using a transect method. Each transect consisted of a straight line. In each population, the length, numbers, and directions of transects were determined depending on the size of the population and other characteristics of the location such as slope, topography, and uniformity. Depending on the size of the population, 1 plant was sampled at every 10 to 30 m of a transect. The collected samples were stored at −20 °C in a freezer until the DNA extraction procedure.

Genomic DNA isolation

Genomic DNA was extracted from leaves and tubers using a protocol optimized according to the methods of Doyle and Doyle (15), Cullings (16), and Sharma et al. (17). According to this optimized protocol, 1 g of tissue (leaf or tuber) was submerged in 5 mL of 96% ethanol for 30 min. After evaporation of the ethanol, the tissue was ground with a mortar and pestle. Another 5 mL of 96% ethanol was then applied to the ground tissue, and the sample was left in the hood for evaporation of the ethanol. After grinding the tissue once more, 500 µL of prewarmed (65 °C) CTAB buffer (0.1 M Tris, pH 8.0; 1.4 M NaCl; 0.02 M EDTA; and 2.0% cetyltrimethylammonium bromide) was added to 0.03 g of the finely powdered tissue, vortexed very well, and incubated in a 65 °C water bath for 60 min by vortexing at the 20th, 40th, and 60th minutes. This was followed by the addition of 500 µL chloroform and isoamyl alcohol (24:1). The samples were mixed well by inverting the tubes and centrifuged for 10 min at 14,000 rpm. Next, the top aqueous layer was transferred to another tube. The volume of the aqueous phase was estimated to be approximately 300-350 µL, and 0.08 volumes of cold 7.5 M ammonium acetate and 0.54 volumes (using the combined volume of aqueous phase and added ammonium acetate) of cold isopropanol were added to precipitate the DNA. After incubation at −20 °C for 30 min, the sample was centrifuged at 14,000 rpm for 4 min to pellet the DNA. The supernatant was removed and 700 µL of cold 70% ethanol was added to wash the pellet, followed by centrifugation at 14,000 rpm for 3 min. The ethanol was then removed and the pellet was dried and resuspended in 100 µL of TE buffer, pH 8.0 (10 mM Tris, 1 mM EDTA). Next, 10 µg of RNase (Fermentas, Lithuania) was added and incubated at 37 °C for 60 min, mixing thoroughly but gently. After incubation, 400 µL of chloroform and isoamyl alcohol (24:1) was added; the solution was mixed thoroughly for 10 min and centrifuged at 14,000 rpm for another 10 min. The top aqueous layer was transferred to a clean tube, and 2 volumes of 96% ethanol were added. This was

followed by precipitation at −20 °C for 30 min and centrifugation at 14,000 rpm for 4 min to pellet the DNA. The supernatant was removed, and 700 µL of 70% ethanol was added to the pellet. This was followed by centrifugation at 14,000 rpm for 3 min. Finally, the ethanol was removed, and the pellet was dried and resuspended in 100 µL of TE, pH 8.0.

Although running test gels indicated that the DNA from the samples was of sufficient quality and quantity, the majority of samples did not produce any bands or produced only a few faint bands in the polymerase chain reaction (PCR). Therefore, they were washed with NaCl to cleanse them of PCR inhibitors such as excess polysaccharides and phenolic compounds in an additional step conducted in the above procedure. In this step, 100 µL of 2 M NaCl and 200 µL of 96% ethanol were added to DNA suspended in 100 µL of TE and incubated on ice for 30 min. Following centrifugation at 12,000 rpm for 10 min, the pellet was washed with 70% ethanol. After drying, the pellet was resuspended in 100 µL of TE, pH 8.0. DNA yields per sample varied from 1800 ng to 24,000 ng. Each template DNA was diluted to 10 ng/µL with TE (pH 8.0) prior to use. However, the problem encountered in PCR continued in the DNA extracted from tubers. The problem was solved with a 1:1 dilution (to 5 ng/µL) by TE (pH 8.0), which most likely diluted the inhibitor substances to ineffective levels in the PCR.

