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# Assessment of genetic variation of natural populations of wild cherry (*Prunus avium* L.) via SSR markers

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**Abstract:** Wild cherry (*Prunus avium* L, syn. *Cerasus avium* L. Moench.) is a widely spread forest tree that has ecological and economical importance. However, the genetic diversity of this species is threatened for many reasons. Therefore, a breeding and conservation program should be established in order to minimise the loss of genetic diversity. In this study, we aimed to understand the genetic structure of 440 individual wild cherries sampled from 22 different populations in Turkey using 10 SSR molecular markers. With the molecular variance analysis, we found that the genetic diversity within the population is approximately 88.5% and the genetic diversity among the populations is approximately 9.8%. Thus, wild cherry genetic diversity within populations is high whereas it is moderate between tested populations ($F_{ST}$ values 0.02-0.16). Phylogeny, principal component, and genetic STRUCTURE analysis showed that populations are divided according to their geographical locations. Moreover, Veliköy and Kemerköprü populations that are located at higher altitudes, Macara population which is the closest sample to Europe, and the Tota population that is sampled from the Mediterranean Region; were found genetically different from the others. Hence, we suggest in-situ conservation to Veliköy, Kemerköprü, Macara, and Tota populations. Our results will contribute to in-situ and ex-situ conservation and breeding programmes to conserve genetic resources of the wild cherries in Turkey.

**Key words:** Wild cherry, *Prunus avium* L., genetic diversity, SSR markers

## 1. Introduction

Wild cherry (*Prunus avium* L.) is a diploid forest tree from the *Rosaceae* family (2n = 16) (Arumuganathan and Earle, 1991). Its matching system is outcrossing (Vaughan et al., 2007) by pollinating with pollens carried by insects and wind, or vegetatively by giving root shoots (Frascaria et al., 1993). The fruits of wild cherry are eaten by birds, and mammals that ensure the spread of the seeds (Russell, 2003). Wild cherry can grow faster than other leafy species that could reach 25 meters height and 50–70 cm in diameter in suitable growing conditions. In optimal conditions, these trees can reach 35 meters in length and 120 cm in diameter (Savill, 1991; Russell, 2003).

Wild cherry (*Prunus avium* L.) spreads in Europe, North Africa and West Asia and it has a scattered distribution (Welk et al., 2016). Although, wild cherry in Turkey is mainly distributed Black Sea and Northern Marmara Regions, it can be seen in other regions of Turkey as well (Yaman, 2003; Welk et al., 2016). Generally, wild cherry prefers low altitude areas (Savill, 1991), and its distribution could go up to 1700 m altitude in Turkey (Yaman, 2003). However, Artvin-Veliköy which is one of the sampled populations in this study was found to be at an altitude of 1900 metres.

Wild cherry has a high economic value that can be used as a source of wood in many areas (Savill, 1991; Russell, 2003). The cultured form of wild cherry is called sweet cherry and it is used as a rootstock for sweet cherry. This species is also important for wildlife, for example fruits are a food source for humans and many animals such as bears, birds, small mammals (Russell, 2003). In addition, its fruit stems have diuretic properties and are boiled and consumed by humans (Ercisli, 2004). Additionally, it is used for landscaping in parks and gardens (Saatçioğlu, 1971).

Wild cherry is protected by European Forest Genetic Resources Programme (EUFORGEN), however, its conservation has been neglected in Turkey (Yaman, 2003; Esen, et al. 2006). Destruction of habitat, transfer of seed from different areas or dubious origins, collection of seed from a small number of seed stands, phenotypic selection for homogenous stands, hybridization with

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sweet cherry, pests and diseases, low natural regeneration, and competition with other species are the main threats for wild cherry genetic diversity (Russell, 2003), all of which caused a considerable decrease in forest areas of wild cherries in Turkey. For this reason, comprehensive breeding and conservation studies should be carried out on wild cherry, which has few resources regarding its genetics in our country (Yaman, 2003; Esen, et al., 2006). The diversity and genetic structure of the species within and between populations should be known before the initiation of breeding and conservation studies.

Molecular markers is a good way to determine the genetic structure of a particular species (Agarwal et al., 2008) and have made great contributions to plant biotechnology researches due to their capability to provide rapid results in plant breeding studies and are not affected by environmental conditions. Among them, simple sequencing repeat (SSR) is one of the widely used markers that have many advantages such as requiring a small amount of DNA, showing high polymorphism, being codominant, producibility a large number of alleles for each locus, repeatability, transferability between not only species belonging to the same genus but also genus belonging to the same family. Because of these advantages, they are widely used in plant identification (Parveen et al., 2016).

The first studies on the genetics of wild cherry were generally carried out in terms of morphology (Weiser, 1996; Santi et al., 1998). However, studies that focused on morphological characters are sensitive to environmental conditions and could take a long time (Mondini et al., 2009; Jiang, 2013). Biochemical and molecular markers were developed to eliminate these limitations (Mohan et al., 1997). To determine the genetic diversity of the wild cherry, isozymes among these markers were initially used (Frascaria et al., 1993; Gömöry, 2004). Then, molecular markers began to be used and many studies have been conducted. The most commonly used molecular marker in population genetics studies of wild cherries is the SSR markers (Schueler et al., 2003; Vaughan and Russell, 2004; Vaughan et al., 2007; Guarino et al., 2009; Avramidou et al., 2010; Ganopoulos et al., 2011; Tanceva-Crmaric et al., 2011; Jarni et al., 2012; Rogatis et al., 2012; Fernandez-Cruz et al., 2014; Khadivi-Khub et al., 2014). In addition to SSR markers, other marker systems were also used to analyse the genetic diversity of wild cherry (Mohanty et al., 2001; Panda et al., 2003). While many molecular studies have been done with the genetics of wild cherry, there are still limited molecular studies that mostly examined a few populations of Turkish wild cherry (Ercisli et al., 2011; Türkoglu et al., 2012; Unsal et al., 2019) together with two recent studies that were conducted on quantitative characters of wild cherry (Temel, 2018; Velioğlu et al., 2020). Thus, we sampled 22 the natural populations of wild cherries in Turkey by using ten nuclear microsatellite (nSSR) markers. Our main aim was not only to examine the levels and distribution of genetic variability of wild cherry natural populations in Turkey, but also to assist in breeding program and conservation practices for *P. avium* natural populations.

## 2. Materials and methods

### 2.1. Plant materials

Twenty-two different wild cherry natural populations from areas in which it is mostly distributed in Turkey were sampled (Figure 1 and Table 1). A total of 440 leaf samples from 20 trees were sampled for each population in the summer and spring (2015). Sampling was performed with a minimum 100 m distance and a maximum 300 m difference in altitude between trees to avoid vegetative clones, and stabilise the variance. One individual from a wild prune tree (*Prunus cerasifera*) was also sampled as an external group for phylogenetic analyses.

