MOLECULAR GENETIC ANALYSES IN TURKISH PLUM *(Prunus cerasifera)*

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ABSTRACT

MOLECULAR GENETIC ANALYSES IN TURKISH PLUM

Plum is an economically important and popular fruit in Turkey, which ranks in sixth place in world plum production. This fruit is attractive to consumers with its beautiful scent and its delicious taste. In addition, it is an important plant species with its wealthy mineral, vitamin and phytochemical content. The genus *Prunus* is classified into three groups: the European, the Asian and the American plums. *P. cerasifera*, which was used in this study, belongs to the European group. *P. cerasifera* (2n=2x=16) is a Turkish plum drupe species. Since there are many subspecies, it can grow naturally in many parts of Turkey. The genetic diversity and population structure of *P. cerasifera* have not been studied using molecular techniques. Here, we studied the genetic diversity of 66 *P. cerasifera* accessions collected from Turkey at the molecular level using 47 sequence related amplified polymorphism (SRAP) primer combinations. The SRAP marker combinations showed reasonable polymorphism. A dendrogram was constructed using the Dice coefficient and unweighted neighbor joining algorithm. The dendrogram revealed three groups and the similarity between accessions ranged from 0.04 to 0.66 with an average dissimilarity of 0.37. Population structure analysis identified three subpopulations (K=3). This is the first genetic diversity analysis of *P. cerasifera* using SRAP markers. Genetic diversity and population structure data can be useful for determining breeding strategies in *P. cerasifera* accessions.

ÖZET

TÜRK ERİKLERİNDE MOLEKÜLER GENETİK ANALİZLER

Erik, dünya erik üretiminde beşinci sırada yer alan, Türkiye'de ekonomik açıdan önemli ve popüler bir meyvedir. Bu meyve, güzel kokusu ve piyasalarda lezzetli tadıyla müşteriler için caziptir. Buna ek olarak, zengin mineral, vitamin ve fitokimyasal içeriği ile önemli bitki türüdür. Prunus cinsi üç gruba ayrılmıştır (Avrupa, Asya ve Amerika erikleri). Bu çalışmada kullanılan *P. cerasifera* Avrupa grubuna aittir. *P. cerasifera* (2n = 2x = 16), bir erik tomurcuk türüdür. Çok sayıda alt tür olduğu için, Türkiye'nin birçok yerinde doğal olarak büyüyebilir. *P.cerasifera*'nın genetik çeşitliliği ve yapı analizi moleküler teknikler kullanılarak incelenmemiştir. Burada, sekansla ilişkili amplifiye polimorfizm (SRAP) kullanarak moleküler düzeyde Türkiye'den toplanan 66 *P. cerasifera* katılımının genetik çeşitliliği üzerinde çalışılmıştır. 66 *P. cerasifera* katılımının genetik çeşitlilik ve nüfus yapısı analizi, 47 SRAP markör kombinasyonu kullanılarak belirlenmiştir. SRAP primer kombinasyonları makul polimorfizm gösterilmiştir. Dice katsayısı ve ağırlıksız komşu birleştirme algoritması kullanılarak bir dendrogram oluşturulmuştur. Dendrogram üç grupta ortaya çıkmış ve dendrogramın benzerliği 0,04 ile 0,66 arasında değişmiş ve ortalama farksızlık 0,37 olmuştur. Nüfus yapısı analizi üç alt popülasyonu belirlenmiştir (K = 3). SRAP belirteçlerini kullanarak *P. cerasifera*'nın ilk genetik çeşitlilik analizi yapılmıştır. Genetik çeşitlilik ve nüfus yapısı verileri, *P.cerasifera* katılımlarında üreme stratejisini belirlemek için yararlı olabilir.

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CHAPTER 1

INTRODUCTION

1.1. Biology and Genetics of Plums

Prunus is a member of the Magnoliopsida class, the Rosales order and Rosaceae family (Potter et al., 2007). The genus *Prunus* is comprised of approximately 400 species of trees and shrubs. The genus contains many economically important stone fruits including plums, cherries, peaches, nectarines, apricots and almonds. Plums are classified into three groups, the European, the Asian, and the American plums (Ayanoğlu et al., 2007; Özçağıran et al., 2011).

European plum consists of the species *Prunus domestica*, *P. spinosa*, *P. avium, P. mahaleb, P. institia* and *P. cerasifera*. European plum can reach 10 m in height. It has a straight trunk and dark brown bark. Leaves have oval shape. The flowers are arranged in groups of 2 to 3 or are solitary. The fruit is usually oblong with yellow flesh covered by a deep purple-blue skin (Walkowiak and Tomczak, 2008).

The Asian group contains *Prunus salicina* and *Prunus mume*. They grow up to 10 m tall and have reddish-brown shoots. The leaves are 6 to 12 cm long and 2.5 to5 cm broad, with a serrated margin. The flowers are produced in early spring with five white petals. The fruit is 4 to 7 cm in diameter with yellow-pink flesh and is harvested in the summer (OECD Environment, Health and Safety Publications, 2002).

The last group is American plum and consists of the best known species, *Prunus americana.* They grow up to 15 m tall. The leaves are alternate and broadly oval in shape. The flowers are white, 5-petaled and borne singly or in clusters. Their fruits are red to yellow and globular (Cobianchi and Watkins, 1984).

Plum species are self-pollinated. Cytogenetic studies reported that *P. domestica, P. institia* and *P. cerasifera* are the leading genetic sources of the genus, and that cultivated plum varieties were formed by the combination of these species (Beppu et al., 2005). *P. cerasifera* and *P. salicina* are fast growing species while *P. domestica* and *P. institia* are slow growing species. The number of *Prunus* sp. chromosomes in a single set is n = x = 8. *P. cerasifera* and *P. salicina* have 2*n*=16 chromosomes. *P. domestica*

and *P. institia* have $2n$=48 chromosomes (Lecouls et al., 2004). *P. domestica* is believed to have arisen as a natural alloploid between *P. cerasifera* (diploid) and *P. spinosa* (tetraploid) (Shimada et al., 1999). *P. domestica* (European plum) and *P. salicina* (Japanese plum) and the hybridization between them results in a diploid chromosome set ($2n$=$6x$=48) (OECD Environment, Health and Safety Publications, 2002) (Table 1.1).

Table 1.1. Chromosome numbers of plum species

Species	Common name	Origin	Chromosome number
Prunus cerasifera	Green plum	West Asia	16
Prunus salicina	Japanese plum	China	16
Prunus domestica	European plum	Europe-West Asia	48
Prunus institia	Damson, Miralla	Europe-West Asia	48
Prunus spinosa	Çakal plum	Europe-West Asia	32

1.2. Ecology and Dispersion

Plum species are native throughout the Northern Hemisphere but are found mostly in the temperate zone. The tree is now distributed worldwide (Öz et al., 2013). Plums have characteristics which affect their dispersion in nature. For example, they have different ecological and soil requirements (Bircan, 2015; Özçağıran et al., 2011). The climate requirements of plum species also differ from each other. *P. cerasifera* are temperate, European plums are adapted to cold temperatures and Japanese plums are most suitable for temperate or warm climates. As with many fruit, plums need chilling. The chilling requirement of the European plum is over 1000 hours, the *P. cerasifera* is 400-500 hours and the Japanese plum is 600 hours (Sedaghathoor et al., 2009). Sensitiveness to cold and frost increase during blooming and when fruit are young. For this reason, it is inadvisable to cultivate *P. cerasifera* and Japanese plums which are known to bloom early, in areas where winter and spring frost are frequent. Although flowers can withstand temperatures from -2.2°C to -0.6°C, the young fruits are harmed at temperatures between -1.1°C and -0.6°C (Bircan, 2015).

P. cerasifera, the subject of this thesis, belongs to the European plum group with some ecogeographical subspecies in the Balkans, Asia Minor, the Caucasian region, and central Asia. The cultivated and wild forms of plums grown in Turkey mostly belong to *P. cerasifera* and are well adapted to the various conditions within Anatolia extending from the south east through central Anatolia to the Mediterranean and Aegean regions. The coastal areas of the Mediterranean region show relatively wide plum diversity including many economically important green plum genotypes (Ayanoğlu et al., 2007).

