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# Determination of the genetic diversity, population structure, and some ecological preferences of the endemic *Muscari adilii*

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**Abstract:** The population structure and genetic diversity of *M. adilii* and some edaphic and climatic preferences of the species were investigated. Although the chemical characteristics such as pH, EC, and gypsum were similar for the three subpopulations, the physical qualities and lime content of the soil were different from each other. In terms of bioclimate, the Nallıhan station (Nallıhan Bird Sanctuary) has a lower annual average temperature and precipitation value, and monthly average values during the germination and flowering periods, than the Beypazarı station (Hırkatepe and Çoban Ahmet Fountain). Amplification with 16 ISSR markers produced 377 bands from 84 individuals belonging to three subpopulations. Three hundred and sixty-six of the 377 bands were polymorphic and 9 ISSR markers were found to be 100% polymorphic. The percentage of polymorphic loci at the species level was determined as $PLY_{SP}$ = 97.08%, whereas the average $PLY_{POP}$ at the population level was 69.67%. Total genetic diversity ($H_T$), genetic diversity within the population ($H_S$), genetic differentation among populations ($G_{ST}$), and gene flow among populations ($N_M$) values were as follows $H_T$ = 0.1888, $H_S$ = 0.1712, $G_{ST}$ = 0.0934, and $N_M$ = 4,8566, respectively. Genetic variance within-population was 89%, whereas among-population it was 11% according to AMOVA. The results of the STRUCTURE analysis (ΔK = 3) were in accord with the UPGMA and PCoA analyses.

**Key words:** Conservation, ecological preferences, endemic, population genetics

## 1. Introduction

*Muscari* Mill. is a genus of the Asparagaceae family with bulbous perennial species known from the Caucasus, temperate Europe, Africa, South-West, and North-West Asia (Jafari and Maassoumi, 2011). According to WCSP (The World Checklist of Asparagaceae) records, *Muscari* genus has worldwide 51 species (Govaerts, 2019). *M. adilii* M.B. Güner & H. Duman is narrowly distributed endemic species for Turkey. The species is bloom in early February or March and usually prefers pine and oak forest openings with white, gravel, and calcareous-clay soils (Eker et al., 2016).

An endemic species which exists naturally and exclusively in a certain geographic area and is highly suited to that area (Anderson, 1994; Işik, 2011) is more sensitive to anthropogenic pressures and natural changes, and hence has a higher risk of extinction (Coelho et al., 2020; Keser et al. 2020).

Conservation biology is a new stage of applied science that works to prevent the destruction of species, communities, and ecosystems, either directly or indirectly, by human activities or other factors. The aim of conservation biology is to develop principles and tools to protect biological diversity (Soulé, 1985). First protection efforts focused only on the number of individuals in the population of endangered species. However, in conservation efforts genetic diversity has a very important role in the long-term continuity of populations as well as the number of individuals (Klug et al., 2006). From the very beginning, conservation genetics has focused largely on the genetic consequences of small populations that may limit the survival of populations and species (Frankel, 1974). The primary factor in genetic diversity among plant populations is the species' ability to distribute genes (Loveless and Hamrick, 1984; Hamrick and Godt, 1989). However, the different geographic locations differ according to some important ecological characteristics such as altitude, temperature, and humidity (Bennett, 1970). Climate influences plant reproductive ecology (including pollination, inbreeding depression, and seed dispersal), population dynamics (including individual densities and sizes, germinating seeds, seedlings, and seedling establishment), herbivory and predation interactions, local adaptations, and geographic distribution (Foll and Gaggiotti, 2006; Kiambi et al., 2008; Temunović et al., 2012; Hamasha et al., 2013).

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In this study, it was considered to determine climatic and edaphic features together with the genetic diversity of the narrowly distributed, endangered endemic species. Thus, by determining the genetic structure and ecological adaptations of the species and comparing them with those of other studied *Muscari* species, improved conservation was aimed in the face of changing climatic conditions.

## 2. Material and method

### 2.1. Plant material

A total of 84 fresh leaves were collected 28 indiviuals from each locality of *M. adilii* for the genetic studies (Figure 1). Fresh leaves were dried in silica gel and then stored at –80 °C.

### 2.2. Ecological features

The climatic data from the closest meteorological stations, Beypazarı and Nallıhan, were obtained from the Turkish State Meteorological Service (TSMS, 2019) and temperature, precipitation, humidity, and wind data were evaluated according to Emberger (1955). The ombrothermic diagrams of the stations were prepared according to the Gaussen method (Gaussen, 1955). For the determination of the soil preferences of the species, soil samples were taken from each locality and analyzed. The texture (Demiralay, 1993), electrical conductivity (EC), lime (Tüzüner, 1990), pH (Richards, 1954), and gypsum (Porta Casanellas et al. 1986) ratios of the soils were determined for each soil sample.

