

Molecular Genetic Characterization of the Turkish National Green Plum (*Prunus cerasifera* Ehrh.) Collection

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ABSTRACT: Plum is an important fruit worldwide and has high nutritional value. *Prunus cerasifera* Ehrh., a type of European plum species, is very popular in Turkey and is usually eaten at the green, unripe stage. In this work for the first time, the genetic diversity and population structure of the 66 accessions housed in the Turkish National *P. cerasifera* collection were investigated using molecular markers. A total of 47 Sequence-Related Amplified Polymorphism (SRAP) primer pairs were used and found to be highly polymorphic with 98% of the 495 amplified alleles providing polymorphism. Average diversity of the accessions was 0.39 as determined using the dice coefficient and was similar to *P. cerasifera* germplasm from France, Iran and Belarus but higher than that from China. This difference was expected as Turkey, Iran and Belarus are within the geographical origin of this species which was distributed to Europe during ancient times. The genetic relationships among accessions of the germplasm collection were assessed using unweighted neighbor joining dendrogram and population structure analyses. The dendrogram and population structure results were strongly correlated as both methods clustered the material into two main groups with a much smaller third admixed group. The analysis also indicated that Can and Papaz types, despite their morphological differences are not genetically distinct and provides information about genetic relationships that can be used in future plum breeding.

Keywords: Plum, *Prunus cerasifera* Ehrh., SRAP, national collection, fruit genetics, green plum diversity.

Türk Ulusal Yeşil Erik (*Prunus cerasifera* Ehrh.) Koleksiyonunun Moleküler Genetik Karakterizasyonu

ÖZ: Erik, dünya çapında önemli bir meyvedir ve yüksek besin değerine sahiptir. Bir tür Avrupa eriği türü olan *Prunus cerasifera* Ehrh., Türkiye'de çok popülerdir ve genellikle yeşil, olgunlaşmamış dönemde yenilmektedir. İlk kez bu çalışmada, Türk Ulusal *P. cerasifera* koleksiyonunda yer alan 66 genotipin genetik çeşitliliği ve popülasyon yapısı moleküler markörler kullanılarak araştırılmıştır. Toplam 47 Sekansla İlişkili Amplifiye Polimorfizm (SRAP) primer çifti kullanılmıştır ve polimorfizm sağlayan 495 adet çoğaltılmış allelin % 98'inin oldukça polimorfik olduğu bulunmuştur. Genotiplerin ortalama çeşitliliği, dice katsayısı kullanılarak, 0,39 olarak belirlenmiştir ve elde edilen bu değer Fransa, İran ve Beyaz Rusya'dan *P. cerasifera*

germplazmlarıyla yapılan çalışmalarla benzer, ancak Çin'de yapılan çalışmadan daha yüksek bulunmuştur. Antik çağlardan beri Avrupa'ya dağılan türlerin coğrafi orijinleri içerisinde Türkiye, İran ve Beyaz Rusya olduğu için bu fark beklenen bir durumdur. Germplazm koleksiyonunu oluşturan genotipler arasındaki genetik ilişkiler, ağırlıksız komşu birleştirme dendrogramı ve popülasyon yapısı analizleri kullanılarak değerlendirilmiştir. Dendrogram ve popülasyon yapısı sonuçları, her iki yöntem de materyali iki ana gruba ve çok daha küçük bir üçüncü, karıştırılmış grupla ayırdığı için güçlü bir şekilde ilişkilendirilmiştir. Ayrıca, analizler, Can ve Papaz erik tiplerinin morfolojik farklılıklarına rağmen genetik olarak farklı olmadıklarını ve gelecekteki erik ıslahında kullanılabilecek genetik ilişkiler hakkında bilgi verdiğini göstermiştir.

Anahtar kelimeler: Erik, *Prunus cerasifera* Ehrh., SRAP, ulusal koleksiyon, meyve genetiği, yeşil erik çeşitliliği.

INTRODUCTION

Prunus is a genus of the Rosaceae family and consists of over 286 approved species including the stone fruit species plums, almonds, cherries and peaches (The Plant List, 2013). In the early classifications of *Prunus* dating back to the 1600s, only plum was included in the genus by John Ray, Joseph Pitton de Tournefort, Johann Jacob Dillenius and Herman Boerhavee (Faust and Surányi, 1999). In 1940, species in *Prunus* were classified into five subgenera as proposed by Alfred Rehder: *Amygdalus* (L.) Focke (peaches and almonds), *Prunus* Focke (plums and apricots), *Cerasus* Pers (sweet and tart cherries), *Padus* (Moench) Koehne (deciduous bird-cherries) and *Laurocerasus* Koehne (evergreen laurel-cherries) (Milošević and Milošević, 2018). Subsequently, *Lithocerasus* (flowering, sand cherries) was added to the list as the sixth *Prunus* subgenus (Ingram, 1948).

Plum species in the subgenus *Prunus* are cultivated worldwide and can be divided into three groups: Japanese, American and European plums. Among the Japanese species (Japon eriği) are *Prunus salicina* and *P. mume*, two species that can grow to 10 meters and produce fruits with yellow-pink flesh. The American plums (Amerikan eriği), *Prunus americana*, can reach 15 meters and their fruits have red skin and yellow flesh (Heiges, 1897; Cobianchi and Watkins, 1984). European plums (Avrupa eriği) include four subspecies: *Prunus domestica*, *P. spinosa*, *P. mahaleb* and *P. cerasifera* that grow up to 10 meters in height, generally producing fruits with yellow flesh covered by deep-purple-blue skin (Walkowiak-Tomczak *et al.*, 2008).

Interestingly, there is a conflict in the common names used for plum species in Turkey as compared to the literature. In Turkey, plum

cultivars are classified into three groups: Can, European, and Japanese (Anonymous, 2012). American plums are not included, perhaps because *P. americana* is not commonly grown in Turkey. According to the Turkish classification, *P. cerasifera* (Myrobalan plum, green plum, cherry plum, or “Kiraz eriği”) falls within the “Can” plums and is represented by several varieties that are named Can. In the literature, *P. cerasifera* is considered to be a European plum species. *P. cerasifera* is one of the most consumed plum species in Turkey. This species is adapted to the varied climatic and soil conditions of Turkey as well as other parts of the world (Ayanoglu *et al.*, 2007). *P. cerasifera* is mostly grown in the coastal areas of the Mediterranean region and the green plum market has a high economic impact and drives production (Ayanoglu *et al.*, 2007).