1.3. Production of *Prunus*

China is the most important producer of plum with 6,256,906 tons production followed by Romania (512,975 tons) and Serbia (463.115 tons) (FAO, 2016) (Table 1.2.). In Turkey, plum production in 2016 was 297,589 tons. (TÜİK, 2016). Turkey ranks sixth in world plum production with 2.5% of the world's production (FAO, 2016).

Table 1.2. Eight main countries producing plum throughout the World (Source: FAO, 2016)

Country	**Production (tons)**
China	6.256.906
Romania	512.975
Serbia	463.115
Iran	401.452
Chile	296.439
Turkey	265.490
Spain	232.765
USA	231.800

In Turkey, the Mediterranean region (100,391 tons) has first place in plum production followed by the Aegean (58,827 tons), East Marmara (44,008 tons), and West Marmara (15,693 tons) regions (TÜİK, 2015) (Table 1.3.). The country's plum exports are 6,693 tons and plum consumption per capita is 2.62 kg per year (TÜİK, 2008). As a result, *Prunus* is an economically important product of the country, especially in coastal regions where most of the plantations are located.

Table 1. 3. Plum production by province in Turkey
(Source: TÜİK, 2015)

Geographical Regions	Production (tons)
Mediterranean	100.391
Aegean	58.827
East Marmara	44.008
West Marmara	15.693
West Black Sea	13.765
Western Anatolia	12.851
Eastern Black Sea	5.092
Southeastern Anatolia	4.623
Middle East Anatolia	4.598
Central Anatolia	3.257
Northeast Anatolia	1.920

1.4. Nutritional Quality and Health Benefits of Plum

Plums are economically valuable and popular fruits because of their delicious taste. They are mostly used as a fresh snack but can also be juiced or dried. The fruits contain relatively large amounts of carbohydrates, constituting a source of readily available energy (Table 1.4.) (USDA National Nutrient Database for Standard Reference, 2016). Plums have phenolic acids, anthocyanins, carotenoids, flavonols, organic acids, (e.g., citric and malic acids), fibre (pectin), tannins, aromatic substances, enzymes, minerals (e.g., potassium, phosphorus, calcium and magnesium, organic) and vitamins A, B, C and K (Birwal et al., 2017). Their fruits constitute a rich source of antioxidant compounds, such as phenolic acids, anthocyanins and other flavonoids (Walkowiak and Tomczak, 2008).

Plums have many beneficial effects for human health (Cantin et al., 2009). For example, they are known to be good for cancer prevention, digestive health, brain health, blood sugar levels, macular degeneration prevention and weight loss. Plums also contain dietary fiber, sorbitol and isatin which are helpful for the smooth functioning of the digestive system because they are effective natural laxatives (Cantin et al., 2009).

Plums do not cause a rapid increase in blood sugar levels due to the high amount of fructose and sorbitol present in these species (Slavin and Lloyd, 2012). Also plums could be helpful for regulation of carbohydrate, protein and fat metabolism because many other beneficial compounds like niacin, vitamin B6, and pantothenic acid are found in their content. Fresh plums are an important source of boron which plays a role in calcium availability and the prevention of osteoporosis (Walkowiak and Tomczak, 2008). In addition, fresh plums contain vitamin A and carotene that are good for eyesight and skin. *P. cerasifera* has many medicinal properties. For example, the antibacterial activity of its bark, seeds and leaves are used for strengthening teeth (Vicente et al., 2014).

Table 1. 4. Plum nutritional content
(**Source:** USDA, 2016)

Nutrients	**Units**	**Nutrient content per 100 grams**
Water	G	123.45
Energy	kcal	147
Protein	G	0.069
Total lipid (fat)	G	0.27
Carbohydrate	G	35.34
'Fiber, total dietary	G	9.7
Sugars, total	G	17.08
Calcium, Ca	mg	18
Iron, Fe	mg	0.27
Magnesium, Mg	mg	13
Phosphorus, P	mg	48
Potassium, K	mg	586
Sodium, Na	mg	6
Vitamin C	mg	16.6
Thiamin	mg	0.008
Riboflavin	mg	0.068
Niacin	mg	0.591
Vitamin B-6	mg	0.150
Vitamin A, IU	IU	5577
Vitamin E	mg	0.85
Vitamin K	mg	18.0
Vitamin A, RAE	mg	279

1.5. Genetic Diversity

Modern plant varieties should be developed for high biotic and abiotic stress tolerance. Landraces, wild types and subspecies have genetic potential for the improvement of such traits (Abdurakhmonov and Abdukarimov, 2008). These germplasm resources should be included in plant breeding programs to contribute to genetic diversity and introgression of suitable alleles into selected cultivars (Tanksley and McCouch, 1997). Therefore, these resources should be preserved and utilized for efficient and sustainable plant breeding.

Genetic resources are being destroyed by many forces including urbanization, climate change, over-exploitation and disease. These resources must be collected in seed banks and other germplasm collections such as in situ and ex situ orchards. Plant breeders need to be able to easily access the genetic potential (including biotic and abiotic tolerance) of these genetic resources. Thus, the first step is collection of plant genotypes which have agronomic potential. The second step is the morphological and molecular characterization of germplasm. These two major components serve different purposes. Breeders use both morphological and molecular characterization to establish breeding strategies.

1.5.1. Molecular Marker Systems

DNA markers are commonly used molecular tools in plant genomic analysis such as genetic mapping and DNA fingerprinting. They are also used to track genes in MAS (Marker-Assisted Selection). RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter-Simple Sequence Repeat) are popular markers used in non-model plants. SRAP (Sequence-Related Amplified Polymorphism) markers have also been developed for such species (Li and Quiros, 2001). SRAPs are molecular markers designed to amplify partially in open reading frames and are applicable for many genera. They are preferred for their ability to detect high allele diversity in species. SRAPs are dominant markers and are evenly distribution across chromosomes (Jones et al., 2009). They combine simplicity, reliability and detection of multiple loci, cost effectiveness. SRAP markers are often used for functional genetic analysis due to their high abundance.

1.5.2. Molecular Marker Systems in Plum

To date, there are only a few molecular genetic diversity studies performed in plum and most of these have been with a limited number of accessions and markers such as ISSR, SSR and RAPD. The studies performed by Dirlewanger et al. (2002); Shimada et al. (1999) and Liu et al. (2007) were more comprehensive. Liu et al. (2007) analyzed the genetic diversity of 104 plum accessions from eight species in China with 103 ISSR markers. Makovics-Zsohár et al. (2017) studied the polymorphism level and determined allelic variation and genetic relationships in 55 *P. domestica* L. accessions with 7 SSR markers. Shimada et al. (1999) investigated the genetic diversity of 42 plum species varieties by RAPD analysis. All these studies revealed that plum species have sufficient genetic diversity.

However, there has not been any SRAP marker analysis in *P cerasifera*. In contrast, there were many SRAP marker analyses in other *Prunus* subspecies. For example, Abedian et al. (2012) determined the genetic diversity and population structure of 47 Mahaleb cherry genotypes (*P. mahaleb*) and six sweet cherry accessions (*P. avium*) with 13 SRAP primer combinations. In another study, Li et al. (2014) investigated the genetic diversity and relationships of 76 accessions (32 Chinese cultivars of *P. armeniaca* and *P.sibirica*, 20 Central Asian cultivars of *P. armeniaca* and *P.dasycarpa*, 3 European cultivars of *P. armeniaca*, 6 North American cultivars of *P. armeniaca*, 2 Iran-Caucasian cultivars of *P. armeniaca*, 5 Kernel-using apricot cultivars and 8 plumcot cultivars of *P.simonii* using 12 SRAP markers.

1.6. Aim of the Study

Prunus species are cultivated worldwide for their economically valuable and popular fruits which have delicious taste and high nutritional content. Although Turkey is a plum producer, production needs to be increased for high economic profit. Thus, molecular tools should be used for development of plum varieties with high yield. This work aimed to integrate molecular tools to plum improvement programs by using 47 SRAP primer combinations. To achieve this, molecular characterization of a *P. cerasifera* population containing 66 accessions collected from Turkey was performed.