### 2.3. DNA extraction

DNAs were obtained from leaf samples dried in silica gel by using NucleoSpin® Plant II DNA (Macherey-Nagel) protocol. The amounts and purity of the DNA samples were determined by the Nanodrop spectrophotometer. The obtained DNA samples were diluted to 8 ng/µL.

### 2.4. PCR amplification

During optimization processes, a total of 20 µL containing 1.5 µL DNA, 2 µL 10X buffer (with 2mM $MgCI_2$ ), 0.4 µL from each 5 mM dNTP, 0.8 µL of 10 µM primer, 0.15 µL of 5U/µL Taq DNA polymerase were prepared for each tube. In amplification processes are 95 °C/5 min 1 cycle prefrontation, then 95 °C/30 s denaturation, 15 cycles each, 57–47 °C-TD (Don et al., 1991) and 72 °C/1 min, 30 s elongation, more. Then PCR products were obtained with 23 cycles of 95 °C/30 s denaturation, 45 °C/30 s annealing and 72 °C/1 min 30 s elongation and finally 72 °C/7 min retention. A total of 54 different ISSR markers were tested for the three subpopulations (set no. 9, University of British Columbia). The bands giving diversity are specified (Table 1). 2% agarose gel with 0.5X TBE buffer solution stained with ethidium bromide (EB) was used to separate the DNA fragments. The agarose gel was run at 90 volts for 3.5 h. The BioRad Molecular Imager DocXR + analyzer was used to record the band profiles.

### 2.5. Data analysis

The data obtained by the ISSR method were scored as


**Figure 1.** Locations of *M. adilii* subpopulations (accessed via Google Earth Pro on 12.12. 2022).

**Table 1.** ISSR primers used for genetic diversity analysis of *M.adilii*.

| Primer | Sequence 5'-3' | Total Number of Bands | Polymorphism ratio (%) | PIC value |
|---|---|---|---|---|
| UBC 807 | AGAGAGAGAGAGAGAGT | 27 | 100 | 0.241 |
| UBC 808 | AGAGAGAGAGAGAGAGC | 20 | 100 | 0.240 |
| UBC 811 | GAGAGAGAGAGAGAGAC | 27 | 100 | 0.243 |
| UBC 818 | CACACACACACACACAG | 27 | 100 | 0.253 |
| UBC 825 | ACACACACACACACACT | 20 | 95 | 0.248 |
| UBC 826 | ACACACACACACACACC | 24 | 95.8 | 0.215 |
| UBC 834 | AGAGAGAGAGAGAGAGYT | 23 | 100 | 0.233 |
| UBC 835 | AGAGAGAGAGAGAGAGYC | 18 | 88.8 | 0.227 |
| UBC 836 | AGAGAGAGAGAGAGAGYA | 22 | 100 | 0.194 |
| UBC 840 | GAGAGAGAGAGAGAGAYT | 26 | 100 | 0.212 |
| UBC 851 | GTGTGTGTGTGTGTGTYG | 24 | 95.8 | 0.178 |
| UBC 856 | ACACACACACACACACYA | 25 | 100 | 0.257 |
| UBC 857 | ACACACACACACACACYG | 35 | 100 | 0.229 |
| UBC 866 | CTCCTCCTCCTC CTCCTC | 17 | 82.3 | 0.244 |
| UBC 880 | GGAGAGGAGAGGAGA | 20 | 90 | 0.279 |
| UBC 886 | VDVCTCTCTCTC TCTCT | 22 | 95.4 | 0.270 |
| **Total** | | 377 | 97.08 | 0.221 |