Beyond their economic impact as a fruit crop, plums are valued for their delicious taste and nutritional content (Walkowiak-Tomczak *et al.*, 2008). Due to their high carbohydrate content, plums constitute a source of “ready to be used” energy (Anonymous, 2019b). In addition, plum fruits are rich in metabolites such as anthocyanins, phenolic acids, carotenoids, and fiber (pectin) (Birwal *et al.*, 2007). One serving (100 g) of fresh plum contains minerals such as potassium (157 mg), phosphorus (16 mg), magnesium (7 mg) and calcium (6 mg) as well as vitamin A (17 µg), vitamin C (9.5 mg), vitamin K (6.4 µg) and vitamin B complex: thiamine (0.028 mg), pantothenic acid (0.135 mg), riboflavin (0.026 mg), niacin (0.417) (Anonymous, 2019b). The purported health benefits of plum fruit include prevention of cancer and heart diseases, regulation of the digestive system, resistance to infectious agents or free radicals, balancing of blood pressure, and reduction in the severity of Alzheimer’s disease (Birwal *et al.*, 2017).

Moreover, because of their low glycemic index, plums do not cause a rapid increase in blood sugar levels (Asif, 2011; Slavin and Lloyd, 2012).

Turkey's plum production increased 19% in the last decade and, with 291.934 tons of production in 2017, Turkey currently ranks 6th worldwide (Anonymous, 2019a; Anonymous, 2019c). *P. cerasifera* is one of the most consumed plum species in Turkey as it is adapted to varied climatic and soil conditions (Ayanoğlu *et al.*, 2007) and is an early spring favorite fruit. In Turkey, there are a number of economically important *P. cerasifera* cultivars including Can, Papaz, Havran, Kebap and Aynalı. Among these cultivars, Can, Papaz and Aynalı are harvested and consumed as green sour plums. In addition to their fresh use, there is increasing interest in dried plum production for industrial (prune powder) and commercial uses (table prunes) in Turkey and elsewhere (Bolat *et al.*, 2017).

P. cerasifera has also been studied for its tolerance to biotic and abiotic stress factors and its potential for use as a rootstock. Stress factors affect the growth and yield of current European, Japanese and American plum cultivars which could be improved by the use of suitable rootstocks (Nasri *et al.*, 2019). *P. cerasifera* can be used as a rootstock for cultivation of other plum species as it is resistant to temperature extremes (Ercisli, 2004; Ercisli *et al.*, 2006). In other work, Lecouls *et al.* (1999, 2004) investigated root-knot nematode (RKN) resistance genes in Myrobalan parental lines with molecular markers. They hypothesized that the RKN resistance genes *Mal*, *Ma2* and *Ma3* might be closely linked and can be used in the generation of new interspecific hybrids and to supply a nematode resistant rootstock source. These researchers also identified molecular markers for these genes and suggested that they may control different RKN resistance mechanisms. In addition to its pest resistance, *P. cerasifera* is a flood sensitive species (Domingo *et al.*, 2002; Amador *et al.*, 2012; Almada *et al.*, 2013; Rubio-Cabetas *et al.*, 2018). The photoperiod responses of somaclonal variants of *P. cerasifera* plantlets under flooding conditions were tested by Iacona *et al.* (2019). This work suggested that some of the variants had flooding stress tolerance independent from photoperiod while other variants had

sensitivity depending on photoperiod. Tolerant variants are, of course, interesting for rootstock development.

The breeding and selection of new, genetically diverse and adaptable *P. cerasifera* species and rootstocks can be aided by the use of molecular tools. These tools can also provide pivotal information for the conservation and management of plum germplasm. As a perennial tree species, plum accessions are expensive and laborious to maintain. Therefore, it is important to characterize this germplasm with all available methods (phenological, horticultural, molecular genetic) to ensure that these valuable resources are maintained and used for optimal benefit. To date, only a limited number of molecular genetic diversity analyses have been done within *P. cerasifera* (Ayanoglu *et al.*, 2007; Horvath *et al.*, 2008; Wöhrmann *et al.*, 2011; Zhao *et al.*, 2015). As a result, there is a need for assessment of the genetic diversity and structure of more plum specimens using different molecular markers. One such marker system is Sequence-Related Amplified Polymorphisms (SRAP). SRAP markers have high levels of polymorphism and reproducibility (Li and Quiros, 2001) indicating that they should be a useful system for investigating plum genetic diversity and population structure. In addition, these markers are not species-specific and are, therefore, cost effective. In our study, 47 SRAP marker combinations were used to assess genetic diversity among 66 plum (*P. cerasifera*) accessions conserved by the Aegean Agriculture Research Institute (AARI) (Menemen, Turkey). This material represents Turkey's national green plum germplasm collection; therefore, it is important to characterize this gene pool for future plum breeding efforts. To our knowledge, this is the first study using SRAP markers for characterization of *P. cerasifera* germplasm. Such work provides a foundation for the development of new high-quality cultivars, as well as conservation and management of the national collection and natural plum populations. In the future, this molecular genetic information can be combined with phenological and horticultural data to identify quantitative trait loci (QTL) and molecular markers that can be used to improve the yield and quality of the plum crop.

MATERIALS and METHODS

Plant materials

Leaf tissue from 66 *P. cerasifera* accessions which were collected in six cities (Balıkesir, İzmir, Aydın, Manisa, Muğla, and Denizli) in two regions of Turkey (Marmara and Aegean) were obtained from the Aegean Agricultural Research Institute (AARI) (Table 1). The germplasm included economically important local cultivars such as cultivars Can and Papaz.

DNA extraction

Genomic DNAs were isolated from fresh leaf tissue with a modified CTAB method (Doyle, 1987). DNA concentrations (ng/μl) of all *Prunus* samples were measured at Abs 260/280 (nm) using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Vantaa, Finland). All genomic DNAs were stored at -20°C.

Table 1. Plum materials used in this study, their PI numbers, local names and collection locations. Note: Sub Pop indicates sub-population membership based on Structure analysis and No indicates genotype number used in data analyses.