This characterization revealed genetic diversity and population structure of population which will provide valuable information to initiate breeding programs in plum.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

Sixty-six *P. cerasifera* germplasm accessions were collected from different geographical regions in Turkey and established in an orchard at Aegean Agricultural Research Institute (AARI), Menemen, İzmir. Plum leaves were obtained from these trees. Accession name, local name and source of each Turkish *Prunus* accession are shown in Table 2.1.

Table 2.1. Sample name, local name and collection location used for this study.

PI Number	Local Name	Location
TUR0010270	Can	Unknown
TUR0010130	Havran	İzmir
TUR0010131	Can	İzmir
TUR0010132	Can	İzmir
TUR0010133	Papaz	Manisa
TUR0010134	Akpapaz	Manisa
TUR0010135	Papaz	Denizli
TUR0010137	Papaz	Aydın
TUR0010139	Can	Aydın
TUR0010140	Kebap	İzmir
TUR0010141	Papaz	İzmir
TUR0010142	Papaz	İzmir
TUR0010143	Can	İzmir

(cont. on next page)

Table 2.1 (Cont.)

PI Number	Local Name	Location
TUR0010144	Bekiroğlu	İzmir
TUR0010145	Papaz	Balıkesir
TUR0010146	Can	Balıkesir
TUR0010147	Havran	Unknown
TUR0010148	Şam	Balıkesir
TUR0010149	Papaz	İzmir
TUR0010150	Can	İzmir
TUR0010151	Can	İzmir
TUR0010152	Papaz	İzmir
TUR0010153	Can	İzmir
TUR0010154	Papaz	İzmir
TUR0010155	Can	İzmir
TUR0010156	Papaz	Aydın
TUR0010157	Havran	İzmir
TUR0010158	Can	İzmir
TUR0010159	Havran	İzmir
TUR0010160	Papaz	İzmir
TUR0010161	Unknown	Manisa
TUR0010162	Kebap	Manisa
TUR0010163	Can	Manisa
TUR0010164	Can	Manisa
TUR0010165	Can	Manisa
TUR0010167	Can	Balıkesir
TUR0010168	Yeşil Şam	Balıkesir
TUR0010169	Sarı Şam	Balıkesir
TUR0010170	Ödemiş	Balıkesir
TUR0010171	Can	Muğla

(cont. on next page)

Table 2.1 (Cont.)

PI Number	Local Name	Location
TUR0010172	Can	Muğla
TUR0010173	Papaz	Muğla
TUR0010174	Papaz	Muğla
TUR0010175	Papaz	Aydın
TUR0010176	Papaz	Aydın
TUR0010177	Papaz	İzmir
TUR0010178	Papaz	İzmir
TUR0010179	Papaz	Manisa
TUR0010180	Papaz	Manisa
TUR0010181	Can	Manisa
TUR0010182	Can	İzmir
TUR0010183	Can	İzmir
TUR0010184	Can	İzmir
TUR0010185	Papaz	İzmir
TUR0010186	Papaz	İzmir
TUR0010187	Havran	İzmir
TUR0010188	Can	İzmir
TUR0010189	Can	İzmir
TUR0010607	Unknown	Unknown
TUR0010190	Can	Aydın
TUR0010608	Can	Unknown
TUR0010191	Halil Efendi	Tokat
TUR0010252	Unknown	Unknown
TUR0010606	Unknown	Unknown
TUR0010604	Unknown	Unknown
TUR0010605	Unknown	İzmir

2.2. Methods

2.2.1. DNA Extraction

Genomic DNA was isolated from fresh leaf tissue bulked from ten leaves per accession using a CTAB method (Doyle and Doyle, 1990). The DNA was quantified on a Nanodrop ND-1000 spectrophotometer (Thermo Scientific™, Vantaa, Finland) following the manufacturer's protocol. DNA concentrations (ng/µl), Abs 260/280 (nm) and Abs 260/230 (nm) of all *Prunus* accessions were measured. All genomic DNAs were stored at -20°C.

2.2.2. Molecular Marker Analysis

2.2.2.1. SRAP Analysis

PCR amplifications were carried out using 47 SRAP primer combinations (Li and Quiros 2001, Lin et al., 2005). Forward and reverse primers of SRAP markers are shown in Table 2.4. Each 25 µl PCR mixture consisted of 2.5 µl Tango Buffer, 1 µl (20 ng) DNA templates, 2 µl Mg^{2+} (25 mM), 1.5 µl dNTP (0.2 mM), 0.5 µl forward primer (10 pmol), 0.5 µl reverse primer and 1 µl *Taq* DNA polymerase (0.25 U). The PCR protocol was as follows: 94°C for 5 min of denaturation followed by 5 cycles of 1 min denaturation at 94°C, 1 min annealing at 35°C, and 1 min elongation at 72°C. This was followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 50°C, and 1 min elongation at 72°C and a final extension step at 72 ^{o}C for 10 min with a hold at 4°C. PCR products were run on 2 % agarose (Lonza, Sea Kem® LE Agarose) gels in 1X TAE buffer by electrophoresis, stained with ethidium bromide and visualized under UV light (BIO-RAD, California, USA).

2.2.2.2. Population Structure and Molecular Genetic Diversity Analysis

Amplified SRAP loci were scored as present (1), absent (0) or missing data (9). The average, maximum and minimum values of gene diversity (GD) for each marker were calculated using Gene Diversity Software (GDdom) (Abuzayed et al., 2017). The marker data were analyzed for population structure with the Structure computer program (Structure 2.3.4) (Pritchard et al., 2000) for model based clustering of the

accessions. First, number of subpopulations (population model) representing the plant population needed to be identified. To achieve this, different models (from 1 to 10) were evaluated after 10,000 burn-in cycles using Markov Chain Monte Carlo (MCMC) replications and ad hoc statistics. Also, each model was tested 20 times with 300,000 iterations. The results were analyzed by Structure Harvester software (Earl et al., 2012) to find the best population model (K) based on ΔK value. The model with the highest ΔK value was considered as the best model for the population. A genetic identity threshold of ≥ 0.70 was selected to determine subpopulation membership as this threshold gave the best fit to the selected K. Accessions that were not assigned to subpopulations based on the threshold were considered as admixed.

The marker data were analyzed for hierarchical clustering. For this, a dendrogram was constructed with the Dice coefficient and unweighted neighbor joining algorithm using DARwin (Dissimilarity Analysis and Representation for Windows) software (Perrier and Jacquemoud, 2006). Also a Mantel test was performed to find the correlation between the dissimilarity matrix and the dendrogram. This program was also used for principal coordinate analysis (PCoA).

Genetic diversity within and between two major plum populations; "Can" and "Papaz"; was evaluated by PhiPT (FST analogue) analysis via GenAlEx plugin. A PhiPT value less than 0.15 was accepted as significant for gene flow between these two populations (Frankham, Briscoe, and Ballou, 2002). The genetic divergence analysis was conducted with "9999" pairwise permutations and P value was accepted as significant below 0.05. Additionally, mean diversity (h), Nei's genetic distance (NGD) and Nei's genetic identities were calculated in the same plugin.

Table 2.2. SRAP markers used in this study.

Marker Combination	Forward Primer(5'-3')	Reverse Primer(5'-3')
Me1-Em1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT
Me2-Em1	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT
Me3-Em1	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTAAT
Me4-Em1	TGAGTCCAAACCGGACC	GACTGCGTACGAATTAAT
Me5-Em1	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTAAT
Me1-Em2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC
Me2-Em2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC
Me3-Em2	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTTGC
Me4-Em2	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTGC
Me5-Em2	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTGC
Me1-Em3	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC
Me2-Em3	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGAC
Me3-Em3	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTGAC
Me4-Em3	TGAGTCCAAACCGGACC	GACTGCGTACGAATTGAC
Me5-Em3	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTGAC
Me1-Em4	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGA
Me2-Em4	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGA
Me3-Em4	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTTGA
Me4-Em4	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTGA
Me5-Em4	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTGA
Me1-Em5	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAC
Me2-Em5	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAC
Me3-Em5	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTAAC
Me4-Em5	TGAGTCCAAACCGGACC	GACTGCGTACGAATTAAC
Me5-Em5	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTAAC

(cont. on next page)

Table 2.2. (cont.)