DNA amplification conditions

In this study, 37 ten-base oligonucleotide primers from Operon Technologies (Alameda, California, USA) including kits from A (OPA) to R (OPR) were used. The conditions reported by Williams et al. (18) for generating RAPD markers by PCR were optimized for DNA from the *C. alpinum* populations used in this study. The PCR mixture (25 µL) included 25 ng template DNA, 5.5 mM $MgCl_2$, 0.2 µM primer, 200 µM dNTPs (for each), 1 unit Taq DNA polymerase, 2.5 µL PCR buffer (from 10X), 0.5 µL bovine serum albumin (BSA), and 0.13 µL Tween 20. The cycling program was as follows: 45 cycles of 1 min at 94 °C (denaturing), 1 min at 37 °C (annealing), and 2 min at 72 °C (extension), with 6 min of initial denaturating at 94 °C and 10 min of postholding at 72 °C for the final extension in an Eppendorf Mastercycler gradient thermal cycler (Hamburg, Germany).

Strategy for identification of RAPDs and data collection

Initially, 37 RAPD primers were screened against DNA from a subset of 6 individuals randomly selected among the 70 individuals from the 6 *C. alpinum* populations. From among these, 15 primers were selected based on the reproducibility and number of polymorphic bands. The primers were then screened with the DNA of all individuals of the studied populations. Each amplification reaction with these 15 primers was performed twice, and highly reproducible results were obtained. Nonreproducible bands were not included in scoring.

In the single-locus segregation data, presence of a band was scored as 1, while absence of the same band was scored as 0. In cases where no bands were detected throughout a lane, the locus was scored as missing data.

Data analysis

In order to evaluate *C. alpinum* populations for intra- and interpopulation genetic diversity, several statistics were used. Polymorphism (*P*), the proportion of polymorphic loci detected (criterion of 99% was used); mean number of observed (*Na*) and effective (*Ne*) alleles per locus (19); Nei's gene diversity (*h*) as a measure of heterozygosity (20); and Shannon's information index (*I*) (21) were calculated. Total genetic variation (H_T), within-population genetic variation (H_S), and Nei's (20) genetic differentiation coefficient (G_{ST}) were determined. Gene flow (*Nm*) was estimated from G_{ST} values using the relationship $Nm = 0.5\ (1 - G_{ST})\ /\ G_{ST}$, in which *N* is the effective population size and *m* is the proportion of the population that are migrants. All calculations were performed using the POPGENE v. 1.32 (22) computer program.

Subsequently, genetic distance (D_N) coefficients among all possible population pairs were calculated (23), and a dendrogram by the neighbor-joining (NJ) method (24) was constructed using the Genetic Data Analysis (GDA) program (25). Regression and correlation analyses between elevations of the populations and their diversity measures (*P%*, *Na*, *Ne*, *h*, and *I*) obtained in the study were performed using SPSS 14.0.

Results

Of the 37 RAPD primers that were initially screened against DNA from a subset of 6 individuals randomly selected from among the 70 total individuals from 6 *C. alpinum* populations, 23 revealed at least 1 polymorphic locus. In this study, the frequency of polymorphic RAPD primer for *C. alpinum* was 62.16%. None of the primers were monomorphic, and the remaining 14 primers produced no bands in the subset from the populations (Table 1).

Screening the DNA from all individuals of the studied populations with 15 polymorphic RAPD primers resulted in a total of 190 loci. Genetic parameters for intra- and interpopulation variability are given in Table 2. The percentage of polymorphic loci (*P%*) in populations varied between 25.26% (Pop-6) and 72.11% (Pop-3). Of all loci detected in the populations, 97.37% were polymorphic. The overall mean number of observed alleles per locus (*Na*) was 1.97 ± 0.16, while the overall mean number

Table 1. Total number of bands with their size ranges and number of polymorphic bands produced by the screened 37 random 10-mer OPERON primers.

