GENETIC DIVERSITY OF EUROPEAN BLACK POPLAR (*Populus nigra*) POPULATIONS FROM TURKEY ASSESSED BY MICROSATELLITE DNA MARKERS

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ABSTRACT

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The genus *Populus* is known as a group of forest trees for commercial exploitation. The European black poplar (*Populus nigra* L.), belonging to the genus *Populus*, is a keystone species of floodplain forests in Europe. *Populus nigra* has also been used as a model tree in the study of genetic resource conservation of wild relatives of cultivated plants. Turkey has over 68.000 ha poplar plantations with *P. nigra* and 77. 000 ha consisting of various hybrids of it. Due to overexplotation, loss of natural distribution area and introgression, *P. nigra* is one of the most threatened tree species in Turkey and Europe.

The important and major genetic resources of European black poplar for Turkey consist of 297 clones and located in Behiçbey clone bank in Ankara with perspective of *ex situ* conservation programme for future plantations. To characterize genetic diversity in European black poplar populations and to provide genetic identity information for these, 297 *P. nigra* clones were analysed by using 12 microsatellite DNA markers. Also, 32 trees from newly discovered two natural populations were included into the analyses to compare them with the clone bank populations with respect to genetic diversity. The number of alleles ranged from 3.36 to 8.08 for 12 populations. The observed heterozygosity was found to be between 0.62 and 0.69. About 8 % of total genetic variation is between clone bank and natural populations. Great portion of total genetic variation (92%) is within the populations present in the clone bank. When all European black poplar populations (clone bank and natural populations) were evaluated with respect to genetic relatedness, clone bank and natural populations formed two separate group in the dendogram. Foreign and open pollinated populations indicated a close association to natural populations.

In the current study, with the help of microsatellite DNA markers, identity problems of clones were solved. Furthermore, magnitute and pattern of genetic diversity in populations from clone bank and natural distribution areas were assessed and clonally duplicated trees were successfully determined. All populations have sufficient genetic diversity to carry out further breeding programmes for European black poplar. There have been found many

clonally duplicated trees in the clone bank. For further breeding studies, these clones have to be replaced with new selected individuals from genetic diversity rich areas.

Key Words: *Populus*, microsatellite marker, genetic diversity, identity problem, natural distribution

ÖZ

TÜRKİYEDEKİ AVRUPA KARA KAVAK POPULASYONLARININ (*Populus nigra*) GENETİK ÇEŞİTLİLİĞİNİN MİKROSATELLİT DNA MARKÖRLERİ İLE DEĞERLENDİRİLMESİ

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Populus cinsi, ticari tüketimi olan orman ağaçlarının bir grubu olarak bilinir. *Populus* cinsine ait olan Avrupa kara kavağı (*Populus nigra* L.), Avrupa' da taşkın yatağında bulunan ormanların önemli türlerinden biridir. *Populus nigra* ayrıca kültüre alınmış bitkilerin yabani formlarının genetik kaynaklarını koruma çalışmalarında model ağaç olarak kullanılmaktadır. Türkiye, 68.000 hektarın üzerinde *Populus nigra* ve 77.000 hektar da kara kavağın çeşitli hibritlerinden oluşan kavak plantasyonuna sahiptir. Aşırı tüketim, doğal dağılım alanının kaybı ve gen alışverişi yüzünden *P.nigra,* Türkiye ve Avrupa'da en çok tehdit edilen ağaç türlerinden biridir.

Türkiye'nin gelecek plantasyonlarını oluşturan önemli ve temel Avrupa kara kavak gen kaynakları 297 ağaçtan oluşmakta ve ex situ koruma çalışmaları çerçevesinde Ankara Behiçbey klon bankasında bulunmaktadır. Avrupa kara kavak populasyonlarının genetik çeşitliliğini belirlemek ve klonların genetik kimlik bilgisini elde etmek için, 297 *P. nigra* klonu 12 mikrosatellit markörü kullanılarak analiz edildi. Ayrıca yeni bulunan iki doğal populasyondan 32 ağaç, klon bankası populasyonları ile aralarındaki genetik çeşitliliği karşılaştırmak amacıyla analize dahil edildi. Oniki populasyon için alel sayısı 3,36 ve 8,08 arasında değişti. Gözlenen heterozigotluk 0,62 ve 0,69 arasında bulundu. Genetik varyasyonun yaklaşık %8'i klon bankası ve doğal populasyonlar arasındadır. Toplam genetik çeşitliliğin büyük kısmı (%92) klon bankasında yer alan populasyonlar içindedir. Bütün kara kavak populasyonları (klon bankası ve doğal populasyonlar) genetik ilişki açısından değerlendirildiğinde klon bankası ve doğal populasyonlar dendogramda iki ayrı grup oluşturdu. Yabancı ve açık tozlaşma ile elde edilen populasyonlar doğal populasyonlarla yakın bir ilişki gösterdi.