Marker Combination	Forward Primer(5'-3')	Reverse Primer(5'-3')
Me6-Em5	TGAGTCCAAACCGGTAA	GACTGCGTACGAATTAAC
Me1-Em6	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGCA
Me2-Em6	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGCA
Me3-Em6	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTGCA
Me4-Em6	TGAGTCCAAACCGGACC	GACTGCGTACGAATTGCA
Me5-Em6	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTGCA
Me1-Em7	TGAGTCCAAACCGGATA	GACTGCGTACGAATTCAA
Me2-Em7	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTCAA
Me3-Em7	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTCAA
Me5-Em7	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAA
Me6-Em7	TGAGTCCAAACCGGTAA	GACTGCGTACGAATTCAA
Me9-Em7	TGAGTCCAAACCGGATG	GACTGCGTACGAATTCAA
Me1-Em8	TGAGTCCAAACCGGATA	GACTGCGTACGAATTCTC
Me2-Em8	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTCTC
Me3-Em8	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTCTC
Me4-Em8	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCTC
Me5-Em8	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCTC
Me1-Em9	TGAGTCCAAACCGGATA	GACTGCGTACGAATTCGA
Me2-Em9	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTCGA
Me3-Em9	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTCGA
Me4-Em9	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCGA
Me6-Em6	TGAGTCCAAACCGGTAA	GACTGCGTACGAATTCGA

CHAPTER 3

RESULTS AND DISCUSSION

3.1. DNA Extraction

DNA concentrations of 66 *P.cerasifera* accessions were measured and found to be suitable for PCR amplification (Table 3.1).

Table 3.1. Quantification and quality of DNA of *P.cerasifera* accessions.

No	DNA Concentration (ng/µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TUR0010270	229.91	1.86	2.15
TUR0010130	387.14	1.9	1.77
TUR0010131	434.01	1.77	1.8
TUR0010132	273.64	1.8	2.11
TUR0010133	493.45	1.82	2.12
TUR0010134	753.95	1.88	2.1
TUR0010135	588.41	1.9	2.15
TUR0010137	564.73	1.77	1.9
TUR0010139	638.02	1.92	1.88
TUR0010140	392.32	1.78	1.98
TUR0010141	288.24	1.8	1.97
TUR0010142	941.65	1.84	2.1
TUR0010143	462.63	1.92	2.05
TUR0010144	463.81	1.75	1.95
TUR0010145	1002.51	1.79	1.97
TUR0010146	177.038	1.82	2.05
TUR0010147	310.97	1.8	2.11
TUR0010148	449.03	1.81	1.91

(cont. on next page)

Table 3.1.(cont.)

No	DNA Concentration (ng/µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TUR0010149	471.06	1.81	1.96
TUR0010150	441.05	1.85	1.8
TUR0010151	402.07	1.87	1.92
TUR0010152	311.38	1.84	1.88
TUR0010153	639.59	1.85	1.93
TUR0010154	540.35	1.9	1.95
TUR0010155	811.26	1.76	1.88
TUR0010156	235.84	1.75	1.85
TUR0010157	213.82	1.82	1.84
TUR0010158	518.38	1.83	1.91
TUR0010159	455.72	1.82	1.89
TUR0010160	2114.14	1.81	2.07
TUR0010161	585.91	1.86	1.94
TUR0010162	246.23	1.78	1.96
TUR0010163	368.71	1.82	1.88
TUR0010164	989.13	1.82	1.85
TUR0010165	372.76	1.8	1.81
TUR0010167	566.17	1.85	1.94
TUR0010168	513.42	1.87	1.93
TUR0010169	722.83	1.84	1.85
TUR0010170	692.65	1.9	1.93
TUR0010171	341.66	1.94	1.99
TUR0010172	507.83	1.87	2.11
TUR0010173	1103.21	1.83	2.01
TUR0010174	413.91	1.82	2.04
TUR0010175	783.94	1.77	1.98
TUR0010176	272.87	1.78	1.87
TUR0010177	375.66	1.98	1.92
TUR0010178	241.24	1.89	1.78
TUR0010179	416.87	1.8	1.86

(cont. on next page)

Table 3.1.(cont.)

No	DNA Concentration (ng/µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TUR0010180	465.23	1.79	1.85
TUR0010181	487.33	1.83	1.77
TUR0010182	265.92	1.9	1.81
TUR0010183	298.11	1.87	1.88
TUR0010184	503.87	1.85	1.76
TUR0010185	303.26	1.93	1.93
TUR0010186	268.55	1.88	1.99
TUR0010187	492.11	1.89	1.93
TUR0010188	233.25	1.84	2.04
TUR0010189	224.22	1.78	1.93
TUR0010607	833.56	1.77	1.95
TUR0010190	574.93	1.82	1.78
TUR0010608	533.44	1.81	1.85
TUR0010191	627.11	1.86	1.76
TUR0010252	409.42	1.88	1.99
TUR0010606	306.87	2.01	1.73
TUR0010604	268.96	1.9	1.8
TUR0010605	396.12	1.91	1.87

3.2. Molecular Marker Analysis

3.2.1. SRAP Marker Analysis

In the thesis, 47 SRAP primer combinations were tested with *P. cerasifera*, which were utilized for evaluating genetic diversity. SRAP analysis was carried out on all 66 accessions. As a result, dominant scoring generated 495 alleles, 485 (98%) of which were polymorphic. Average number of alleles of the primers was 10.4 while combinations em1-me1 and em1-me2 had the highest number of alleles, 13 and 12, respectively. The combination em9-me3 had the lowest number with 8 alleles. All the primers except em1-me2 and em1-me5 (83.3% and 62.5%) had more than 90% polymorphism. The maximum average gene diversity (GD) was 0.46 for em3-me4

primer combination. The minimum average gene diversity was 0.19, which was shared by two different primer pairs: em1-me5 and em4-me3 (Table 3.2.).

Table 3.2. Average GD for each primer

Primer	Average GD value ± SE	Primer	Average GD value ± SE
em3me4	0.46 ± 0.01	**em4me5**	0.32 ± 0.03
em7me3	0.44 ± 0.02	**em4me4**	0.32 ± 0.04
em5me5	0.43 ± 0.03	**em8me4**	0.31 ± 0.05
em3me1	0.40 ± 0.04	**em1me4**	0.31 ± 0.05
em7me8	0.39 ± 0.04	**em8me3**	0.31 ± 0.04
em3me2	0.39 ± 0.04	**em6me3**	0.31 ± 0.04
em2me5	0.38 ± 0.04	**em6me2**	0.30 ± 0.05
em5me1	0.38 ± 0.05	**me6em1**	0.29 ± 0.05
em5me2	0.38 ± 0.04	**em1me3**	0.29 ± 0.04
em6me4	0.37 ± 0.03	**em3me3**	0.29 ± 0.05
em7me6	0.37 ± 0.04	**em6me5**	0.29 ± 0.04
em4me2	0.37 ± 0.05	**em1me2**	0.28 ± 0.05
em7me1	0.36 ± 0.05	**em4me1**	0.27 ± 0.04
em2me3	0.36 ± 0.10	**em2me4**	0.27 ± 0.05
em8me2	0.35 ± 0.05	**em9me3**	0.27 ± 0.06
em5me3	0.35 ± 0.06	**em6me6**	0.26 ± 0.05
em8me1	0.34 ± 0.05	**em9me2**	0.25 ± 0.06
em8me5	0.34 ± 0.05	**em2me1**	0.24 ± 0.04
em3me5	0.34 ± 0.05	**em5me4**	0.24 ± 0.06
em7me5	0.33 ± 0.06	**em5me6**	0.20 ± 0.05
em9me4	0.33 ± 0.07	**em9me1**	0.20 ± 0.04
em2me2	0.33 ± 0.05	**em1me5**	0.19 ± 0.07
em7me2	0.33 ± 0.04	**em4me3**	0.19 ± 0.05
em1me1	0.32 ± 0.05		

Notes: GD= Gene Diversity; SE= Standard Error.

