MOLECULAR GENETIC ANALYSIS IN OPIUM POPPY

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
in Molecular Biology and Genetic

by
İBRAHİM ÇELİK

June 2017
İZMİR

We approve the thesis of **İbrahim ÇELİK**

Examining Committee Members

____
Prof. Dr. Sami DOĞANLAR
Department of Molecular Biology and Genetics, Izmir Institute of Technology

____
Prof. Dr. Hülya İLBİ
Department of Horticultural, Ege University

____
Assoc. Prof. Dr. Hüseyin Çağlar KARAKAYA
Department of Molecular Biology and Genetics, Izmir Institute of Technology

____
Assoc. Prof. Dr. Engin ÖZÇİVİCİ
Department of Bioengineering, Izmir Institute of Technology

____
Assist. Prof. Dr. Seçkin EROĞLU
Department of Genetics and Bioengineering, Izmir University of Economics

6 June 2017

____
Prof. Dr. Sami DOĞANLAR
Supervisor, Department of Molecular Biology and Genetics,
Izmir Institute of Technology

____
Prof. Dr. Volkan SEYRANTEPE
Head of Department of Molecular
Biology and Genetics

____
Prof. Dr. Aysun SOFUOĞLU
Dean of the Graduate School of
Engineering and Sciences

ACKNOWLEDGEMENTS

I would like to thank to my supervisor **Prof. Dr. Sami DOĞANLAR** for giving opportunity to study in his research group, close interest, guidance and motivation. At the same time, I would like to thank to **Prof. Dr. Anne FRARY** for her patience, understanding and endless support while analyzing data and writing this thesis and other manuscripts.

I would like to thank to **Fatih LEBLEBİCİ** from the Turkish Soil Product Office (TMO) for cultivation of opium poppy world collection. Also, I would like to thank to **Dr. Hüseyin CAMCI**, **Dr. Arzu KÖSE** and **Dr. Ferda KOŞAR** from the Anatolia Agricultural Research Institute (Eskişehir) for morphological characterization of Turkish opium poppy germplasm.

I would like to thank to **The Scientific and Technological Research Council of Turkey (TUBITAK)** (project no. 109O797) for financial support of the project.

I want to thank to my friend **Pelin TEKİN** for helping me collecting leaf samples of the plant material in Eskişehir and **ÖZTÜRK** family (Süleyman Can **ÖZTÜRK** and **Saniye Elvan ÖZTÜRK**) for friendship, especially during the my hard times in İzmir Atatürk Hospital.

Furthermore, I would like to thank to my father **Halil ÇELİK**, my mother **Selvinaz ÇELİK** and my sister **Dilek ÇAKMAK** for their support and understanding.

Finally, I also would like to express my thankfulness to my wife **Gökcen ÇELİK** for her deepest love and encouragement.

ABSTRACT

MOLECULAR GENETIC ANALYSIS IN OPIUM POPPY

As the sole plant source of many potent alkaloids, opium poppy (*Papaver somniferum* L.) is an important medicinal crop. Nevertheless, few studies have characterized opium poppy germplasm with crop-specific molecular markers. In this study, recently developed SSR markers were validated for diversity analysis and tested in an opium poppy world collection. The limited diversity of the world collection suggested that other genetic resources such as those from Turkey, a diversity center for the crop, should be explored. Thus, molecular and morphological characterization of Turkish opium poppy germplasm were performed. As a result, Turkish germplasm (11%) was found to have higher diversity than the world collection (5%). Also potentially useful morphological variation was observed for morphine content, plant height, and capsule index. However, the landraces exhibited limited breeding potential for stigma number, and seed and straw yields. Two core sets containing 22 and 21 accessions were selected from the world and Turkish germplasm, respectively, for effective management of collections in seed banks and breeding programs. The primary findings showed that Turkish germplasm is a valuable genetic resource to identify QTLs controlling morphine content and agronomic traits using an association mapping approach. Thus, a total of 164 SSR and 367 AFLP polymorphic loci were applied to an opium poppy association mapping panel composed of 95 opium poppy landraces which were grown for two seasons. One SSR and three AFLP loci were found to be significantly associated with morphine content ($P < 0.01$ and LD value (r^2) = 0.10-0.32) and six SSR and 14 AFLP loci were significantly associated with five agronomic traits (plant height, stigma number, capsule index, seed and straw yield) ($P < 0.01$ and LD value (r^2) = 0.08-0.35). This is the first report of association mapping in this crop. The identified markers provide initial information for marker-assisted selection of important traits in opium poppy.

ÖZET

HAŞHAŞ'TA MOLEKÜLER GENETİK ANALİZLER

Birçok alkaloidin yegane kaynağı konumunda olan haşhaş önemli bir tıbbi bitki olmasına rağmen, haşhaş gen kaynaklarının moleküler markörlerle karakterize edildiği sınırlı sayıda araştırma mevcuttur. Bu çalışmada, yeni geliştirilen SSR markörlerinin genetik çeşitlilik analizleri bakımından validasyonu yapılmış ve bu markörler haşhaş dünya koleksiyonunda testlenerek düşük genetik çeşitlilik belirlenmiştir. Bu sonuçlar Türkiye gibi haşhaşın çeşitlilik merkezlerinin önemini ortaya koymuş ve Türk haşhaş çeşitleri morfolojik ve moleküler olarak karakterize edilmiştir. Sonuç olarak Türk haşhaş populasyonunun (%11) dünya koleksiyonundan (%5) daha fazla çeşitliliğe sahip olduğu belirlenmiştir. Ayrıca Türk populasyonunun stigma sayısı, tohum ve kapsül verimi bakımından düşük çeşitliliğe sahip olmasına rağmen, morfin içeriği, bitki boyu ve kapsül indeksi bakımından yüksek çeşitliliğe sahip olduğu belirlenmiştir. Haşhaş gen kaynaklarının tohum bankalarında ve ıslah programlarında etkin bir şekilde yönetilebilmesi için, dünya ve Türk haşhaş populasyonlarından sırasıyla 22 ve 21 adet haşhaş genotipinden oluşan iki adet çekirdek koleksiyon oluşturulmuştur. Bu bulgular Türk haşhaş populasyonunun, morfin içeriğini ve agronomik karakterleri kontol eden QTL'lerin ilişkilendirme haritalaması yaklaşımıyla belirlenebilmesi için değerli bir gen kaynağı olduğunu ortaya koymuştur. Bundan dolayı polimorfik bulunan 164 SSR ve 367 AFLP markörü iki yılda yetiştirilen ve 95 haşhaş genotipinden oluşan ilişkilendirme panelinde testlenmiştir. Sonuç olarak, bir SSR ve üç AFLP markörünün morfin içeriğiyle ($P < 0.01$ ve LD değeri (r^2) = 0.10-0.32), altı SSR ve 14 AFLP markörünün de beş adet agronomik karakterle (bitki boyu, stigma sayısı, kapsül indeksi, tohum ve kapsül verimi) ($P < 0.01$ ve LD değeri (r^2) = 0.08-0.35) ilişkili olduğu belirlenmiştir. İlişkilendirme haritalaması yaklaşımı bu çalışmada ilk defa haşhaşta uygulanmıştır. Belirlenen markörler, haşhaşta önemli karakterlerin markör destekli seleksiyon uygulamaları için ilk bilgileri sağlamaktadır.

To my Wife Gökcen ÇELİK

TABLE OF CONTENTS

LIST OF THE FIGURES xi

LIST OF TABLES xii

CHAPTER 1. INTRODUCTION 1
1.1. Opium Poppy as a Medicinal Plant.. 1
1.2. Origin and Distribution 1
1.3. Opium Poppy Breeding and Cultivation in Turkey 2
1.4. Crop Specific Genomic Tools.. 2
1.5. Opium Poppy Diversity 3
1.5.1. Importance of Diversity for Plant Breeding 3
1.5.2. Molecular Diversity 4
1.5.3. Diversity of Phytochemical Traits 4
1.5.4. Diversity of Agronomic Traits... 5
1.5.5. Plant Core Set Selection 5
1.6. Association Mapping Approach for Opium Poppy Breeding................ 6
1.7. Goals 7

CHAPTER 2. MATERIALS AND METHODS 8
2.1. Materials 8
2.1.1. Plant Materials for Validation of Genomic SSR Markers 8
2.1.2. Opium Poppy World Collection 10
2.1.3. Turkish Opium Poppy Germplasm 13
2.2. Methods 16
2.2.1. DNA Extraction 16
2.2.2. Molecular Marker Analysis 17
2.2.2.1. Marker Analysis for Validation of Genomic SSR markers….17
2.2.2.2. Marker Analysis of World Collection……….......……………17
2.2.2.3. Marker Analysis of Turkish Germplasm……………………17
2.2.3. Population Structure and Molecular Genetic Diversity Analysis... 18
2.2.4. Core Set Selection... 19

2.2.5. Morphological Trait Analysis 19
2.2.6. Association Mapping 20

CHAPTER 3. RESULTS 21
3.1. Validation of Genomic SSR Markers 21
3.1.1. Polymorphism of Genomic SSR markers 21
3.1.2. Genetic Diversity Revealed by Genomic SSR markers 23
3.2. Molecular Characterization of Opium Poppy World Collection 25
3.2.1. Population Structure 25
3.2.2. Genetic Diversity 28
3.2.3. Core Set Selection 29
3.3. Molecular and Morphological Characterization of Turkish Opium Poppy Accessions 30
3.3.1 Population Structure 30
3.3.2. Molecular Genetic Diversity 33
3.3.3. Morphological Trait Diversity 36
3.3.4. Core Set Selection 38
3.4. Association Mapping 42

CHAPTER 4. DISCUSSION 46
4.1. Validation of Genomic SSR Markers 46
4.1.1. Polymorphism of Genomic SSR Markers 46
4.1.2 Genetic Diversity Revealed by Genomic SSR Markers 47
4.2. Molecular Characterization of Opium Poppy World Collection 48
4.2.1. Population Structure 48
4.2.2. Genetic Diversity and Core Set Selection 48
4.3. Molecular and Morphological Characterization of Turkish Opium Poppy Accessions 49
4.3.1 Population Structure 49
4.3.2. Molecular Genetic Diversity 50
4.3.3. Morphological Trait Diversity 50
4.3.4. Core Set Selection 53
4.4. Association Mapping.. 53

CHAPTER 4. CONCLUSION 55

REFERENCES 56

LIST OF THE FIGURES

Figure **Page**

Figure 3.1. Unweighted neighbor-joining dendrogram of Turkish poppy accessions.. ……………………………………………………………24

Figure 3.2. Q-plot of world collection based on SSR markers……………………28

Figure 3.3. Hierarchical clustering of opium poppy world collection…………….29

Figure 3.4. Q-plot of Turkish germplasm based on genomic and EST SSR data... 30

Figure 3.5. Unweighted neighbor-joining dendrogram of the Turkish germplasm... 35

Figure 3.6. Principal component analysis (PCA) plot for morphological traits…..38

LIST OF TABLES

Table **Page**

Table 2.1. Opium poppy accessions and Papaver species used for validation of SSR markers……………………..……………………………………….. ..8
Table 2.2. Opium poppy accessions in world collection……………………………… 10
Table 2.3. Turkish opium poppy accessions……………………………………….... 13
Table 3.1. Polymorphism information content of genomic markers………………….. 22
Table 3.2. Subpopulation and cluster assignments of accessions in world collection………………………………………………………………25
Table 3.3. Subpopulation assignments of Turkish landraces and cultivars…………… 31
Table 3.4. Morphine content and morphological traits for the opium poppy landraces and cultivars……………………………………………….... 36
Table 3.5. Principal component analysis for morphological traits……………………. 37
Table 3.6. Genotypes of selected core sets…………………………………………… 39
Table 3.7. Means, SE, CV and range of the morphological traits for the landraces and the three core sets…………………………………………… 40
Table 3.8. Shannon diversity indices of the morphological data for all landraces and the three core sets……………………………………….... 40
Table 3.9. Average molecular genetic diversity for each core set……………………. 41
Table 3.10. SSR and AFLP locus pairs with significant LD………………………….. 42
Table 3.11. Association models…………………………………………………….. 43
Table 3.12. AFLP and SSR markers associated with morphine content and agronomic traits……………..…………………………………………43

CHAPTER 1

INTRODUCTION

1.1. Opium Poppy as a Medicinal Plant

Opium poppy (2n = 22, *Papaver somniferum* L.) from the Papaveraceae family is a self-pollinating angiosperms with a maximum of 37 % outcrossing due to insects (Patra et al. 1992; Acharya et al. 2009). The crop belongs to the Papaver genus which containing 11 sections classified based on morphological traits such as capsule characteristics (Bernath 2003). The crop is a major source of many pharmaceutically valuable benzylisoquinoline alkaloids with numerous medicinal properties, including analgesic and narcotic (morphine), antitumor (noscapine), antitussive (codeine) and muscle relaxant (papaverine) effects (Facchini and De Luca 2008; Ziegler et al. 2009; Winzer et al. 2012). Thus, it is an important medicinal crop for many poppy-growing countries such as Turkey, India and Bulgaria. In addition, poppy seeds and their oil are edible (Schulz et al. 2004).

1.2. Origin and Distribution

European archaeological excavations demonstrated that the crop was first cultivated in the Neolithic period (before 4000 B.C.); however, it was not domesticated until the 12th century in western Anatolia (Tétényi 1997). Although its center of origin is Irano-Anatolian region (Asia Minor), opium poppy is now distributed worldwide due to its adaptability to different climates. This trait is reportedly due to the aneuallopolyploid nature of its genome *P. omniferum* (2n = 22) which was derived from three species with x = 7 (Tétényi 1997; Lavania and Srivastava 1999).

1.3. Opium Poppy Breeding and Cultivation in Turkey

Turkey is a historically significant opium poppy producer with the Irano-Anatolian region accepted as the center of origin of this crop (TMO 2009; Tétényi 1997). Although half of the legal opium poppy production area in the world lies within Turkey (TMO 2009), the country ranks only third in morphine production (18%) due to the prevalence of low morphine cultivars. Efforts to improve alkaloid content have been hampered by a lack of information about the genetic diversity and breeding potential of Turkish germplasm (Gumuscu and Arslan 2008) as well as the difficulty of breeding for biochemical traits.

1.4. Crop Specific Genomic Tools

Molecular research in opium poppy has mainly involved non-specific markers such as amplified fragment length polymorphism (AFLP) (Saunders et al. 2001; Dittbrenner et al. 2008), random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) (Acharya and Sharma 2009; Parmaksiz and Ozcan 2011) markers. There is only one genetic linkage map for opium poppy constructed with 77 AFLP and 48 RAPD markers (Straka and Nothnagel 2002).

Recently, crop specific SSR markers were developed for opium poppy genome analysis. The first set of SSR (Simple Sequence Repeat) markers was reported by Selale et al. (2013). In this study, 2,147 SSR markers were developed from publicly available opium poppy specific expressed sequence tag (EST) sequences and 67 markers were tested on 37 accessions and seven Papaver species. The most comprehensive crop specific markers were reported in my Master of Science thesis (MSc) (Celik et al. 2014). In that study, 23,126 SSR markers were developed from 166,724 contigs representing 105 Mb of the genome generated by next generation pyrosequencing technology. Although amplification of 100 markers was tested in six accessions and seven *Papaver* species, the SSR markers were not further characterized for polymorphism. Thus, in the present study, a total of 53 genomic SSR markers were tested in 37 opium poppy accessions and seven *Papaver* species for determination of intra- and inter-specific polymorphism.

1.5. Opium Poppy Diversity

1.5.1. Importance of Diversity for Plant Breeding

Although high genetic diversity is essential for efficient plant breeding, the diversity of modern breeding materials decreased due to domestication and selection. Thus, conservation and utilization of plant genetic resources are essential for sustainable plant breeding (Sørensen et al. 2007; Friedt et al. 2007). Despite the high breeding value of modern cultivars, they need to be improved to meet the changing needs of both humans and the environment. Thus, fruit quality traits and tolerances to biotic and abiotic stresses have become more important (Friedt et al. 2007). Landraces, wild individuals and sub-species have major contributions to genetic diversity and have high genetic potential for resistance against abiotic and biotic stresses. Thus, such resources should be integrated into plant breeding programs to increase genetic diversity and for introgression of favorable alleles into elite cultivars (Tanksley and McCouch 1997).

The primary step for maintaining diversity is conservation of plant genotypes in seed banks and local collections (Tanksley and McCouch 1997; Zhou et al. 2014). As far as we know, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Seeland, Germany) and Anatolia Agricultural Research Institute (AARI) (Eskisehir, Turkey) are two institutes containing opium poppy world and Turkish collections, respectively (Dittbrenner et al. 2008). The next step is molecular and morphological characterization of the germplasm to assess genetic diversity and agronomic potential. While molecular characterization enables breeders to determine breeding strategies using the current gene pool or increasing the gene pool, morphological characterization provides information for efficient parental selection (Sørensen et al. 2007).

Molecular characterization involves the use of molecular marker systems such as SSR, AFLP and SNP (Single Nucleotide Polymorphism) to detect sequence variation (Indels or single nucleotide changes) (Sørensen et al. 2007). Molecular diversity is analyzed using two clustering methods (hierarchical and model-based clustering). In hierarchical clustering, a distance matrix is generated from marker scores using a coefficient such as DICE or Jaccard. Then, a dendrogram is constructed using algorithms such as UPGMA (Unweighted Pair Group Method with Arithmetic Mean) or NJ (Neighbor-Joining). In model-based clustering, the ancestry of the individuals is inferred

using a MCMC (Markov Chain Monte Carlo) algorithm based on the selected model (K, number of clusters) (Friedt et al. 2007). The best model representing the population is determined from ΔK values and a plot of Ln (probability of data) of repeated runs of each model (K from 1 to 10) (Dent and Bridgett 2012).

1.5.2. Molecular Diversity

To date, there are only a few molecular genetic diversity studies performed in opium poppy and most of these have been done with a limited number of accessions and non-specific markers such as AFLP, RAPD and ISSR (Saunders et al. 2001; Acharya and Sharma 2009; Parmaksiz and Ozcan 2011). Other studies performed by Dittbrenner et al. (2008) and Verma et al. (2016) were more comprehensive. While Dittbrenner et al. (2008) analyzed the genetic diversity of an opium poppy world collection containing 300 accessions with AFLP markers. Verma et al. (2016) characterized 95 Indian accessions with AFLP markers. All these studies revealed the low genetic diversity of opium poppy populations. Despite the importance of Turkey as the center of origin for the crop, Turkish germplasm has not been molecularly characterized to date. One of the goal of the present study was to assess the molecular diversity of Turkish opium poppy germplasm.