### 2.2. DNA extraction, gel electrophoresis, and PCR amplification

Genomic DNA from young leaves (20 mg of tissue) was extracted using the *i-genomic plant DNA Extraction Mini Kit (iNtRON Biotechnology)*, according to the manufacturer's protocol. Isolated DNAs were analysed on a gel electrophoresis system with 1% agarose for 30 minunder 80 volts.

The SSR protocol was performed using 10 labelled primers. Seven of these primers (Empas01, Empas02, Empas06, Empas10, Empas11, Empas12, and Empas14) are specific for wild cherry (Vaughan and Russell, 2004); the other three (Empa004, Empa005, and Empa015) were originally designed previously for sweet cherry (Clarke and Tobutt, 2003). The information of the primers is given in Table 2. Amplification reaction was carried out in a total reaction volume of 25 µL with 0.3 µM fluorescent labelled forward primer, 0.3 µM reverse primer, 10–50 ng DNA template and 5 µL Ready 5xFIREPol® Master Mix (Solis Biodyne). PCR reactions were performed on a Peltier thermal cycler, by following the conditions reported by Clarke and Tobutt (2003), and Vaughan and Russell (2004). Amplified products were analysed in an automatic sequencer, 3730XL DNA Analyzer (Applied Biosystems). The size of fragments was estimated using the Peak Scanner Software v1.0.

### 2.3. Population genetics analysis

R statistical programming language (3.3.2) was used to conduct population genetic analyses of analysed SSR markers (R Core Team, 2020). Following population genetic analysis libraries were used: *poppr* (Kamvar et al., 2014), *ape* (Paradis et al., 2004), *adegenet* (Jombart,

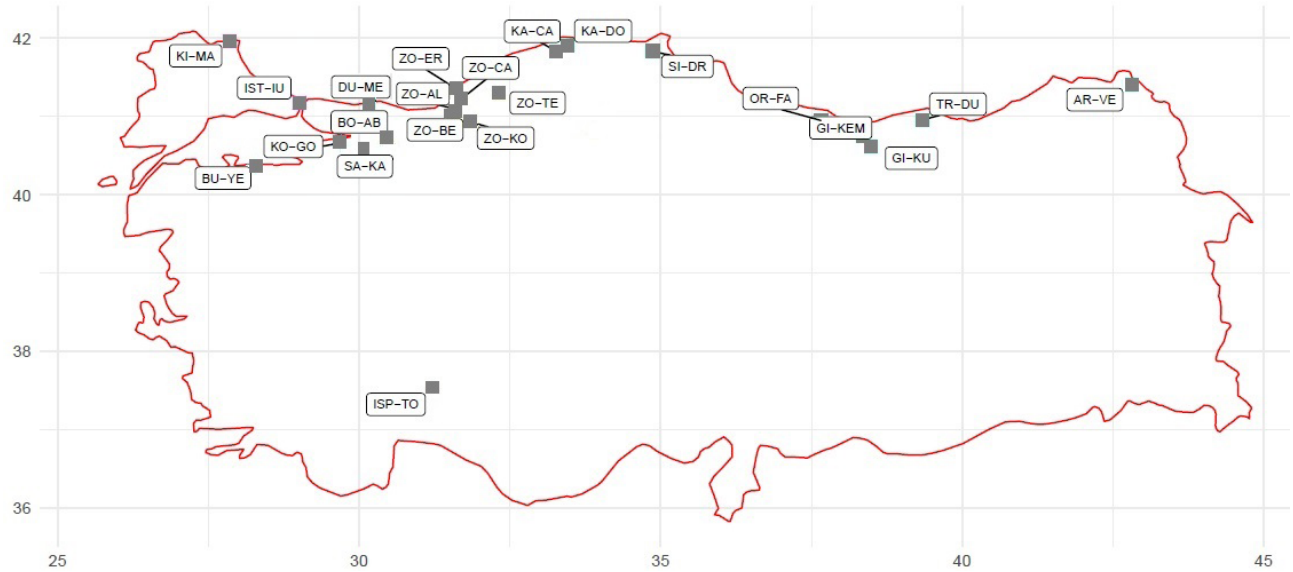


**Figure 1.** Geographic locality of 22 wild cherry populations in this study.

**Table 1.** Characteristics of 22 wild cherry populations in this study.

| No | Population | Code | Altitude | Latitude | Longitude |
|---|---|---|---|---|---|
| 1 | Artvin-Veliköy | AR-VE | 1900 | 41° 23’ 80” | 42° 46’ 63” |
| 2 | Trabzon-Düzköy | TR-DU | 600 | 40° 50’ 27.2” | 39° 20’ 22.9” |
| 3 | Giresun-Kümbet | GI-KU | 1348 | 40° 37’ 5.76” | 38° 28’ 38.00” |
| 4 | Giresun-Kemerköprü | GI-KEM | 1699 | 40° 45’ 24.41” | 38° 21’ 24.11” |
| 5 | Ordu-Fatsa | OR-FA | 950 | 40° 57’ 4” | 37° 39’ 18.1” |
| 6 | Sinop-Dranos–GCF | SI-DR | 850 | 41° 49’ 44.40” | 34° 52’ 9.42” |
| 7 | Kastamonu-Doganyurt | KA-DO | 1073 | 41° 53’ 59.93” | 33° 26’ 52.83” |
| 8 | Kastamonu-Camlıbük-GCF | KA-CA | 1116 | 41° 49’ 17.21” | 33° 17’ 44.04” |
| 9 | Zonguldak -Tefen | ZO-TE | 592 | 41° 17’ 2.67” | 32° 18’ 53.89” |
| 10 | Zonguldak - Ereğli | ZO-ER | 250 | 41° 20’ 50.62” | 31° 37’ 26.14” |
| 11 | Zonguldak-Çaylıoğlu-GCF | ZO-CA | 300 | 41° 13’ 46” | 31° 41’ 26” |
| 12 | Zonguldak -Kozdere | ZO-KO | 1250 | 40° 55’ 59.14” | 31° 50’ 22.83” |
| 13 | Zonguldak -Bendere | ZO-BE | 1120 | 41° 3’ 35.74” | 31° 36’ 46.32” |
| 14 | Zonguldak -Alaplı-GCF | ZO-AL | 550 | 41° 5’ 12.79” | 31° 38’ 24.43” |
| 15 | Bolu-Abant | BO-AB | 950 | 40° 39’ 30.50” | 31° 24’ 19.97” |
| 16 | Düzce-Melen | DU-ME | 320 | 40° 47’ 32.06” | 30° 53’ 26.52” |
| 17 | Sakarya -Karapürçek | SA-KA | 744 | 40° 37’ 5” | 30° 29’ 28” |
| 18 | Kocaeli-Gölcük | KO-GO | 670 | 40° 39’ 43.96” | 29° 41’ 59.59” |
| 19 | Bursa -Yeniköy | BU-YE | 130 | 40° 23’ 40” | 28° 18’ 16” |
| 20 | İstanbul-İstanbul University | IST-IU | 50 | 41° 10’ 39.77” | 29° 0’ 20.27” |
| 21 | Kırklareli-Macara | KI-MA | 200 | 41° 57’ 40” | 27° 50’ 55” |
| 22 | Isparta-Tota | ISP-TO | 880 | 37° 31’ 16” | 31° 12’ 43” |