3.2.1.1. Population Structure

Population structure analysis was performed for the population using the 495 fragments generated by 47 SRAP primer combinations. Results of the analysis indicated that three subpopulations (Table 3.3) (K=3) were the best model for the population based on Δ*K* values (Figure 3.1). A subpopulation identity threshold of ≥ 0.70 was selected for better analysis of clustering results generated by structure software. The Standard Deviation (SD) is important for each value of K when deciding the correct number of subpopulations and these results supported K=3 (Figure 3.2). As a result, while a total of 4 individuals were assigned to subpopulation A, 27 individuals were assigned to subpopulation C and 28 individuals were assigned to subpopulation B. Also 7 individuals were found to be admixed (Table 3.2., Figure 3.3). There was no origin specific clustering in structure analysis.

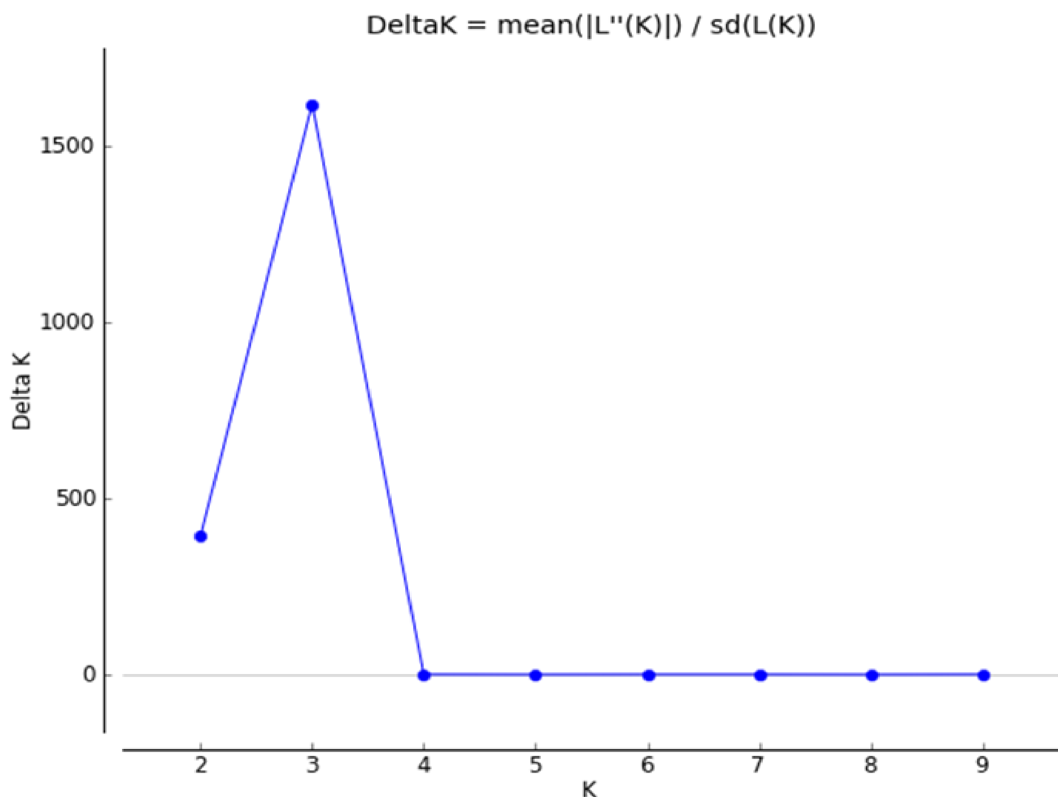

Figure 3.1. ΔK values for each number of subpopulations (K) for *P. cerasifera*

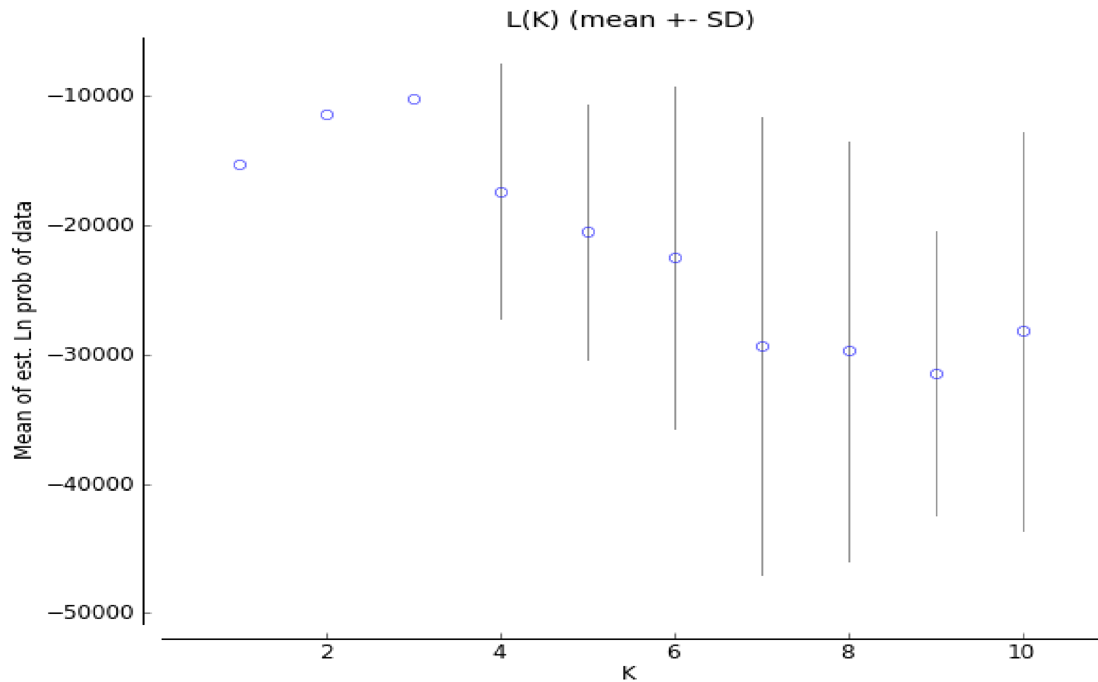

Figure 3.2. SD values for each number of *P.cerasifera* subpopulations

Although there have been no previous population structure studies in *P. cerasifera*, population structures of two different plum species (*P. salicina, P. domestica*) were reported. The study performed by Mnejja et al. (2004), used a small population containing 29 plum accessions (*P. salicina* Lindl.). The study was performed using a different marker system (SSR markers), and they found that the germplasm fell into two subpopulations. A more recent study was performed by Makovics-Zsohár et al. (2017) using a population containing 55 European Plum genotypes (mainly Hungary and Germany) (*P. domestica* L.). The study reported that the population fell into three subpopulations. Our study showed that population structure of Turkish Plum (three subpopulations) genotypes had similar population model with European Plums and a simple population structure.

3.2.2.2. Molecular Genetic Diversity

The SRAP data were analyzed using hierarchical clustering method. As a result, a dendrogram was generated using the Dice coefficient and neighbor-joining method. The dendrogram contained three clusters (Cluster A, B, C). Mantel tested showed that

there was a strong correlation (r = 0.99) between the distance matrix and reconstructed tree. Cluster A contained 28 accessions, cluster B contained 33 accessions and cluster C contained 5 accessions. Genetic diversity ranged from 0.04 to 0.66 with an average dissimilarity of 0.37. Highest diversity was observed between TUR0010167 (Can plum) and TUR0010142 (Papaz plum) (66%). The lowest was observed between TUR0010175 (Papaz plum) and TUR0010608 (Can plum) (4%). There was no cultivar type specific clustering in the dendrogram. All three clusters (A, B, C) contained Papaz Plum (dark blue color in dendrogram). Both cluster A and cluster B contained Can plum (red color) (Figure 3.4.). The genetic diversity of Cluster C (0.31) was higher than that of Cluster A (0.25) and Cluster B (0.23). All the subclusters (A, B, C) generated by Darwin program had individuals from all subpopulations and admixed groups of structure result indicating that the results of the two analyses were not strongly correlated. Subcluster A contained three individuals from subpopulation A, two individuals from admixed groups, 12 individuals from subpopulation B and 10 individuals from subpopulation C. Subcluster C contained two individuals from subpopulation B, two individuals from two C groups and one individual from admixed group. Subcluster B contained 14 individuals from subpopulation B, one individual from A group, four individuals from admixed group and 15 individuals from subpopulation C (Table 3.3.). There was no origin specific clustering in DARwin analysis.