Y = C/T, V = A/C/G; Annealing Temperature (AT) for all primers = TD-57–47 °C

present (1) and absent (0) in a binary matrix. Data files were prepared in formats suitable for analysis programs. Observed allel number (Na), effective allele number (Ne), polymorphic locus number (PLS), percentage of polymorphic locus (PLY), Nei (1973, 1987) gene diversity (H), Shannon (Lewontin, 1972) information index (I), genetic diversity ratios of taxa at population and species levels were calculated by POPGENE ver 1.32 (Yeh et al., 1997) and GenAlExVer 6.5 (Peakall and Smouse, 2012) programs. Genetic diversity ($H_S$) within populations, genetic differentation among populations ($G_{ST}$) and total genetic diversity ($H_T$) values were calculated according to statistical data generated by the Nei gene diversity method (Nei, 1987). Also, gene flow ($N_M$) among populations was calculated (McDermott and McDonald, 1993). The polymorphism information content (PIC) for each ISSR marker was calculated (Abuzayed et al., 2017). The genetic relationship between individuals was established with the UPGMA based on the binary genetic distance between genotypes through the Jaccard similarity coefficient (Jaccard, 1908). The correlation of the cluster analysis formed with the UPGMA dendrogram was examined by the PCoA (Principal Coordinate Analysis) cluster analysis (Orlóci, 1978) created by the GenAlEx program. Genetic variances among and within populations was examined by AMOVA (Excoffier et al., 1992). The MANTEL Test (Mantel, 1967) program was used in the GenAlEx program to determine the correlation between genetic distance and geographical distance between populations. STRUCTURE version 2.3.4 was used to assign individuals to genetic groups (Pritchard et al., 2000). The Admixture model was used to determine subsequent probabilities of the data for each K ((Pr(X|K)) or L(K) cluster for K = 2 through K = 20 clusters, allowing for possible recombination between inferred clusters. For each K, 10 runs with three iterations were done after a burn-in period of 20,000 and 200,000 McMC replications. As previously stated, the amount of variance in each K.'s likelihood was calculated (Evanno et al., 2005). K was used to calculate the bestfit number of groups in STRUCTURE HARVESTER v.0.6.8 (Earl and vonHoldt, 2012).

## 3. Results

### 3.1. Ecological data

#### 3.1.1. Bioclimatic data

According to anlysis of the meteorological data on distribution area of *M. adilii* was under the influence of "semiarid lower Mediterranean" bioclimate. Annual average temperatures were measured as 13.0 °C in Beypazarı and 12.6 °C in Nallıhan. Annual average precipitation, average max and min temperatures, precipitation-temperature coefficient (Q) according to

Emberger (1955), drought index (S), precipitation regime can be seen from Table 2. According to the S value the distribution area is under the influence of Mediterranean bioclimate. According to the temperature data, frost occur at January and February in Beypazarı and January, February and December in Nallıhan. However, the possible frost occurrences occur in March, April, October, and November at both meteorological stations. The rainfall regime of the distribution areas is Eastern Mediterranean Type 1. As it can be seen from Figure 2 dry season shows similar patterns at both Beypazarı and Nallıhan from May to October. According to all these values the bioclimatic of the distribution areas is found as semiarid "lower", Mediterranean bioclimate.

### 3.1.2. Soil characteristics of the subpopulations distribution areas

The sand ratios of the locations belonging to the subpopulations were determined between 45.78% and 81.71% and the texture structures were classified as loamy-sand, sandy-loam, and clayey, respectively (Table 3).

The pH values of soils in Nallıhan Bird Sanctuary, Çoban Ahmet Fountain, and Hırkatepe localities were found between 8.07 and 8.19. The electrical conductivity (EC) values, $CaCO_3$ (lime) and $CaSO_4$-$2H_2O$ (gypsum) contents of each distribution areas soils can be seen from Table 4.

### 3.2. Genetic data

In the study, 377 bands were obtained from a total of 84 individuals belonging to 3 subpopulations with 16 ISSR primers determined for *M. adilii*. $PLY_{SP}$ was 97.08% and the average of $PLY_{POP}$ was determined as 69.67%. $PLY_{POP}$ values were determined as 69.76% (263 bands) in the Nallıhan Bird Sanctuary, 70.03% (264 bands) in the Hırkatepe and 69.23% (261 bands) in the Çoban Ahmet Fountain. The number of alleles observed and the number of effective alleles at the species level were calculated as Na = 1.9708

**Table 2.** Bioclimatic analysis of the stations.

| Station | P (mm) | M (°C) | m (°C) | Q | PE (mm) | S | Rainfall regime | Bioclimatic layers |
|---|---|---|---|---|---|---|---|---|
| Beypazarı | 410.1 | 32.2 | -1.8 | 41.85 | 58.2 | 1.80 | Eastern Mediterranean Type 1 | Semiarid "lower", Mediterranean |
| Nallıhan | 350 | 31.6 | -2.4 | 35.79 | 45.1 | 1.42 | Eastern Mediterranean Type 1 | Semiarid "lower", Mediterranean |

**P:** Mean total annual rainfall, **M:** Mean max. temperature of the warmest month, **m:** Mean min. temperature of the coldest month, **Q:** Rainfall-temperature coefficient **PE:** Summer rainfal total **S:** Drought index.