GCF Gene conservation forest

**Table 2.** Genetic diversity values calculated at ten microsatellite loci.

| Locus | Allelic range (bp) | $n_a$ | $H_o$ | $H_e$ | $F_{ST}$ | $F_{IS}$ | $N_m$ | Null Allel |
|---|---|---|---|---|---|---|---|---|
| **Empas01** | 219–259 | 18 | 0.702 | 0.779 | 0.088 | 0.017 | 2.604 | 0.052 |
| **Empas02** | 131–146 | 8 | 0.72 | 0.793 | 0.096 | 0 | 2.356 | 0.048 |
| **Empa004** | 171–198 | 13 | 0.716 | 0.836 | 0.086 | 0.067 | 2.662 | 0.077 |
| **Empa005** | 233–264 | 15 | 0.768 | 0.836 | 0.093 | –0.008 | 2.445 | 0.042 |
| **Empas06** | 202–241 | 20 | 0.773 | 0.833 | 0.076 | 0.001 | 3.052 | 0.038 |
| **Empas10** | 148–192 | 21 | 0.608 | 0.773 | 0.103 | 0.128 | 2.178 | 0.119 |
| **Empas11** | 62–114 | 18 | 0.673 | 0.752 | 0.148 | –0.042 | 1.442 | 0.055 |
| **Empas12** | 123–155 | 11 | 0.709 | 0.77 | 0.096 | –0.014 | 2.342 | 0.041 |
| **Empas14** | 188–210 | 6 | 0.518 | 0.543 | 0.105 | –0.06 | 2.121 | 0.024 |
| **Empa015** | 203–253 | 21 | 0.688 | 0.804 | 0.078 | 0.075 | 2.94 | 0.078 |
| **Mean** | | 15.1 | 0.688 | 0.772 | 0.097 | 0.016 | 2.414 | 0.057 |

$n_a$ number of alleles; $H_o$ average observed heterozygosity; $H_e$ average expected heterozygosity; $F_{ST}$ gene differentiation coefficient; $F_{IS}$ inbreeding coefficient; $N_m$ gene flow values

2008), *pegas* (Paradis, 2010), *PopGenReport* (Adamack and Gruber, 2014), *hierfstat* (Goudet, 2005), *diveRsity* (Keenan et al., 2013), *vegan* (Oksanen et al., 2012).

### 2.4. Descriptive analysis of SSR markers

For descriptive analysis of the SSR markers, we calculated number of alleles ($n_a$), expected heterozygosity ($H_e$), observed heterozygosity ($H_o$) and $F_{IS}$ values (Weir and Cockerham, 1984) for each SSR marker system. $H_o$ and $H_e$ values were calculated based on Nei (1978) using the *poppr* (Kamvar et al., 2014) library. To assess the authenticity of the SSR amplification, we carried out null allele analysis based on Brookfield (1996) in *PopGenReport* (Adamack and Gruber, 2014) library. Null alleles are unwanted amplicons that are amplified due to variations in the SSR primer binding site and could cause loss of heterozygosity.

### 2.5. Population differentiation of wild cherries

To understand the differentiation between the populations, we used calculated F-statistics and gene flow values ($N_m$) values for each population. $F_{IS}$ indices describe the deviation of Hardy-Weinberg equilibrium (HWE) for the tested population. Values closer to +1 means excess homozygosity, values closer to -1 means excess heterozygosity and values closer to zero means that the population is in HWE. $F_{ST}$ describes the differentiation of the population pairs. Generally, $F_{ST}$ values higher than 0.05 to 0.15 are defined as medium differentiation and $F_{ST}$ values between 0.15–0.25 are high differentiation (Hartl and Clark, 2007). We calculated pairwise population $F_{ST}$ values by the method described Nei (1973) on *hierfstat* (Goudet, 2005). Also, we calculated $N_m$ values between each population. $N_m$ value describes the number of individuals that are migrated between populations and it is calculated by the formula described by Slatkin and Barton (1989). In addition, Mantel test was used to evaluate the relationship between the geographic distance and the pairwise population $F_{ST}$ values by using *vegan* analysis library (Oksanen et al., 2012) that works on R statistical programming language (3.3.2). At last, we calculated analysis of molecular variance (AMOVA) with 1000 bootstrap replicates to assess the total population variation and intra-population variation. For this analysis, we used *poppr* library with the function AMOVA based on Excoffier et al. (2005).

### 2.6. Phylogenetic and population structure analysis

To create phylogenetic trees, we used UPGMA method (Sokal and Michener, 1958), and tree was drawn with *poppr* analysis library (Kamvar et al., 2014) working on R statistical programming language (3.3.2). To understand the population structure in sampled wild cherries, we used discriminant analysis of principal components (DAPC) described by Jombart et al. (2010). The idea of this method is to apply discriminate analysis to principal components to find the allele contributions for each population group. Moreover, we used the Bayesian framework, STRUCTURE version 2.3.4 (Pritchard et al., 2000) tool to understand the population structure. Without giving any prior population labels, this tool computes the number of groups (denoted as K) in the given set by using a predefined population model. We tested K values between 2 – 20 and used $\Delta K$ method described by Evanno et al. (2005) to select the best possible configuration.

## 3. Results

### 3.1. Descriptive statistics of SSR markers

A total of 151 alleles were detected according to the analysis with 10 SSR primer pairs. The highest number of alleles were Empas10 (21) and Empa015 (21), and the primer with lowest number of alleles was Empas14 (6). Mean $H_o$ and $H_e$ heterozygous values were 0.688 and 0.772, respectively. It was determined that value ranges on the basis of loci were for $H_o$ 0.518–0.773 and for $H_e$ 0.543–0.836 (Table 2) .

$F_{IS}$ value gives the degree of deviation from the Hardy-Weinberg equilibrium (HWE) in each locus. According to this indice, the highest $F_{IS}$ (0.128) value was determined to be at Empas10. These values indicate an excess homozygosity. The lowest $F_{IS}$ (–0.06) value was found to be at Empas14. This also indicates the excess heterozygosity in this locus. However, the loci were generally close to zero in terms of $F_{IS}$ value, and it was observed that loci were in the HWE (Table 2).