In the dendrogram, Havran Plum, Kebab Plum, Halil Efendi Plum, Akpapaz Plum and Bekiroğlu Plum were closely related to Can Plum groups. Although Şam Plum was not closely related to Yeşil Şam Plum and Sarı Şam Plum, it was related to Can Plum and Havran Plum. Yeşil Şam Plum and Sarı Şam Plum were closely clustered in a Can Plum group. Papaz tended to form small clusters within larger clusters (A1, B1, C1) (Figure 3.4).

Population diversity was examined for Can and Papaz types. The Dice coefficient of variation for Can Plum varied from 0.11 to 0.63 with an average of 0.38. The Papaz Plum varied from 0.08 to 0.63 with an average of 0.40. Based on AMOVA, nearly all of the variation in the Can and Papaz type were found within the types (98%) rather than between the two types (2%). Within each type, the level of diversity was identical with a heterozygosity value (h) of 0.48. Thus, neither type was more genetically variable than the other. Genetic identity (Nei) was very high between the Can and Papaz Plums with a value of 0.96. Indeed, gene flow between the two types

was very high with a PhiPT value of 0.018. Overall these results indicate that Can and Papaz Plums are not genetically defined from each other as was also observed in the dendrogram and population structure results.

The population analyzed in the present study had higher variation (ranged from 0.04 to 0.66) than the population containing 20 P. cerasifera accessions collected from Turkey (ranged from 0.02 to 0.16) and studied with AFLP markers (Ayanoğlu, et al., 2007). The reason for the different genetic diversity of the population might be due to the use of different marker systems (SRAP and AFLP). In general, the two markers systems have different polymorphism level (Ferriol et al., 2003). Also, the present study had more sub clusters than the AFLP study (two sub clusters) (Ayanoğlu et al., 2007). The reason for this difference is probably due to the higher number of accessions of the present study. Thus, the present study determined that Prunus accessions collected from seaside regions of Turkey had more diversity than the P. cerasifera accessions collected from the study of Ayanoğlu (Ayanoğlu et al., 2007).

Principal coordinate analysis (PCoA) of SRAP data showed that there was clear separation between A, B and C populations (Figure 3.5). There were three clusters in the PCoA plot. Cluster A contained two Kebab Plums, three Havran Plums, Halil Efendi Plum, Sarı Şam Plum, Akpapaz Plum, Ödemiş Plum, Yeşil Şam Plum, some of the Can Plums and Papaz Plums. Cluster B consisted of Bekiroğlu Plum, two Havran Plums, some of the Can Plums and Papaz Plums. Cluster C consisted of four Papaz Plums. According to this analysis, the Can and Papaz Plums were dispersed in all populations and, as a result, had high genetic diversity. Some of the Can and Papaz Plums were closely related to Kebab Plum, Havran Plum, Halil Efendi Plum, Sarı Şam Plum, Akpapaz Plum, Ödemiş Plum, Yeşil Şam Plum, Bekiroğlu Plum. PCoA of molecular data showed that the first, second and third eigenvectors explained 40.25%, 17.53% and 5.39% of the variation, respectively. There was no region specific clustering in the plot (Figure 3.5).

Tablo 3.3. Subpopulation and cluster assignments of accessions

Genotype number	**PI number**	**Local name**	**Location of collection**	**Subpop. A**	**Subpop. B**	**Subpop. C**	**Structure Assign.**	**DARwin Assgn.**
1	TUR0010185	Papaz	İzmir	0.165	0.003	0.832	C	Cluster B
2	TUR0010177	Papaz	İzmir	0.001	0.003	0.996	C	Cluster B
3	TUR0010142	Papaz	İzmir	0.001	0.001	0.998	C	Cluster C
4	TUR0010607	Unknown	Unknown	0.004	0.057	0.939	C	Cluster B
5	TUR0010149	Papaz	İzmir	0.396	0.002	0.602	Admixed	Cluster A
6	TUR0010179	Papaz	Manisa	0.996	0.003	0.001	A	Cluster B
7	TUR0010135	Papaz	Denizli	0.003	0.003	0.994	C	Cluster A
8	TUR0010270	Can	Unknown	0.002	0.001	0.997	C	Cluster A
9	TUR0010152	Papaz	İzmir	0.982	0.017	0.001	A	Cluster A
10	TUR0010154	Papaz	İzmir	0.001	0.002	0.997	C	Cluster A

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	PI number	Local name	Location of collection	Subpop. A	Subpop. B	Subpop. C	Structure Assign.	DARwin Assgn.
11	TUR0010174	Papaz	Muğla	0.002	0.003	0.994	C	Cluster B
12	TUR0010141	Papaz	İzmir	0.997	0.002	0.001	A	Cluster A
13	TUR0010139	Can	Aydın	0.046	0.001	0.952	C	Cluster C
14	TUR0010144	Bekiroğlu	İzmir	0.008	0.001	0.991	C	Cluster A
15	TUR0010170	Ödemiş	Balıkesir	0.053	0.007	0.94	C	Cluster B
16	TUR0010191	Halil Efendi	Tokat	0.001	0.041	0.958	C	Cluster B
17	TUR0010161	Unknown	Manisa	0.208	0.001	0.791	C	Cluster A
18	TUR0010155	Can	İzmir	0.002	0.004	0.994	C	Cluster A
19	TUR0010150	Can	İzmir	0.001	0.001	0.998	C	Cluster A
20	TUR0010171	Can	Muğla	0.053	0.001	0.946	C	Cluster B

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	PI number	Local name	Location of collection	Subpop. A	Subpop. B	Subpop. C	Structure Assign.	DARwin Assgn.
21	TUR0010160	Papaz	İzmir	0.413	0.002	0.585	Admixed	Cluster C
22	TUR0010182	Can	İzmir	0.002	0.037	0.962	C	Cluster B
23	TUR0010188	Can	İzmir	0.179	0.001	0.82	C	Cluster B
24	TUR0010181	Can	Manisa	0.326	0.003	0.671	Admixed	Cluster B
25	TUR0010159	Havran	İzmir	0.001	0.001	0.998	C	Cluster A
26	TUR0010180	Papaz	Manisa	0.001	0.002	0.997	C	Cluster B
27	TUR0010187	Havran	İzmir	0.003	0.003	0.994	C	Cluster B
28	TUR0010184	Can	İzmir	0.019	0.002	0.979	C	Cluster B
29	TUR0010133	Papaz	Manisa	0.993	0.006	0.001	A	Cluster A
30	TUR0010172	Can	Muğla	0.284	0.002	0.715	C	Cluster B

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	PI number	Local name	Location of collection	Subpop. A	Subpop. B	Subpop. C	Structure Assign.	DARwin Assgn.
31	TUR0010604	Unknown	Unknown	0.645	0.019	0.336	Admixed	Cluster B
32	TUR0010252	Unknown	Unknown	0.002	0.151	0.846	C	Cluster B
33	TUR0010156	Papaz	Aydın	0.246	0.004	0.749	C	Cluster A
34	TUR0010132	Can	İzmir	0.227	0.007	0.766	C	Cluster A
35	TUR0010153	Can	İzmir	0.323	0.677	0.001	Admixed	Cluster A
36	TUR0010145	Papaz	Balıkesir	0.001	0.998	0.001	B	Cluster A
37	TUR0010169	Sarı Şam	Balıkesir	0.001	0.998	0.001	B	Cluster B
38	TUR0010163	Can	Manisa	0.019	0.978	0.003	B	Cluster A
39	TUR0010130	Havran	İzmir	0.132	0.867	0.001	B	Cluster A
40	TUR0010608	Can	Unknown	0.001	0.999	0.001	B	Cluster B