Primer code	Sequence of the primer (5'to 3')	Total # of bands produced	Range of bands produced (bp)	Total # of polymorphic bands produced
OPA-01	CAGGCCCTTC	-	-	-
OPA-02*	TGCCGAGCTG	6	600-2500	2
OPB-01	GTTTCGCTCC	-	-	-
OPB-04	GGACTGGAGT	7	500-1600	3
OPC-03	GGGGGTCTTT	-	-	-
OPC-09	CTCACCGTCC	8	600-2500	2
OPD-02*	GGACCCAACC	7	800-3000	4
OPD-07	TTGGCACGGG	7	400-1500	3
OPE-01	CCCAAGGTCC	-	-	-
OPE-02*	GGTGCGGGAA	12	650-2000	2
OPF-01	ACGGATCCTG	12	300-1900	5
OPF-07	CCGATATCCC	-	-	-
OPF-10*	GGAAGCTTGG	13	400-1900	5
OPG-03*	GAGCCCTCCA	9	750-2400	2
OPG-10*	AGGGCCGTCT	9	750-1900	4
OPH-01	GGTCGGAGAA	-	-	-
OPH-03*	AGACGTCCAC	11	750-2000	2
OPH-08	GAAACACCCC	-	-	-
OPI-02*	GGAGGAGAGG	10	650-2000	3
OPI-03	CAGAAGCCCA	-	-	-
OPI-07	CAGCGACAAG	8	650-1800	2
OPJ-02	CCCGTTGGGA	8	650-2000	2
OPK-06	CACCTTTCCC	9	400-1500	4
OPK-07*	AGCGAGCAAG	13	650-2100	3
OPK-08	GAACACTGGG	-	-	-
OPL-01	GGCATGACCT	-	-	-
OPL-05	ACGCAGGCAC	-	-	-
OPL-06*	GAGGGAAGAG	12	500-1900	5
OPM-07*	CCGTGACTCA	13	500-2000	4
OPN-06*	GAGACGCACA	14	600-2100	6
OPN-09	TGCCGGCTTG	-	-	-
OPP-01	GTAGCACTCC	7	750-1900	3
OPP-08*	ACATCGCCCA	14	500-2000	5
OPP-09	GTGGTCCGCA	-	-	-
OPQ-01*	GGGACGATGG	12	500-2000	3
OPR-04	CCCGTAGCAC	-	-	-
OPR-08*	CCCGTTGCCT	10	750-1800	4

*Primers screened with the DNA of all individuals.

Table 2. Genetic parameters for intra- and interpopulation variability in *C. alpinum* populations.

Populations	Mean sample size per locus	Percentage of polymorphic loci (*P%*)	Mean number of alleles per locus		*h*	*I*
			Observed (*Na*)	Effective (*Ne*)		
Pop-1	11	64.21	1.64 ± 0.48	1.33 ± 0.36	0.20 ± 0.19	0.30 ± 0.27
Pop-2	7	50.00	1.50 ± 0.50	1.28 ± 0.35	0.17 ± 0.19	0.25 ± 0.27
Pop-3	14	72.11	1.72 ± 0.45	1.38 ± 0.37	0.23 ± 0.19	0.34 ± 0.27
Pop-4	3	27.37	1.27 ± 0.45	1.19 ± 0.34	0.11 ± 0.18	0.16 ± 0.26
Pop-5	15	60.53	1.61 ± 0.49	1.32 ± 0.37	0.19 ± 0.20	0.28 ± 0.28
Pop-6	5	25.26	1.27 ± 0.45	1.17 ± 0.31	0.10 ± 0.17	0.15 ± 0.25
Overall mean	55	97.37	1.97 ± 0.16	1.46 ± 0.31	0.28 ± 0.15	0.44 ± 0.20

of effective alleles per locus (*Ne*) was 1.46 ± 0.31. Pop-4 and Pop-6 had the same lowest value for *Na* (1.27 ± 0.45), and Pop-3 had the highest value (1.72 ± 0.45). *Ne* values of the populations varied between 1.17 ± 0.31 (Pop-6) and 1.38 ± 0.37 (Pop-3). The overall mean of Nei's (20) gene diversity (*h*) was 0.28 ± 0.15, ranging from 0.10 ± 0.17 in Pop-6 to 0.23 ± 0.19 in Pop-3; the overall mean of Shannon's information index (*I*) was 0.44 ± 0.20, with values ranging from 0.15 ± 0.25 in Pop-6 to 0.34 ± 0.27 in Pop-3.

Gene diversity, population differentiation, and gene flow estimates for the *C. alpinum* populations are given in Table 3. Total genetic variation (H_T) was 0.27 ± 0.02. A high proportion of this variation, 0.16 ± 0.01 (59.26%), was due to within-population genetic variation (H_S). The genetic differentiation coefficient (G_{ST}) was 0.41. Mean gene flow (*Nm*) within a generation among the 6 populations was 0.73.

Genetic distance coefficients (D_N) (23) ranged from 0.1301 to 0.3604 among population pairs. The minimum distance was detected between Pop-1 and Pop-2, and the maximum distance was detected between Pop-4 and Pop-5 (Table 4). A NJ dendrogram is presented in Figure 2.