$F_{ST}$ value was found to be 0.097 on average, and it is observed that the genetic variation between populations was moderate since this value was above 0.05. In addition, $N_m$ value calculated according to $F_{ST}$ values was found to be 2.414 on average and this value indicates that approximately 2–3 wild cherry individuals migrate in each generation (Table 2).

The null allele frequency was found to be between 0.024 (Empas14) and 0.111 (Empas10) and the fact that these values which were close to zero reduced the possibility of the presence of null allele (Table 2).

The diagram showing the populations that diverge significantly from the HWE in terms of loci was given in Supplemental Material - Figure S1. While Düzköy, Kümbet, Fatsa, Dranos, Kozdere, Bendere, and İstanbul populations were determined to be in HWE in terms of all loci examined. Macara population (6) had the highest number of loci that diverge from HWE. According to the results of the analysis with ten SSR loci, it was observed that the wild cherry populations were generally in HWE.

### 3.2. Analysis of populations and population differentiation among wild cherries

Fatsa (8.6) and Veliköy (8) populations have the highest average allele numbers, Kemerköprü (4.4) and Karapürçek (4.5) have the lowest average allele. In addition, the maximum number of private alleles was determined in the Veliköy (6) population. $H_o$ and $H_e$ values were found to be 0.69 and 0.74, respectively. $F_{IS}$ values of 22 populations were determined as negative, and the average $F_{IS}$ value was found to be –0.07. The negativity of $F_{IS}$ values indicates the excess heterozygous in populations (Table 3).

Pairwise $F_{ST}$ values were determined between 0.02–0.16 (Supplemental Material–Table S1). The differentiation between the populations was generally at low and medium level. The highest differentiation was observed between the Kemerköprü population and the Tota and Macara populations (0.16). In addition, Kemerköprü was found to be the most differentiated population. $N_m$ values that were calculated based on the $F_{ST}$ values showed that the minimum numbers of individuals migrating were between Kemerköprü-Tota (1.35) and Kemerköprü-Macara (1.33) populations, while the highest number of migrating individuals was between Doğanyurt - Çamlıbük populations (17.32) (Table 4). As a result of the Mantel test, a Pearson correlation of 0.59 was found that indicates a P-value below 0.001. Next, the correlation between genetic and geographic distances were assessed to reveal out the spatial pattern of genetic variation with the Mantel test. The Mantel correlation between genetic and

**Table 3.** Genetic diversity values calculated at population levels in 22 wild cherry populations.

| Population | N | A | $H_o$ | $H_e$ | $F_{IS}$ | $P_a$ |
|---|---|---|---|---|---|---|
| **AR-VE** | 20 | 8 | 0.75 | 0.79 | –0.03 | 6 |
| **TR-DU** | 20 | 7 | 0.68 | 0.73 | –0.04 | 0 |
| **GI-KU** | 20 | 6.3 | 0.72 | 0.72 | –0.09 | 2 |
| **GI-KE** | 20 | 4.4 | 0.64 | 0.63 | –0.18 | 0 |
| **OR-FA** | 20 | 8.6 | 0.78 | 0.8 | –0.03 | 3 |
| **SI-DR** | 20 | 7 | 0.72 | 0.76 | –0.04 | 1 |
| **KA-DO** | 20 | 7.8 | 0.71 | 0.76 | –0.03 | 1 |
| **KA-CA** | 20 | 6.7 | 0.7 | 0.79 | –0.06 | 2 |
| **ZO-TE** | 20 | 5.7 | 0.63 | 0.73 | –0.08 | 0 |
| **ZO-ER** | 20 | 5.6 | 0.59 | 0.72 | –0.07 | 0 |
| **ZO-CA** | 20 | 5.8 | 0.68 | 0.63 | –0.08 | 1 |
| **ZO-KO** | 20 | 6.2 | 0.66 | 0.8 | –0.05 | 1 |
| **ZO-BE** | 20 | 5.4 | 0.7 | 0.76 | –0.07 | 0 |
| **ZO-AL** | 20 | 5.6 | 0.62 | 0.76 | –0.1 | 0 |
| **BO-AB** | 20 | 5.9 | 0.72 | 0.79 | –0.05 | 2 |
| **DU-ME** | 20 | 5.7 | 0.69 | 0.73 | –0.04 | 0 |
| **SA-KA** | 20 | 4.5 | 0.57 | 0.72 | –0.07 | 0 |
| **KO-GO** | 20 | 5.9 | 0.72 | 0.63 | –0.08 | 1 |
| **BU-YE** | 20 | 6.5 | 0.68 | 0.8 | –0.07 | 2 |
| **IST-IU** | 20 | 5.6 | 0.72 | 0.76 | –0.1 | 1 |
| **KI-MA** | 20 | 5.2 | 0.8 | 0.76 | –0.12 | 0 |
| **ISP-TO** | 20 | 5.5 | 0.66 | 0.79 | –0.12 | 2 |
| **Mean** | 20 | 6.1 | 0.69 | 0.74 | –0.07 | 1.14 |

N sample size; A mean number of alleles per locus; $H_o$ average observed heterozygosity; $H_e$ average expected heterozygosity; $F_{IS}$ average inbreeding coefficient; $P_a$ number of private alleles