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	PI number	Local name	Location of collection	Subpop. A	Subpop. B	Subpop. C	Structure Assign.	DARwin Assgn.
41	TUR0010175	Papaz	Aydın	0.001	0.999	0.001	B	Cluster B
42	TUR0010178	Papaz	İzmir	0.001	0.999	0.001	B	Cluster B
43	TUR0010140	Kebap	İzmir	0.214	0.785	0.001	B	Cluster A
44	TUR0010168	Yeşil Şam	Balıkesir	0.003	0.997	0.001	B	Cluster B
45	TUR0010143	Can	İzmir	0.002	0.99	0.009	B	Cluster A
46	TUR0010183	Can	İzmir	0.028	0.971	0.001	B	Cluster B
47	TUR0010137	Papaz	Aydın	0.003	0.995	0.001	B	Cluster A
48	TUR0010134	Akpapaz	Manisa	0.131	0.868	0.001	B	Cluster C
49	TUR0010186	Papaz	İzmir	0.018	0.98	0.001	B	Cluster B
50	TUR0010165	Can	Manisa	0.002	0.997	0.001	B	Cluster B

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	PI number	Local name	Location of collection	Subpop. A	Subpop. B	Subpop. C	Structure Assign.	DARwin Assgn.
51	TUR0010173	Papaz	Muğla	0.001	0.998	0.002	B	Cluster B
52	TUR0010157	Havran	İzmir	0.001	0.998	0.001	B	Cluster A
53	TUR0010190	Can	Aydın	0.001	0.999	0.001	B	Cluster B
54	TUR0010605	Unknown	Unknown	0.001	0.998	0.001	B	Cluster B
55	TUR0010131	Can	İzmir	0.002	0.997	0.001	B	Cluster A
56	TUR0010147	Havran	Unknown	0.013	0.987	0.001	B	Cluster A
57	TUR0010148	Şam	Balıkesir	0.002	0.996	0.002	B	Cluster A
58	TUR0010158	Can	İzmir	0.036	0.962	0.002	B	Cluster C
59	TUR0010176	Papaz	Aydın	0.002	0.996	0.001	C	Cluster B
60	TUR0010167	Can	Balıkesir	0.599	0.453	0.001	Admixed	Cluster B

(cont. on next page)

Tablo 3.3. (Cont.)

Genotype number	**PI number**	**Local name**	**Location of collection**	**Subpop. A**	**Subpop. B**	**Subpop. C**	**Structure Assign.**	**DARwin Assgn.**
61	TUR0010164	Can	Manisa	0.547	0.453	0.001	Admixed	Cluster B
62	TUR0010146	Can	Balıkesir	0.002	0.995	0.003	B	Cluster A
63	TUR0010151	Can	İzmir	0.002	0.997	0.001	B	Cluster A
64	TUR0010162	Kebap	Manisa	0.004	0.995	0.001	B	Cluster A
65	TUR0010606	Unknown	Unknown	0.008	0.991	0.001	B	Cluster B
66	TUR0010189	Can	İzmir	0.006	0.992	0.002	B	Cluster B

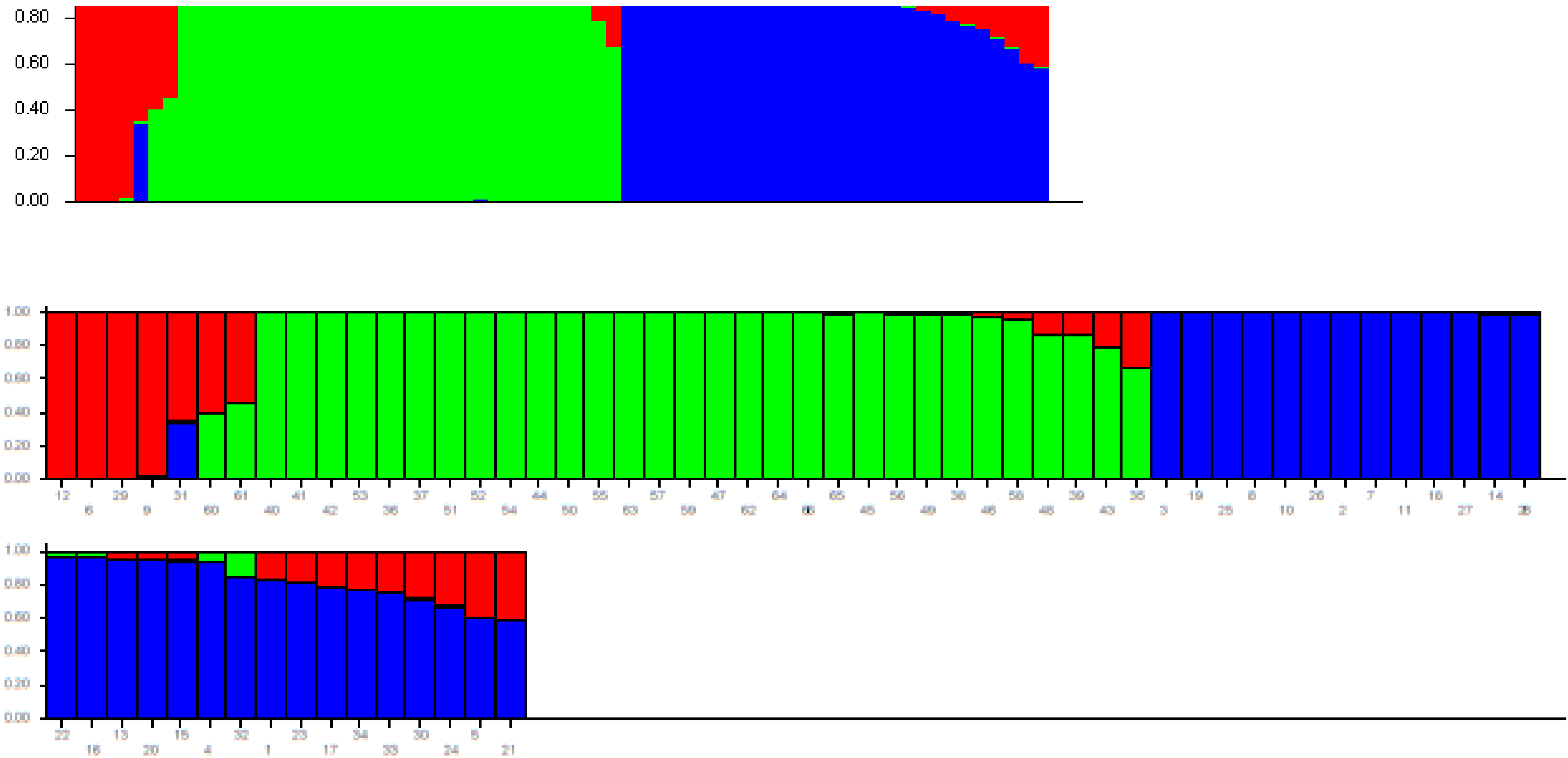

Figure 3.3. Q-plot of *P. cerasifera* population based on SRAP markers. The bar plot for K=3. Each accession is represented by a vertical bar. Red, green and dark blue colored sections within each vertical bar indicate membership coefficient (Q on the y-axis) of the accession to subpopulation.