Çizelge 1. Bu çalışmada kullanılan erik materyallerinin PI numaraları, yerel adlandırmaları ve koleksiyon toplanma yerleri. Not: Sub Pop Structure analizinden elde edilen istatistiksel verilere dayandırılarak aynı alt popülasyona dahil olma durumunu göstermektedir ve No genotiplerin veri analizlerindeki sırasını temsil etmektedir.

| No | PI-Number § (TUR0010) | Local Name | Sampling Location | Structure (Sub Pop) | Sub Pop A | Sub Pop B | Darwin (Cluster) |
|----|---------------------------|---------------|----------------------|------------------------|--------------|--------------|---------------------|
| 39 | 130 | Havran | İzmir | A | 0.999 | 0.001 | A |
| 55 | 131 | Can | İzmir | A | 0.999 | 0.001 | A |
| 48 | 134 | Akpapaz | Manisa | A | 0.994 | 0.006 | A |
| 47 | 137 | Papaz | Aydın | A | 0.999 | 0.001 | A |
| 43 | 140 | Kebap | İzmir | A | 0.999 | 0.001 | A |
| 45 | 143 | Can | İzmir | A | 0.994 | 0.006 | A |
| 36 | 145 | Papaz | Balıkesir | A | 0.999 | 0.001 | A |
| 62 | 146 | Can | Balıkesir | A | 0.998 | 0.002 | A |
| 56 | 147 | Havran | Unknown | A | 0.999 | 0.001 | A |
| 57 | 148 | Şam | Balıkesir | A | 0.999 | 0.001 | A |
| 63 | 151 | Can | İzmir | A | 0.999 | 0.001 | A |
| 35 | 153 | Can | İzmir | A | 0.997 | 0.003 | A |
| 52 | 157 | Havran | İzmir | A | 0.999 | 0.001 | A |
| 58 | 158 | Can | İzmir | A | 0.998 | 0.002 | A |
| 64 | 162 | Kebap | Manisa | A | 0.999 | 0.001 | A |
| 38 | 163 | Can | Manisa | A | 0.997 | 0.003 | A |
| 61 | 164 | Can | Manisa | A | 0.871 | 0.129 | A |
| 50 | 165 | Can | Manisa | A | 0.999 | 0.001 | A |
| 60 | 167 | Can | Balıkesir | A | 0.842 | 0.158 | A |
| 44 | 168 | Yeşil Şam | Balıkesir | A | 0.999 | 0.001 | A |
| 37 | 169 | Sarı Şam | Balıkesir | A | 0.999 | 0.001 | A |
| 51 | 173 | Papaz | Muğla | A | 0.999 | 0.001 | A |
| 41 | 175 | Papaz | Aydın | A | 0.999 | 0.001 | A |
| 59 | 176 | Papaz | Aydın | A | 0.999 | 0.001 | A |
| 42 | 178 | Papaz | İzmir | A | 0.999 | 0.001 | A |
| 46 | 183 | Can | İzmir | A | 0.999 | 0.001 | A |
| 49 | 186 | Papaz | İzmir | A | 0.999 | 0.001 | A |
| 66 | 189 | Can | İzmir | A | 0.999 | 0.001 | A |
| 53 | 190 | Can | Aydın | A | 0.999 | 0.001 | A |
| 54 | 605 | Unknown | İzmir | A | 0.999 | 0.001 | A |
| 65 | 606 | Unknown | Unknown | A | 0.999 | 0.001 | A |
| 40 | 608 | Can | Unknown | A | 0.999 | 0.001 | A |
| 34 | 132 | Can | İzmir | B | 0.086 | 0.914 | B |
| 7 | 135 | Papaz | Denizli | B | 0.002 | 0.998 | B |
| 13 | 139 | Can | Aydın | B | 0.001 | 0.999 | B |
| 3 | 142 | Papaz | İzmir | B | 0.001 | 0.999 | B |
| 14 | 144 | Bekiroğlu | İzmir | B | 0.001 | 0.999 | B |
| 5 | 149 | Papaz | İzmir | B | 0.024 | 0.976 | B |
| 19 | 150 | Can | İzmir | B | 0.001 | 0.999 | B |

Table 1. Continued
Çizelge 1. Devam

| No | PI-Number § (TUR0010 ___) | Local Name | Sampling Location | Structure (Sub Pop) | Sub Pop A | Sub Pop B | Darwin (Cluster) |
|----|------------------------------|---------------|----------------------|------------------------|--------------|--------------|---------------------|
| 10 | 154 | Papaz | İzmir | B | 0.001 | 0.999 | B |
| 18 | 155 | Can | İzmir | B | 0.002 | 0.998 | B |
| 33 | 156 | Papaz | Aydın | B | 0.108 | 0.892 | B |
| 25 | 159 | Havran | İzmir | B | 0.001 | 0.999 | B |
| 21 | 160 | Papaz | İzmir | B | 0.067 | 0.933 | B |
| 17 | 161 | Unknown | Manisa | B | 0.002 | 0.998 | B |
| 15 | 170 | Ödemiş | Balıkesir | B | 0.003 | 0.997 | B |
| 20 | 171 | Can | Muğla | B | 0.001 | 0.999 | B |
| 30 | 172 | Can | Muğla | B | 0.005 | 0.995 | B |
| 11 | 174 | Papaz | Muğla | B | 0.002 | 0.998 | B |
| 2 | 177 | Papaz | İzmir | B | 0.002 | 0.998 | B |
| 26 | 180 | Papaz | Manisa | B | 0.002 | 0.998 | B |
| 24 | 181 | Can | Manisa | B | 0.056 | 0.944 | B |
| 22 | 182 | Can | İzmir | B | 0.008 | 0.992 | B |
| 28 | 184 | Can | İzmir | B | 0.001 | 0.999 | B |
| 1 | 185 | Papaz | İzmir | B | 0.016 | 0.984 | B |
| 27 | 187 | Havran | İzmir | B | 0.002 | 0.998 | B |
| 23 | 188 | Can | İzmir | B | 0.001 | 0.999 | B |
| 16 | 191 | Halil Efendi | Tokat | B | 0.021 | 0.979 | B |
| 32 | 252 | Unknown | Unknown | B | 0.12 | 0.88 | B |
| 8 | 270 | Can | Unknown | B | 0.001 | 0.999 | B |
| 31 | 604 | Unknown | Unknown | B | 0.3 | 0.7 | B |
| 4 | 607 | Unknown | Unknown | B | 0.011 | 0.989 | B |
| 29 | 133 | Papaz | Manisa | ADX | 0.518 | 0.482 | C |
| 12 | 141 | Papaz | İzmir | ADX | 0.514 | 0.486 | C |
| 9 | 152 | Papaz | İzmir | ADX | 0.534 | 0.466 | C |
| 6 | 179 | Papaz | Manisa | ADX | 0.532 | 0.468 | C |

§The accessions have the same standard initial letters (TUR0010 ___); therefore, these initials were eliminated from the abbreviated accession names.