1.5.3. Diversity of Phytochemical Traits

Most opium poppy research focused on phytochemical traits such as morphine, codeine, thebaine, papaverine, and noscapine content. The most comprehensive phytochemical study was performed by Dittbrenner et al. (2008). In this study, major alkaloids (morphine, codeine, thebaine, papaverine, and noscapine) in the world collection containing 300 accessions were quantified by high performance liquid chromatography (HPLC). Prevalently, Indian and Turkish accessions were phytochemically characterized. In a different study, a total of 115 Indian opium poppy genotypes were characterized for eight alkaloids (papaverine, reticuline, narcotine, thebaine, codeinone, codeine, morphine and oripavine) (Prajapati et al. 2002). In another study, 122 Indian genotypes were characterized for five alkaloids (morphine, codeine, thebaine, narcotine and papaverine) (Shukla et al. 2010). Both studies indicated that

Indian genetic resources had considerable variation for phytochemical traits. Also Turkish landraces and cultivars have been examined for their phytochemical trait variation (Gumuscu and Arslan 1999; Arslan et al. 2000; Gumuscu et al. 2008). The largest study analyzed morphine content of 353 Turkish opium poppy accessions (Arslan et al. 2000). In other work, morphine content of 20 opium poppy cultivars was analyzed (Gumuscu and Arslan 1999). These studies demonstrated that Turkish opium poppy genetic resources had a high level of variation and phytochemical breeding value.

1.5.4. Diversity of Agronomic Traits

Seed and straw yield are the most important agronomic traits in the crop. Poppy seeds are edible and straw yield is directly related to morphine production because morphine is extracted from the poppy straw (Schulz et al. 2004). Other traits such as plant height, capsule index and stigma number are also related to morphine production. Plants with medium height and more leaves were more efficient opium producers and may have increased opium latex (Singh et al. 2004). In addition, opium poppy cultivars with medium height are more resistant to lodging and make straw harvesting easier (Singh et al. 2004). Stigma number is strongly correlated with morphine content (Trivedi et al. 2006). Seed and morphine yield are reported to be maximized in globular capsules (capsule index = 1) (Brezinová et al. 2009). The largest study was performed by Brezinová et al. (2009). In this study, an opium poppy world collection containing 404 accessions was evaluated for 22 agronomic traits. Also Gumuscu and Arslan (1999) characterized 20 Turkish opium poppy cultivars for four traits (flowering period, seed yield, capsule yield, and capsule number) and reported high levels of variation.

1.5.5. Plant Core Set Selection

Although seed banks and local plant collections have been constructed worldwide, managing them is difficult due to the large number of accessions. Thus, selection of a core collection to represent the overall genetic diversity as proposed by Frankel (1984) is essential for efficient conservation of genetic stocks. Core sets not only decrease the cost of seed banks but also increase plant breeding efficiency because core collections are

often more extensively morphologically and molecularly characterized than entire collections (Brown 1989; Van Hintum 2000).

1.6. Association Mapping Approach for Opium Poppy Breeding

Marker assisted selection (MAS) is a widely used method that facilitates crop selection and breeding. MAS must be integrated into opium poppy breeding for efficient improvement of complex biochemical and agronomic traits such as seed and straw yield. The first requirement for implementation of MAS in breeding is identification of quantitative trait loci (QTLs) for the characters of interest and linked molecular markers (Collard et al. 2005). Association mapping (AM), also called linkage disequilibrium or LD mapping, is a powerful strategy for identification of QTLs in plant genomes (Zhu et al. 2008). AM has higher resolution than mapping based on biparental populations because it takes advantage of recombination events that have accumulated during evolution and the resulting greater allelic diversity in natural plant germplasm (Gómez et al. 2011). Although AM has been extensively applied in major crop species, relatively little work has been done in non-model plants such as poppy. Some exceptions include work in teosinte, oat, peanut, loblolly pine and sesame that used LD to identify significant associations between molecular markers and agronomic traits (Weber et al. 2008; Achleitner et al. 2008; Wang et al. 2011; Eckert et al. 2012; Wei et al. 2013). Recently, after the publication of our results of AM, another AM study in opium poppy was reported by Verma et al. (2016). In this study, an AM panel containing 95 Indian accessions was tested with AFLP markers. As a result, 27 AFLP markers were found to be associated with six alkaloids (morphine, codeine, thebaine, narcotine, papaverine and opium yield). Unfortunately, AFLP markers cannot be directly used for MAS. The linked fragment must first be cloned and sequenced.

The main problem in AM is the occurrence of false positive associations due to population structure and the relatedness of individuals. Such factors are determined by the crop's mating system and breeding history (Stich et al. 2005; Semagn et al. 2010). False positive associations may be detected in opium poppy because it is self-pollinating with a low but significant amount of outcrossing (10 – 37 %) in the presence of insects (Patra et al. 1992). Detection of false positive associations can be corrected by using models such as the GLM (General Linear Model) and MLM (Mixed Linear Model) which

take into account population structure (Q), kindship matrix (K) and principal component (PC) information (Bradbury et al. 2007; Price et al. 2006; Yu et al. 2006). The association model with the best fit to the experimental data will have the lowest false discovery rate (FDR) (Akhatar et al. 2015).

1.7. Goals

The present thesis is composed of several goals to develop molecular breeding methods in opium poppy. The first aim was determination of intra- and inter-specific polymorphism of newly developed crop-specific genomic SSR markers. To achieve this aim, 53 genomic SSR markers were tested in 37 opium poppy accessions and seven *Papaver* species. Secondly, SSR markers (genic and genomic) were used for molecular characterization of two opium poppy germplasm sets: a world collection and Turkish germplasm. The morphological traits of the Turkish germplasm were also evaluated. The thesis also aimed to select core set for each population for efficient conservation of these plant genetic resources. Characterization of these resources will enable informed selection of parental lines for the development of opium poppy cultivars with improved alkaloid content and seed yield.

Another major goal of the thesis was to identify QTLs for morphine content and agronomic traits. To achieve this, molecular (SSR and AFLP markers) and morphological data (morphine content and agronomic traits) for the Turkish germplasm were associated to identify loci significantly associated with the traits using an AM approach. The identified markers provide initial information for marker-assisted selection of important traits in opium poppy breeding.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials for Validation of Genomic SSR Markers

A total of 37 opium poppy accessions from Turkey and seven *Papaver* species were used (Table 2.1). Eight opium poppy accessions were obtained from the Turkish Soil Products Office (TMO) and 29 accessions were obtained from the Anatolia Agricultural Research Institute (AARI), Eskisehir, Turkey. The related species were: *Papaver orientale* (Iran), *Papaver pseudoorientale* (Iran), *Papaver bracteatum* (Iran), *Papaver rhoeas* (Bulgaria), *Papaver umbonatum* (Turkey), *Papaver nudicaule* (Mongolia) and *Papaver armeniacum* (Armenia). These accessions were obtained from USDA-ARS Plant Germplasm Inspection Station, Beltsville MD, USA. Each accession was planted in seedling plates. Plants were grown in the greenhouse (24–25 °C, approximately 33 % humidity).

Table 2.1. Opium poppy accessions and *Papaver* species used for validation of SSR markers.

Name	Source	Location	Landrace/Cultivar/Species
1290	AARI	-	Cultivar
1061	AARI	-	Cultivar
1259	AARI	-	Cultivar
1065	AARI	-	Cultivar
Kemerkaya	AARI	-	Cultivar
Tınaztepe	AARI	-	Cultivar
Zaferyolu	AARI	-	Cultivar
Anayurt	AARI	-	Cultivar
Afyon95	AARI	-	Cultivar

(Cont. on the next page)

Table 2.1. (cont.)

Ofis3	TMO	-	Cultivar
Ofis4	TMO	-	Cultivar
Ofis8	TMO	-	Cultivar
Ofis95	TMO	-	Cultivar
TM01	TMO	-	Cultivar
TM02	TMO	-	Cultivar
TM03	TMO	-	Cultivar
TM04	TMO	-	Cultivar
7	AARI	Sandıklı, Alagöz, Afyon	Landrace
10	AARI	Çeltik, Burdur	Landrace
14	AARI	Sivas	Landrace
15	AARI	Koçyatağı, Şuhut, Afyon	Landrace
19	AARI	Dişli, Afyon	Landrace
22	AARI	Anayurt, Şuhut, Afyon	Landrace
32	AARI	Afyon	Landrace
33	AARI	Ekinhisar, Sandıklı, Afyon	Landrace
37	AARI	Höyüklü, Yalvaç, Isparta	Landrace
45	AARI	Simav, Kütahya	Landrace
59	AARI	Sülümenli, Afyon	Landrace
60	AARI	Koçyatağı, Afyon	Landrace
61	AARI	Şuhut, Afyon	Landrace
67	AARI	Alacami, Sandıklı, Afyon	Landrace
76	AARI	Göğen, Uşak	Landrace
89	AARI	Güre, Uşak	Landrace
92	AARI	Bolvadin, Afyon	Landrace
95	AARI	Acıpayam, Denizli	Landrace
96	AARI	Kütahya	Landrace
103	AARI	Çay, Afyon	Landrace
PI 229617	AARI	Iran	*P. orientale*
PI 381612	AARI	Iran	*P. pseudoorientale*
PI 414784	AARI	Iran	*P. bracteatum*
W6 10919	AARI	Bulgaria	*P. rhoeas*
W6 11444	AARI	Turkey	*P. umbonatum*
W6 18131	AARI	Mongolia	*P. nudicaule*
W6 23866	AARI	Armenia	*P. armeniacum*

2.1.2. Opium Poppy World Collection

An opium poppy population containing 62 landraces and 33 cultivars from 30 countries was obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Seeland, Germany) for molecular genetic analysis (Table 2.1). Seeds of each accession were germinated in seedling plates after cold treatment and were grown in the greenhouse under controlled conditions at a temperature of 24 - 25 °C and 33% relative humidity.

Table 2.2. Opium poppy accessions in world collection.

Genotype	Country of collection	Continent	Landrace/Cultivar
PAP152	Morocco	Africa	Cultivar
PAP775	Poland	Eastern Europe	Cultivar
PAP862	Bulgaria	Southeast Europe	Cultivar
PAP892	Czech Republic	Central Europe	Cultivar
PAP586	Slovakia	Central Europe	Landrace
PAP696	Spain	Western Europe	Landrace
PAP733	Vietnam	Southeast Asian	Landrace
PAP749	Portugal	Southern Europe	Landrace
PAP760	Italy	Southern Europe	Landrace
PAP766	Korean	East Asia	Landrace
PAP815	China	East Asia	Landrace
PAP833	France	Western Europe	Landrace
PAP1050	Hungary	Central Europe	Cultivar
PAP200	Japan	East Asia	Cultivar
PAP246	Ukraine	Eastern Europe	Cultivar
PAP328	Bulgaria	Southeast Europe	Cultivar
PAP452	United Kingdom	northwestern Europe	Cultivar
PAP773	Denmark	Scandinavia	Cultivar
PAP776	Poland	Eastern Europe	Cultivar
PAP859	Former USSR	Former USSR	Cultivar
PAP873	Czech Republic	Central Europe	Cultivar
PAP879	Former USSR	Former USSR	Cultivar
PAP881	Germany	Western Europe	Cultivar

(Cont. on the next page)

Table 2.2. (cont.)

PAP889	Poland	Eastern Europe	Cultivar
PAP891	Former USSR	Former USSR	Cultivar
PAP937	Hungary	Central Europe	Cultivar
PAP943	Hungary	Central Europe	Cultivar
PAP144	Netherlands	Western Europe	Landrace
PAP160	Yugoslavia	Southeast Europe	Landrace
PAP167	China	East Asia	Landrace
PAP336	Switzerland	Central Europe	Landrace
PAP337	Switzerland	Central Europe	Landrace
PAP457	Germany	Western Europe	Landrace
PAP496	Germany	Western Europe	Landrace
PAP499	France	Western Europe	Landrace
PAP506	Germany	Western Europe	Landrace
PAP511	Spain	Western Europe	Landrace
PAP513	Germany	Western Europe	Landrace
PAP518	Belgium	Western Europe	Landrace
PAP556	Netherlands	Western Europe	Landrace
PAP584	Slovakia	Central Europe	Landrace
PAP585	Slovakia	Central Europe	Landrace
PAP630	Vietnam	Southeast Asian	Landrace
PAP656	Bulgaria	Southeast Europe	Landrace
PAP657	Bulgaria	Southeast Europe	Landrace
PAP719	Kazakhstan	Central Asia	Landrace
PAP748	Portugal	Southern Europe	Landrace
PAP750	Vietnam	Southeast Asian	Landrace
PAP757	Italy	Southern Europe	Landrace
PAP758	Italy	Southern Europe	Landrace
PAP764	Korean	East Asia	Landrace
PAP765	Korean	East Asia	Landrace
PAP767	Korean	East Asia	Landrace
PAP786	Romania	Central Europe	Landrace
PAP791	Romania	Central Europe	Landrace
PAP831	Spain	Western Europe	Landrace
PAP848	Finland	Western Europe	Landrace
PAP865	Romania	Central Europe	Landrace
PAP883	Japan	East Asia	Landrace
PAP920	Poland	Eastern Europe	Landrace
PAP928	Austria	Central Europe	Landrace

(Cont. on the next page)

Table 2.2. (cont.)

PAP938	Austria	Central Europe	Landrace
PAP939	Austria	Central Europe	Landrace
PAP945	Germany	Western Europe	Landrace
PAP946	Germany	Western Europe	Landrace
PAP114	Sweden	Scandinavia	Cultivar
PAP151	Morocco	Africa	Cultivar
PAP169	Morocco	Africa	Cultivar
PAP327	Bulgaria	Southeast Europe	Cultivar
PAP49	Netherlands	Western Europe	Cultivar
PAP761	Japan	East Asia	Cultivar
PAP816	USA	USA	Cultivar
PAP857	France	Western Europe	Cultivar
PAP860	Czech Republic	Central Europe	Cultivar
PAP905	Czech Republic	Central Europe	Cultivar
PAP910	Czech Republic	Central Europe	Cultivar
PAP932	Hungary	Central Europe	Cultivar
PAP934	Netherlands	Western Europe	Cultivar
PAP935	Former USSR	Former USSR	Cultivar
PAP180A	Mongolia	East Asia	Landrace
PAP332	France	Western Europe	Landrace
PAP64	Australia	Australia	Landrace
PAP663	Slovakia	Central Europe	Landrace
PAP674	Spain	Western Europe	Landrace
PAP714	Poland	Eastern Europe	Landrace
PAP715	Belgium	Western Europe	Landrace
PAP734	Vietnam	Southeast Asian	Landrace
PAP742	Italy	Southern Europe	Landrace
PAP746	Portugal	Southern Europe	Landrace
PAP747	Portugal	Southern Europe	Landrace
PAP768	Korean	East Asia	Landrace
PAP792	Romania	Central Europe	Landrace
PAP872	Germany	Western Europe	Landrace
PAP888	Former USSR	Former USSR	Landrace
PAP930	Austria	Central Europe	Landrace

2.1.3. Turkish Opium Poppy Germplasm

A total of 103 opium poppy landraces collected from eight cities (Afyon, Burdur, Usak, Eskisehir, Isparta, Denizli, Kutahya, Sivas) in the central Anatolian and southern Aegean regions of Turkey were obtained from the Anatolia Agricultural Research Institute (AARI) (Table 2.3). In addition, eight and seven cultivars were obtained from the Turkish Soil Products Office (TMO) and AARI, respectively. TMO and AARI are responsible for all opium poppy breeding in Turkey. *P. umbonatum* was used as outgroup and was obtained from the U.S. Department of Agriculture, Agricultural Research Service Plant Germplasm Inspection Station, Beltsville MD, USA. The opium poppy landraces were grown in the field in Eskisehir during the 2011 and 2012 growing seasons.

Table 2.3. Turkish opium poppy accessions used for molecular and morphological characterization.

Genotype	Location of collection	Landrace/Cultivar
3	Alacami, Sandikli, Afyon	Landrace
5	Celtik, Burdur	Landrace
6	Alagoz, Sandikli, Afyon	Landrace
7	Alagoz, Sandikli, Afyon	Landrace
8	Alacami, Sandıklı, Afyon	Landrace
9	Cobanlar, Afyon	Landrace
10	Celtik, Burdur	Landrace
11	Afyon	Landrace
13	Yalvachoyuklu, Isparta	Landrace
14	Sivas	Landrace
15	Kocyatagi, Suhut, Afyon	Landrace
16	Ulubey, Camlibel, Usak	Landrace
17	Kocyatagi, Suhut, Afyon	Landrace
18	Isparta	Landrace
19	Disli, Afyon	Landrace
20	Bolvadin, Afyon	Landrace
21	Kocyatagi, Suhut, Afyon	Landrace
22	Anayurt, Suhut, Afyon	Landrace
23	Afyon	Landrace
24	Alagoz, Sandikli, Afyon	Landrace

(Cont. on the next page)

Table 2.3. (cont.)

25	Kocyatagi, Suhut, Afyon	Landrace
26	Deresenek, Afyon	Landrace
27	Banaz, Usak	Landrace
28	Kocyatagi, Suhut, Afyon	Landrace
29	Yukarisogut, Eskisehir	Landrace
30	Isabey, Cal, Denizli	Landrace
31	Karayahsiler, Civril, Denizli	Landrace
32	Afyon	Landrace
33	Ekinhisar, Sandikli, Afyon	Landrace
35	Kocyatagi, Suhut, Afyon	Landrace
36	Sivas	Landrace
37	Yalvachoyuklu, Isparta	Landrace
39	Kocyatagi, Suhut, Afyon	Landrace
40	Isparta	Landrace
41	Kocyatagi, Suhut, Afyon	Landrace
43	Celtik, Burdur	Landrace
45	Simav, Kutahya	Landrace
46	Celtik, Burdur	Landrace
48	Karayahsiler, Civril, Denizli	Landrace
49	Cevrekoyu, Usak	Landrace
50	Mamat, Suhut, Afyon	Landrace
51	Civril, Karayahsiler, Denizli	Landrace
52	Yukarısogutonu, Eskisehir	Landrace
53	Civril, Denizli	Landrace
54	Erice, Usak	Landrace
55	Ekinhisar, Sandikli, Afyon	Landrace
56	Deresenek, Afyon	Landrace
57	Sivas	Landrace
59	Sulumenli, Afyon	Landrace
60	Kocyatagi, Afyon	Landrace
62	Sulumenli, Afyon	Landrace
63	Hoyuklu, Yalvac, Isparta	Landrace
65	Cobanlar, Afyon	Landrace
66	Cobanlar, Afyon	Landrace
67	Alacami,Sandikli, Afyon	Landrace
68	Alacami,Sandikli, Afyon	Landrace
69	Sivas	Landrace
70	Cobanlar, Afyon	Landrace

(Cont. on the next page)

Table 2.3. (cont.)