Bu çalışmada mikrosatellit DNA markörleri yardımıyla klonların kimlik karışıklığı çözüldü. Ayrıca, klon bankası ve doğal dağılım alanlarındaki populasyonların genetik çeşitliliğinin boyutu ve şekli değerlendirildi ve klonal olarak çoğaltılmış ağaçlar başarılı bir şekilde belirlendi. Bütün populasyonlar Avrupa kara kavağı için dizayn edilecek gelecek ıslah programları için yeterli genetik çeşitliliğe sahiptir. Klon bankasında çok sayıda klonal olarak

çoğaltılmış ağaç bulundu. Gelecek ıslah çalışmaları için bu klonlar genetik çeşitliliğin zengin olduğu bölgelerden seçilmiş yeni bireylerle değiştirilmelidir.

Anahtar Kelimeler: *Populus*, mikrosatellit markör, genetik çeşitlilik, kimlik problemi, doğal dağılım

to my love...

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
βME	Beta Mercapto Ethanol
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose Triphosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
EUFORGEN	European Forest Genetic Resources Programme
GDA	Genetic Data Analysis
IPC	International Poplar Commission
IPGC	International Populus Genome Consortium
IUCN	International Union for Conservation of Nature
MCMC	Markov Chain Monte Carlo
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymoprhic DNA
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TBE	Tris Boric Acid EDTA (Ethylene Diamine Tetra Acetic Acid Disodium Salt)
UPGMA	Unweighted Pair Group Method using Arithmethic Averaging

CHAPTER 1

INTRODUCTION

1.1. Genus *Populus*

The genus *Populus*, including poplars, cottonwoods and aspens, is known as one of the most commercially exploited groups of forest trees (Hamzeh and Dayanandan, 2004). Members of the genus *Populus* (Salicaceae) are major suppliers of industrial wood worldwide and are suitable for clonal forestry. *Populus* contains deciduous flowering plants belonging to the family Salicaceae. Eckenwalder (1996) divided the genus *Populus* into 29 species in six sections (Abaso, Aigeiros, Leucoides, Populus, Tacamahaca, Turanga*).* Its distribution area is in the temperate and subtropical zones of the northern hemisphere from *20°* to *70°* N latitude and many other parts of the world. It is known as the most abundant woody plant genus in temperate forests around the world (Rae *et al*., 2007).

Populus species are predominantly dioecious and obligatory outcrossers. These trees with alternate leaves have rapid growth. The flowers of poplar occur in catkins and yield large amounts of pollen and small, cotton-tufted seed. Its seeds are dispersed by wind and water in light hydrophobic cotton (Legionnet *et al*., 1997). The genus has the potential to undergo reproduction asexually and sexually.

Poplars are recognized as an excellent choice for intensive-culture management due to their rapid juvenile growth, immediate response to cultural practices and coppicing property (Stettler, 1996). The genus *Populus* is recognized as a model forest tree because of their potential to grow fast, easy production of clonal propagation and small genome size (X = 19, 2n = 2X = 38, 450-550 mega base pairs) (Bradshaw *et al*., 2000; Taylor, 2002). Also physical and molecular genetic maps which link between phenotypic traits and genes are available. In 2003, the poplar genome was the first tree genome that was fully sequenced (Taylor, 2002). Poplar species are used increasingly in physiology, biochemistry, agronomy, biotechnology, genetic engineering and genomics studies around the world (Rae *et al*., 2007).

1.1.1. *Populus nigra*

1.1.1.1 Biology and ecology

Floodplain forests are known as among the most diverse and important ecosystems in Europe. *Populus nigra* is the most representative tree species of these riparian habitats (Vietto *et al*., 2008). *Populus nigra* (European black poplar) is single-stemmed, deciduous tree with a rounded outline. It can grow 30 m (rarely 40 m) height and 1.5 m in diameter

(Figure 1.1.A). Wide canopy has large and dispersed branches. Alternate leaves are oval or triangular in shape, 5-10 cm long and 6-8 cm broad, also green on both surfaces. The blade margins are crenate. Flattened leaf-stalks are 3-7 cm long (Figure 1.1.B).