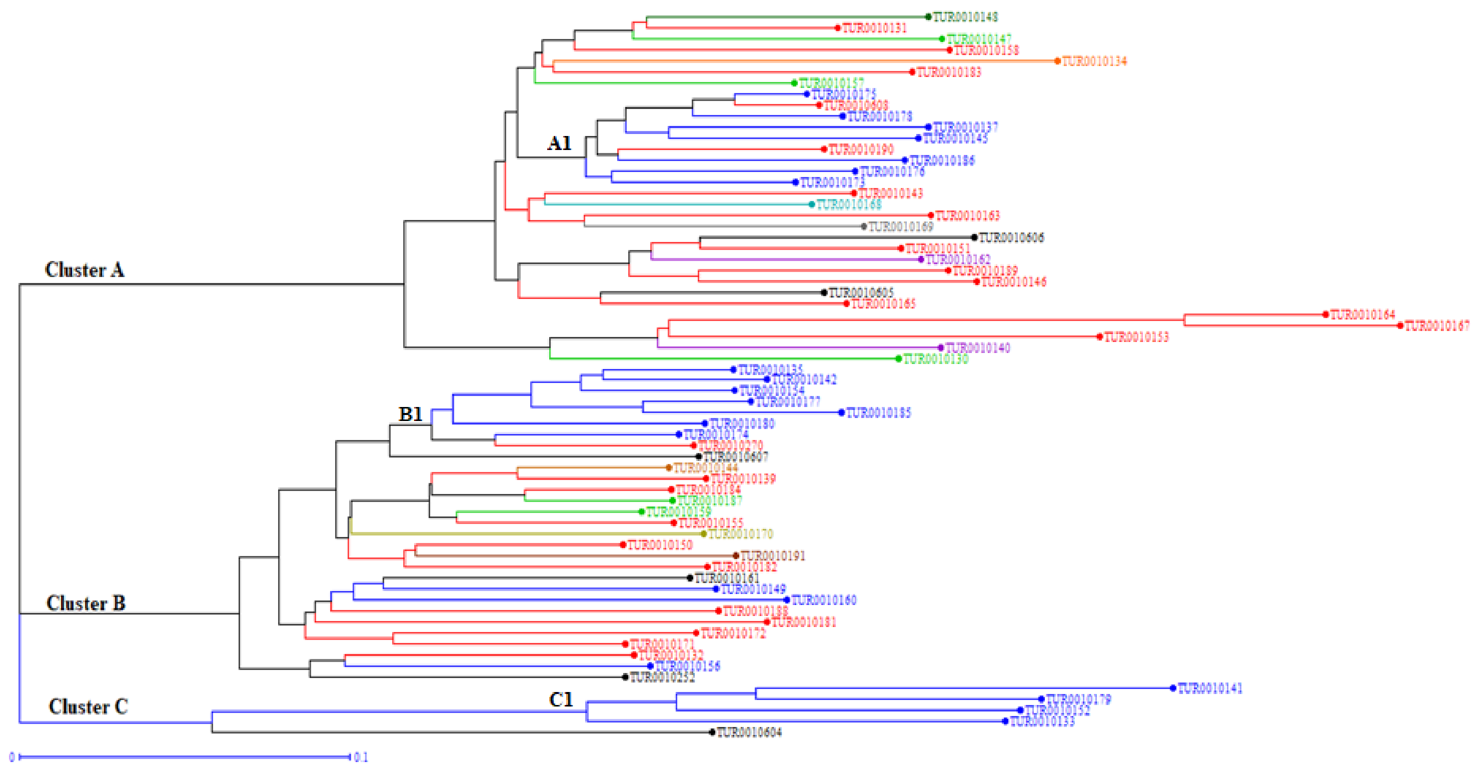

Figure 3.4. Dendrogram showing genetic diversity of *P.cerasifera* accessions. Hierarchical clustering of *P. cerasifera* accessions based on the Dice coefficient and unweighted neighbor-joining method. *P.cerasifera* accessions are color-coded by localı name. Accessions are color coded by Local name: Can Plum: red, Papaz Plum: dark blue, Havran Plum: green, Kebab Plum: purple, Unknown: black, Halil Efendi Plum: brown, Akpapaz Plum: orange, Ödemiş Plum: dark green, Yeşil Şam Plum: light blue, Sarı Şam Plum: dark grey, Bekiroğlu Plum: yellow, Şam Plum: dark green

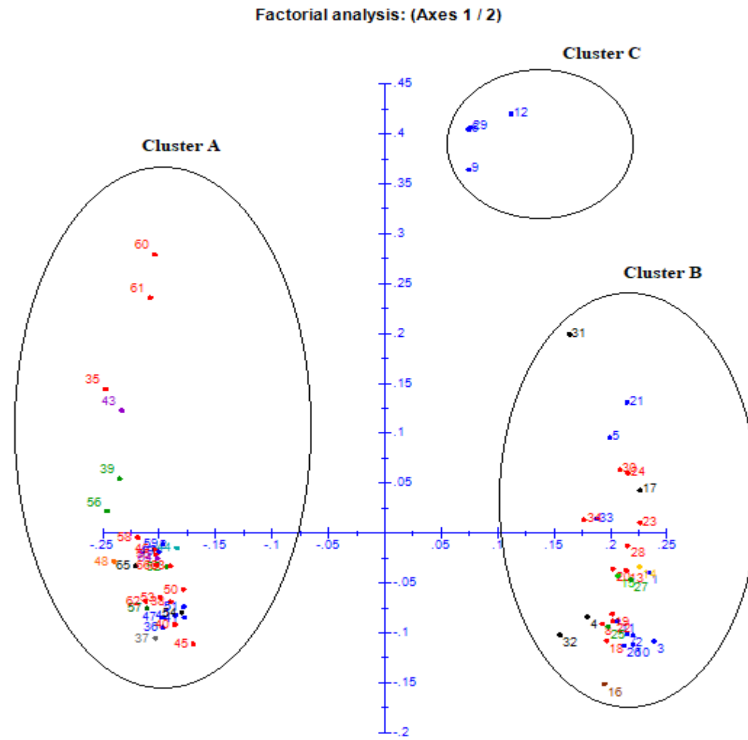

Figure 3.5. PCoA for *P. cerasifera* accessions. Red, dark blue, green, purple, brown, orange, light green, dark green, dark grey, light yellow and dark green brown represent genotypes from the most abundant.Accessions are color coded by Local name: Can Plum: red, Papaz Plum: dark blue, Havran Plum: green, Kebab Plum: purple, Unknown: black, Halil Efendi Plum: brown, Akpapaz Plum: orange, Ödemiş Plum: dark green, Yeşil Şam Plum: light blue, Sarı Şam Plum: dark grey, Bekiroğlu Plum: yellow, Şam Plum: dark green

CHAPTER 4

CONCLUSION

Plum (*P. cerasifera*) is an important fruit crop in Turkey. It has wealthy mineral, vitamin and phytochemical content. There are many of subspecies of *P. cerasifera* and it can grow naturally in many parts of Turkey. Turkey ranks sixth in the world with its production rate. The main goal of this study was to determine the genetic diversity of 66 Turkish *P. cerasifera* accessions collected from Turkey using 47 SRAP primer combinations and to analyze their population structure. A total of 495 fragments were obtained and among them 4 98% were polymorphic and 2% were monomorphic. The maximum average GD was 0.46 (em3me4 primers) and the minimum average gene diversity was 0.19 (em1me5 and em4me3 primers). These results indicated that SRAP markers are useful for analysis of genetic diversity in Turkish plums. Genetic diversity of the 66 *P.cerasifera* accessions as measured by the Dice coefficient ranged from 0.04 to 0.66 with an average dissimilarity of 0.37. The data were analyzed using the Neighbor-Joining algorithm PCoA. The dendogram divided accesions into three clusters (A, B, C). Cluster A contained 28 accessions, cluster B contained 33 accessions and cluster C contained 5 accessions in the present study. Additionally, the population structure of the Turkish plum species was determined. Population structure analysis indicated that there were three subpopulations. A total of four individuals were assigned to subpopulation A, 28 individuals were assigned to subpopulation B and 27 individuals were assigned to subpopulations C. Also, 7 individuals were found to be admixed. Overall diversity was found to be moderate but higher than that of Turkish plums collected from other regions. Clustering of the plums was not related to origin or to type indicating that Papaz and Can plums are not genetically distinct classes. This is the first molecular characterization of a *P. cerasifera* population that was performed using SRAP markers. Molecular genetic data presented in this thesis can be useful for molecular breeding of plum. The accumulation of genomic information about plum wil facilitate genetics research and molecular breeding of plum itself plum breeding should include worldwide germplasm to maximize diversity, improve adaptation and increase productivity of the plant. Therefore, germplasm management is fundamental for providing well characterized material for crop improvement.

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