72	Kocyatagi, Suhut, Afyon	Landrace
73	Sulumenli, Afyon	Landrace
74	Sulumenli, Afyon	Landrace
75	Afyon	Landrace
76	Gogen, Uşak	Landrace
77	Disli, Afyon	Landrace
78	Alacami, Sandikli, Afyon	Landrace
79	Emet, Hisarcik, Kutahya	Landrace
80	Cobanlar, Afyon	Landrace
82	Disli, Afyon	Landrace
83	Kocyatagi, Suhut, Afyon	Landrace
84	Cobanlar, Afyon	Landrace
85	Alacami, Sandikli, Afyon	Landrace
86	Kocyatagi, Suhut, Afyon	Landrace
87	Kocyatagi, Suhut, Afyon	Landrace
88	Kocyatagi, Suhut, Afyon	Landrace
89	Gure, Usak	Landrace
91	Kocyatagi, Suhut, Afyon	Landrace
92	Bolvadin, Afyon	Landrace
93	Suhut, Afyon	Landrace
94	Acipayam, Denizli	Landrace
95	Acipayam, Denizli	Landrace
96	Kutahya	Landrace
97	Suhut, Afyon	Landrace
99	Anayurt, Suhut, Afyon	Landrace
101	Anayurt, Suhut, Afyon	Landrace
102	Deresenek, Afyon	Landrace
103	Cay, Afyon	Landrace
104	Mahmut, Suhut, Afyon	Landrace
105	Kocyatagi, Suhut, Afyon	Landrace
106	Anayurt, Suhut, Afyon	Landrace
107	Acipayam, Denizli	Landrace
108	Anayurt, Suhut, Afyon	Landrace
109	Acipayam, Denizli	Landrace
110	Deresenek, Afyon	Landrace
111	Suhut, Afyon	Landrace
112	Sulumenli, Afyon	Landrace
113	Arizlar, Sandikli, Afyon	Landrace

(Cont. on the next page)

Table 2.3. (cont.)

114	Sulumenli, Afyon	Landrace
116	Deresenek, Afyon	Landrace
117	Deresenek, Afyon	Landrace
118	Anayurt, Suhut, Afyon	Landrace
119	Eber, Afyon	Landrace
120	Anayurt, Suhut, Afyon	Landrace
122	Anayurt, Suhut, Afyon	Landrace
1061	-	Cultivar
1065	-	Cultivar
Afyon95	-	Cultivar
Anayurt	-	Cultivar
Kemerkaya	-	Cultivar
Tinaztepe	-	Cultivar
Zaferyolu	-	Cultivar
Ofis3	-	Cultivar
Ofis4	-	Cultivar
Ofis8	-	Cultivar
Ofis95	-	Cultivar
Ofis96	-	Cultivar
Tmo1	-	Cultivar
Tmo2	-	Cultivar
Tmo3	-	Cultivar

2.2. Methods

2.2.1. DNA Extraction

Genomic DNA was isolated from fresh leaf tissue bulked from ten (for Turkish germplasm) and three plants (for world collection) per accession using a CTAB method (Doyle and Doyle 1990).

2.2.2. Molecular Marker Analysis

2.2.2.1. Marker Analysis for Validation of Genomic SSR markers

Amplification of the opium poppy DNA with genomic SSR primers was carried out in 25 µL reaction mixtures containing 1X PCR buffer, 3 mM MgCl, 0.125 nM deoxyribo-nucleotide triphosphates (dNTPs), 1 U Taq Polymerase, 2 pmol forward and reverse primers and 80 ng template DNA. The PCR profile was: one step of 5 min at 94°C; 35 cycles with 45 sec at 94°C, 1 min at 55 °C for annealing, 1 min at 72°C; and a final extension step of 5 min at 72°C. To prepare PCR product for analysis by capillary electrophoresis, 3 µl of the PCR product was added to 27 µl sample loading buffer (Beckman). In all runs, 0.5 µl 600 bp size standard (Beckman) was used per reaction. Finally the mixture for each accession was run on a Beckman CEQ8800 capillary electrophoresis device using the frag3 method (capillary temperature 50 °C, denaturation at 90 °C for 120 s, injection voltage 2.0 kV for 3 s, separation voltage 4.8 kV for 60 min).

2.2.2.2. Marker Analysis of World Collection

A total of 25 SSR markers (6 EST and 19 genomic SSRs) were tested in the 95 accessions according to the amplification protocols of Selale et al. (2013) and Celik et al. (2014). PCR fragments were analyzed using a Fragment Analyzer^{TM} (Advanced Analytical Technologies, Inc.) with a DNF-900 dsDNA Reagent Kit (Advanced Analytical) and appropriate method (sample injection voltage 7.5 kV for 15 s, separation voltage 8 kV for 80 min).

2.2.2.3. Marker Analysis of Turkish Germplasm

For molecular genetic diversity analysis, 13 EST SSR markers (Selale et al. 2013) and 33 genomic SSR markers (Celik et al. 2014) were tested in the 118 *P. somniferum* accessions and *P. umbonatum* according to the amplification protocols in the respective publications.

For association mapping, both SSR and AFLP markers were assayed. In addition to the SSRs described above, a further 34 SSR markers (Selale et al. 2013; Celik et al. 2014) were tested on the panel to increase genome coverage. Thus, a total of 80 SSR markers were used which consisted of 14 EST SSR and 66 genomic SSR markers. Amplified PCR fragments were separated using a Fragment Analyzer^{TM} (Advanced Analytical Technologies, Inc.) with a DNF-900 dsDNA Reagent Kit (Advanced Analytical) and appropriate method (sample injection voltage 7.5 kV for 15 s, separation voltage 8 kV for 80 min).

AFLP markers were also used in AM to increase genome coverage. For AFLP analysis, 10 primer combinations (E-ACT + M-CAG, E-AAC + M-CTT, E-AAC + M-CTC, E-ACG + M-CAA, E-ACC + M-CAC, E-AGC + M-CTA, E-ACA + M-CTG, E-ACC + M-CAG, E-ACA + M-CAG, E-AGG + M-CAT) were used in selective amplification using the AFLP Core Reagent and AFLP Starter Primer Kits from Invitrogen (Carlsbad, CA) according to the manufacturer's protocol. AFLP primers were labelled with blue fluorescent dye for separation in the CEQ8800 Sequencer (Beckman-Coulter, Fullerton, CA). The default Frag 4 separation method was used: capillary temperature 50°C, denaturation temperature 90°C for 120 s, injection voltage 2.0 kV for 30 s, and separation voltage of 4.8 kV for 60.0 min.

2.2.3. Population Structure and Molecular Genetic Diversity Analysis

Amplified SSR loci were scored as present (1) or absent (0). Rare PCR fragments (those that occurred at less than 10% frequency) were potentially unreliable and excluded from analysis. The binary data generated by EST and genomic SSR markers were analyzed for population structure with the computer program Structure (Pritchard et al. 2000a) which uses a model-based Bayesian method to assign accessions to subpopulations. To find the best model representing population structure, the program performs calculations using different models (K= 1 to 10, where K = number of subpopulations) after 50,000 burn-in cycles. Each model was tested 20 times with 100,000 iterations per K. The population structure results were analyzed with the Structure Harvester program (Dent and Bridgett 2012) to find the best model with the highest ΔK value. Thresholds of ≥0.70 and ≥0.80 inferred ancestry were used to assign accessions to subpopulation clusters. Accessions that did not meet the threshold value

were considered to be admixed. For hierarchical clustering, a dissimilarity matrix generated using the Dice coefficient was used to construct a dendrogram with the unweighted neighbor joining method as implemented by the DARwin computer program. The correlation between the dissimilarity matrix and the dendrogram was calculated using a Mantel test as provided in the DARwin computer program.

2.2.4. Core Set Selection

A core set for each population was selected with PowerCore 1.0 software (Kim et al. 2007) which uses the M strategy and a modified heuristic algorithm.

2.2.5. Morphological Trait Analysis

The opium poppy landraces were grown in the field in Eskisehir during the 2011 and 2012 growing seasons. Plants were in 5 m long, three-row plots with a row spacing of 0.45 cm. The plants were fertilized with 6 kg ha^{-1} phosphorus and 6 kg ha^{-1} nitrogen. Morphine content of landraces was measured by HPLC (high-performance liquid chromatography). A total of 50 g poppy straw (capsule without seed) bulked from several plants per accession was ground with activated acidic Al_2O_3 for 30 minutes. The filtered solution was used for HPLC analysis with mobile phase containing 1% TFA in water-acetonitrile-methanol (46:40:14, v/v). These results were used to calculate the percentage of morphine content in straw.

Morphological traits were measured on 10 randomly selected plants of each accession and included: height (cm), stigma number, capsule index, and seed and straw yield. Capsule index was calculated by dividing capsule height by capsule diameter. Seed and straw yield were measured as gram per plot and then converted to kg per hectare.

The two years' data were averaged for each trait. In addition, means and coefficients of variation for cultivars and landraces were calculated separately for comparison. Principal component analysis (PCA) was performed with DARwin and PASW software (Norusis 2010). Basic statistics such as correlation analysis between traits and paired sample Student's t-tests were performed using PASW software.

2.2.6. Association Mapping

The binary data generated for the SSR and AFLP markers assayed on the panel of 93 poppy accessions were used for AM of morphine content and agronomic traits using the GLM and MLM models of TASSEL v2.1 (Trait Analysis by aSSociation, Evolution and Linkage software) software (Bradbury et al. 2007). Linkage disequilibrium (LD) values (r^2 and *P*-values) between SSR and AFLP loci were calculated using TASSEL v2.1 software. Different models were tested to determine the model with the best fit for AM analysis. Analysis was first performed using the GLM model without any correction. Then the GLM model was corrected with: the Q-matrix of population structure [GLM (Q)], principal components (PC) [GLM (PC)] and both Q and PC [GLM (Q+PC)]. Similarly, the MLM model corrected with kindship matrix (K) [MLM (K)] was used and further corrected with: the Q-matrix [MLM (K+Q)], principal components [MLM (K+PC)] and both [MLM (K+Q+PC)] The Q-matrix generated at K=2 (subgroup number=2) was used as covariate. Principal components (PC) were calculated in TASSEL software. To determine the best model, the *P* values generated by the eight models were analyzed with QVALUE (Storey 2002) software using a false discovery rate (FDR) of 0.05 (Storey and Tibshirani 2003). Bootstrap analysis was performed to calculate $\pi 0$ (%) which indicates the probability that a given hypothesis is truly null, and $\pi 1$ (%) [100 - $\pi 0$ (%)] which represents the probability of significant results. The model with the highest $\pi 1$ value was accepted as the one with the best fit and its results are reported herein. Marker trait associations with *P*-values lower than 0.01 were selected as significant associations.

CHAPTER 3

RESULTS

3.1. Validation of Genomic SSR Markers

3.1.1. Polymorphism of Genomic SSR markers

A total of 100 genomic SSR primer pairs were first tested on five opium poppy accessions (1290, 1061, 1259, 1065, Kemerkaya). Of these primers, 96 (96%) amplified products. A total of 53 genomic SSR markers which showed clear amplification on agarose gel, were then tested in 37 opium poppy accessions and seven *Papaver* species for determination of polymorphism (Table 3.1). Seventeen of the opium poppy accessions were named varieties while the others were landraces collected in Turkey. The 53 SSR primers generated 209 polymorphic fragments in all accessions and 90 polymorphic fragments in *P. somniferum* accessions. The average number of amplified fragments per genomic SSR marker was 5 ± 0.01 (SE) with a range of 1 to 13 fragments. A total of 48 SSR primers (95%) were polymorphic in all accessions with average fragment polymorphism of 84%. For all accessions, average polymorphism information content (PIC) values ranged from 0.05 for psgSSR076 to 0.47 for psgSSR022 with an average PIC of 0.19. Fewer (60.4%) SSR primers showed intraspecific polymorphism in *P. somniferum* with an average fragment polymorphism of 63%. The average intraspecific polymorphism information content (PIC) value decreased to 0.17. Intraspecific PIC values ranged from 0.05 for five different markers to 0.49 for psgSSR22 (Table 3.1). In all analyses there was no significant correlation between PIC values and SSR motif types or lengths.

Table 3.1. Polymorphism information content of genomic markers. * Markers that amplified more than one locus in *P. somniferum*.

SSR marker	Repeat motif	All accessions		*P.somniferum*	
		#Poly./total # fragments (%)	Average PIC ± SE	#Poly./total # fragments (%)	Average PIC ± SE
psgSSR002*	$(ATG/TAC)_4$	5/7 (71)	0.12 ± 0.02	1/7 (14)	0.06
psgSSR005	$(CATCTG/GTAGC)_3$	2/2 (100)	0.21 ± 0.01	2/2 (100)	0.21 ± 0.02
psgSSR006	$(AACA/TTGT)_3$	4/5 (80)	0.31 ± 0.06	4/5 (80)	0.25 ± 0.09
psgSSR008*	$(AAG/TTC)_8$	8/8 (100)	0.28 ± 0.05	6/8 (75)	0.20 ± 0.08
psgSSR013*	$(ATA/TAT)_4$	13/13 (100)	0.18 ± 0.01	1/8 (13)	0.05
psgSSR015	$(CAG/GTC)_4$	5/6 (83)	0.17 ± 0.03	0/5 (0)	0
psgSSR021	$(TA/AT)_6$	3/3 (100)	0.15 ± 0.09	3/3 (100)	0.15 ± 0.10
psgSSR022	$(TGG/ACC)_4$	1/3 (33)	0.47	1/2 (50)	0.49
psgSSR023*	$(TGTCA/ACAGT)_3$	7/7 (100)	0.29 ± 0.05	5/7 (71)	0.22 ± 0.09
psgSSR024	$(TTC/AAG)_6$	9/9 (100)	0.16 ± 0.03	3/5 (60)	0.1 ± 0.03
psgSSR027	$(TA/AT)_6$	1/3 (33)	0.20	0/2 (0)	0
psgSSR029	$(TCAT/AGTA)_3$	0/2 (0)	0	0/2 (0)	0
psgSSR030	$(AACA/TTGT)_3$	1/1 (100)	0.13	1/1 (100)	0.10
psgSSR033	$(CAAA/GTTT)_3$	3/4 (75)	0.22 ± 0.02	1/3 (33)	0.10
psgSSR034	$(TGG/ACC)_4$	4/5 (80)	0.21 ± 0.09	2/3 (67)	0.27 ± 0.22
psgSSR036	$(CCAA/GGTT)_3$	1/3 (33)	0.20	0/6 (0)	0
psgSSR037*	$(GAA/CTT)_{10}$	9/9 (100)	0.20 ± 0.06	5/9 (56)	0.25 ± 0.08
psgSSR038	$(TGAT/ACTA)_3$	6/7 (86)	0.24 ± 0.04	2/4 (50)	0.23 ± 0
psgSSR039*	$(ACAAC/TGTTG)_4$	11/11 (100)	0.21 ± 0.02	5/10 (50)	0.18 ± 0.01
psgSSR040	$(TGT/ACA)_4$	3/4(75)	0.18 ± 0.03	0/3 (0)	0
psgSSR041*	$(TCTTA/AGAAT)_3$	10/10 (100)	0.29 ± 0.03	10/10 (100)	0.13 ± 0.04
psgSSR042	$(TTCA/AAGT)_4$	5/5 (100)	0.16 ± 0.03	0/4 (0)	0
psgSSR046	$(TGAT/ACTA)_3$	3/4 (75)	0.17 ± 0.07	2/4 (50)	0.19 ± 0
psgSSR047	$(AGA/TCT)_4$	5/5 (100)	0.19 ± 0.08	2/5 (40)	0.30 ± 0.20
psgSSR050	$(GAA/CTT)_4$	0/3 (0)	0	0/3 (0)	0
psgSSR053	$(TA/AT)_7$	2/5 (40)	0.12 ± 0.08	1/5 (20)	0.05
psgSSR054	$(TCGT/AGCA)_3$	4/4 (100)	0.13 ± 0.02	0/2 (0)	0
psgSSR055	$(TC/AG)_7$	3/5 (60)	0.13 ± 0.05	0/4 (0)	0
psgSSR058	$(AATA/TTAT)_3$	1/2 (50)	0.13	0/2 (0)	0
psgSSR059	$AAAT/TTTA)_3$	5/6 (83)	0.20 ± 0.02	0/1 (0)	0

(Cont. on the next page)

Table 3.1. (cont.)

psgSSR060	$(TCT/AGA)_4$	2/3 (67)	0.30 ± 0.17	1/2 (50)	0.44
psgSSR061	$(AAAT/TTTA)_3$	3/3(100)	0.27 ± 0.02	3/3 (100)	0.1 ± 0.05
psgSSR062	$(AGAC/TCTG)_3$	2/3 (67)	0.07 ± 0.02	0/3 (0)	0
psgSSR064	$(ATA/TAT)_4$	1/1(100)	0.20	1/1 (100)	0.05
psgSSR067*	$(TTCT/AAGA)_3$	9/9 (100)	0.23 ± 0.02	3/7 (43)	0.19 ± 0.07
psgSSR068	$(TTCT/AAGA)_3$	0/1 (0)	0	0/1 (0)	0
psgSSR069	$(CAAT/GTTA)_4$	5/6 (83)	0.20 ± 0.02	0/5 (0)	0
psgSSR070	$(ATT/TAA)_5$	2/2 (100)	0.22 ± 0.05	0/1 (0)	0
psgSSR071	(TATTC/ATAAG)	2/2 (100)	0.13 ± 0.00	0/2 (0)	0
psgSSR074*	$(GTTT/CAAA)_3$	12/12 (100)	0.22 ± 0.03	11/11 (100)	0.19 ± 0.03
psgSSR076	$(AATG/TTAC)_3$	1/1 (100)	0.05	1/1(100)	0.05
psgSSR077	$(ATC/TAG)_4$	5/5 (100)	0.14 ± 0.04	1/4 (25)	0.05
psgSSR078	$(GTATT/CATAA)_3$	3/4 (75)	0.15 ± 0.02	1/3 (33)	0.15
psgSSR079	$(GGAA/CCTT)_3$	0/2 (0)	0	0/2 (0)	0
psgSSR080	$(GGAA/CCTT)_3$	6/6 (100)	0.29 ± 0.05	4/4 (100)	0.29 ± 0.08
psgSSR082	$(AAT/TTA)_4$	1/2(50)	0.20	0/2 (0)	0
psgSSR085	$(ATTT/TAAA)_3$	5/5 (100)	0.18 ± 0.04	1/4 (25)	0.1
psgSSR90	$(TGT/ACA)_4$	5/6(83)	0.16 ± 0.04	0/2	0
psgSSR093	$(AAT/TTA)_4$	2/2 (100)	0.07 ± 0.02	2/2 (100)	0.05 ± 0
psgSSR094	$(GCA/CGT)_4$	3/3 (100)	0.28 ± 0.03	3/3 (100)	0.15 ± 0.04
psgSSR098	$(TGTTG/ACAAC)_3$	0/1 (0)	0	0/1 (0)	0
psgSSR099	$(ATC/TAG)_4$	2/4 (50)	0.22 ± 0.05	0/2 (0)	0
psgSSR100	$(GTG/CAC)_4$	4/5 (80)	0.14 ± 0.04	1/4 (25)	0.15