§Bireyler aynı standart başlangıç harflerine sahiptir (TUR0010 ___); bu nedenle, genotip ismi kısaltmalarında bu başlangıç işaretleri göz ardı edilmiştir.

Molecular marker analysis

SRAP analysis

Polymerase Chain Reactions (PCRs) were performed with 47 combinations of SRAP primers (Table 2) (Li and Quiros, 2001, Lin *et al.*, 2005). The components of each 25 µl PCR reaction were: 2 µl 10x buffer, 1 µl (20 ng) DNA sample, 2 µl (25 mM) MgCl₂, 1.5 µl (20 mM) dNTP, 0.5 µl (10 pmol) forward primer, 0.5 µl (10 pmol) reverse primer and 1 µl (0.25 U) *Taq* DNA polymerase. The PCR reaction had two main stages. Stage I (5x cycles) was: 5 min of initial denaturation at 94 °C, followed by denaturation at 94 °C for 1 min, then 1 min annealing at 35 °C, then followed by 1 min extension at 72 °C for 5 cycles. Stage II (35x cycles) was: denaturation 94 °C for 1 min, 1 min annealing at 50 °C and 1 min extension at 72 °C followed by a final extension at 72 °C for 10 min. A hold at 4 °C ended the reactions. PCR products were electrophoresed at 110 volts through 3%

agarose gels (Lonza, Sea Kem® LE Agarose) and imaged under UV light after ethidium bromide staining.

Table 2. SRAP primer sequences.

Çizelge 2. SRAP primer dizileri.

| Forward | Primer Sequences (5' – 3') |
|---------|----------------------------|
| me1 | TGAGTCCAAACCGGATA |
| me2 | TGAGTCCAAACCGGAGC |
| me3 | TGAGTCCAAACCGGAAT |
| me4 | TGAGTCCAAACCGGACC |
| me5 | TGAGTCCAAACCGGAAG |
| me6 | TGAGTCCAAACCGGTAA |
| Reverse | Primer Sequences (5' – 3') |
| em1 | GACTGCGTACGAATTAAT |
| em2 | GACTGCGTACGAATTTC |
| em3 | GACTGCGTACGAATTGAC |
| em4 | GACTGCGTACGAATTTGA |
| em5 | GACTGCGTACGAATTAAC |
| em6 | GACTGCGTACGAATTGCA |
| em7 | GACTGCGTACGAATTCAC |
| em8 | GACTGCGTACGAATTCCTC |
| em9 | GACTGCGTACGAATTCGA |

Data analyses

Alleles obtained as a result of SRAP analysis were scored dominantly as present (1), absent (0) or missing data (9). The mean, maximum and minimum genetic diversity values per marker were determined by Gene Diversity software (GDdom) (Abuzayed *et al.*, 2017). Population structure based on the markers was analyzed with the computer program Structure (Structure 2.3.4) in order to classify genotypes (Pritchard *et al.*, 2000). During this analysis, different models ($K = 1$ to 10) were tested and evaluated after 10,000 Markov Chain Monte Carlo (MCMC) burn-in cycles and ad hoc statistics to determine the optimal population number. In addition, each model was tested 20 times with 300,000 repetitions. The results were analyzed with Structure Harvester software to determine the best population model (K) (Earl, 2012). The model with the highest ΔK value was accepted as the best model for the population. To assign individuals to subpopulations within the selected model (K), a genetic identity threshold of ≥ 0.70 for the genotypes was used. Genotypes with identity values below this threshold were not included in a subpopulation and were considered genetically admixed genotypes.

The marker data were analyzed by hierarchical classification. For this purpose, a dendrogram was

generated in the program DARwin (Perrier and Jacquemoud-Collet, 2006) using the Dice coefficient and unweighted neighbor joining algorithm. In addition, a Mantel test was performed to determine the correlation between the distance matrix and the dendrogram. DARwin was also used for PCoA analysis (Principal Coordinate Analysis).

The populations of Can and Papaz which had more than one individual, were analyzed for their within and between population genetic diversity in GenAlEx plugin (Peakall and Smouse, 2006) with construction of a random haploid binary data matrix and AMOVA analysis. Genetic separation analysis was performed with 9999 paired permutations and P values less than 0.05 were accepted as significant.

RESULTS

Allelic profile of plum species

In this work, 47 SRAP primer combinations were assayed on 66 *P. cerasifera* accessions. Example gel images are provided in Figure 1.

A total of 495 alleles displaying 98% polymorphism (485 alleles) in the 66 accessions was generated by dominant scoring. The mean number of alleles per primer combination was 10.4, with the most alleles

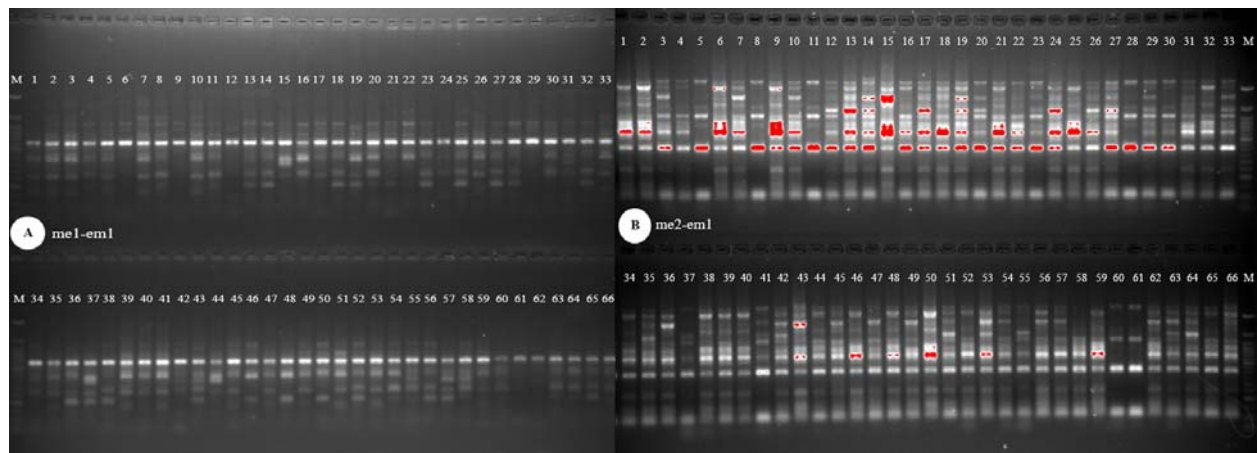


Figure 1. Sample allelic patterns obtained from two SRAP markers. A. PCR products showing a highly similar allelic pattern for the me1-em1 SRAP combination. B. PCR products showing a more polymorphic allelic pattern for the me2-em1 SRAP combination. The numbers from 1 to 66 represent the specimen number for genotypes (first column) as given in Supplementary Table 1.