**Table 4.** Gene flow rate ($N_m$) among 22 wild cherry populations.

| | ArVe | TrDu | GiKu | GiKe | OrFa | SiDr | KaDo | KaCa | ZoTe | ZoEr | ZoCa | ZoKo | ZoBe | ZoAl | BoAb | DuMe | SaKa | KoGo | BuYe | IstIU | KıMa | IspTo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ArVe** | 0 | 2.88 | 3.32 | 1.99 | 4.79 | 3.67 | 3.56 | 3.63 | 2.09 | 2.06 | 2.14 | 2.44 | 2.43 | 2.81 | 2.62 | 3.03 | 2.17 | 2.76 | 3.35 | 2.76 | 2.58 | 2 |
| **TrDu** | 2.88 | 0 | 7.06 | 2.84 | 7.63 | 4.58 | 4.53 | 4.16 | 3.14 | 2.62 | 2.65 | 3.17 | 3.7 | 3.91 | 3.35 | 4.57 | 3.15 | 3.28 | 3.53 | 3.71 | 1.83 | 2 |
| **GiKu** | 3.32 | 7.06 | 0 | 3.45 | 10.9 | 4.73 | 4.08 | 4.04 | 2.59 | 2.6 | 3.2 | 3.49 | 3.31 | 3.09 | 3.15 | 4.12 | 2.78 | 3.26 | 3.41 | 3.04 | 1.94 | 1.78 |
| **GiKe** | 1.99 | 2.84 | 3.45 | 0 | 3.27 | 2.28 | 2.02 | 1.87 | 1.44 | 1.51 | 1.79 | 1.82 | 1.94 | 1.65 | 1.63 | 2.02 | 1.55 | 1.73 | 1.63 | 1.6 | 1.33 | 1.35 |
| **OrFa** | 4.79 | 7.63 | 10.9 | 3.27 | 0 | 11.76 | 8.89 | 8.17 | 4.54 | 5.05 | 4.84 | 5.77 | 6.6 | 5.8 | 5.38 | 6.42 | 3.96 | 5.8 | 5.74 | 4.62 | 2.87 | 2.98 |
| **SiDr** | 3.67 | 4.58 | 4.73 | 2.28 | 11.76 | 0 | 11.28 | 11.17 | 5.7 | 6.03 | 4.74 | 7.25 | 6.93 | 7.48 | 6.21 | 7.05 | 4.2 | 11.43 | 5.81 | 5.52 | 3.53 | 3.39 |
| **KaDo** | 3.56 | 4.53 | 4.08 | 2.02 | 8.89 | 11.28 | 0 | 17.32 | 4.91 | 4.92 | 4.47 | 5.33 | 5.98 | 7.13 | 5.59 | 6.09 | 3.76 | 8.79 | 5.65 | 4.36 | 3.1 | 3.26 |
| **KaCa** | 3.63 | 4.16 | 4.04 | 1.87 | 8.17 | 11.17 | 17.32 | 0 | 5.13 | 5.7 | 4.33 | 5.55 | 5.58 | 8.16 | 6.9 | 7.02 | 3.69 | 9 | 7.45 | 4.75 | 3.59 | 2.93 |
| **ZoTe** | 2.09 | 3.14 | 2.59 | 1.44 | 4.54 | 5.7 | 4.91 | 5.13 | 0 | 7.76 | 7.79 | 9.52 | 10.49 | 12.63 | 8.87 | 7.63 | 4.49 | 4.81 | 3.92 | 3.74 | 1.98 | 3.19 |
| **ZoEr** | 2.06 | 2.62 | 2.6 | 1.51 | 5.05 | 6.03 | 4.92 | 5.7 | 7.76 | 0 | 10.32 | 7.32 | 9.68 | 8.47 | 11.74 | 5.75 | 3.4 | 5.22 | 4.09 | 2.98 | 2.54 | 2.62 |
| **ZoCa** | 2.14 | 2.65 | 3.2 | 1.79 | 4.84 | 4.74 | 4.47 | 4.33 | 7.79 | 10.32 | 0 | 9.42 | 10.08 | 8.07 | 9.56 | 5.56 | 5.15 | 4.33 | 3.27 | 2.86 | 2.2 | 2.47 |
| **ZoKo** | 2.44 | 3.17 | 3.49 | 1.82 | 5.77 | 7.25 | 5.33 | 5.55 | 9.52 | 7.32 | 9.42 | 0 | 10.53 | 8.97 | 8.92 | 8.25 | 5.83 | 5.48 | 4.77 | 4.19 | 2.5 | 3.27 |
| **ZoBe** | 2.43 | 3.7 | 3.31 | 1.94 | 6.6 | 6.93 | 5.98 | 5.58 | 10.49 | 9.68 | 10.08 | 10.53 | 0 | 10.04 | 8.21 | 7.56 | 4.65 | 5.76 | 4.21 | 3.73 | 2.34 | 2.69 |
| **ZoAl** | 2.81 | 3.91 | 3.09 | 1.65 | 5.8 | 7.48 | 7.13 | 8.16 | 12.63 | 8.47 | 8.07 | 8.97 | 10.04 | 0 | 13.49 | 8.45 | 4.86 | 5.8 | 5.05 | 5.86 | 2.41 | 3.67 |
| **BoAb** | 2.62 | 3.35 | 3.15 | 1.63 | 5.38 | 6.21 | 5.59 | 6.9 | 8.87 | 11.74 | 9.56 | 8.92 | 8.21 | 13.49 | 0 | 10.99 | 5.06 | 6.14 | 5.97 | 4.44 | 2.69 | 3.3 |
| **DuMe** | 3.03 | 4.57 | 4.12 | 2.02 | 6.42 | 7.05 | 6.09 | 7.02 | 7.63 | 5.75 | 5.56 | 8.25 | 7.56 | 8.45 | 10.99 | 0 | 5.19 | 6.84 | 6.48 | 5.25 | 2.61 | 3.49 |
| **SaKa** | 2.17 | 3.15 | 2.78 | 1.55 | 3.96 | 4.2 | 3.76 | 3.69 | 4.49 | 3.4 | 5.15 | 5.83 | 4.65 | 4.86 | 5.06 | 5.19 | 0 | 3.77 | 2.93 | 3.03 | 1.84 | 2.21 |
| **KoGo** | 2.76 | 3.28 | 3.26 | 1.73 | 5.8 | 11.43 | 8.79 | 9 | 4.81 | 5.22 | 4.33 | 5.48 | 5.76 | 5.8 | 6.14 | 6.84 | 3.77 | 0 | 5.3 | 4.64 | 3.88 | 3.28 |
| **BuYe** | 3.35 | 3.53 | 3.41 | 1.63 | 5.74 | 5.81 | 5.65 | 7.45 | 3.92 | 4.09 | 3.27 | 4.77 | 4.21 | 5.05 | 5.97 | 6.48 | 2.93 | 5.3 | 0 | 5.85 | 3.15 | 2.95 |
| **IstIU** | 2.76 | 3.71 | 3.04 | 1.6 | 4.62 | 5.52 | 4.36 | 4.75 | 3.74 | 2.98 | 2.86 | 4.19 | 3.73 | 5.86 | 4.44 | 5.25 | 3.03 | 4.64 | 5.85 | 0 | 2.22 | 3.95 |
| **KıMa** | 2.58 | 1.83 | 1.94 | 1.33 | 2.87 | 3.53 | 3.1 | 3.59 | 1.98 | 2.54 | 2.2 | 2.5 | 2.34 | 2.41 | 2.69 | 2.61 | 1.84 | 3.88 | 3.15 | 2.22 | 0 | 1.9 |
| **IspTo** | 2 | 2 | 1.78 | 1.35 | 2.98 | 3.39 | 3.26 | 2.93 | 3.19 | 2.62 | 2.47 | 3.27 | 2.69 | 3.67 | 3.3 | 3.49 | 2.21 | 3.28 | 2.95 | 3.95 | 1.9 | 0 |

geographic matrices was equal to 0.398 that indicates a P-value below 0.001 meaning 39.8% of the genetic distance can be explained by the geographical distance. The scatter plot showed that there is positively a linear relationship between genetic and geographic distances (Figure 2). The total genetic differentiation values were calculated separately according to its components using AMOVA with 1000 bootstrap replicates. Result showed that the genetic difference between populations was 9.768%, and the genetic difference within the population was 88.46% (Table 5). Most of genetic differentiation was seen to be within the population.