3.1.2. Genetic Diversity Revealed by Genomic SSR markers

Low frequency fragments (observed in less than 10% of the poppy accessions) were excluded from all analyses because these low frequency fragments can be unreliable. A total of 209 high quality, reproducible polymorphic fragments were used for diversity analysis of opium poppy and related species. The binary presence/absence data were used to generate a distance matrix using the Dice coefficient to draw a dendrogram with the unweighted Neighbor-joining algorithm. As expected, a Mantel test showed a strong correlation (r = 0.998) between the distance matrix and dendrogram. Dissimilarity between accessions ranged from 0.008 to 0.48 (52% similarity) with average dissimilarity

of 0.14 (Figure 3.1). Dissimilarity between opium poppy and related species ranged from 0.23 to 0.48. As expected, *Papaver* species clustered separately from *P. somniferum* accessions. The *P. somniferum* accessions fell into three clusters. Cluster 1 contained 13 landraces and 13 varieties/breeding lines. In Cluster 1, dissimilarity ranged from 0.01 to 0.08 with average dissimilarity of 0.04. Cluster 1 had six subclusters (subclusters A to F). Cluster 1B contained only named varieties and cluster 1C contained only landraces of *P. somniferum*. Varieties and landraces were intermixed in the other subclusters. Cluster 2 contained eight opium poppy accessions (four landraces and four varieties). Average dissimilarity of Cluster 2 was 0.06 with minimum and maximum dissimilarities of 0.03 and 0.16, respectively. Cluster 3 was comprised of three opium poppy landraces (59, 22 and 76) which were the most genetically distinct opium poppy accessions in the study.

Figure 3.1. Unweighted neighbor-joining dendrogram of Turkish poppy accessions constructed by genomic SSR markers.

3.2. Molecular Characterization of Opium Poppy World Collection

3.2.1. Population Structure

Population structure of the opium poppy world collection containing 95 accessions was determined using 107 fragments generated by the 25 EST and genomic SSR markers. The results showed that the data were best represented by two subpopulations (K = 2) (Figure 3.2). A total of 42 (44.2%) accessions were assigned to subpopulations. Subpopulation A contained 12 accessions (8 landraces and 4 cultivars). Subpopulation B contained 30 accessions (16 landraces and 14 cultivars). Based on a membership threshold of 70 %, 53 accessions (55.8 %) (38 landraces and 15 cultivars) had admixed ancestry (Table 3.2). When the membership threshold was increased to 80%, none of the accessions could be assigned to any subpopulation as all of them fell into the admixed category. Clustering based on region or accession type (landrace or cultivar) was not observed.

Table 3.2. Subpopulation and cluster assignments of accessions in world collection.

				Inferred ancestry			
Genotype	Country	Continent	Landrace/CV.	Subpop. A	Subpop. B	Structure Assign.	DARwin Assign.
PAP152*	Morocco	Africa	Cultivar	0.732	0.268	A	Cluster 1
PAP775*	Poland	Eastern Europe	Cultivar	0.722	0.278	A	Cluster 1
PAP862*	Bulgaria	Southeast Europe	Cultivar	0.752	0.248	A	Cluster 1
PAP892*	Czech Republic	Central Europe	Cultivar	0.72	0.28	A	Cluster 1
PAP586*	Slovakia	Central Europe	Landrace	0.777	0.223	A	Cluster 1
PAP696*	Spain	Western Europe	Landrace	0.722	0.278	A	Cluster 2
PAP733*	Vietnam	Southeast Asian	Landrace	0.743	0.257	A	Cluster 1
PAP749*	Portugal	Southern Europe	Landrace	0.76	0.24	A	Cluster 1
PAP760*	Italy	Southern Europe	Landrace	0.711	0.289	A	Cluster 2
PAP766	Korean	East Asia	Landrace	0.703	0.297	A	Cluster 1
PAP815	China	East Asia	Landrace	0.755	0.245	A	Cluster 1
PAP833*	France	Western Europe	Landrace	0.749	0.251	A	Cluster 1
PAP1050	Hungary	Central Europe	Cultivar	0.593	0.407	admixed	Cluster 1
PAP200	Japan	East Asia	Cultivar	0.668	0.332	admixed	Cluster 1
PAP246	Ukraine	Eastern Europe	Cultivar	0.627	0.373	admixed	Cluster 1

(Cont. on the next page)

Table 3.2. (cont.)

PAP328	Bulgaria	Southeast Europe	Cultivar	0.626	0.374	admixed	Cluster 1
PAP452	United Kingdom	northwestern Europe	Cultivar	0.563	0.437	admixed	Cluster 1
PAP773	Denmark	Scandinavia	Cultivar	0.344	0.656	admixed	Cluster 1
PAP776	Poland	Eastern Europe	Cultivar	0.585	0.415	admixed	Cluster 1
PAP859*	Former USSR	Former USSR	Cultivar	0.404	0.596	admixed	Cluster 2
PAP873*	Czech Republic	Central Europe	Cultivar	0.675	0.325	admixed	Cluster 1
PAP879	Former USSR	Former USSR	Cultivar	0.385	0.615	admixed	Cluster 1
PAP881	Germany	Western Europe	Cultivar	0.421	0.579	admixed	Cluster 1
PAP889	Poland	Eastern Europe	Cultivar	0.347	0.653	admixed	Cluster 1
PAP891	Former USSR	Former USSR	Cultivar	0.45	0.55	admixed	Cluster 1
PAP937	Hungary	Central Europe	Cultivar	0.339	0.661	admixed	Cluster 1
PAP943	Hungary	Central Europe	Cultivar	0.466	0.534	admixed	Cluster 2
PAP144	Netherlands	Western Europe	Landrace	0.391	0.609	admixed	Cluster 2
PAP160*	Yugoslavia	Southeast Europe	Landrace	0.359	0.641	admixed	Cluster 1
PAP167*	China	East Asia	Landrace	0.44	0.56	admixed	Cluster 1
PAP336	Switzerland	Central Europe	Landrace	0.664	0.336	admixed	Cluster 2
PAP337	Switzerland	Central Europe	Landrace	0.614	0.386	admixed	Cluster 1
PAP457	Germany	Western Europe	Landrace	0.304	0.696	admixed	Cluster 2
PAP496*	Germany	Western Europe	Landrace	0.583	0.417	admixed	Cluster 1
PAP499	France	Western Europe	Landrace	0.51	0.49	admixed	Cluster 1
PAP506	Germany	Western Europe	Landrace	0.607	0.393	admixed	Cluster 1
PAP511*	Spain	Western Europe	Landrace	0.61	0.39	admixed	Cluster 1
PAP513*	Germany	Western Europe	Landrace	0.59	0.41	admixed	Cluster 1
PAP518	Belgium	Western Europe	Landrace	0.312	0.688	admixed	Cluster 1
PAP556*	Netherlands	Western Europe	Landrace	0.563	0.437	admixed	Cluster 2
PAP584	Slovakia	Central Europe	Landrace	0.567	0.433	admixed	Cluster 1
PAP585	Slovakia	Central Europe	Landrace	0.578	0.422	admixed	Cluster 1
PAP630	Vietnam	Southeast Asian	Landrace	0.689	0.311	admixed	Cluster 2
PAP656*	Bulgaria	Southeast Europe	Landrace	0.527	0.473	admixed	Cluster 1
PAP657	Bulgaria	Southeast Europe	Landrace	0.589	0.411	admixed	Cluster 1
PAP719	Kazakhstan	Central Asia	Landrace	0.471	0.529	admixed	Cluster 2
PAP748*	Portugal	Southern Europe	Landrace	0.666	0.334	admixed	Cluster 1
PAP750	Vietnam	Southeast Asian	Landrace	0.419	0.581	admixed	Cluster 1
PAP757	Italy	Southern Europe	Landrace	0.454	0.546	admixed	Cluster 1
PAP758	Italy	Southern Europe	Landrace	0.313	0.687	admixed	Cluster 1
PAP764	Korean	East Asia	Landrace	0.512	0.488	admixed	Cluster 1
PAP765	Korean	East Asia	Landrace	0.527	0.473	admixed	Cluster 1
PAP767	Korean	East Asia	Landrace	0.31	0.69	admixed	Cluster 2
PAP786	Romania	Central Europe	Landrace	0.615	0.385	admixed	Cluster 1
PAP791*	Romania	Central Europe	Landrace	0.566	0.434	admixed	Cluster 1
PAP831	Spain	Western Europe	Landrace	0.428	0.572	admixed	Cluster 2
PAP848	Finland	Western Europe	Landrace	0.377	0.623	admixed	Cluster 1

(Cont. on the next page)

Table 3.2. (cont.)

PAP865*	Romania	Central Europe	Landrace	0.433	0.567	admixed	Cluster 1
PAP883	Japan	East Asia	Landrace	0.406	0.594	admixed	Cluster 1
PAP920	Poland	Eastern Europe	Landrace	0.666	0.334	admixed	Cluster 1
PAP928	Austria	Central Europe	Landrace	0.58	0.42	admixed	Cluster 1
PAP938	Austria	Central Europe	Landrace	0.374	0.626	admixed	Cluster 1
PAP939	Austria	Central Europe	Landrace	0.522	0.478	admixed	Cluster 1
PAP945	Germany	Western Europe	Landrace	0.32	0.68	admixed	Cluster 1
PAP946	Germany	Western Europe	Landrace	0.53	0.47	admixed	Cluster 1
PAP114	Sweden	Scandinavia	Cultivar	0.24	0.76	B	Cluster 1
PAP151	Morocco	Africa	Cultivar	0.275	0.725	B	Cluster 2
PAP169	Morocco	Africa	Cultivar	0.264	0.736	B	Cluster 1
PAP327	Bulgaria	Southeast Europe	Cultivar	0.3	0.7	B	Unclustered
PAP49	Netherlands	Western Europe	Cultivar	0.265	0.735	B	Unclustered
PAP761	Japan	East Asia	Cultivar	0.257	0.743	B	Cluster 1
PAP816	USA	USA	Cultivar	0.273	0.727	B	Cluster 2
PAP857	France	Western Europe	Cultivar	0.261	0.739	B	Unclustered
PAP860	Czech Republic	Central Europe	Cultivar	0.251	0.749	B	Cluster 1
PAP905	Czech Republic	Central Europe	Cultivar	0.285	0.715	B	Cluster 2
PAP910	Czech Republic	Central Europe	Cultivar	0.29	0.71	B	Cluster 2
PAP932	Hungary	Central Europe	Cultivar	0.236	0.764	B	Cluster 1
PAP934	Netherlands	Western Europe	Cultivar	0.28	0.72	B	Cluster 2
PAP935	Former USSR	Former USSR	Cultivar	0.289	0.711	B	Cluster 1
PAP180A	Mongolia	East Asia	Landrace	0.269	0.731	B	Cluster 1
PAP332	France	Western Europe	Landrace	0.251	0.749	B	Cluster 1
PAP64	Australia	Australia	Landrace	0.257	0.743	B	Cluster 1
PAP663	Slovakia	Central Europe	Landrace	0.234	0.766	B	Cluster 1
PAP674	Spain	Western Europe	Landrace	0.297	0.703	B	Cluster 1
PAP714	Poland	Eastern Europe	Landrace	0.257	0.743	B	Cluster 1
PAP715	Belgium	Western Europe	Landrace	0.296	0.704	B	Cluster 1
PAP734	Vietnam	Southeast Asian	Landrace	0.288	0.712	B	Cluster 2
PAP742	Italy	Southern Europe	Landrace	0.255	0.745	B	Cluster 1
PAP746	Portugal	Southern Europe	Landrace	0.239	0.761	B	Cluster 1
PAP747	Portugal	Southern Europe	Landrace	0.253	0.747	B	Cluster 1
PAP768	Korean	East Asia	Landrace	0.299	0.701	B	Cluster 2
PAP792	Romania	Central Europe	Landrace	0.241	0.759	B	Cluster 1
PAP872	Germany	Western Europe	Landrace	0.238	0.762	B	Cluster 1
PAP888	Former USSR	Former USSR	Landrace	0.258	0.742	B	Cluster 1
PAP930	Austria	Central Europe	Landrace	0.297	0.703	B	Cluster 1

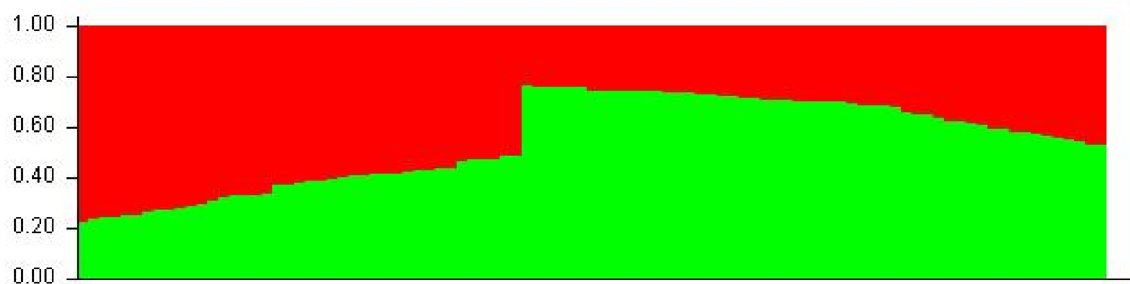

Figure 3.2. Q-plot of world collection based on SSR markers.

3.2.2. Genetic Diversity

In addition to model-based clustering, hierarchical clustering of the individuals was performed using the combined data of both marker types due to the limited number of EST SSR markers available. The Mantel test showed a strong correlation (r = 0.95) between the distance matrix and dendrogram. Dissimilarity between all accessions ranged from 0 to 0.18 with mean of 0.05 (95% similarity). The dendrogram contained seven nodes with at least two identical accessions at each node (Figure 3.3). The opium poppy accessions fell into two clusters (Figure 3.3). Three accessions (PAP49, PAP327 and PAP857) were not clustered with the rest. Most of the accessions (77%) were in cluster 1 (51 landraces and 22 cultivars) and had the same range (0 - 0.18) and mean diversity (0.05) as the entire collection. Although cluster 1 contained 4 subclusters (subclusters 1A-1D), there was no region and accession type clustering. Cluster 2 contained 19 accessions (12 landraces and 7 cultivars) and had lower mean diversity (0.03) than cluster 1. Opium poppy landraces (0.05) had slightly higher genetic diversity than cultivars (0.04) ($P < 0.05$, as determined by a Student's *t* test). When compared to the population structure results, most cluster 1 accessions (43 accessions, 45.3%) belonged to the admixed subgroup. The remaining accessions belonged to subpopulation A (10 accessions, 10.5 %) and B (20 accessions, 21 %). Cluster 2 contained accessions from the admixed and B subpopulations (10 and 7 accessions, respectively) with the exception of two accessions from subpopulation A. Three unclustered accessions belonged to subpopulation B. Student's *t* test ($P < 0.05$) demonstrated that accessions from Asia (0.06) had slightly higher mean genetic diversity than accessions from Europe (0.05).

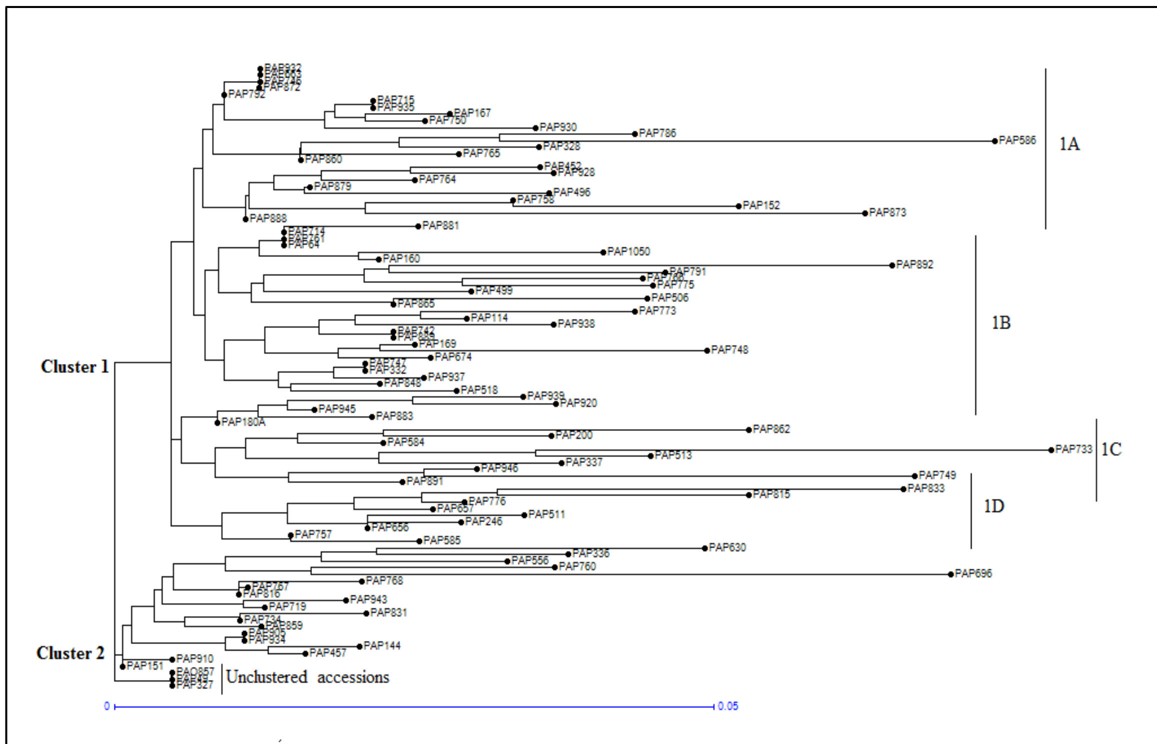

Figure 3.3. Hierarchical clustering of opium poppy world collection based on the Dice coefficient and unweighted neighbor-joining method.