Şekil 1. İki SRAP marköründen elde edilen alelik görünüm örnekleri. A. me1-em1 primer kombinasyonu için monomorfliğe yakın alelik görünüm veren PCR ürünleri. B. me2-em1 SRAP kombinasyonu için polimorfik alelik görünüm örneği veren PCR ürünleri. 1'den 66'ya kadar olan numaralar Ek Çizelge 1'deki genotip numaralarını göstermektedir.

obtained from primer combination me1-em1 (13 alleles) (Table 3). The fewest alleles (8) were obtained from combination me3-em9. For each primer pair, approximately 90% polymorphism was observed except for the me5-em1 (63%) primer combination. The random haploid binary data matrix for Can and Papaz populations was constructed with an assumption of an average equal number of individuals for each population (N) and a high level of gene flow (Nm) was

observed between these two main populations with a value of 13.27 (Table 4).

Genetic diversity

Gene diversity (GD) values were calculated for each SRAP primer combination (Table 3) and the maximum average GD was 0.46 for the me4-em3 primer combination. The minimum average GD was 0.19, which was shared by two different primer pairs: me5-em1 and me3-em4.

Table 3. Numbers of alleles, mean gene diversity (GD) values and standard deviations (SD) for each primer combination. Markers are sorted from highest to lowest GD. ΣNa : Total number of alleles per primer combination.

Çizelge 3. Her bir primer kombinasyonu için ortalama genetik çeşitlilik değerleri ve standard sapmaları. Markörler en yüksek genetik çeşitlilik değerinden en düşük genetik çeşitlilik değerine göre sıralanmıştır. ΣNa : Primer kombinasyonu başına elde edilen toplam allel sayısı.

| Primer pair | ΣNa | Mean GD \pm SD | Primer pair | ΣNa | Mean GD \pm SD |
|-------------|-------------|------------------|-------------|-------------|------------------|
| me4-em3 | 13 | 0.46 \pm 0.01 | me5-em4 | 19 | 0.32 \pm 0.03 |
| me3-em7 | 10 | 0.44 \pm 0.02 | me4-em4 | 9 | 0.32 \pm 0.04 |
| me5-em5 | 12 | 0.43 \pm 0.03 | me4-em8 | 14 | 0.31 \pm 0.05 |
| me1-em3 | 8 | 0.40 \pm 0.04 | me4-em1 | 14 | 0.31 \pm 0.05 |
| me8-em7 | 12 | 0.39 \pm 0.04 | me3-em8 | 9 | 0.31 \pm 0.04 |
| me2-em3 | 10 | 0.39 \pm 0.04 | me3-em6 | 13 | 0.31 \pm 0.04 |
| me5-em2 | 10 | 0.38 \pm 0.04 | me2-em6 | 9 | 0.30 \pm 0.05 |
| me1-em5 | 10 | 0.38 \pm 0.05 | me6-em1 | 12 | 0.29 \pm 0.05 |
| me2-em5 | 12 | 0.38 \pm 0.04 | me3-em1 | 9 | 0.29 \pm 0.04 |
| me4-em6 | 11 | 0.37 \pm 0.03 | me3-em3 | 10 | 0.29 \pm 0.05 |
| me6-em7 | 10 | 0.37 \pm 0.04 | me5-em6 | 11 | 0.29 \pm 0.04 |
| me2-em4 | 12 | 0.37 \pm 0.05 | me2-em1 | 12 | 0.28 \pm 0.05 |
| me1-em7 | 10 | 0.36 \pm 0.05 | me1-em4 | 10 | 0.27 \pm 0.04 |
| me3-em2 | 4 | 0.36 \pm 0.10 | me4-em2 | 10 | 0.27 \pm 0.05 |
| me2-em8 | 8 | 0.35 \pm 0.05 | me3-em9 | 8 | 0.27 \pm 0.06 |
| me3-em5 | 11 | 0.35 \pm 0.06 | me6-em6 | 12 | 0.26 \pm 0.05 |
| me1-em8 | 9 | 0.34 \pm 0.05 | me2-em9 | 6 | 0.25 \pm 0.06 |
| me5-em8 | 9 | 0.34 \pm 0.05 | me1-em2 | 18 | 0.24 \pm 0.04 |
| me5-em3 | 10 | 0.34 \pm 0.05 | me4-em5 | 10 | 0.24 \pm 0.06 |
| me5-em7 | 8 | 0.33 \pm 0.06 | me6-em5 | 9 | 0.20 \pm 0.05 |
| me4-em9 | 7 | 0.33 \pm 0.07 | me1-em9 | 8 | 0.20 \pm 0.04 |
| me2-em2 | 9 | 0.33 \pm 0.05 | me5-em1 | 8 | 0.19 \pm 0.07 |
| me2-em7 | 18 | 0.33 \pm 0.04 | me3-em4 | 6 | 0.19 \pm 0.05 |
| me1-em1 | 13 | 0.32 \pm 0.05 | | | |

Table 4. Number of randomly selected accessions for Can and Papaz populations (N), Number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index value (I), diversity (h) and unbiased diversity (uh), $PhiPT$ (F_{ST} analogue) and Nm (number of migrants reflecting to gene flow) values, and Nei 's genetic distance and identities.

Çizelge 4. Can ve Papaz popülasyonlarının herbiri için rastgele seçilen bireylerin sayısı (N), farklı allellerin sayısı (Na), etkili allellerin sayısı (Ne), Shannon'un Bilgi Kataloğu değeri (I), çeşitlilik (h) ve sapmasız çeşitlilik (uh), $PhiPT$ (F_{ST} analoğu) ve Nm (gen akışına katkıda bulunan göç edenlerin sayısı) değerleri ve Nei 'ye ait genetik uzaklık ve kimlikler.

| | N | Na | Ne | I | h | uh | $PhiPT$ | Nm | $Nei D$ | $Nei I$ |
|------|-------|------|------|------|------|------|---------|-------|---------|---------|
| Mean | 23.00 | 2.00 | 1.93 | 0.67 | 0.48 | 0.50 | 0.02 | 13.27 | 0.04 | 0.96 |
| SE | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Population structure

Population structure analysis was performed in Structure software and the results were used to determine the best model for the population based on ΔK values via Structure Harvester program. Consequently, the optimal number of subpopulations in the national collection was determined to be $K = 2$ (Figure 2). For assignment of accessions to each subpopulation, the identity threshold was set at ≥ 0.70 (Table 1). The number of individuals in subpopulations A and B were 32 and 30, respectively. In addition, four accessions (6% of the total) were classified as admixed. Slightly more than half (56%) of the Can plums fell into subpopulation A accounting for 44% of the cultivars in this group. Papaz plums accounted for 22% of subpopulation A. Can and Papaz plums represented 37% and 33% of subpopulation B accessions, respectively. The four admixed

individuals were Papaz accessions. None of the subpopulations reflected the geographical origins of the plums.