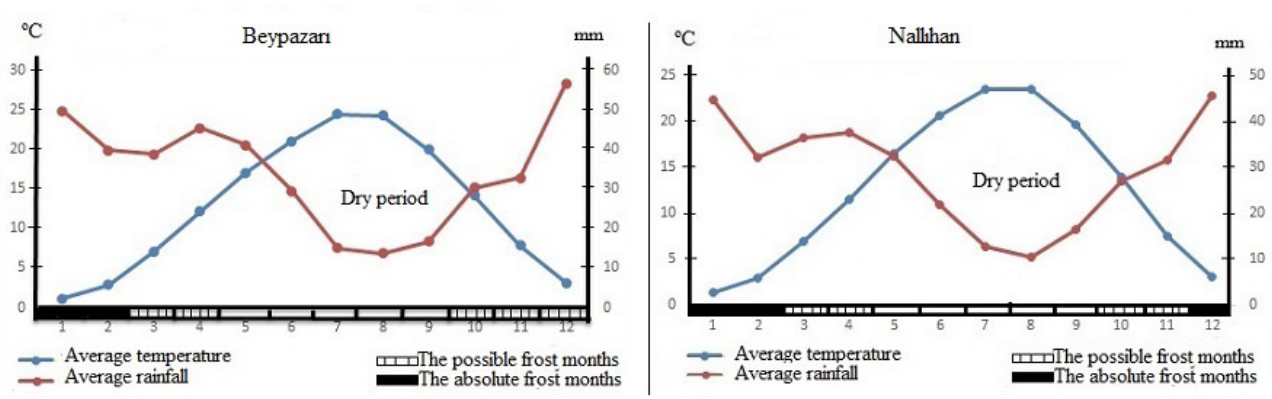


**Figure 2.** Ombrothermic climate diagrams of Beypazarı and Nallıhan.

**Table 3.** Results of physical parameter analyses of soil samples.

| Species | Locality | Clayey (%) | Silt (%) | Sand (%) | Texture |
|---|---|---|---|---|---|
| *M. adilii* | Nallıhan Bird Sanctuary | 9.15 | 9.14 | 81.71 | Loamy-sand |
| | Çoban Ahmet Fountain | 14.36 | 17.93 | 67.71 | Sandy-loam |
| | Hırkatepe | 40.43 | 13.78 | 45.78 | Clayey |

and Ne = 1.3078, respectively. Also Na and Ne values at the population level in Nallıhan Bird Sanctruary were 1.6976 and 1.2694, Hırkatepe were 1.7003 and 1.2948, and Çoban Ahmet Fountain 1.6923 and 1.2761, respectively (Table 5).

The number of bands specific to the subpopulations was calculated as 37 in Nallıhan Bird Sanctuary, 30 in Hırkatepe and 32 in Çoban Ahmet Fountain, respectively. The Nei's genetic diversity (H) value at the population level were determined as H = 0.1661 in Nallıhan Bird Sanctuary, H = 0.1785 in Hırkatepe and H = 0.1690 in Çoban Ahmet Fountain.

Total genetic diversity ($H_T$) in loci, genetic diversity within the population ($H_S$), genetic differentation among populations ($G_{ST}$) and gene flow among populations ($N_M$) values for *M. adilii* were $H_T$ = 0.1888, $H_S$ = 0.1712, $G_{ST}$ = 0.0934, and $N_M$ = 4.8566 respectively. Also, within-population variation was calculated as 89%, and among-population variation was calculated as 11% by AMOVA (Figure 3). The value of genetic differentiation among populations was calculated as ΦPR = 0.114. The number of permutations based on 999 repetitions and the value of variation within populations ($p < 0.001$) were found to be significant.

The genetic distance and geographic distance between the subpopulations of Nallıhan Bird Sanctuary and Çoban Ahmet Fountain were calculated as 0.0300 and 22.25 km, and between subpopulations of Nallıhan Bird Sanctuary and Hırkatepe were as 0.0305 and 16.55 km, and the genetic distance and geographic distance between the subpopulations of Hırkatepe and Çoban Ahmet Fountain from Beypazarı were calculated as 0.0253 and 5.78 km by using unbiased genetic distance and genetic similarity parameters (Nei, 1978) (Table 6).

Hırkatepe and Çoban Ahmet Fountain formed a group, however Nallıhan Bird Sanctuary was separated from the other two subpopulations by UPGMA. Similarly, at the population level, Hırktepe and Çoban Ahmet Fountain subpopulations were located separately from the Nallıhan Bird Sanctuary subpopulation by PCoA (Figure 4).