3.2.3. Core Set Selection

The molecular genetic data were used to select a core set from the world collection. A total of 22 accessions were selected as the core set (marked with asterisks in Table 3.2). Genetic diversity of the core set ranged from 0.01 to 0.18 with a mean of 0.08 and contained 16 landraces and 6 cultivars from 16 countries. In terms of population structure, the core set contained materials from two subpopulations: A and admixed with 10 and 12 accessions, respectively.

3.3. Molecular and Morphological Characterization of Turkish Opium Poppy Accessions

3.3.1 Population Structure

Analysis of population structure was performed for the 118 Turkish individuals using 200 high-quality, reproducible fragments generated by the 13 EST and 33 genomic SSR markers (the outgroup, *P. umbonatum,* was excluded from population structure analysis). The best model representing population structure was identified as two subpopulations (K=2) based on Δ*K* values and a plot of Ln (probability of data). Of the 118 accessions, 110 (93%) could be assigned to subpopulations with 33 individuals in subpopulation A and 77 individuals in subpopulation B. Based on an 80% membership threshold, the remaining eight accessions were considered to be admixed (Figure 3.4 and Table 3.3). Average distance between individuals in the same cluster was calculated as 0.115 for subpopulation A and 0.133 for subpopulation B. All but one of the cultivars belonged to subpopulation A. Tmo3 was the only exception and was found to belong to subpopulation B. There was no region-specific clustering of landraces. Stepwise discriminant analysis showed that only straw yield and morphine content made significant contributions to the multivariate discrimination of the three subpopulations (A, B and admixed) with high correlations for straw yield and morphine content (r = 0.77 and r = 0.71, respectively).

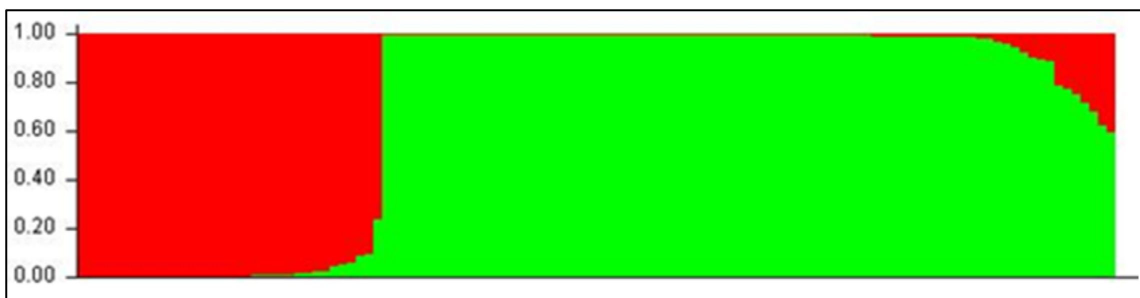

Figure 3.4. Q-plot of Turkish germplasm based on genomic and EST SSR data.

Table 3.3. Subpopulation assignments of Turkish landraces and cultivars.

			Inferred ances.			
Genotype	Location of collection	Landrace/Cultivar	Subpop. A	Subpop. B	Structure Assign.	DARwin Assign.
3	Alacami, Sandikli, Afyon	Landrace	0.002	0.998	B	cluster 1
5	Celtik, Burdur	Landrace	0.979	0.021	A	cluster 2A
6	Alagoz, Sandikli, Afyon	Landrace	0.013	0.987	B	cluster1
7	Alagoz, Sandikli, Afyon	Landrace	0.997	0.003	A	cluster 2A
8	Alacami, Sandıklı, Afyon	Landrace	0.002	0.998	B	cluster 1
9	Cobanlar, Afyon	Landrace	0.976	0.024	A	cluster 2A
10	Celtik, Burdur	Landrace	0.002	0.998	B	cluster 1
11	Afyon	Landrace	0.007	0.993	B	cluster 1
13	Yalvachoyuklu, Isparta	Landrace	0.003	0.997	B	cluster 1
14	Sivas	Landrace	0.002	0.998	B	cluster 1
15	Kocyatagi, Suhut, Afyon	Landrace	0.008	0.992	B	cluster 1
16	Ulubey, Camlibel, Usak	Landrace	0.002	0.998	B	cluster 1
17	Kocyatagi, Suhut, Afyon	Landrace	0.004	0.996	B	cluster 1
18	Isparta	Landrace	0.002	0.998	B	cluster 1
19	Disli, Afyon	Landrace	0.993	0.007	A	cluster 2A
20	Bolvadin, Afyon	Landrace	0.003	0.997	B	cluster 1
21	Kocyatagi, Suhut, Afyon	Landrace	0.002	0.998	B	cluster 1
22	Anayurt, Suhut, Afyon	Landrace	0.989	0.011	A	cluster 2A
23	Afyon	Landrace	0.002	0.998	B	cluster 1
24	Alagoz, Sandikli, Afyon	Landrace	0.002	0.998	B	cluster 1
25	Kocyatagi, Suhut, Afyon	Landrace	0.004	0.996	B	cluster 1
26	Deresenek, Afyon	Landrace	0.015	0.985	B	cluster 1
27	Banaz, Usak	Landrace	0.009	0.991	B	cluster 1
28	Kocyatagi, Suhut, Afyon	Landrace	0.004	0.996	B	cluster 1
29	Yukarisogut, Eskisehir	Landrace	0.01	0.99	B	cluster 1
30	Isabey, Cal, Denizli	Landrace	0.003	0.997	B	cluster 1
31	Karayahsiler, Civril, Denizli	Landrace	0.005	0.995	B	cluster 1
32	Afyon	Landrace	0.072	0.928	B	cluster 1
33	Ekinhisar, Sandikli, Afyon	Landrace	0.998	0.002	A	cluster 2A
35	Kocyatagi, Suhut, Afyon	Landrace	0.004	0.996	B	cluster 1
36	Sivas	Landrace	0.002	0.998	B	cluster 1
37	Yalvachoyuklu, Isparta	Landrace	0.003	0.997	B	cluster 1
39	Kocyatagi, Suhut, Afyon	Landrace	0.002	0.998	B	cluster 1
40	Isparta	Landrace	0.996	0.004	A	cluster 2A
41	Kocyatagi, Suhut, Afyon	Landrace	0.005	0.995	B	cluster 1
43	Celtik, Burdur	Landrace	0.098	0.902	B	cluster 1
45	Simav, Kutahya	Landrace	0.998	0.002	A	cluster 2A
46	Celtik, Burdur	Landrace	0.01	0.99	B	cluster 1
48	Karayahsiler, Civril, Denizli	Landrace	0.005	0.995	B	cluster 1

(Cont. on the next page)

Table 3.3. (cont.)

49	Cevrekoyu, Usak	Landrace	0.002	0.998	B	cluster 1
50	Mamat, Suhut, Afyon	Landrace	0.005	0.995	B	cluster 1
51	Civril, Karayahsiler, Denizli	Landrace	0.002	0.998	B	cluster 1
52	Yukarısogutonu, Eskisehir	Landrace	0.003	0.997	B	cluster 1
53	Civril, Denizli	Landrace	0.002	0.998	B	cluster 1
54	Erice, Usak	Landrace	0.006	0.994	B	cluster 1
55	Ekinhisar, Sandikli, Afyon	Landrace	0.002	0.998	B	cluster 1
56	Deresenek, Afyon	Landrace	0.005	0.995	B	cluster 1
57	Sivas	Landrace	0.015	0.985	B	cluster 1
59	Sulumenli, Afyon	Landrace	0.997	0.003	A	cluster 2A
60	Kocyatagi, Afyon	Landrace	0.003	0.997	B	cluster 1
62	Sulumenli, Afyon	Landrace	0.032	0.968	B	cluster 1
63	Hoyuklu, Yalvac, Isparta	Landrace	0.002	0.998	B	cluster 1
65	Cobanlar, Afyon	Landrace	0.003	0.997	B	cluster 1
66	Cobanlar, Afyon	Landrace	0.002	0.998	B	cluster 1
67	Alacami,Sandikli, Afyon	Landrace	0.997	0.003	A	cluster 2A
68	Alacami,Sandikli, Afyon	Landrace	0.101	0.899	B	cluster 1
69	Sivas	Landrace	0.002	0.998	B	cluster 1
70	Cobanlar, Afyon	Landrace	0.006	0.994	B	cluster 1
72	Kocyatagi, Suhut, Afyon	Landrace	0.223	0.777	admixed	cluster 1
73	Sulumenli, Afyon	Landrace	0.245	0.755	admixed	cluster 1
74	Sulumenli, Afyon	Landrace	0.003	0.997	B	cluster 1
75	Afyon	Landrace	0.01	0.99	B	cluster 1
76	Gogen, Uşak	Landrace	0.039	0.961	B	cluster 1
77	Disli, Afyon	Landrace	0.003	0.997	B	cluster 1
78	Alacami, Sandikli, Afyon	Landrace	0.006	0.994	B	cluster 1
79	Emet, Hisarcik, Kutahya	Landrace	0.008	0.992	B	cluster 1
80	Cobanlar, Afyon	Landrace	0.981	0.019	A	cluster 2A
82	Disli, Afyon	Landrace	0.002	0.998	B	cluster 1
83	Kocyatagi, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
84	Cobanlar, Afyon	Landrace	0.005	0.995	B	cluster 1
85	Alacami, Sandikli, Afyon	Landrace	0.003	0.997	B	cluster 1
86	Kocyatagi, Suhut, Afyon	Landrace	0.011	0.989	B	cluster 1
87	Kocyatagi, Suhut, Afyon	Landrace	0.905	0.095	A	cluster 2A
88	Kocyatagi, Suhut, Afyon	Landrace	0.007	0.993	B	cluster 1
89	Gure, Usak	Landrace	0.998	0.002	A	cluster 2A
91	Kocyatagi, Suhut, Afyon	Landrace	0.113	0.887	B	cluster 1
92	Bolvadin, Afyon	Landrace	0.996	0.004	A	cluster 2A
93	Suhut, Afyon	Landrace	0.056	0.944	B	cluster 1
94	Acipayam, Denizli	Landrace	0.004	0.996	B	cluster 1
95	Acipayam, Denizli	Landrace	0.95	0.05	A	cluster 2A
96	Kutahya	Landrace	0.997	0.003	A	cluster 2A
97	Suhut, Afyon	Landrace	0.989	0.011	A	cluster 2A
99	Anayurt, Suhut, Afyon	Landrace	0.002	0.998	B	cluster 1

(Cont. on the next page)

Table 3.3. (cont.)

101	Anayurt, Suhut, Afyon	Landrace	0.992	0.008	A	cluster 2A
102	Deresenek, Afyon	Landrace	0.014	0.986	B	cluster 1
103	Cay, Afyon	Landrace	0.954	0.046	A	cluster 2A
104	Mahmut, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
105	Kocyatagi, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
106	Anayurt, Suhut, Afyon	Landrace	0.406	0.594	admixed	cluster 1
107	Acipayam, Denizli	Landrace	0.002	0.998	B	cluster 1
108	Anayurt, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
109	Acipayam, Denizli	Landrace	0.003	0.997	B	cluster 1
110	Deresenek, Afyon	Landrace	0.013	0.987	B	cluster 1
111	Suhut, Afyon	Landrace	0.318	0.682	admixed	cluster 1
112	Sulumenli, Afyon	Landrace	0.013	0.987	B	cluster 1
113	Arizlar, Sandikli, Afyon	Landrace	0.372	0.628	admixed	cluster 1
114	Sulumenli, Afyon	Landrace	0.007	0.993	B	cluster 1
116	Deresenek, Afyon	Landrace	0.003	0.997	B	cluster 1
117	Deresenek, Afyon	Landrace	0.012	0.988	B	cluster 1
118	Anayurt, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
119	Eber, Afyon	Landrace	0.279	0.721	admixed	cluster 1
120	Anayurt, Suhut, Afyon	Landrace	0.214	0.786	admixed	cluster 1
122	Anayurt, Suhut, Afyon	Landrace	0.003	0.997	B	cluster 1
1061[Λ]	-	Cultivar	0.997	0.003	A	Cluster 2B
1065[Λ]	-	Cultivar	0.997	0.003	A	Cluster 2B
Afyon95[Λ]	-	Cultivar	0.997	0.003	A	Cluster 2B
Anayurt[Λ]	-	Cultivar	0.997	0.003	A	Cluster 2B
Kemerkaya[Λ]	-	Cultivar	0.977	0.023	A	Cluster 2B
Tinaztepe[Λ]	-	Cultivar	0.997	0.003	A	Cluster 2B
Zaferyolu[Λ]	-	Cultivar	0.998	0.002	A	Cluster 2B
Ofis3[Ψ]	-	Cultivar	0.914	0.086	A	cluster 2A
Ofis4[Ψ]	-	Cultivar	0.994	0.006	A	cluster 2A
Ofis8[Ψ]	-	Cultivar	0.996	0.004	A	Cluster 2B
Ofis95[Ψ]	-	Cultivar	0.997	0.003	A	Cluster 2B
Ofis96[Ψ]	-	Cultivar	0.991	0.009	A	Cluster 2B
Tmo1[Ψ]	-	Cultivar	0.99	0.01	A	cluster 2A
Tmo2[Ψ]	-	Cultivar	0.997	0.003	A	cluster 2A
Tmo3[Ψ]	-	Cultivar	0.764	0.236	admixed	Cluster 2B

3.3.2. Molecular Genetic Diversity

The EST and genomic SSR marker data were also used to assess the genetic diversity of Turkish opium poppy germplasm. The binary data were used to generate a distance matrix using the Dice coefficient which was then used to draw a dendrogram

using the unweighted neighbor joining algorithm. As expected, a Mantel test showed a strong correlation (r = 0.993) between the distance matrix and dendrogram. Dissimilarity between accessions ranged from 0.005 to 0.25 (75% similarity) with average dissimilarity of 0.11. As expected, *P. umbonatum* clustered separately from *P. somniferum* accessions with a maximum dissimilarity of 0.67 (33% similarity) to the opium poppy accessions. The *P. somniferum* accessions fell into two clusters (Figure 3.5). Cluster 1 contained 84 landraces and dissimilarity ranged from 0.015 to 0.20 with an average dissimilarity of 0.075. Cluster 2 was comprised of 19 landraces and 15 cultivars and dissimilarity ranged from 0.006 to 0.19, with an average dissimilarity of 0.092. Cluster 2 consisted of two sub-clusters (subclusters A and B). Cluster 2B contained only named varieties, however, varieties and landraces were intermixed in cluster 2A (Figure 3.5). Average genetic diversity of landraces (0.10) was significantly ($p \leq 0.05$ as determined by a Student's t-test) higher than average genetic diversity of cultivars (0.07). The hierarchical clustering results showed that there was no region-specific clustering of Turkish opium poppy accessions.

Genetic diversity of the poppy accessions based on EST and genomic SSR markers was also calculated separately. For EST SSR markers, genetic diversity ranged from 0 to 0.23 with average diversity of 0.07. For the genomic SSR markers, the range of genetic diversity was significantly ($p \leq 0.05$) higher than for EST SSRs and ranged from 0 to 0.33 with an average dissimilarity of 0.13.

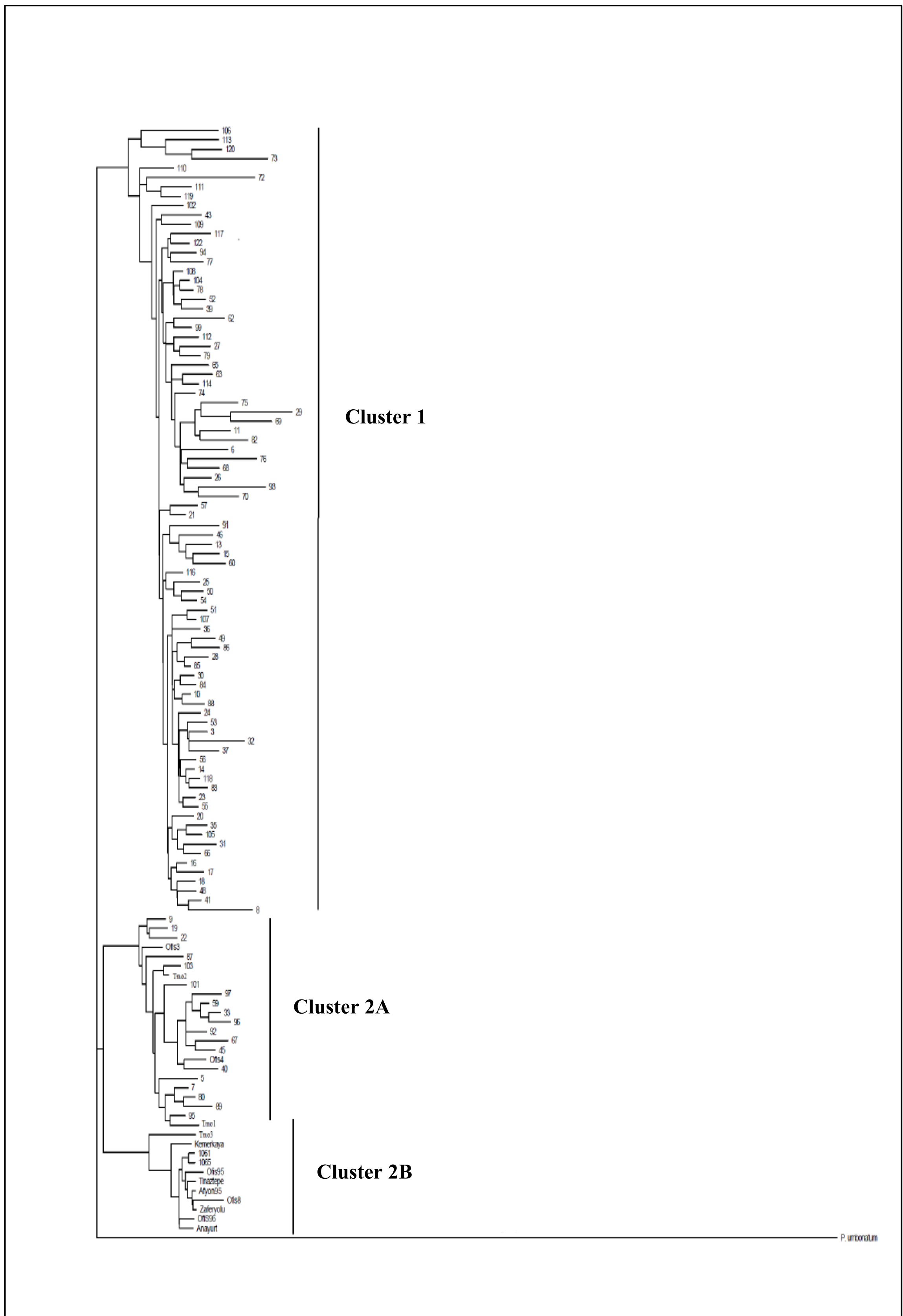

Figure 3.5. Unweighted neighbor-joining dendrogram of the Turkish germplasm constructed with genomic and EST simple sequence repeat data.