Unweighted neighbor joining dendrogram

The mean dissimilarity value for the unweighted neighbor joining dendrogram was 0.39 with a maximum of 0.68 and a minimum of 0.05. The dendrogram consisted of three main clusters: A, B and C (Figure 3). The dendrogram clusters reflected the observed subpopulations in population structure analysis. The first cluster, cluster A, consisted of 32 individuals in total while cluster B consisted of 30 accessions. Cluster C, contained only one unknown and four Papaz accessions which were determined to be admixed in the population structure analysis. Clustering did not coincide with geographic origin.

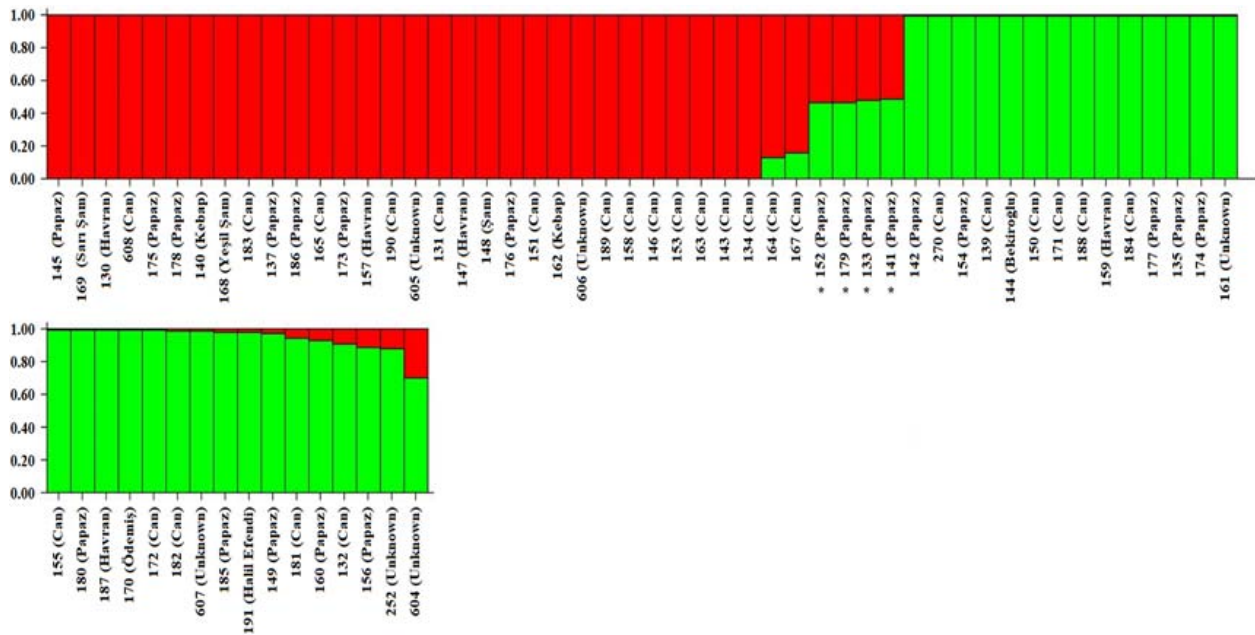


Figure 2. Q-plot of *P. cerasifera* accessions based on SRAP markers. The x-axis represents individuals sorted according to subpopulation. The y-axis reflects the genetic contribution from each subpopulation to single individuals. The bar plot for $K=2$ is shown. Each accession is represented by a vertical bar. Red and green colored sections within each vertical bar indicate membership coefficient (Q on the y-axis) of the accession to each subpopulation. Subpopulation A is colored red and subpopulation B is colored green. Accession codes are abbreviated into three digits. For example, TUR0010145 is shortened to 145. Admixed accessions that do not belong to either subpopulation are indicated with *.

Şekil 2. *P. cerasifera* bireylerinin SRAP markörlerine dayalı Bar grafiği $K=2$ için gösterilmiştir. Her birey düşey bir çubukla gösterilmiştir. x-ekseni, alt popülasyonlara göre sıralanmış bireyleri temsil etmektedir. y-ekseni, her alt popülasyonun her bir bireye ne kadar genetik katkıda bulunduğu bir göstergesidir. Her düşey çubuktaki kırmızı ve yeşil renkli kısımlar bireylerin her bir alt popülasyona üyelik katsayısını (y-ekseninde Q) belirtmektedir. Alt popülasyon A kırmızı ve alt popülasyon B yeşil renktedir. Birey kodları üç basamak olarak kısaltılmıştır. Örneğin, TUR0010145, 145 olarak kısaltılmıştır. Herhangi bir alt popülasyona dahil olmayıp karışık genetik çeşitliliğe sahip olan bireyler * ile belirtilmiştir.