According to the genetic relationship between individuals, the subpopulations of *M. adilii* were divided into two subclusters by UPGMA. The first of these is the cluster where the Hırkatepe subpopulation is located, the other is the cluster divided into two subunits as Nallıhan Bird Sanctuary and Çoban Ahmet Fountain subpopulations. However, Nallıhan Bird Sanctuary subpopulation's individuals were located on the first axis, while the Hırkatepe and Çoban Ahmet Fountain subpopulations were located on the second axis by PCoA. The genetic structure of the three subpopulations was calculated using the STRUCTURE software to determine the reliability of the putative cluster groupings already investigated. Evanno's technique revealed that the number of genetic groups (ΔK) peaked at 3 (Figure 5).

**Table 4.** Results of chemical parameter analyses of soil samples.

| Taxa | Locality | pH (insaturation sludge) | EC ds/m | Lime (%) | Gypsum (%) |
|---|---|---|---|---|---|
| *M. adilii* | Nallıhan Bird Sanctuary | 8.19 | 1.69 | 0.74 | 0.086 |
| | Çoban Ahmet Fountain | 8.10 | 1.43 | 4.75 | 0.109 |
| | Hırkatepe | 8.07 | 1.04 | 29.67 | 0.107 |

**Table 5.** Genetic diversity analysis of *M. adilii*.

| Subpopulation | N | Na ± S | Ne ± S | H ± S | I ± S | PLS | PLY (%) |
|---|---|---|---|---|---|---|---|
| Nallıhan Bird Sanctuary | 28 | 1.6976 ± 0.4599 | 1.2694 ± 0.3333 | 0.1661 ± 0.1787 | 0.2623 ± 0.2523 | 263 | 69.76 |
| Hırkatepe | 28 | 1.7003 ± 0.4588 | 1.2948 ± 0.3473 | 0.1785 ± 0.1863 | 0.2775 ± 0.2626 | 264 | 70.03 |
| Çoban Ahmet Fountain | 28 | 1.6923 ± 0.4622 | 1.2761 ± 0.3383 | 0.1690 ± 0.1816 | 0.2651 ± 0.2566 | 261 | 69.23 |
| Average of subpopulation | 28 | 1.6967 ± 0.4603 | 1.2801 ± 0.3396 | 0.1712 ± 0.1822 | 0.2683 ± 0.2571 | 262.6 | 69.67 |
| Species | 84 | 1.9708 ± 0.1685 | 1.3078 ± 0.3435 | 0.1888 ± 0.1797 | 0.3005 ± 0.2446 | 366 | 97.08 |

**N:** Sample sizes, **Na:** Number of observed allel, **Ne:** Number of effective allel, **H:** Nei' genetic diversity (1973), **I:** Shannon's Information Index, **PLS:** The number of polymorphic locus, **PLY:** Percentage of polymorphic locus, **S:** Standard deviation.

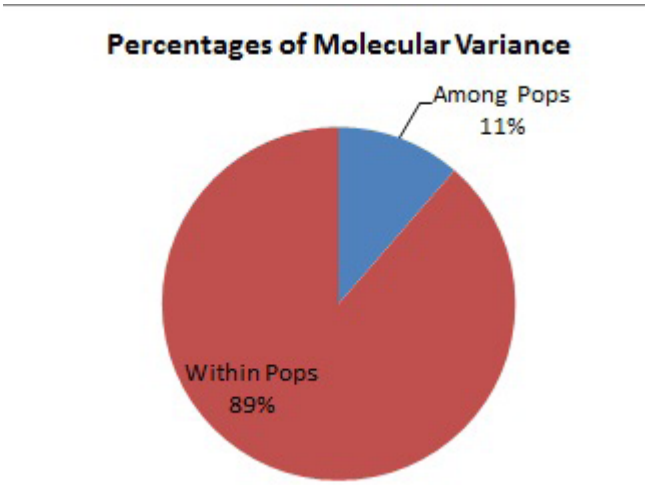


**Figure 3**. Molecular variance analysis of *M. adilii*.

**Table 6.** The genetic distance values between the subpopulations.

| Subpopulation | Nallıhan Bird Sanctuary | Hırkatepe | Çoban Ahmet Fountain |
|---|---|---|---|
| Nallıhan Bird Sanctuary | 0 | | |
| Hırkatepe | 0.0305 | 0 | |
| Çoban Ahmet Fountain | 0.0300 | 0.0253 | 0 |