### 3.3. Phylogenetic analysis

UPGMA tree showed two main branches. Wild cherry populations formed the first major branch and the outgroup, *Prunus cerasifera*, formed the second major branch. It was determined that the Kemerköprü population was grouped separately with a parsimony rate of 99.4% from other populations. Veliköy and Macara populations were grouped separately from others with 30%, and 25.8% parsimony rate. Düzkoy, Kümbet, and Fatsa populations in the Eastern Black Sea were grouped together, and samples in the Western Black Sea were also grouped together. Also, Western and Central Black Sea populations were clustered together. Karapürçek, İstanbul and Yeniköy populations in Marmara Region were also observed to be closely related to Western and Central Black Sea populations. However, Gölcük population was grouped closer to the Dranos, Çamlıbük and Doğanyurt populations. In the UPGMA analysis, populations were observed to be partitioned into groups corresponding generally to geography (Figure 3).

### 3.4. Population structure analysis

According to the PCA analysis; Veliköy, Kemerköprü, and some individuals of the Macara populations grouped separately from other populations as three different groups. While Düzköy, Kümbet and Fatsa populations grouped between Kemerköprü population and others, the remaining populations grouped together (Supplemental Material–Figure S2).

STRUCTURE analysis with 10 SSR markers was performed without prior information on the geographic origin of samples, and the highest likelihood of the data was obtained for K = 9 following the method described by Evanno et al. (2005), (Supplemental Material–Figure S3). According to the population structure at K = 9; Veliköy, Kemerköprü, Macara, and Tota populations were determined to be classified differently from each other and all other populations. In addition, Düzköy and Kümbet populations formed a different group than other populations. While Fatsa, Dranos, Doğanyurt and Çamlıbük populations were in the same group, Tefen, Eregli, Çaylıoğlu, Kozdere, Bendere, Alaplı, Abant, Melen and Karapürçek populations were also grouped together. It was determined that the Istanbul and Yeniköy populations were also grouped similarly, and Gölcük population was similar to the Central Black Sea populations, but it was

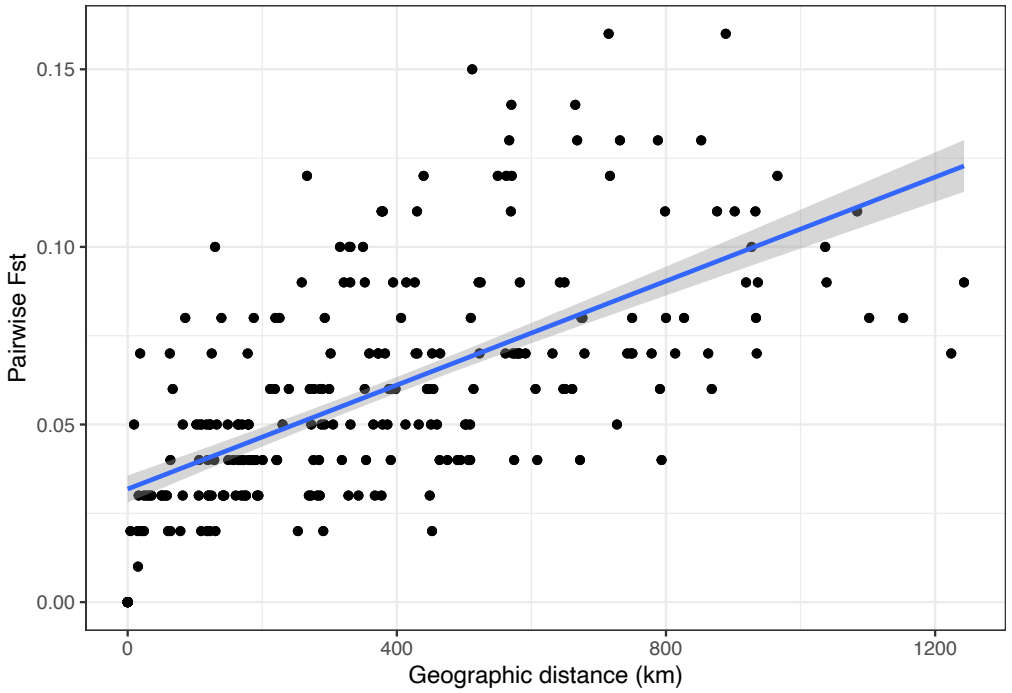


**Figure 2.** Mantel test results indicating the positive correlation between pairwise $F_{ST}$ and geographic distances of the wild cherry populations (r = 0.3983803, P < 0.001).

**Table 5.** Analysis of molecular variance results for 22 wild cherry populations based on ten SSR markers.

| Source of variation | df | SSD | MSD | Sigma | Total variance (%) | P |
|---|---|---|---|---|---|---|
| Between population | 22 | 797.4908 | 36.249580 | 0.7600234 | 9.768006 | <0.05 |
| Between samples within population | 418 | 2992.2253 | 7.158434 | 0.1377143 | 1.769937 | <0.05 |
| Within samples | 441 | 3035.4053 | 6.883005 | 6.8830053 | 88.462057 | <0.05 |
| Total | 881 | 6825.1214 | 7.747016 | 7.7807429 | 100 | |

df degrees of freedom; SSD sum of squared deviations; MSD mean squared deviations; P probability of obtaining a larger component estimate. Number of permutations = 1000.

different from the populations in its region. Populations were partitioned into clusters or gene pools corresponding generally to geography (Figure 4).

## 4. Discussion

In order to reveal the genetic variation of natural populations of wild cherry in Turkey, we analysed the efficacy of the marker systems using descriptive statistics and null allele analyses. We found 151 total alleles and 15.1 mean number of alleles per loci. Our results showed more alleles compared to other studies where allele count per loci generally changes between 3.27– 12.7 (Schueler et al., 2003; Vaughan et al., 2007; Avramidou et al., 2010; Ganopoulos et al., 2011; Ercisli et al., 2011; Tanceva-Crmaric et al., 2011; Türkoglu et al., 2012), except Rogartis et al. (2012). To authenticate the SSR amplification, null allele analysis was used. The null allele indices varied between 0.024–0.119 which is below the suggested threshold of 0.19 (Chapuis et al., 2008), thus we concluded that no null alleles exist in our data set.

To describe the genetic diversity in wild cherry populations, we used observed, expected heterozygosity values and inbreeding coefficient. We found 0.69 $H_o$ and 0.74 $H_e$ values, which are greater than published studies (Schueler et al., 2003; Vaughan and Russell, 2004; Avramidou et al., 2010; Tanceva-Crmaric et al., 2011; Jarni et al., 2012; Rogatis et al., 2012), except (Vaughan et al., 2007; Ganopoulos et al., 2011; Türkoglu et al., 2012). Considering these heterozygosity values, it is evident that genetic variation is high in the studied populations.