3.3.3. Morphological Trait Diversity

A total of 103 opium poppy landraces and 15 cultivars were analyzed for morphine content and five quantitative traits (plant height, stigma number, capsule index, straw and seed yield). Germplasm and cultivars were analyzed separately to assess the breeding potential of the landraces (Table 3.4). The cultivars had nearly two-fold higher mean morphine content (0.84%) than the landraces (0.43%). Cultivars Ofis 3, Ofis 4 and Ofis 8 had the highest morphine content with more than 1.1% morphine for each. Cultivar Anayurt had the lowest morphine content with only 0.55%. A total of 11% of the landraces had higher morphine content than Anayurt including accession 116 which had the highest morphine content (0.8%) among landraces. Morphine content did not showed any correlation with morphological traits.

Table 3.4. Morphine content and morphological traits for the opium poppy landraces and cultivars.

Trait	Cultivars			Landraces		
	Mean ± SE	CV	Range	Mean ± SE	CV	Range
		%			%	
Morphine content, %	0.84 ± 0.06	26	0.55-1.35	0.43 ± 0.01	21	0.24-0.80
Plant height, cm	117.5 ± 4.4	14	89.0-160.0	101.7 ± 0.5	5	89.5-111.5
Stigma number	4.5 ± 0.4	36	2.0-8.0	4.8 ± 0.1	24	2.8-8.5
Capsule index	1.08 ± 0.06	20	0.43-1.46	0.95 ± 0.01	9	0.55-1.12
Seed yield, kg ha^{-1}	132.8 ± 4.2	12	110.0-160.0	100.7 ± 1.4	14	66.5-133.5
Straw yield, kg ha^{-1}	127.8 ± 3.8	11	109.0-160.0	76.5 ± 1.2	17	53.0-105.0

Plant height varied more in the cultivars (CV = 14%) than in the landraces (CV = 5%) with 71 and 22 cm ranges for cultivars and landraces, respectively. On average, cultivars (117.5 cm) were taller than landraces (101.7 cm). Accessions 14, 86 and 88 were tallest among landraces but were still shorter than the cultivar mean. Plant height showed low correlation with seed and straw yields ($r = 0.22$, $r = 0.32$, respectively).

Variation for stigma number was similar for both cultivars and landraces with two to eight stigmas per flower. Mean stigma number was also similar for cultivars and landraces. Capsule index indicates capsule shape with perfectly round capsules having an

index of 1.0. Capsule index for cultivars (1.08) was slightly higher than for landraces (0.95) with a tendency of the cultivars to have more elongated capsules as indicated by the range which varied up to an index value of 1.46. Five landraces had higher capsule index than the mean capsule index of cultivars. Stigma number and capsule index did not showed any correlation with other morphological traits.

Mean seed and straw yield of cultivars (132.8 and 127.8 kg ha^{-1}, respectively) were significantly ($p < 0.05$) higher than for landraces (100.7 and 76.5 kg ha^{-1}. Kemerkaya had highest seed yield among cultivars. Most of the landraces had lower seed yield than the cultivar with the lowest seed yield (Ofis 8, 110 kg ha^{-1}). Landraces 3 and 43 had the highest seed yield among landraces, however, their seed yields were only average when compared to the cultivars. All of the landraces had lower straw yield than the cultivar with lowest straw yield (Tmo2, 109 kg ha^{-1}). As might be expected, there was high positive correlation between seed yield and straw yield ($r = 0.82$).

Principal component analysis (PCA) of the quantitative traits was performed and showed that the first two Eigen vectors explained 61.9% of the morphological variation. A total of 44% of the variation was explained by PC1 with high positive correlations to straw and seed yield, morphine content and plant height (Table 3.5). PC2 explained 17.9% of variation with high positive correlation to stigma number and capsule index and low negative correlation to straw and seed yield. Euclidean distances of landraces and cultivars were between 0.32 and 52.48 with an average distance of 13.33. The two-dimensional PCA plot showed that, despite their relatively low number, cultivars had very high morphological diversity compared to the landraces. All the cultivars were in the upper left of the PCA plot with no overlap between cultivars and landraces (Figure 3.6).

Table 3.5. Principal component analysis for morphological traits. Eigen values were calculated for the first two principal component axes (PC).

Trait	PC1	PC2
Morphine content, %	0.72	0.30
Plant height, cm	0.72	0.16
Stigma number	-0.12	0.73
Capsule index	0.34	0.54
Seed yield, kg ha^{-1}	0.82	-0.32
Straw yield, kg ha^{-1}	0.88	-0.19

Stepwise discriminant analysis was performed using landrace collection location as a grouping variable and all morphological traits as independents. None of the morphological traits made significant contributions to the multivariate discrimination of the eight provinces.

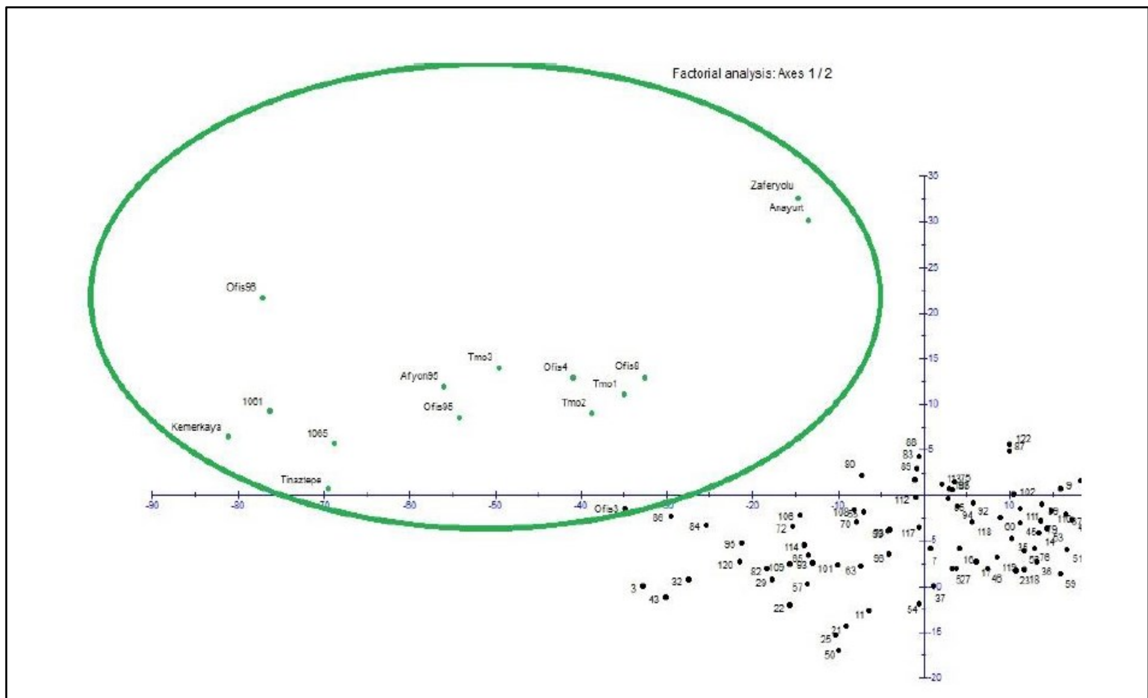

Figure 3.6. Principal component analysis (PCA) plot for morphological traits. Green color indicates the cultivars. All the cultivars clustered together.

3.3.4. Core Set Selection

Core sets of opium poppy landraces were selected using both morphological and molecular data. The core set selected using the morphological data (M-Core) contained 13 landraces: three from cluster A and 10 from cluster B (Table 3.6). The M-Core set contained landraces from four provinces (Afyon, Burdur, Sivas and Usak). Landraces from M-Core had similar means and coefficients of variation as all landraces for all traits (Table 3.7) but Shannon diversity indices showed that M-Core had lower morphological diversity than all landraces for all traits (Table 3.8). Although average molecular genetic

diversity of landraces from M-Core was similar to average diversity of all landraces, maximum diversity of M-Core set was lower than all landraces (Table 3.9).

Table 3.6. Genotypes of selected core sets using SSR markers (S-core), morphological traits (M-core), and both of core sets (G&M-core).

M-Core			S-Core			S&M-Core		
Genotype	Structure Assign.	Darwin Assign.	Genotype	Structure Assign.	Darwin Assign.	Genotype	Structure Assign.	Darwin Assign.
3	B	cluster 1	8	B	cluster 1	8	B	cluster 1
6	B	cluster1	15	B	cluster 1	11	B	cluster 1
28	B	cluster 1	22	A	cluster 2A	22	A	cluster 2A
39	B	cluster 1	31	B	cluster 1	28	B	cluster 1
46	B	cluster 1	32	B	cluster 1	31	B	cluster 1
54	B	cluster 1	35	B	cluster 1	32	B	cluster 1
57	B	cluster 1	40	A	cluster 2A	35	B	cluster 1
62	B	cluster 1	43	B	cluster 1	40	A	cluster 2A
80	A	cluster 2A	49	B	cluster 1	43	B	cluster 1
87	A	cluster 2A	66	B	cluster 1	49	B	cluster 1
101	A	cluster 2A	69	B	cluster 1	59	A	cluster 2A
116	B	cluster 1	72	admixed	cluster 1	66	B	cluster 1
117	B	cluster 1	73	admixed	cluster 1	69	B	cluster 1
			76	B	cluster 1	72	admixed	cluster 1
			87	A	cluster 2A	73	admixed	cluster 1
			93	B	cluster 1	76	B	cluster 1
			96	A	cluster 2A	80	A	cluster 2A
			101	A	cluster 2A	86	B	cluster 1
			106	admixed	cluster 1	87	A	cluster 2A
			107	B	cluster 1	93	B	cluster 1
			113	admixed	cluster 1	97	A	cluster 2A
						101	A	cluster 2A
						107	B	cluster 1
						111	admixed	cluster 1
						116	B	cluster 1
						117	B	cluster 1

Table 3.7. Means, SE, CV and range of the morphological traits for the landraces and the three core sets.

Trait	Landraces		M-Core		S-Core		S&M-Core	
	Mean ± SE	CV %	Mean±SE	CV %	Mean±SE	CV %	Mean±SE	CV %
Morphine content , %	0.43 ± 0.01	21	0.49 ± 0.04	30	0.44 ± 0.02	19	0.46 ± 0.02	25
Plant height, cm	101.7 ± 0.5	5	101.4 ± 1.6	6	103.2 ± 0.8	3	102 ± 1	5
Stigma number	4.8 ± 0.1	24	5 ± 0.4	32	5 ± 0.3	26	4.8 ± 0.2	25
Capsule index	0.95 ± 0.01	9	0.91 ± 0.04	15	0.93 ± 0.02	7	0.93 ± 0.02	9
Seed yield, kg ha^{-1}	100.7 ± 1.4	14	100.5 ± 4.7	17	101.5 ± 3.7	17	100 ± 3.6	18
Straw yield, kg ha^{-1}	76.5 ± 1.2	17	76.7 ± 3.9	18	77.5 ± 3.1	19	76.4 ± 2.9	19

Table 3.8. Shannon diversity indices of the morphological data for all landraces and the three core sets.

	All landraces	M-Core	S-Core	S&M-Core
Morphine content , %	4.61	2.52	3.03	3.23
Plant height, cm	4.63	2.56	3.04	3.26
Stigma number	4.61	2.52	3.01	3.23
Capsule index	4.63	2.55	3.04	3.25
Seed yield, kg ha^{-1}	4.62	2.55	3.03	3.24
Straw yield, kg ha^{-1}	4.62	2.55	3.02	3.24

Table 3.9. Average molecular genetic diversity and range of diversity for each core set.

	Average diversity	Range
All landraces	0.109	0.005-0.246
M-Core	0.098	0.033-0.171
S-Core	0.136	0.029-0.23
S&M-Core	0.126	0.029-0.227

The core set of opium poppy landraces selected using the SSR markers (S-Core) contained 21 landraces: five from Cluster A, 12 from Cluster B and four from the admixed accessions (Table 3.6). S-Core contained landraces from all poppy-growing provinces except Isparta. No significant ($p < 0.05$) differences were observed between morphological means and coefficients of variation for S-Core and all landraces (Table 3.7). However, Shannon diversity indices for traits showed that S-Core had lower morphological diversity than all landraces for all traits (Table 3.8). Although maximum molecular genetic diversity of S-Core was similar to maximum genetic diversity of all landraces (0.24), average and minimum diversity of S-Core was higher (0.14 and 0.03, respectively) than average and minimum diversity of all landraces (0.11 and 0.005) (Table 3.9).

The S&M-Core selected using both SSR and morphological data contained 26 landraces: seven from cluster A, 16 from cluster B and three from admixed landraces (Table 3.6). Landraces of S&M-Core contained accessions from all provinces except for Eskisehir and Kutahya. Mean and range of variation of morphological traits for landraces from S&M-Core was similar to the mean and range of variation of all landraces for all traits ($p < 0.05$) (Table 3.7). Coefficients of variation for S&M-Core were similar to all landraces for plant height, stigma number and capsule index. However, coefficients of variation for S&M-Core were higher than all landraces for straw and seed yield and morphine content. Shannon diversity indices of S&M-Core were similar to those for S-Core, higher than M-Core but lower than all landraces (Table 3.8). Average molecular genetic diversity of S&M-Core was higher than M-core and all landraces (Table 3.9).

3.4. Association Mapping

A total of 80 SSR (14 EST-SSR and 66 genomic SSR) markers and 10 AFLP primer combinations were assayed on an AM panel of 95 Turkish opium poppy accessions. Rare loci were deleted (< 10%) as potentially unreliable. As a result, 282 and 412 loci were generated with the SSR and AFLP markers, respectively. Among these loci, 164 (58%) and 367 (89%) were found to be polymorphic for SSR and AFLP markers, respectively. Thus, 531 polymorphic marker loci were used in AM analysis.

A total of 13,160 (6.5%) SSR and AFLP locus pairs showed significant LD ($P <$ 0.001). LD values (r^2) of these locus pairs ranged from 0.03 to 1 with a mean of 0.21. Most (82.9%) of the locus pairs that showed significant LD were AFLP locus pairs with a mean of r^2 of 0.21 (Table 3.10). AFLP-SSR locus pairs accounted for 14.8% (1954) of the comparisons with LD and had a mean of r^2 of 0.18. Only 2.3% (300) of the comparisons with LD were SSR locus pairs with a mean r^2 of 0.25. Student's t test ($P <$ 0.05) showed that the mean LD value (r^2) for the SSR locus pairs was significantly higher than the means for the AFLP and AFLP-SSR locus pairs.

Table 3.10. SSR and AFLP locus pairs with significant LD.

Locus pairs	Number	%	LD value (r^2)	
			Average ± SE	Range
SSR-SSR locus pairs	300	2.3	0.25±0.01	0.07-1
AFLP locus pairs	10906	82.9	0.21±0.0008	0.03-0.81
AFLP-SSR locus pairs	1954	14.8	0.18±0.002	0.08-1
Total	13160	100	0.21±0.002	0.03-1

Different AM models [GLM, GLM (Q), GLM (PC), GLM (Q+PC), MLM (K), MLM (Q+K), MLM (PC+K), MLM (Q+PC+K)] were compared and used to calculate the proportion of significant results. The GLM model corrected with Q-matrix of population structure had highest proportion of significant results among the eight association models (π1 (%) = 9) and was used for AM of morphine content and agronomic traits (plant height, stigma number, capsule index, seed yield and straw yield) in opium

poppy (Table 3.11). It is important to note that the trait means for the association panel were not significantly different from those for the entire set (Student`s t test $P < 0.05$).

Table 3.11. Association models tested to determine best model for association analysis. * Overall proportion of true null hypotheses (FDR). ** Proportion of significant results.

	π0 (%) *	π1 (%) **
GLM	99.7	0.3
GLM (Q)	91	9
GLM (PC)	99.6	0.4
GLM (Q+PC)	93.2	6.8
MLM (K)	99.7	0.3
MLM (Q+K)	91.2	8.8
MLM (PC+K)	99.6	0.4
PC+Q+K	96.2	3.8

One SSR and three AFLP loci were significantly associated with morphine content (*P*-value < 0.01) (Table 3.12). SSR marker psgSSR853 was most strongly associated with the trait (*P*-values = 0.002) and had an LD value of 0.18. AFLP locus E-ACA + M-CAG-63 had the highest LD value, 0.32 while the other two AFLP loci (E-ACC + M-CAC-146 and E-AGC + M-CTA-138) associated with morphine content had low LD values of 0.10 each.

Table 3.12. AFLP and SSR markers associated with morphine content and agronomic traits.

Trait	Marker*	Marker type	P-value	LD value (r^2)
Morphine content, %	psgSSR853	Genomic SSR	0.002	0.18
Morphine content, %	E-ACA + M-CAG-63	AFLP	0.004	0.32
Morphine content, %	E-ACC + M-CAC-146	AFLP	0.009	0.10
Morphine content, %	E-AGC + M-CTA-138	AFLP	0.005	0.10
Plant height, cm	E-ACC + M-CAG-215	AFLP	0.0001	0.19
Plant height, cm	E-AAC + M-CTC-84	AFLP	0.008	0.13
Plant height, cm	E-ACC + M-CAC-134	AFLP	0.002	0.11

(Cont. on the next page)

Table 3.12. (cont.)