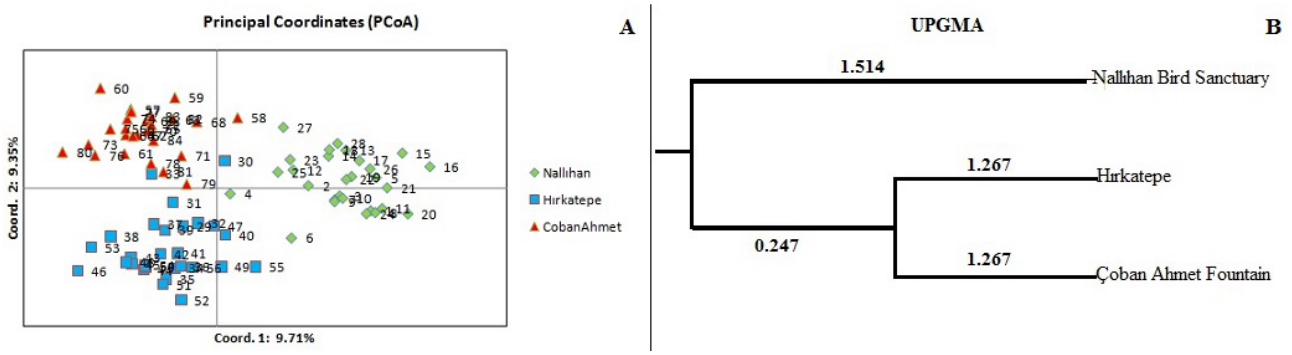


**Figure 4. A:** PCoA of *M. adilii* individuals and **B:** UPGMA dendrogram of *M. adilii* subpopulations.

## 4. Discussion

### 4.1. Ecologicgal features

#### 4.1.1. Climate

The annual mean temperatures of the distribution areas of *M. adilii* changes between 13.0–12.6 °C, and the average precipitation was in between 410.1–350 mm. The flowering period of *M. adilii* was in March and April and monthly average temperature changes between 6.9 and 12.2 °C and monthly precipitations were in between 36.6 and 45.5 mm. According to the climatological values from meteorological stations, Nallıhan was drier than Beypazarı with larger distribution area and has higher number of individuls of *M. adilii* (Keser et al., 2020). The correlation between the genetic differentiation and climatic values changes in different plants (Foll and Gaggiotti, 2006). For instance, the genetic diversity of *Oryza longistaminata* A. Chev. & Roehr. was a function of annual rainfall (Kiambi et al., 2008), however, species of *Stipa* L. were higher genetic variation in drier environments (Hamasha et al., 2013). Climate and environmental heterogeneity influence plant distribution on a global scale, whereas environmental heterogeneity controls plant distribution on a local scale (Lavers and Field, 2006). The input of resources required for plant development, such as moisture, radiation, and temperature, is influenced by

climate, whereas environmental variability (topography, aspect) dictates the number of actual environmental gradient combinations in a given landscape (Huston and DeAngelis, 1994). At both of the distribution areas the precipitation regime is determined as Eastern Mediterranean Type 1 precipitation regime. *M. adilii* prefers long and intense dry period from May to October (Figure 2). Since the S values of the stations are less than 5 (S < 5), study areas are under the influence of the Mediterranean bioclimate (Akman, 1990). As a result of the Q values specified by Emberger (1955), Beypazarı and Nallıhan fall into the lower semiarid Mediterranean bioclimate level.

#### 4.1.2. Soil

*M. adilii* prefers slightly alkaline and salt-free soils. Gypseous soils contain more than 2% of gypsum and the lower layers of this structure generally contain more than 14% gypsum (Van Alphen and de los Ríos Romero, 1971). In subpopulations areas exist gypsum values were detected at very low rates. Also, the species were not sensitive to changing lime conditions and texture ratios.