Regression and correlation analysis was not significant between elevations of the populations and their diversity measures (*P%*, *Na*, *Ne*, *h*, and *I*) obtained in the study (data not shown).

Discussion

Virtually all of Earth's ecosystems have been significantly affected by intense human activities. A change in an ecosystem necessarily affects the included species. Human activities have caused an estimated 50 to 1000 times more extinctions in the last 100 years than would have occurred due to natural processes (26). According to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List released on 4 May 2006, the IUCN being the main authority on the conservation status of species, 40,168 species as a whole plus an additional 2160 subspecies, varieties, aquatic stocks, and subpopulations have been evaluated. Among the species evaluated, 16,118 were considered threatened; 8390 of these threatened species were plants.

Cyclamen species occupy the Mediterranean region and have their highest levels of diversity in Greece and Turkey, areas that support their

Table 3. Gene diversity, population differentiation, and gene flow estimates for the *C. alpinum* populations.

Total # of loci	H_T	H_S	G_{ST}	*Nm*
190	0.27 ± 0.02	0.16 ± 0.01	0.41	0.73

Table 4. Estimates of Nei's genetic distance (D_N) coefficients (23) among the *C. alpinum* populations.

	Pop-1	**Pop-2**	**Pop-3**	**Pop-4**	**Pop-5**	**Pop-6**
Pop-1	****					
Pop-2	0.1301	****				
Pop-3	0.1886	0.1729	****			
Pop-4	0.2881	0.2809	0.2122	****		
Pop-5	0.2487	0.2310	0.3037	0.3604	****	
Pop-6	0.3216	0.2885	0.3024	0.3174	0.2581	****

phenological preference for dry summers and wet winters. Although the entire genus is in the Convention on International Trade in Endangered Species of Wild Fauna and Flora-Appendix II (CITES II), no species has yet been red-listed (27). A recent study developed models for the climatic niches of *Cyclamen* and projected future climate scenarios for 2050 (28). According to this study, the area of climatic suitability for every *Cyclamen* species is predicted to decrease, and a limited dispersal capacity places them at high risk for extinction. Half of the species are threatened, with potential extinction due to projected loss of habitat. In a subsequent study, Ciotir et al. (27) proposed the following IUCN categories for the *Cyclamen* species according to a phylogenetic pattern based on species distribution, speciation, and the previously predicted area loss: CR (critically endangered), area loss of ≥80%, for *C. colchicum*, *C. intaminatum*, *C. libanoticum*, *C. creticum*, *C. cyprium*, *C. elegans*, *C. mirabile*, *C. parviflorum*, *C. rohlfsianum*, *C. somalense*, *C. cilicium*, *C. africanum*, *C. balearicum*, *C. pseudibericum*, *C. coum*, and *C. trochopteranthum*; EN (endangered), area loss of ≥50%, for *C. hederifolium*, *C. graecum*, and *C. persicum*; VU (vulnerable), area loss of ≥30%, for *C. purpurascens* and *C. repandum*; and LC (least concern) for none of the species. This means that the 16 CR-proposed species have a high probability of becoming extinct in the wild, and the EN group may become critically endangered without human intervention. Therefore, these species were recommended for addition to the IUCN Red List under the threatened categories.

The focus of this study was *C. alpinum* (formerly *C. trochopteranthum*). As Ciotir et al. (27) indicated and according to our own and other researchers' experiences during field observations (personal communications), habitat fragmentation, environmental degradation, and overharvesting of

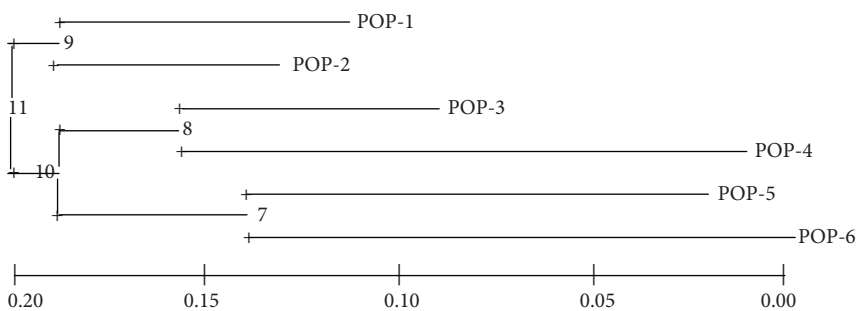

Figure 2. Dendrogram based on NJ of the genetic distances between *C. alpinum* populations.