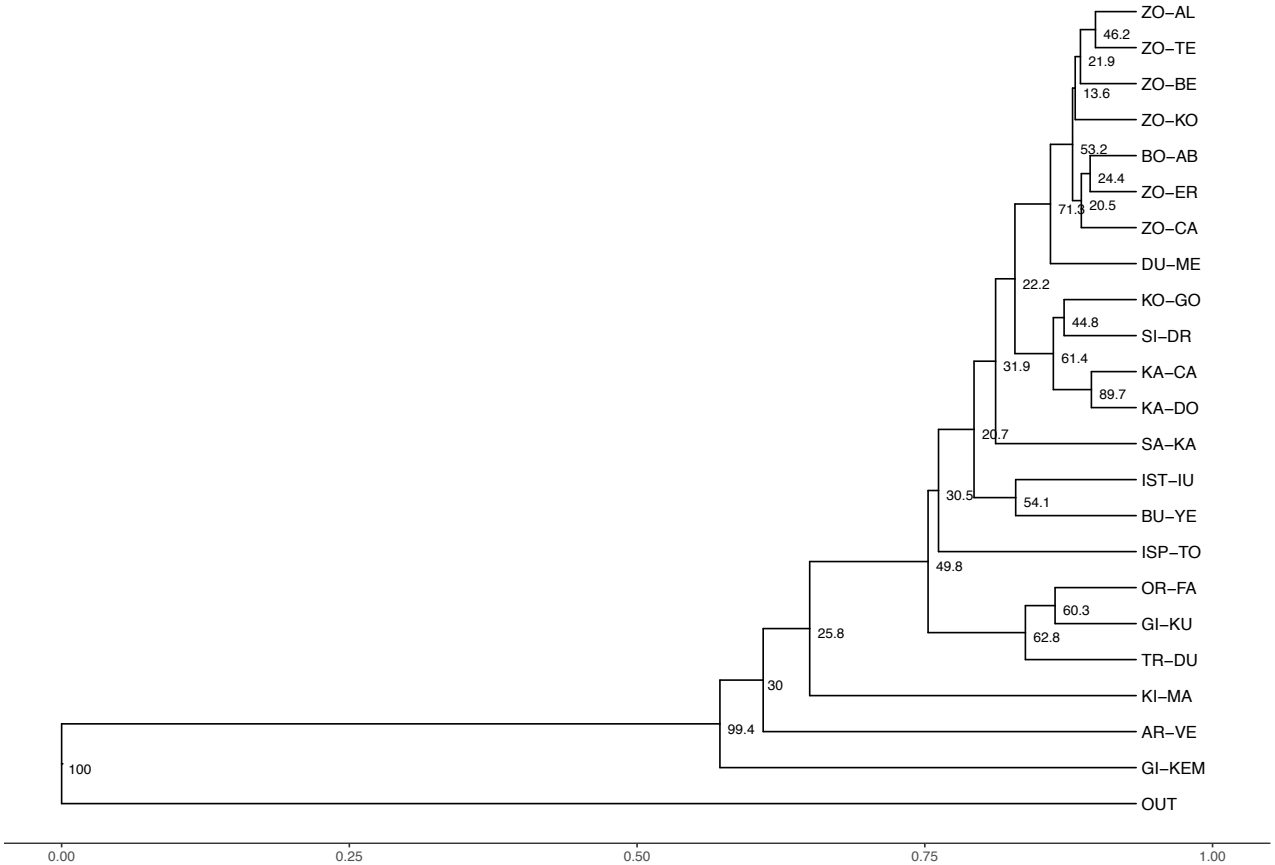


**Figure 3.** Phylogenetic dendrogram of wild cherry populations obtained using UPGMA method. Numbers above branches indicate the bootstrap value of that branch based on 1000 permutations.

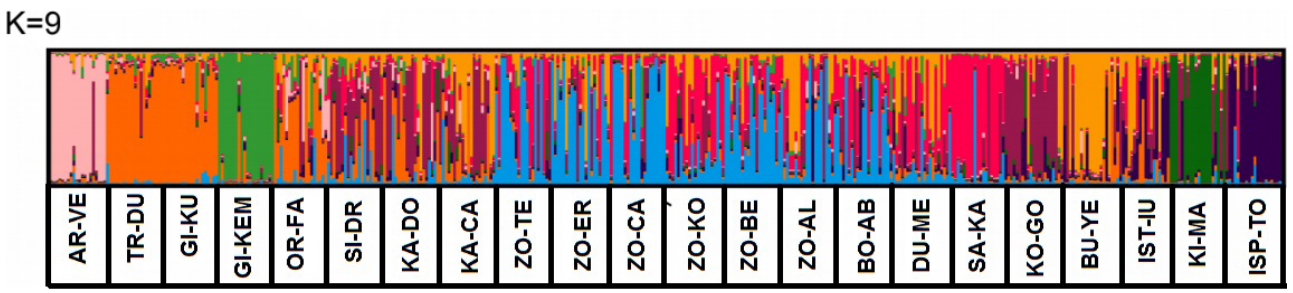


**Figure 4.** The scheme showing the clustering of 22 wild cherry populations, obtained using STRUCTURE method with genetic data of 10 SSR loci. Number of clusters, K= 9. Each vertical bar corresponds with a distinct genotype and different colours indicate the part of its genome assigned to each cluster.

Additionally, the mean inbreeding coefficient ($F_{IS}$) in wild cherry populations is found to have a negative value which suggests high heterozygosity, and low inbreeding among the sampled populations. In the literature, some studies have shown negative $F_{IS}$ values (Frascaria et al., 1993; Vaughan et al., 2007; Guarino et al., 2009), and others varied between 0.001–0.185 (Avramidou et al., 2010; Ganopoulos et al., 2011; Jarni et al., 2012; Rogatis et al., 2012). Wild cherry is a self-incompetent species, meaning only different plants could pollinate. So, most of the alleles remain in heterozygous form (Vaughan et al., 2007) and it is expected to observe an increase in heterozygosity in noninbreeding plants (Ledig, 1998). This case is consistent with results of in our study, so, negative $F_{IS}$ values indicate an increase in heterozygosity.

Overall, we found a medium or low level of differentiation between populations. Among the tested populations, Kemerköprü , Veliköy , Tota , and Macara

are the most differentiated populations in the sample set. Kemerköprü and Veliköy populations were sampled from elevation 1699 to 1900 meters. Therefore, we think that the higher $F_{ST}$ values could be due to the population isolation from lower altitudes. So, they are mostly pollinated within their own population and thus they differentiate from other populations by creating a population isolation. Generally, the distribution of wild cherries is mostly restricted to northern parts of Turkey. However, Tota, which is the most southern population in our sample set is located in Mediterranean region. Thus, we think that the geographical distance is the major factor for differentiation in this population. As a similar example, Macara population is the most western sample which is closer to European region and a possible gene flow from European species could be responsible for the medium differentiation. Also, we observed that the $F_{ST}$ values are generally compatible with the published studies and shows the correlation between geographical distance and higher $F_{ST}$ values. For example, geographically closer populations have lower $F_{ST}$ values (Frascaria et al., 1993; Tanceva-Crmaric et al., 2011; Jarni et al., 2012; Rogatis et al., 2012), and distant populations have higher $F_{ST}$ (Ganopoulos et al., 2011). We think that our sampling strategy that involves a wide range of geographic locations (distance and elevation) is responsible for the higher $F_{ST}$ values. This correlation between pairwise $F_{ST}$ values and geographic distance was also detected by the Mantel test. Generally, nearby wild cherry populations tend to be genetically more similar and genetic differences increase linearly with geographic distances.