### 4.2. Genetic features

Amplification of 16 ISSR markers gave 377 bands from 84 individuals belonging to 3 subpopulations. Along with 366 of 377 bands were polymorphic, 9 of the ISSR markers were determined to be 100% polymorphic. $PLY_{SP}$ and $PLY_{POP}$ were found as 97.08% and 69.67%, respectively. For *M. neglectum* Guss. ex Ten., which has a cosmopolitan distribution, $PLY_{SP}$ was determined as 96.4% and $PLY_{POP}$ was determined as 64.75% (Iabbaf et al., 2020). In widely distributed *M. tenuiflorum* Tausch $PLY_{POP}$ was determined as 55.81% by AFLPs. The results obtained for isozyme markers were found to be in correlation with the AFLP marker results (Hornemann et al., 2012). The $PLY_{SP}$ and $PLY_{POP}$ values of *Dracaena konaensis* (H.St. John) Jankalski (name in original publication: *Chrysodracon hawaiiensis* (O. Deg. & I. Deg.) P.L. Lu & Morden) and *Dracaena rockii* (H.St. John) Jankalski (name in original publication: *Chrysodracon auwahiensis* (H. St. John) P.L. Lu & Morden) which belong to the Asparagacaae family were as 92% and 60%, and 88% and 54.2%, respectively (Lu et al., 2016). The percentage of polymorphic locus of the cosmopolitan *Polygonatum odoratum* (Mill.) Druce were determined as $PLY_{SP}$ = 91.67% and $PLY_{POP}$ = 80.18% (Hao et al., 2017). In the study conducted for *Asparagus acutifolius* L., the mean percentage of polymorphic locus was $PLY_{POP}$ = 44.51% (Sica et al., 2005).

(H) and (I) values of *M. adilii* were $H_{SP}$ = 0.1888 and $H_{POP}$ = 0.1712, respectively, while $I_{SP}$ = 0.3005 and $I_{POP}$ = 0.2683. These values were respectively $H_{POP}$ = 0.23 $I_{POP}$ = 0.35 in *M. neglectum* (Iabbaf et al., 2020), however, $H_{SP}$ =

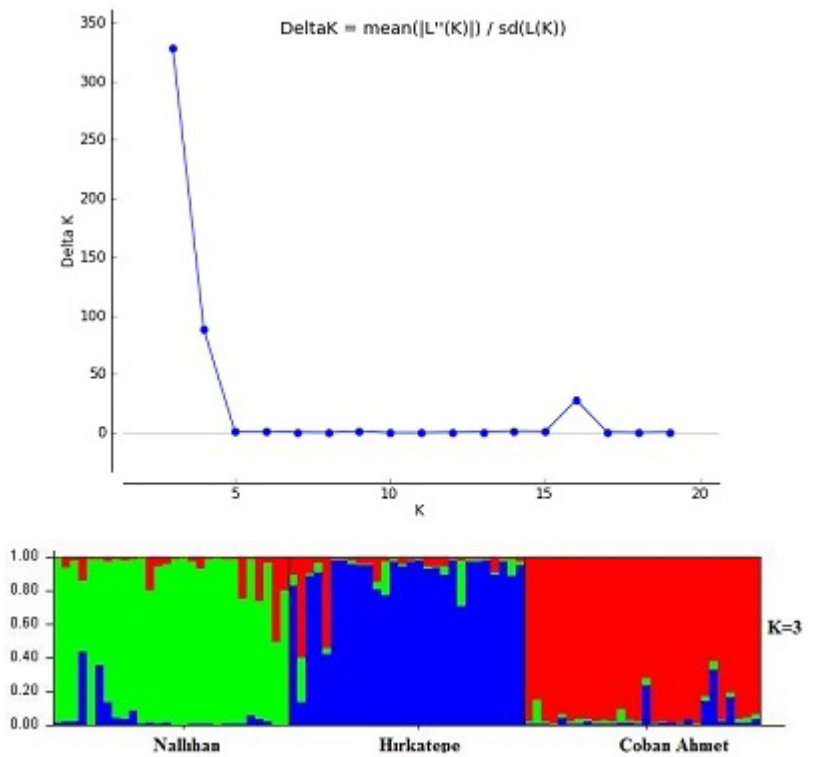


**Figure 5.** Bayesian model-based clustering STRUCTURE analysis of *M. adilii*.

0.346 and $H_{POP}$ = 0.3206 and $I_{SP}$ = 0.5108 and $I_{POP}$ = 0.4490 in *P. odoratum,* (Hao et al., 2017). (I) values of the *D. konaensis* were $I_{SP}$ = 1.450 and $I_{POP}$ = 1.3945, respectively, (I) values of the *D. rockii* were $I_{SP}$ = 1.397 and $I_{POP}$ = 1.3954, respectively (Lu et al., 2016). *Agave sisalana* Perrine were $I_{POP}$ = 0.279 (Santos et al., 2015).

Generally, the genetic diversity of small populations of plant species is thought to lower than the the genetic diversity of large population (Willi et al., 2006; Li et al., 2012). *M. adilii* has higher values in terms of polymorphic locus at the level of species and population average, but Shannon information index (I), has lower averages from *Dracaena konaensis* and *D. rockii* (Lu et al., 2016). Shannon information index values of *M. adillii* were lower compared to other endemic taxa, the probable reason for this results, *M. adilii* were known with only 3 subpopulations in a very small distribution area of approximately 28 km.