Plant height, cm	E-ACC + M-CAC-156	AFLP	0.003	0.10
Plant height, cm	E-ACC + M-CAG-96	AFLP	0.008	0.10
Stigma number	psgSSR910	Genomic SSR	0.008	0.14
Stigma number	E-ACA + M-CAG-103	AFLP	0.006	0.35
Stigma number	E-ACC + M-CAG-124	AFLP	0.004	0.15
Stigma number	E-ACC + M-CAG-153	AFLP	0.006	0.10
Stigma number	E-AGG + M-CAT-195	AFLP	0.004	0.10
Capsule index	psgSSR484	Genomic SSR	0.003	0.20
Capsule index	psgSSR849	Genomic SSR	0.00006	0.17
Capsule index	EST-SSR23	EST-SSR	0.001	0.13
Capsule index	E-ACG + M-CAA-109	AFLP	0.007	0.08
Seed yield, kg ha^{-1}	E-AAC + M-CTC-96	AFLP	0.004	0.15
Seed yield, kg ha^{-1}	E-AAC + M-CTC-107	AFLP	0.005	0.14
Seed yield, kg ha^{-1}	E-AGC + M-CTA-166	AFLP	0.008	0.10
Straw yield, kg ha^{-1}	E-ACC + M-CAG-153	AFLP	0.002	0.13
Straw yield, kg ha^{-1}	E-AGG + M-CAT-213	AFLP	0.004	0.10
Straw yield, kg ha^{-1}	psgSSR909	Genomic SSR	0.004	0.16
Straw yield, kg ha^{-1}	psgSSR910	Genomic SSR	0.005	0.16

Five AFLP loci were associated with plant height (Table 3.12). Among these, AFLP locus E-ACC + M-CAG-215 was the most significant (*P*-value = 0.0001) with a LD value of 0.19. The other AFLP loci had similar levels of statistical significance (*P*-values ranging from 0.002 to 0.008) and had LD values ranging from 0.10 to 0.13.

One SSR marker and four AFLP fragments generated from three AFLP primer combinations were significantly associated with stigma number (Table 3.12). PsSSR910 had a LD value of 0.14. AFLP locus E-ACA + M-CAG-103 had the highest LD value, 0.35, while the other three AFLP loci had LD values ranging from 0.10 to 0.15.

Three SSR markers and one AFLP locus were significantly associated with the capsule index trait (Table 3.12). SSR marker psgSSR849 had the highest significance level (*P*-value=0.00006) with a LD value of 0.17. PsgSSR484 had the highest LD value, 0.20. The other two markers had LD values of 0.13 and 0.08.

A total of three AFLP loci generated by two AFLP primer combinations were significantly associated with seed yields (Table 3.12). LD values of these loci were 0.15, 0.14 and 0.10 for E-AAC + M-CTC-96, E-AAC + M-CTC-107 and E-AGC + M-CTA-

166, respectively. Two AFLP and two SSR markers were significantly associated with straw yield. LD values of these loci were 0.13 and 0.10 for E-ACC + M-CAG-153 and E-AGG + M-CAT-213, respectively. The SSR markers (psgSSR909 and psgSSR909) had slightly higher LD values of 0.16 each.

CHAPTER 4

DISCUSSION

Opium poppy is one of the most important crops for the world's pharmaceutical industry and Turkish agriculture due to its high alkaloids content. Despite its importance, opium poppy is not yet amenable to molecular breeding methods due to limited crop specific molecular tools. Thus, the present study aimed to provide these resources for initiation of molecular breeding. To achieve this aim, molecular and morphological characterization of poppy germplasm were performed for efficient parental selection and conservation. Also the first QTLs for morphine content and agronomic traits were identified in Turkish opium poppy germplasm using an association mapping approach.

4.1. Validation of Genomic SSR Markers

4.1.1. Polymorphism of Genomic SSR Markers

Most of the primers for genomic SSRs (96%) amplified PCR products. The amplification success of the genomic SSR markers was slightly higher than for the genic-SSR markers (82%) developed by Selale et al. (2013). A high rate of successful amplification can be due to high quality sequence data and appropriate primer parameters such as high GC content. In our study, genomic SSR markers detected an intermediate level of polymorphism with an average PIC value of 0.19 among Papaver species and opium poppy accessions and a slightly lower level of intraspecific polymorphism (average PIC of 0.17). Polymorphism of the genomic SSR markers was lower than the previously developed genic SSR markers (Selale et al. 2013, Lee at al. 2011). Although genomic SSRs are often reported to have higher levels of polymorphism than genic SSRs (Varshney et al. 2005), Tian et al. (2012) recently showed that genic-SSR markers were more polymorphic than genomic SSR markers in *Coreoperca whiteheadi.*

Many of the genomic SSR markers amplified multiple fragments (average of 5 fragments per marker). This is most likely the result of polyploidy in opium poppy.

P. somniferum (2n = 22) is an aneuallopolyploid and is hypothesized to have formed from species with x = 7 (Lavania and Srivastava 1999). Therefore, a single copy SSR locus may amplify up to six fragments. In our study, nine SSR markers (17%) had more than six fragments indicating that these SSR markers primed from multiple loci. The number of fragments amplified by genomic SSR markers was slightly higher than for the six genic SSR markers developed by Lee et al. (2011) who obtained an average of 2.8 ± 0.5 fragments (mean ±SE, range 2–5). However, Lee et al. (2011) did not use markers that produced more than three fragments, thereby limiting fragment number. Average fragment number for the genomic SSRs was lower than for the genic SSRs which amplified an average of 8.4 fragments per SSRs (Selale et al. 2013). This result can be explained by the fact that many of the genic SSR markers (23 SSR markers) were multiallelic while only nine genomic SSRs were multiallelic in this study. Additional genic and genomic markers should be tested to determine if this difference is real or a sampling artifact.

4.1.2 Genetic Diversity Revealed by Genomic SSR Markers

The genomic SSR markers developed in this study can be used in opium poppy identification. Although rare alleles were excluded, a total of 32 SSR primers (60.4%) were useful for Turkish opium poppy identification. Retesting of rare alleles is needed to confirm whether or not they are reproducible and suitable for opium poppy identification. Dendrogram analysis also showed that genomic SSR markers were suitable for differentiating opium poppy from *Papaver* species. Thus, these markers can be used to analyze intra- and interspecific genetic diversity of opium poppy. Landraces and named varieties were intermixed in the dendrogram of genomic SSR markers. This differs from the results obtained with nearly the same accessions using genic SSR markers in which named varieties clustered separately from landraces (Selale at al. 2013). This may be the result of artificial selection pressure on genic SSRs. Chabane et al. (2005) reported that genic SSR markers provided clearer separation between wild and cultivated barley than genomic SSR markers as was observed in our studies. Although the topology of the dendrogram for genomic SSR markers was different from the dendrogram based on genic SSR markers, Mantel test results showed that there was a very high correlation (r = 0.98)

between the distance matrices for the two marker types. Therefore genomic and genic SSR markers give consistent results in opium poppy.

4.2. Molecular Characterization of Opium Poppy World Collection

4.2.1. Population Structure

The present study is the first time that population structure of an opium poppy world collection was analyzed using SSR markers. The number of subpopulations present in the world collection was equal to that identified in Turkish germplasm containing 118 accessions using SSRs (Celik et al. 2016) but fewer than the four subpopulations detected in Indian germplasm containing 95 accessions using AFLP markers (Verma et al. 2016). Similar population structure in world and Turkish populations was expected because the crop was distributed to the rest of the world from Anatolia. Opium poppy has low genetic diversity due to its self-pollinated nature and the single origin (Turkey) of accessions in Europe, Asia and Africa (Tétényi 1997). More subpopulations were probably identified in the Indian germplasm because the two studies used different marker systems. Although the level of genetic diversity determined by SSR and AFLP was correlated in other species, the two systems had different levels of polymorphism and generated different fixation indices (F_{st}) with values higher for AFLP markers (Pejic et al. 1998; Woodhead et al. 2005). The high level of admixture identified in the present study was consistent with the results of Dittbrenner et al. (2008) who reported that none of the accessions (300) from world collection clustered based on their locations. The high level of admixture may be due to the aneuallopolyploid nature of the crop (Tétényi 1997).

4.2.2. Genetic Diversity and Core Set Selection

The present study revealed lower genetic diversity in the world collection than in Turkish and Indian (0.11 and 0.16 mean Dice coefficients, respectively) germplasm collections (Celik et al. 2016; Verma et al. 2016). Anatolia is known to be the center of origin and diversity for opium poppy. Thus, the results suggest that the diversity of accessions from other counties has decreased due to the adaptation and selection of

genotypes for various conditions such as the colder climate of northern Europe (Tétényi 1997).This adaptation and selection may also explain the lower genetic diversity of cultivars as compared to landraces. In the present study, the Indian germplasm had similar, moderate levels of genetic diversity as the Anatolian accessions. These results indicate that India might be another center of diversity for this crop; however, this hypothesis must be tested by molecular characterization of additional germplasm from other countries. The diversity analysis results of the present study were consistent with Dittbrenner et al. (2008) in terms of the absence of region-specific clustering. Despite the low genetic diversity of the opium poppy germplasm, the present study allowed selection of a core set containing 22 accessions which preserve the genetic diversity of the entire collection. Inclusion of the core set in local seed banks should be useful to increase efficiency in opium poppy breeding and conservation.

4.3. Molecular and Morphological Characterization of Turkish Opium Poppy Accessions

4.3.1 Population Structure

Structure analysis indicated a low level of diversity as expected based on the previous molecular genetic diversity studies performed using EST (Selale et al. 2013) and genomic SSR (Celik et al. 2014) markers. As with the morphological traits, molecular genetic analysis indicated separate clustering of cultivars and landraces. Moreover, the subpopulations detected by population structure analysis agreed with the clustering results of the neighbor-joining dendrogram. All of the accessions in subpopulation A were in cluster 2 of the dendrogram. In addition, all of the accessions in subpopulation B were in cluster 1 of the dendrogram. All admixed individuals except for Tmo3 clustered in cluster 1 in the neighbor-joining dendrogram. Although the overall genetic diversity of Turkish opium poppy germplasm was low, the quantitative clustering results generated by population structure analysis can be used in association mapping for QTLs (quantitative trait loci) with low magnitudes of effect and for single gene identification because single gene effects can be reduced in highly diverse, structured populations (Tyagi et al. 2014).

Straw yield and morphine content had significant contributions to multivariate discrimination of the three subpopulations (A, B and admixed) with high correlations because subpopulation A had all the cultivars (except Tmo3) which had higher straw yield and morphine content than landraces.

4.3.2. Molecular Genetic Diversity

This study confirmed that Turkish opium poppy germplasm has low genetic diversity. The low diversity determined in this study was consistent with values calculated using EST (Selale et al. 2013) and genomic SSR (Celik et al. 2014) markers in other material. Landraces (0.1) had higher average genetic diversity than cultivars (0.07), therefore, landraces could be a source of molecular genetic diversity to overcome the bottleneck of Turkish opium poppy cultivars. In addition, the introduction of genetic material from other countries may be useful for increasing molecular diversity in Turkish cultivars. The dendrogram results agreed with previous molecular marker studies in that most of the cultivars clustered separately (Selale et al. 2013) but others were intermixed with landraces (Celik et al. 2014). Although the dendrogram topology generated by genomic SSR markers was similar to the topology for both marker types, the EST SSR-based dendrogram did not exhibit the same clustering as the genomic SSR-based dendrogram (data not shown). This might be due to the limited number of EST- SSR markers (13) used in this study. Because the Mantel test results showed a moderate correlation ($r = 0.71$) between the distance matrices for the two types of markers, we decided to combine the data to provide coverage of both genic and non-genic areas of the genome. The moderate level of correlation between the genomic and EST SSR markers may be due to the different selection pressure on genomic and genic SSR markers with genic markers being more likely to be affected by negative selection.

4.3.3. Morphological Trait Diversity

Opium poppy breeding is focused on a few morphological traits including alkaloid content and seed and straw yield. Morphine is the main narcotic alkaloid of opium poppy and remains in high demand (Yadav et al. 2006). Mean morphine content of the Turkish cultivars was nearly two-fold higher than landraces. Cultivars also had more variation in

morphine content (0.84%) than landraces (0.44%). Gumuscu and Arslan (1999) analyzed morphine content of 20 opium poppy breeding materials from seven countries including Turkey and reported that mean morphine content was 0.65% with 0.45% range variation for winter planting and 0.48% with 0.43% variation for spring planting. Thus the average morphine content of opium poppy breeding materials by Gumuscu and Arslan (1999) was lower than the average found in this study with less variation in the trait.

Mean morphine content of the landraces examined in this work (0.44%) was similar to that reported for 353 Turkish accessions which had average content of 0.47% (Arslan et al. 2000). Gumuscu et al. (2008) also found similar morphine content in 99 Turkish opium poppy genotypes with a mean of 0.48%. The Turkish landraces analyzed in the current work had higher mean morphine content than 115 Indian opium poppy genotypes which had a mean of only 0.23% (Prajapati et al. 2002). The Turkish landraces also had a slightly higher coefficient of variation than 122 Indian genotypes (CV=17%) (Shukla et al. 2010). The moderate level of variation for morphine content in the landraces analyzed in this study showed that this population can be used as an association mapping panel to find genes controlling morphine synthesis. Although the landraces analyzed in this study had low breeding potential for morphine content improvement, the top five landraces (genotypes 116, 85, 117, 101, 46) had morphine content more than 0.6% and might be useful in opium poppy breeding. Although (Trivedi et al. 2006) observed a high positive correlation between morphine content and stigma number, no correlation was seen in the current work.

A previous study performed by Gumuscu and Arslan (1999) found that the mean plant height was 76.86 cm with a range variation of 31.6 cm. Plant height of a world collection containing 404 opium poppy accessions had similar mean plant height (105 cm) but a five-fold higher coefficient of variation (25%) than the landraces in this study (Brezinová et al. 2009). Plant height is an important morphological trait because path analysis of nine agronomic traits indicated a positive direct effect of plant height and leaf number on opium yield (Singh et al. 2004). That study showed that plants with medium height and more leaves were more efficient opium producers and may have increased opium latex. In addition, opium poppy cultivars with medium height are more resistant to lodging and make straw harvesting easier. Thus, the opium poppy germplasm used in this study could be good source of alleles for plant height improvement in opium poppy.

The plant germplasm analyzed in this study had lower mean stigma number than found in previous work, 4.5 vs. 11.2 stigmas per flower (Gumuscu et al. 1999). These

results indicate that the landraces analyzed in this study are not good material for improvement of stigma number. Bhandari (1990) suggested that landraces with more stigmas should be selected for morphine content improvement in opium poppy breeding and it was later found that stigma number is strongly correlated with morphine content in opium poppy (Trivedi et al. 2006).

Capsule index of the Turkish poppy landraces was 0.95, indicating almost perfectly globular capsules. The population used in this study had sizable variation for capsule index which was similar with that observed in the world collection of opium poppy containing 404 genotypes (coefficient of variation of 10.19) (Brezinová et al. 2009). Turkish opium poppy landraces had lower capsule index than the world collection (mean of 1.19). These results show that Turkish opium poppy landraces are a good source of alleles for capsule index because the breeding aim for capsule index is a perfectly globular shape. This is because the lamellas of globular capsules can reach the inner space of the capsule as much as possible. As a result, seed and morphine yield increase in globular capsules (Brezinová et al. 2009). In addition, this material can be used as an association mapping panel to understand the genetic basis of capsule shape.

Straw and seed yield are two of the most important traits in opium poppy breeding. Selected opium poppy breeding materials from seven countries including Turkey analyzed by Gumuscu and Arslan (1999) had mean seed yield of approximately 101 kg ha^{-1}. This yield was similar to that calculated for the landraces studied in our work but much lower than for the TMO and AARI cultivars (132.8 kg ha^{-1}). For straw yield, the opium poppy lines analyzed by Gumuscu and Arslan (1999) had higher average straw yield (92.12 kg ha^{-1}) than the Turkish landraces (76.5 kg ha^{-1}) but straw yield was lower than the cultivars (127.8 kg ha^{-1}) analyzed in this study. These results indicate that the TMO and AARI cultivars were more improved than the opium poppy breeding materials analyzed by Gumuscu and Arslan (1999). Although there was diversity for seed and straw yield in the landraces (67 and 52 kg ha^{-1} range diversity for seed and straw yield, respectively), none of the landraces appeared to be suitable as parental material for improvement of seed and straw yield.

The PCA analysis based on the morphological traits of the poppy accessions indicated complete separation of the cultivars with no overlap with landraces. As might be expected, most of the variance in the first axis was explained by morphine content and yield traits. Surprisingly, the cultivars did not show reduced morphological variability compared to the landraces. Overall, the results from morphological analysis suggest that

Turkish landraces may not be the best source of alleles for opium poppy improvement due to their low breeding potential as compared to current Turkish cultivars. However, it must be noted that Turkish landraces had similar breeding potential to the world collection for plant height and capsule index and for morphine content when compared to Indian landraces. The low breeding potential of the Turkish landraces for stigma number, seed and straw yield indicates that germplasm from other countries should be assessed to see how they could contribute to Turkish cultivar breeding.

4.3.4. Core Set Selection

The present study also aimed to test the efficiency of morphological, molecular and both data sets in core set selection. Comparison of the three core sets selected using morphological traits (M-Core), molecular genetic marker data (S-Core), and both datasets (S&M-Core) showed that the molecular genetic data was more efficient than morphological data for core set selection because it allowed maintenance of both morphological and molecular genetic variability in the core set. Frary et al. (2015) reported that core set selection using molecular markers was more reliable in sesame because core sets selected using morphological data could contain genetically identical accessions. S-Core was composed of 21 (20.4%) of the opium poppy landraces from all poppy-growing regions except Isparta. The opium poppy core sets (S-Core and S&M-Core) selected in this study can also be useful to develop association mapping populations such as nested association mapping (NAM) populations (Tyagi et al. 2014).