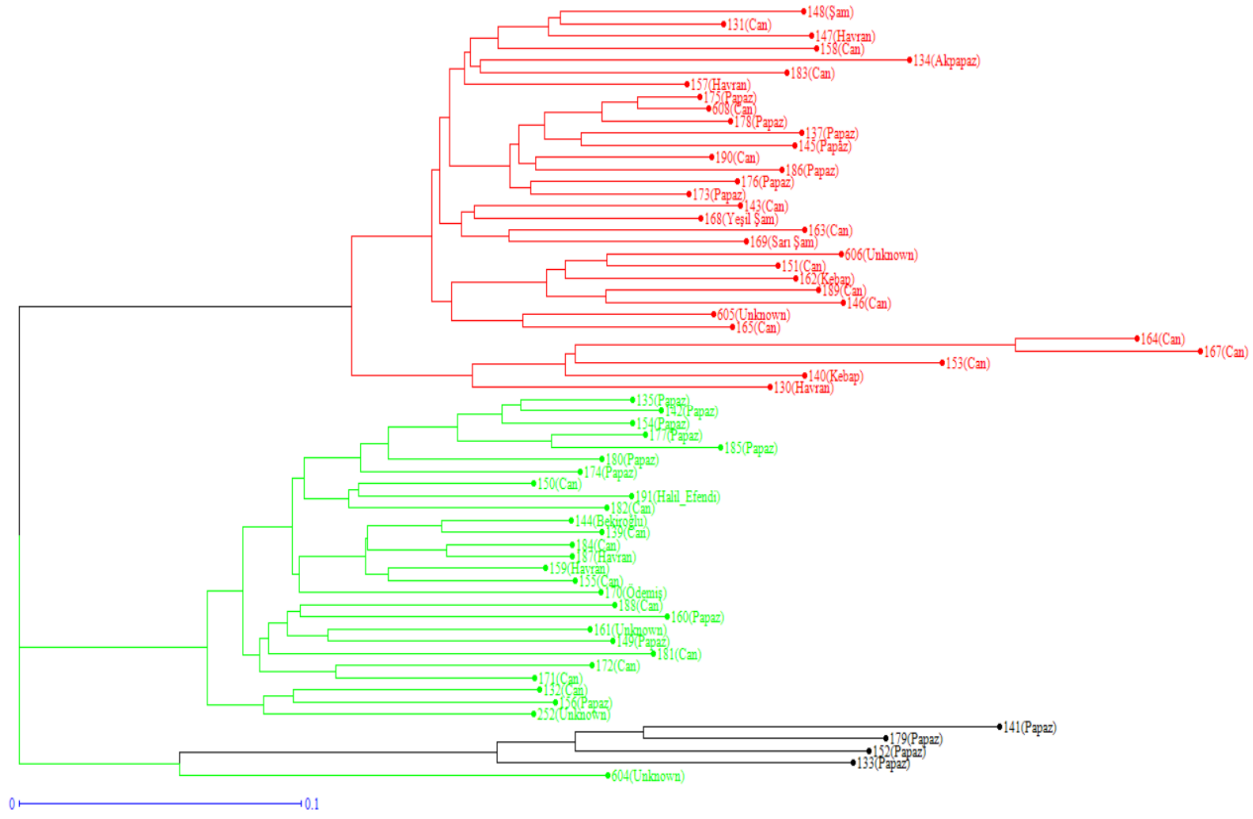


Figure 3. Dendrogram showing genetic diversity among *P. cerasifera* genotypes as revealed by SRAP marker analysis. Accessions are color coded according to Structure subpopulations ($K=2$): subpopulation A in red, subpopulation B in green, and admixed individuals in black. Accession codes are abbreviated into three digits as in Figure 2.

Şekil 3. *P. cerasifera* genotipleri arasında SRAP markör analizi ile elde edilen genetik çeşitliliği gösteren dendrogram. Bireyler Structure'daki alt popülasyonlara ($K=2$) göre renklerle kodlanmıştır: alt popülasyon A kırmızı, alt popülasyon B yeşil ve karışık bireyler siyah ile renklendirilmiştir. Birey kodları Şekil 2'deki gibi üç basamağa kısaltılmıştır.

Principle coordinate analysis

Principle coordinate analysis (PCoA) revealed three main clusters: A, B and C (Figure 4). The first, second and third eigen vectors explained 40.25%, 17.53% and 5.39% of the variation, respectively. This analysis agreed with the population structure and dendrogram results in that the Papaz plums were dispersed in all three clusters with the Can plums found in clusters A and B. Some of the Can and Papaz accessions were closely related to other plum types including Kebab, Havran, and Şam plums. As with the other analyses, no clustering based on collection location was observed.

Analysis of molecular variance (AMOVA)

AMOVA was carried out for the Can and Papaz types because they had sufficient numbers of accessions (25 and 21, respectively) for such analysis. Among population/type (Can vs. Papaz) diversity represented only 2% of total genetic diversity for these plums while 98% of diversity occurred within types (data not shown). These results indicated that Can and Papaz plums are each genetically diverse types with very little genetic separation between them.

Factorial analysis: (Axes 1 / 2)

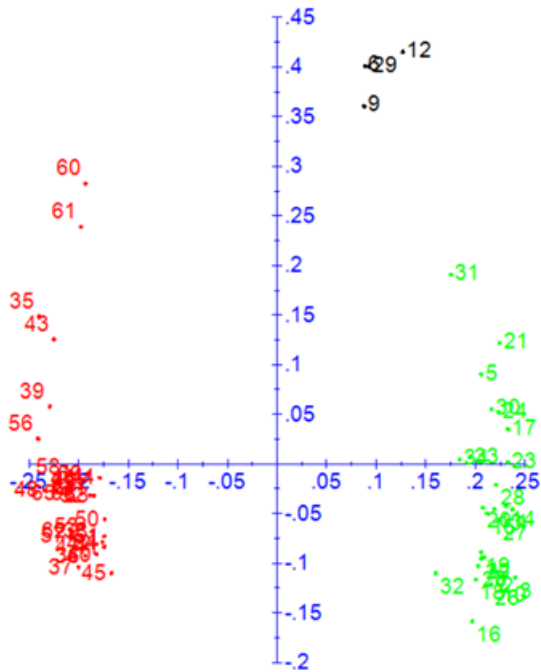


Figure 4. PCoA graph for *P. cerasifera* accessions. Accessions are color coded according to Structure subpopulations ($K=2$), with subpopulation A in red, subpopulation B in green and admixed individuals in black. The numbers from 1 to 66 represents the specimen number for genotypes (first column) in Table 1.

Şekil 4. *P. cerasifera* bireyleri için PCoA grafiği. Bireyler Structure altpopulasyonlarına göre ($K=2$); alt populasyon A kırmızı, alt populasyon B yeşil ve karışık bireyler siyah renklerle kodlandırılmıştır. 1'den 66'ya kadar olan numaralar Çizelge 1'deki genotip numaralarını göstermektedir.

DISCUSSION

Characterization of Turkey's *P. cerasifera* germplasm resources is essential for more informed germplasm conservation, collection and management. Because *P. cerasifera* is very adaptable and a useful rootstock, its proper conservation is crucial to meet future biotic, abiotic and climatic challenges to this species. Genetic characterization is also an important step toward the improvement of this species via plant breeding as it allows selection of genetically distinct parents and opens the door to marker-assisted selection.