*Muscari* species are pollinated with bees; however, some seeds are formed from self-pollination (Garrido-Ramos et al., 1998). Likewise, the pollens of *M. comosum* are carried by insects (Turner et al., 1982) and seeds do not have a special adaptation for long-range distribution (López Alonso and Pascual Reguera, 1989).

In terms of polymorphic loci percentages (PLP), the order of the populations is determined as Hırkatepe > Nallıhan > Çoban Ahmet Çeşmesi, while the Shannon diversity index (I) and Nei gene diversity (H) parameters order is seen as Hırkatepe > Çoban Ahmet Çeşmesi > Nallıhan. When the populations are compared in terms of distribution areas, the order of Nallıhan > Hırkatepe = Çoban Ahmet Fountain is seen, while the populations are examined in terms of the number of individuals, they are ranked from largest to smallest as Nallıhan > Hırkatepe > Çoban Ahmet Fountain (Keser et al. 2020). Although Nallıhan Bird Sanctuary is larger than the other two populations in terms of both population size and number of individuals (Keser et al. 2020), it has the lowest Nei gene diversity and Shannon diversity index values. The area where the Nallıhan Bird Sanctuary population is located is quite far away from the area where the other two populations are located and the low genetic diversity can be explained by either founder effect or former genetic drift caused by nearby dam lake which flooded many suitable habitats for the species approximately 70 years ago.

Assuming the absence of selection, the genetic make up of the populations shows the mating systems and gene flow size among the populations. The high rate of gene flow among populations and random mating explain the low level of genetic differentiation among the populations, and this situation determines the good fit with Hardy-Weinberg expectations. In case of prevention of gene flow, the genetic differentiation increases among populations (Wright, 1978; Knight and Waller, 1987). At the same time, high gene flow between populations increases the level of heterozygosity (Qiu et al., 2004).

It is seen that the differentiation among populations with the value of 0.0934 is low, due to high gene flow among populations. The within population variance was 89% and the among populations variance was 11% by AMOVA. The population differentiation rate ($G_{ST}$ = 0.0934) of *M. adilii* was higher than the $G_{ST}$ = 0.04 of *M. comosum* L. studied with isozyme enzymes (López Alonso and Pascual Reguera, 1989) but lower than the $G_{ST}$ = 0.117 of *P. odoratum* (Hao et al., 2017), the $G_{ST}$ = 0.4176 of *A. acutifolius* (Sica et al., 2005) and the $G_{ST}$ = 0.235 of *A. sisalana* (Santos et al., 2015).

*M. adilii* with a within-population variance value of 89% was higher than *M. neglectum* (Iabbaf et al., 2020), *M. tenuiflorum* (Hornemann et al., 2012), *Dracaena konaensis* and *D. rockii* (Lu et al., 2016), *Agave sisalana* (Santos et al., 2015).

Hırkatepe and Çoban Ahmet Fountain formed the first main group, while Nallıhan Bird Sanctuary formed the second main group by UPGMA. At the population level, both UPGMA and PCoA analyses have found correlation. There was a statistically significant positive correlation between geographical distance and genetic distance values ($r = 0.349$; $p < 0.001$) by Mantel test.

Hırkatepe and Çoban Ahmet Fountain individuals were positioned on one axis and partially individuals of these subpopulations stand close to each other, Nallıhan Bird Sanctuary subpopulation individuals were located on the other axis by PCoA. The individuals have been observed in different subpopulations' location in very rare numbers. Structure analysis results ($\Delta K = 3$) were in correlation with both UPGMA and PCoA analyses.

## 5. Conclusion

This species has three subpopulations: Hırkatepe, Çoban Ahmet Fountain, and Nallıhan Bird Sanctuary. Genetic diversity and expected heterozygosity were found to be the lowest in the subpopulation of Nallıhan with the highest number of individuals, which may be explained by founder effect. The high expected heterozygosity and genetic diversity of the Hırkatepe and Çoban Ahmet Fountain subpopulations, although they have very few individuals, can most likely be explained by the fact that these subpopulations have rapidly shrunk due to afforestation and road construction. In the Hırkatepe and Çoban Ahmet Fountain subpopulations where the genetic diversity is high, the low number of individuals may reduce the attractiveness of pollinators, thus creating a risk of going through a genetic bottleneck, so measures should be taken to increase the number of individuals for these subpopulations.

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**Conflict of interest**
The authors declare that they have no conflicts of interest.

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