tubers have exerted pressures on native populations of *C. alpinum*. This has resulted in declines in density and abundance and created isolation of relatively small populations. In Turkey, the collection, growth, and exportation of tuberous geophytes are controlled according to the governing statute released in 1991. This statute prohibits the exportation of 6 *Cyclamen* species (*C. graecum*, *C. mirabile*, *C. intaminatum*, *C. parviflorum*, *C. pseudibericum*, and *C. alpinum*) from Turkey (14). Although the presence of this governing statute is promising in the context of species survival, these species are still at risk due to the continuing direct and indirect effects of human activities. The level and pattern of genetic diversity in native plant species can be changed by human activities and this, in turn, may lead to loss of genetic diversity, reducing the evolutionary potential of a species to respond to changes in environmental conditions (29). It was shown by Godt et al. (30) and Godt and Hamrick (31) that in many species genetic diversity is directly related to population size. Newman and Pilson (32) and Fischer and Matteis (33) indicated that individual fitness and the potential of a population to persist may be affected by levels of genetic diversity.

The current study has provided information about the relative amounts of genetic variation present within and among 6 *C. alpinum* populations. A higher proportion (59.26%) of genetic variation was due to differences within the populations, while a relatively small portion (40.74%) of variation was distributed among the populations. Gene flow among the 6 studied populations was consistently low, the rate being $Nm = 0.73$ migrants per generation, which is below the critical value of $Nm = 1.0$, indicating the start of genetic differentiation among populations due to genetic drift. In support of this finding, the genetic differentiation coefficient (G_{ST}) was 0.41, which is considerably higher than the critical value of 0.25. Compared to widespread and abundant species, endemic and rare taxa often contain significantly less genetic variability (34-36). According to Hamrick and Godt (37), the mean value of G_{ST} for short-lived perennial and narrowly distributed species was 0.22. In the evaluation of combined categories of seed dispersal mechanisms and geographic range by the same authors, the mean value of G_{ST} was again 0.22 for narrowly distributed species dependent on animals for seed dispersal. Having the same combined life history traits, the G_{ST} value determined for *C. alpinum* populations in our study is almost 2 times higher than these mean values, indicating a serious level of genetic differentiation among the populations. Gene flow patterns due to reduction in pollen and seed movement between populations may be modified by population fragmentation and isolation and lowered population densities, increasing the inbreeding within populations (38). Although many recent studies (39-42) indicated significant correlations between elevation and genetic diversity parameters in plant populations, we did not find any significant correlation between the elevations of the *C. alpinum* populations studied and their diversity measures (*P%*, *Na*, *Ne*, *h*, and *I*) obtained in the study.

The results of this study support the recommendation of Ciotir et al. (27) related to the addition of *C. alpinum* (formerly *C. trochopteranthum*) to the IUCN Red List under the CR category. Since the endemism status of this species (endemic or not) is controversial under the name of *C. alpinum*, its inclusion in this list seems most critical. The findings of our study indicate that all populations should be conserved, giving priority to Pop-3, Pop-1, and Pop-5, due to their relatively higher levels of polymorphism. We propose in situ combined with ex situ conservation, because of their individual and mutual advantages. Most importantly, by applying an in situ conservation strategy, populations may be able to continue on their natural evolutionary paths. However, because natural distribution areas of *C. alpinum* populations are located in important tourism and agricultural areas of the country, the importance of ex situ conservation has become more apparent.

RAPD markers have been used mainly for testing genetic purity in *Cyclamen* seeds (43) and somaclonal variation within *C. persicum* calli (44), and for determining inter- and intraspecific genetic diversity among *C. persicum* and *C. com* accessions (45). This is the first attempt to characterize the populations of *C. alpinum*, a valuable ornamental species, by DNA marker system, specifically RAPDs. This is important given the taxonomically confusing and

controversial status of *C. alpinum*. However, there is a need to compare samples of *C. alpinum* from the other natural distribution areas in Mediterranean countries with the samples obtained from the south and southwest of Turkey. Additionally, other DNA loci as well as isozyme data need to be analyzed to clarify the taxonomic name and endemism status of populations naturally distributed in the south and southwest of Turkey.

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