In this work, the molecular genetic diversity of the national *P. cerasifera* plum germplasm collection

was determined. The 66 accessions in the collection were originally sampled from the Aegean (51 accessions), Marmara (7) and Black Sea (1) and unknown (7) regions of Turkey and were assessed with 47 SRAP marker combinations. The effectiveness of SRAP markers in other *Prunus* species was shown in the work of Zubair *et al.* (2016) who investigated wild and local *Prunus* germplasm including *P. arabica* (wild species), *P. argentea* (wild species) and *P. dulcis* (local species) with 16 primer combinations and found that SRAPs were an effective marker system. In our work, a total of 495 alleles were generated from the SRAP markers and the percentage of polymorphic alleles was very high (98%).

In other work, Ayanoğlu *et al.* (2007) obtained 580 alleles but only 20.7% polymorphism with six AFLP markers on 20 *P. cerasifera* accessions collected from the Antalya, Mersin and Hatay provinces in the Mediterranean region of Turkey. Comparison between the present work and that of Ayanoğlu *et al.* (2007) indicates that, within this species, the polymorphism information content of SRAP markers (495 alleles, 98% percentage of polymorphism) is higher than for AFLPs. The disparity in results could partially be due to the smaller number of accessions used by Ayanoğlu *et al.* (2007). However, SRAP markers have the additional advantages of being easier and cheaper to assay than AFLPs.

When SSR markers were used to determine genetic diversity in *P. cerasifera*, they were found to have similarly high levels of polymorphism as SRAP markers with 96% of fragments showing polymorphism in 40 Chinese accessions (Zhao *et al.*, 2015). As compared to SSR markers, SRAPs are somewhat more economical as the same markers can be used for many different species while SSR markers are usually developed on a species-specific level.

The mean genetic variation across the accessions was 0.39 as determined using a measure of genetic diversity for dominant markers that ranges from 0 to a maximum of 0.50. All other studies evaluated genetic diversity on a scale of 0 to 1.0. According to this range, our mean genetic diversity value was 0.78. Ayanoğlu *et al.* (2007) and Zhao *et al.* (2015) found the lowest levels of genetic dissimilarity in

their studies which examined Turkish (0.09) (this extremely low value may be the result of the small number of accessions studied) and Chinese (0.33) germplasm. Other research using *P. cerasifera* accessions from France, Iran and Belarus had mean diversity values of 0.65, 0.68 and 0.72, respectively (Horvath *et al.*, 2008; Wöhrmann *et al.*, 2011; Urbanovich *et al.*, 2017), which agree with the level of diversity seen in our Turkish material. Thus, our data suggest that Turkish *P. cerasifera* germplasm has a high genetic diversity that is similar to that seen in nearby countries. In general, other studies have indicated that *P. cerasifera* germplasm population structure is simple with two to three subpopulations (Ayanoglu *et al.*, 2007; Zhao *et al.*, 2015; Urbanovich *et al.*, 2017). In our work, the dendrogram analysis revealed two main clusters with a small third group of five accessions. This agreed with the population structure analysis which also placed the accessions in two main subpopulations and a small, admixed group. On the other hand, Wöhrmann *et al.* (2011) were not able to distinguish different clusters in Iranian material: all individuals in the population displayed an admixed genetic structure.

The high level of diversity observed in Turkish *P. cerasifera* germplasm (and also seen in accessions from Belarus and Iran) reflects the species geographic origin and gene center in Western and Central Asia (Milošević and Milošević, 2018). Early historical evidence suggests that *P. cerasifera* was cultivated even before the Neolithic period in the Caucasus region. The species spread via trade and later became an important contributor to the development of European societies with stones from a few plum species discovered at ancient sites (4000-6000 BCE) in Germany and the Ukraine (Faust and Suranyi, 1999). In addition to being grown for its fruits, *P. cerasifera* was also used as rootstocks in ancient times (Zhebentyayeva *et al.*, 2019). While *P. cerasifera* is still grown in many different geographic regions due to its high adaptability to biotic and abiotic stress factors such as climate, soil moisture, drought and nematodes (Anonymous, 2012), it has not been subject to the same interspecific breeding and artificial selection pressures as *P. domestica*, a much more popular species which has low genetic diversity (Zhebentyayeva *et al.*, 2019).

According to Erbil and Ozturk (2000), green *P. cerasifera* plums are mostly grown in the Aegean, Marmara and Mediterranean regions of Turkey. The current study examined the national collection housed at the AARI which primarily contains individuals sampled from the Aegean region. In contrast, Ayanoglu *et al.* (2007) examined 20 accessions sampled from Mediterranean locations. Comparison of these results suggests that Aegean materials are more diverse than Mediterranean ones; however, a true comparison cannot be made because of the different marker systems and number of accessions used in the two studies.

Can and Papaz plums are very popular varieties of *P. cerasifera* in Turkey. Can plums mature earlier, have smaller, sourer fruit and higher yields than Papaz plums. Can plums are yellow when fully ripe whereas Papaz are red (Andaç Çavdar, personal communication). Despite these phenological and horticultural differences, our results indicate that Can and Papaz are not genetically distinct types. In addition, AMOVA analysis indicated that within type (Can vs. Papaz) diversity (98%) was much higher than among type diversity (2%), reinforcing the conclusion that they are not genotypically distinct varieties. The shared genetic diversity among these varieties is most likely due to their origins from natural crosses within and between different parental materials. *P. cerasifera* is described as self-fertile, half self-fertile or even out-crossing (Anonymous, 2012). These types of mating behavior could result in genetic diversity which is perpetuated by clonal propagation.

CONCLUSION

The Turkish National *P. cerasifera* germplasm collection consisting of different plum accessions including the most economically valuable types, Can and Papaz, was investigated with molecular markers for the first time. There was no distinct genetic clustering in the germplasm according to geographical origin or type, however, a high level of diversity was observed in the collection. These results were expected based on the origin of this species in Western and Central Asia. The findings are promising for the conservation of *P. cerasifera* and the development of new cultivars to meet the challenges of future consumer demands and

cultivation conditions. The genotypic data generated in this work can be combined with phenotyping of this valuable collection to allow genetic mapping of phenological and horticultural traits.

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AUTHOR CONTRIBUTIONS

GÇ and TT provided equal contributions; GÇ performed research and analysis and wrote first draft; TT performed analysis, interpreted data and revised manuscript; AÇ provided plant material and information about accessions; SD conceptualized research; AF and AF conceptualized research, interpreted data and revised manuscript.

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