Generally, gene flow rate ($N_m$) correlated to geographical proximity, except Gölcük and Yeniköy. In these populations, gene flow was found to be from Dranos, Çamlıbük and Doğanyurt populations to Gölcük, and Çamlıbük to Yeniköy populations. Wild cherry fruits are eaten by birds and mammals, thus the seeds could be transported to long distances (Russell, 2003). This genetic flow we observe in some populations even if they are geographically separate, may be responsible for these seed distribution mechanisms of wild cherries. We also think that the high $N_m$ values observed in other populations of wild cherry are due to insect-wind pollination and seed transport strategy. Also, the critical $N_m$ value is 0.5, and values above this threshold prevents genetic drift (Hamrick, 1989). All of the $N_m$ values are above 0.5, that means there is no genetic drift in the sampled populations.

To understand intra- and interpopulation differentiation percentages we used AMOVA test. Using this data, we observed that the major genetic differentiation is in intra-population level. In a similar study with 15 different SSR primers, Tanceva-Crimaric et al. (2011) reported that the intra-population genetic diversity is 95.88% and inter-population genetic diversity is 4.12% that support our results. This high genetic diversity at wild cherry has also been reported in other previous studies in Turkey (Ercisli et al., 2011; Türkoglu et al., 2012; Temel, 2018; Unsal et al., 2019; Velioğlu et al., 2020). It is known that, cross pollination creates new gene recombination and intra-population variation (Conkle et al., 1988). Since wild cherry is a self-incompatible plant, most of the new alleles will remain in heterozygous form. Thus, intraspecific genetic variation will increase (Ledig, 1998). Also, wild cherry fruits are the food source for wild life and the seeds could be transported to longer distances which could allow new genetic recombination (Breitbach et al., 2010). Also, it is known that gene flow in wind pollinated plants is higher than insect pollinated plants (Ledig, 1998). Since wild cherries are pollinated through both wind and insects, both mechanisms contribute to the high genetic diversity within the population. Since the intra-population genetic diversity in wild cherries is high, as a result, total genetic diversity is also high. The high intra-population genetic diversity implies that a breeding program could increase the genetic gain (Işık and Kaya, 1995).

Principal component analysis (PCA), genetic structure analysis (STRUCTURE) and phylogenetic analysis (UPGMA) showed that Veliköy and Kemerköprü populations, which were sampled at higher altitudes, are the most genetically distant samples in our population set. These results suggest that the genetic differentiation of wild cherries increased along with the elevation which supports $F_{ST}$ values. Due to the different climatic conditions, pollination times are much later than the lower altitude samples. So, this case restricts the gene flow via pollination. Also, the Macara population which is the most western population that was sampled from a narrow geographical area is found to be genetically different from the rest of the sample set. This isolated geographic location restricts gene flow from the Eastern populations. In this area, winds are mostly coming from north west direction creating a suitable environment for pollination from European species. This effect could cause a relatively high genetic differentiation in comparison with Eastern wild cherries. According to STRUCTURE analysis, Tota population which is in the Mediterranean region is also differentiated from the rest of the set because of the geographical distance. PCA, STRUCTURE, and phylogenetic trees suggest that other remaining populations are all grouped close to their geographic location. Interestingly, Gölcük and Dranos populations and Yeniköy and Çamlıbük populations are clustered together. Similarly, Unsal et al. (2019) reported that the Gölcük population has a different genetic diversity from other populations that were sampled in close proximity. This could be due to transfer of seeds via mammals and birds, and it should be noted that Gölcük

and Dranos populations are on the bird migration path.

Evanno's ΔK analysis suggest a K value of 9, and we observed that the population structure is closely correlated to the sampling locations. Different studies showed smaller K values, for example Fernandez-Cruz et al. (2014) found 2 different population groups of wild cherries in Spain. Ganopoulos et al. (2011) divided Greek wild cherries in 5 different groups. We think that our large scale sampling strategy enabled us to stratify the sample set into 9 groups. Also, Rogatis et al. (2012), found 11 groups in Italian wild cherries. However, it was reported that no specific geographical stratification was found in this study. Although this value is higher than the K value that we found, a general geographic structuring was detected in the populations that we sampled. The biggest threat to the wild cherry diversity is hybridisation between sweet cherries, which contaminates the gene pool (Russell, 2003). For this reason, it has been reported that there is no specific geographical structuring in the study conducted in Italy (Rogatis et al., 2012). In our study, we see that wild cherries are often found with cultured sweet cherries. However, we did not see any sign of genetic contamination. Since the loss of naturalness in wild cherry populations is not as much as in Europe, it is thought that a geo-graphical structuring is observed in the sampled populations.

In conclusion, we successfully employed genetic characterization of selected P. avium populations in Turkey using the 10 SSR markers and we found a high genetic diversity. This high genetic variation is a result of cross-breeding, transfer of seeds through long distance and geographically connected spread. It is shown that the genetic diversity of the forest trees should be high to be adapted for the future climatic changes (Wei, 1995). Thus, genetic diversity will create a defence mechanism for the unpredicted future climatic changes (Ledig, 1998). For this reason, a conservation and breeding program for wild cherry should be developed with it of high genetic diversity despite the many risks it faces. In our country, wild cherries are conserved in-situ in four different conservation forests that are in Zonguldak-Çaylıoğlu, Zonguldak-Alaplı, Kastamonu-Çamlıbük, and Sinop-Dranos. In our study, we found that Kemerköprü, Veliköy, Macara, and Tota populations are genetically different from the current conservation forests and also, Veliköy population has six different private alleles. Thus, these populations should be conserved in-situ to maintain the wild cherry high genetic diversity. In addition to in-situ conservation, these populations should also be conserved in ex-situ to prevent from the risks they will face in the future.

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**Author Contributions**

BUE, EK, EV, and YÖÇ designed research; BUE performed research; BUE carried out the sampling; EV authorized the sampling; BUE, EK, and YÖÇ analyzed data; BUE, EK, and YÖÇ wrote the paper; EV provided financial support; all authors edited, revised, and provided comments to the manuscript.

**Conflict of interest**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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