4.4. Association Mapping

Association mapping is a mapping technique which analyzes linkage disequilibrium (LD) between markers and traits of interest. Molecular characterization revealed significant LD between markers in opium poppy. Mating system, population type and genomic region affect the proportion of marker pairs showing significant LD (Ranc et al. 2012). Opium poppy is self-pollinated with a small proportion of outcrossing. In addition the opium poppy germplasm studied in the present study had low genetic diversity. Thus, it was expected that only a low proportion of marker pairs would have significant LD. In comparison, SSR markers in upland cotton which has a similar mating

system as opium poppy, had slightly higher LD (11% and 9.4%) than the present study (6.5%) (Abdurakhmonov et al. 2008; Qin et al. 2015, respectively). This difference was probably due to population size because the upland cotton studies used much larger populations (241 and 286 individuals). Thus, the smaller population and low diversity of the opium poppy germplasm analyzed in the present study might lead to an underestimation of opium poppy LD. LD can also be due to marker linkage but this could not be confirmed because the SSR and AFLP loci used in this study are not mapped in the opium poppy genome. LD can also be due to selection and relatedness of opium poppy landraces which can lead false positive associations between markers and traits (Pritchard et al. 2000b; Stich et al. 2005). To avoid these false positive associations, AM analysis was corrected using the Q-matrix of population structure generated by STRUCTURE software.

The mean LD values (r^2) of the SSR locus pairs was higher than the means for the AFLP and AFLP-SSR locus pairs due to the different source of the two marker systems. The SSR markers used in the present study were designed from EST and genomic sequences with limited coverage in the opium poppy genome: 0.4% and 2.83% coverage for EST and genomic sequences, respectively (Selale et al. 2013; Celik et al. 2014). In contrast, AFLP markers are random markers with high genome coverage (Jones et al. 2009).

The most economically important trait of opium poppy is morphine content. SSR and AFLP loci associated with this trait had low LD value (r^2). This indicated that each QTL explained a small proportion of the phenotypic variation of morphine content which might be due to dispersion of multiple genes for morphine synthesis in the opium poppy genome. This must be further tested by determining the locations of the markers in the genome and by the addition of more markers. Despite these limitations, these markers are valuable because they can be used to improve the morphine content of opium poppy by 10 to 32%.

A total of five SSR and 14 AFLP markers were found to be associated with five agronomic traits in opium poppy. The greater number of AFLP loci associated with the traits compared to SSR loci was due to the higher number of polymorphic fragments and higher variation of AFLP markers than SSR markers. LD values (r^2) of the SSR and AFLP loci ranged from 0.08 to 0.35. These results indicated that the agronomic traits of opium poppy studied in this study were controlled by loci with minor effect rather than major QTL.

CHAPTER 4

CONCLUSION

Opium poppy (*Papaver somniferum* L.) is major source of many pharmaceutically important alkaloids such as morphine, codeine and noscapine. Although Turkey encompasses half of the legal opium poppy production area in the world, it ranks third in morphine production (18%). The prevalence of low morphine cultivars in Turkey is because of the difficulty of breeding for biochemical traits and a lack of molecular data such as QTL identification and molecular characterization. In the present thesis, recently developed crop specific genomic SSR markers were tested in opium poppy germplasm to demonstrate that they are suitable for efficient genome analysis. Thus, the markers were used to assess the genetic diversity of two populations: a world collection and Turkish germplasm. Also a core set was selected from each population for efficient conservation of the crop diversity. Low genetic diversity and high levels of admixture were revealed and the study suggested that other breeding approaches such as the use of wild species or induced mutagenesis should be employed in opium poppy breeding programs to increase diversity and maximize genetic gain. Morphological diversity of Turkish opium poppy germplasm was analyzed and the results suggested that Turkish landraces had good sources of alleles for morphine content, plant height and capsule index traits. In addition, molecular (SSR and AFLP markers) and morphological data (morphine content and agronomic traits) were associated to identify loci significantly associated with the traits using an AM approach. As a result, QTLs for morphine content and agronomic traits (plant height, stigma number, capsule index, seed and straw yield) were identified. This is the first report of association mapping in this crop. The identified markers provide initial information for marker-assisted selection of important traits in opium poppy breeding

REFERENCES

Acharya HS, Sharma V. 2009. Molecular characterization of opium poppy (*Papaver somniferum*) germplasm. Am J Infect Dis. 5: 148-153.

Achleitner A, Tinker NA, Zechner E, Buerstmayr H. 2008. Genetic diversity among oat varieties of worldwide origin and associations of AFLP markers with quantitative traits. Theor Appl Genet. 117(7): 1041-1053.

Abdurakhmonov IY, Kohel RJ, Yu JZ, Pepper AE, Abdullaev AA, Kushanov FN, Abdukarimov A. 2008. Molecular diversity and association mapping of fiber quality traits in exotic *G. hirsutum* L. germplasm. Genomics. 92(6): 478-487.

Akhatar J, Banga SS. 2015. Genome-wide association mapping for grain yield components and root traits in *Brassica juncea* (L.) Czern & Coss. Mol Breeding. 35: 48.

Arslan N, Buyukgocmen R, Gumuscu A. 2000. Türk haşhaş populasyonlarının yağ ve morfin muhtevaları. Journal of Field Crops Central Research Institute. 9: 1-2.

Bernath J. 2003. Poppy: the genus Papaver. Florida: CRC Press. 405 p.

Bhandari MM. 1990. Out-crossing in opium poppy *Papaver somniferum* L. Euphytica. 48: 167-169.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 23(19): 2633-2635.

Brown AHD. 1989. Core collections: a practical approach to genetic resources management. Genome. 31(2): 818-824.

Brezinová B, Macák M, Eftimová J. 2009. The morphological diversity of selected traits of world collection of poppy genotypes (genus Papaver). J Cent Eur Agric. 10: 183-192.

Celik I, Gultekin V, Allmer J, Doganlar S, Frary A. 2014. Development of genomic simple sequence repeat markers in opium poppy by next-generation sequencing. Mol Breed. 34: 323-334.

Chabane K, Ablett GA, Cordeiro GM,Valkoun J, Henry RJ. 2005. EST versus genomic derived microsatellite markers for genotyping wild and cultivated barley. Gen Resour Crop Evol. 52: 903-909.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica. 142: 169-196.

Dent AE, Bridgett MV. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conserv Genet Resour. 4: 359-361.

Dittbrenner A, Lohwasser U, Mock HP, Borner A. 2008. Molecular and phytochemical studies of *Papaver somniferum* in the context of infraspecific classification. Acta Hortic. 799: 81-88.

Doyle JJ, Doyle JE. 1990. Isolation of plant DNA from fresh tissue. Focus. 12: 13-15.

Eckert AJ, Wegrzyn JL, Cumbie WP, Goldfarb B, Huber DA, Tolstikov V, Fiehn O, Neale DB. 2012. Association genetics of the loblolly pine (*Pinus taeda, Pinaceae*) metabolome. New Phytol. 193: 890-902.

Facchini PJ, De Luca V. 2008. Opium poppy and Madagascar periwinkle: model non-model systems to investigate alkaloid biosynthesis in plants. Plant J Cell Mol Biol. 54: 763-784.

Frankel OH. 1984. Genetic perspective of germplasm conservation. In: Arber W, Llimensee K, Peacock WL, Starlinger P, editors. Genetic manipulation: Impact on man and society. 1st ed. Cambridge: Cambridge University Press. p 161-170.

Frary A, Tekin P, Celik I, Furat S, Uzun B, Doganlar S. 2015. Morphological and Molecular Diversity in Turkish Sesame Germplasm and Core Set Selection. Crop Sci. 55: 702-711.

Friedt W, Snowdon R, Ordon F, Ahlemeyer J. 2007. Plant breeding: assessment of genetic diversity in crop plants and its exploitation in breeding. Prog Bot. 68: 151-178.

Gómez G, Álvarez MF, Mosquera T. 2011. Association mapping, a method to detect quantitative trait loci: statistical bases. Agronomía Colombiana. 29(3): 367-376.

Gumuscu A, Arslan N. 1999. Comparing yield and yield components of some selected poppy (Papaver somniferum L.) lines. Turk J Agric For. 4: 991-997.

Gumuscu A, Arslan N. 2008. Researches on heterosis on yield and yield components of some poppy hybrid lines. J Agric Sci. 14: 365-373.

Gumuscu A, Arslan N, Sarıhan EO. 2008. Evaluation of selected poppy (*Papaver somniferum* L.) lines by their morphine and other alkaloids contents. Eur Food Res Technol. 226: 1213-1220.

Jones N, Ougham H, Thomas H, Pašakinskienė I. 2009. Markers and mapping revisited: finding your gene. New Phytol. 183(4): 935-966.

Kim KW, Chung HK, Cho GT, Ma KH, Chandrabalan D, Gwag JG, Tae-San K, Eun-Gi C, Park YJ. 2007. PowerCore: a program applying the advanced M strategy with a heuristic search for establishing core sets. Bioinformatics. 23(16): 2155-2162.

Lee EJ, Jin GN, Lee KL, Han MS, Lee YH, Yang MS. 2011. Exploiting expressed sequence tag databases for the development and characterization of gene-derived simple sequence

repeat markers in the opium poppy (*Papaver somniferum* L.) for forensic applications. J Forensic Sci. 56: 1131-1135.

Lavania, UC,Srivastava S. 1999. Quantitative delineation of karyotype variation in Papaver as a measure of phylogenetic differentiation origin. Curr Sci. 77: 429-435.

Norusis MJ. 2010. PASW statistics 18 advanced statistical procedures. New Jersey: Prentice Hall Press. 432 p.

Patra NK, Ram RS, Chauhan SP, Singh AK. 1992. Quantitative studies on the mating system of opium poppy (*Papaver somniferum* L.). Theor Appl Genet. 84: 299-302.

Parmaksiz I, Ozcan S. 2011. Morphological, chemical and molecular analyses of Turkish Papaver accessions (Sect. Oxytona). Turk J Bot. 35: 1-16.

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor Appl Genet. 97(8): 1248-1255.

Prajapati S, Bajpai S, Singh D, Luthra R, Gupta MM, Kumar S. 2002. Alkaloid profiles of the Indian land races of the opium poppy *Papaver somniferum* L. Genet Resour Crop Evol. 49: 183-188.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 38: 904-909.

Pritchard JK, Stephens M, Donnelly P. 2000a. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. 2000b. Association mapping in structured populations. Am J Hum Genet. 67: 170-181.

Ranc N, Muños S, Xu J, Le Paslier MC, Chauveau A, Bounon R, Rolland S, Bouchet JP, Brunel D, Causse M. 2012. Genome-wide association mapping in tomato (*Solanum lycopersicum*) is possible using genome admixture of *Solanum lycopersicum* var. cerasiforme. G3 Genes Genom Genet. 2(8): 853-64.

Qin H, Chen M, Yi X, Bie S, Zhang C, Zhang Y, Lan J, Meng Y, Yuan Y, Jiao C. 2015. Identification of associated SSR markers for yield component and fiber quality traits based on frame map and upland cotton collections. PloS one. 10(1): 1-16.

Saunders JA, Pedroni MJ, Penrose LDJ, Fist AJ. 2001. AFLP analysis of opium poppy. Crop Sci. 41: 1596-1601.

Schulz H, Baranska M, Quilitzsch R, Schütze W. 2004. Determination of alkaloids in capsules, milk and ethanolic extracts of poppy (Papaver somniferum L.) by ATR-FT-IR and FT-Raman spectroscopy. The Analyst. 129: 917–920.

Selale H, Celik I, Gultekin V, Allmer J, Doganlar S, Frary A. 2013. Development of EST-SSR markers for diversity and breeding studies in opium poppy (*Papaver somniferum* L.). Plant Breed. 132: 344-351.

Semagn K, Bjørnstad Å, Xu Y. 2010. The genetic dissection of quantitative traits in crops. Electron J Biotechn 13(5): 16-17.

Shukla S, Yadav HK, Rastogi A, Mishra BK, Sant Singh P. 2010. Alkaloid Diversity in Relation to Breeding for Specific Alkaloids in Opium Poppy (*Papaver somniferum* L.). Czech J Genet Plant Breed. 46: 164-169.

Singh SP, Shukla S, Yadav HK. 2004. Genetic studies and their implication to breed desired plant type in opium poppy, *Papaver somniferum* L. Genetika. 36: 69-81.

Sørensen AP, Stuurman J, Voort JRVD, Peleman J. 2007. Molecular breeding: maximizing the exploitation of genetic diversity. In: Varsey RK, Tuberosa R, editors. Genomics-assisted crop improvement. 1st ed. Netherlands: Springer. p 31-56.

Stich B, Melchinger AE, Frisch M, Maurer HP, Heckenberger M, Reif JC. 2005. Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor Appl Genet. 111(4): 723-730.

Storey JD. 2002. A direct approach to false discovery rates. J R Stat Soc Ser B. 64: 479-498.

Storey JD, Tibshirani R. 2003. Statistical significance for genome-wide experiments. Proc Natl Acad Sci. 100: 9440-9445.

Straka P, Nothnagel T. 2002. A genetic map of *Papaver somniferum* L. based on molecular and morphological markers. J Herbs Spices Medic Plants. 9: 235-241.

Tanksley SD, McCouch SR. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science. 277: 1063-1066.

Tétényi P. 1997. Opium poppy (*Papaver somniferum*) botany and horticulture. Hortic Rev. 19: 373-408.

Tian CX, Liang XF, Yang M, Zheng HZ, Dou YQ, Cao L. 2012. Isolation and characterization of novel genomic and EST-SSR markers in *Coreoperca whiteheadi* Boulenger and cross-species amplification. Int J Mol Sci. 13: 13203–13211.

Trivedi M, Tiwari RK, Dhawan OP. 2006. Genetic parameters and correlations of collar rot resistance with important biochemical and yield traits in opium poppy (*Papaver somniferum* L.). J Appl Genet. 47: 29-38.

[TMO] Turkish Soil Products Office. 2009. Opium poppy. Report of a study by Turkish Soil Products Office. Ankara: Turkish Soil Products Office.

Tyagi P, Gore MA, Bowman DT, Campbell BT, Udall JA, Kuraparthy V. 2014. Genetic diversity and population structure in the US Upland cotton (*Gossypium hirsutum* L.). Theor Appl Genet. 127: 283-295.

Van Hintum TJ, Brown AHD, Spillane C, Hodkin T. 2000. Core collections of plant genetic resources. IPGRI Technical Bulletin. 3: 1-48.

Varshney RK, Graner A, Sorrells ME. 2005. Genic microsatellite markers in plants: features and applications. Trends Biotech. 23: 48–55.

Verma N, Jena SN, Shukla S, Yadav K. 2016. Genetic diversity, population structure and marker trait associations for alkaloid content and licit opium yield in India-wide collection of poppy (*Papaver somniferum* L.). Plant Gene. 7: 26-41.

Wang, ML, Sukumaran S, Barkley NA, Chen Z., Chen CY, Guo B, Pittman RN, Stalker HT, Holbrook CC, Pederson GA, Yu J. 2011. Population structure and marker–trait association analysis of the US peanut (Arachis hypogaea L.) mini-core collection. Theor Appl Genet. 123(8): 1307-1317.

Weber AL, Briggs WH, Rucker J, Baltazar, BM., Sánchez-Gonzalez JDJ, Feng P, Bucler ES, Doebley J. 2008. The genetic architecture of complex traits in teosinte (Zea mays ssp. parviglumis): new evidence from association mapping. Genetics. 180(2): 1221-1232.

Wei W, Zhang Y, Lü H, Li D, Wang L, Zhang X. 2013. Association analysis for quality traits in a diverse panel of Chinese sesame (*Sesamum indicum* L.) germplasm. J Integr Plant Biol. 55(8): 745-758.

Winzer T, Gazda V, He Z, Kaminski F, Kern M, Larson TR, Li Y, Meade F, Teodor R, Vaistij FE, Walker C, Bowser TA, Graham IA. 2012. A *Papaver somniferum* 10-gene cluster for synthesis of the anticancer alkaloid noscapine. Sciencexpress. 336: 1704-1708.

Woodhead M, Russell J, Squirrell J, Hollingsworth PM, Mackenzie K, Gibby M, Powell W. 2005. Comparative analysis of population genetic structure in *Athyrium distentifolium* (Pteridophyta) using AFLPs and SSRs from anonymous and transcribed gene regions. Mol Ecol. 14(6): 1681-1695.

Yadav HK, Shukla S, Singh SP. 2006. Genetic variability and interrelationship among opium and its alkaloids in opium poppy (*Papaver somniferum* L.). Euphytica. 150: 207-214.

Yu J, Buckler ES. 2006. Genetic association mapping and genome organization of maize. Curr Opin Biotechnol. 17(2): 155-160.

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Holland JB, Kresovich S, Buckler ES. 2006. A unified mixed-model method for association mapping accounting for multiple levels of relatedness. Nat Genet. 38: 203–208.

Zhou X, Zhang HD, Li ZH, Zhang C, Li JL, Li YX, Zhang YM. 2014. Plant Genetic Resources Management for Sustainable Development. Adv Mat Res. 864: 2528-2531.

Zhu C, Gore M, Buckler ES, Yu J. 2008. Status and prospects of association mapping in plants. The Plant Genome. 1(1): 5-20.

Ziegler J, Facchini PJ, Geissler R, Schmidt J, Ammer C, Kramell R,Voigtländer S, Gesell A, Pienkny S, Brandt W. 2009. Evolution of morphine biosynthesis in opium poppy. Phytochem. 70: 1696-1707.

VITA

İbrahim Çelik

Date and Place of Birth: 1984 Gölhisar/ Burdur

E-mail and Web: İbrahimcelikmbg@gmail.com /www.icelik.net

Education

Ph.D. Molecular Biology and Genetics
20011-2017 İzmir Institute of Technology,
Molecular Biology and Genetics Department, İzmir, Turkey

MSc. Molecular Biology and Genetics
2008-2011 İzmir Institute of Technology,
Molecular Biology and Genetics Department, İzmir, Turkey

BSc. Molecular Biology and Genetics
2003 – 2007 Istanbul University,
Molecular Biology and Genetics Department, Istanbul, Turkey

Teaching and Professional Experience

Research assistant

2008-2017 İzmir Institute of Technology,
Molecular Biology and Genetics Department, İzmir, Turkey

Selected Publications

Celik I Leblebici F, DoganlarS, Frary A. Submitted. SSR markers reveal high level of admixture in opium poppy world collection.

Celik I, Sogut MA, Ercan Ozkaynak, Doganlar S, Frary A. 2016. Physical mapping of NBS-coding resistance genes to the *Me*-gene cluster on chromosome P9 reveals markers tightly linked to the *N* gene for root-knot nematode resistance in pepper. Molecular breeding. 36(10): 1-7.

Celik I, Camci H, Kose A, Kosar FC, Doganlar S, Frary A. 2015. Molecular genetic diversity and association mapping of morphine content and agronomic traits in Turkish opium poppy (*Papaver somniferum*) germplasm. Molecular breeding. 36(4): 1-13.