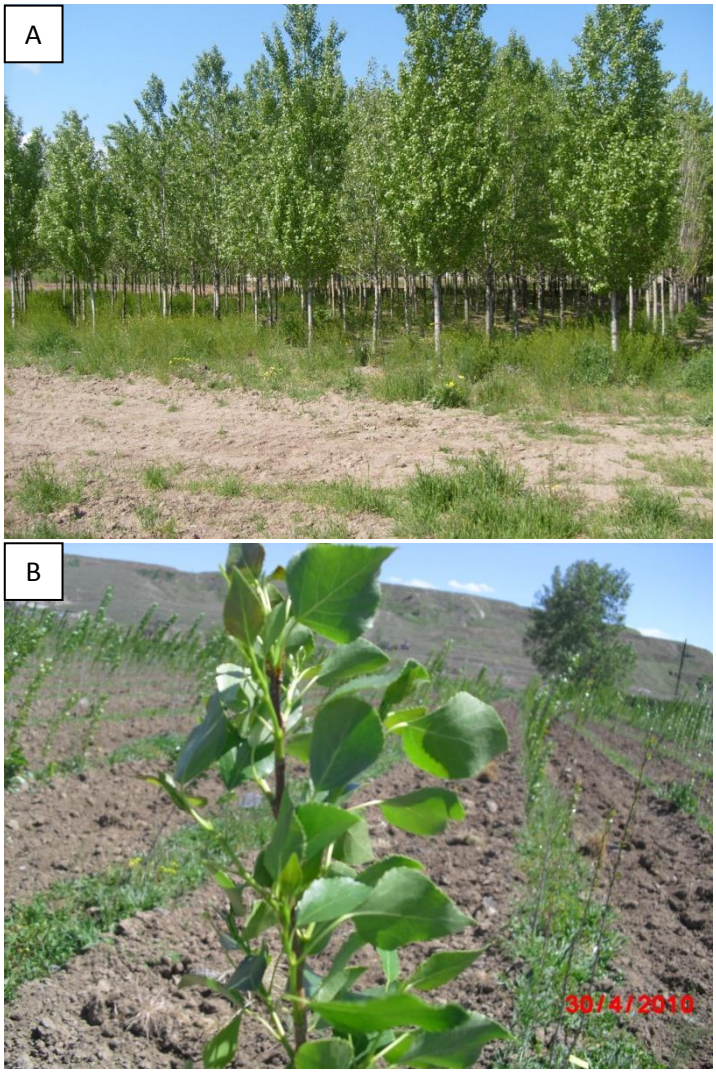

Figure 1.1. A. General apperance of *Populus nigra* in Behiçbey nursery. B .The leaf of European black poplar

The species is dioecious (male and female flowers on different plants). Male and female flowers are clustered in pendulous catkins on separate male and female trees. The catkins (<10 cm long) are reddish-purple in males and green in females (Figure 1.2)

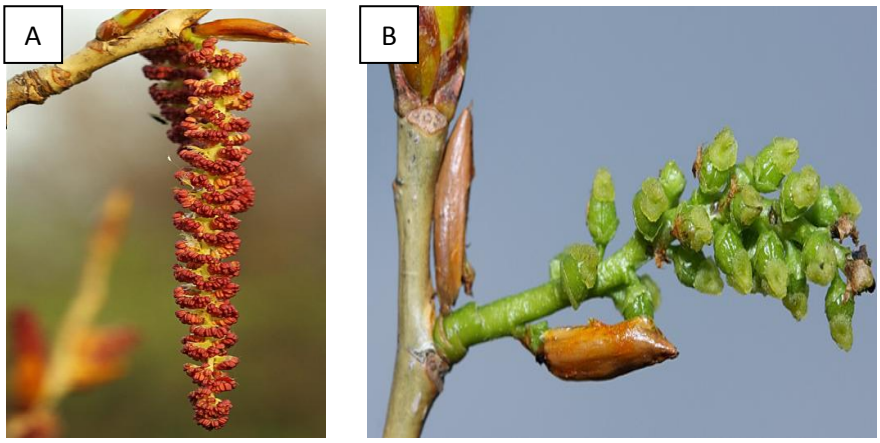

Figure 1.2. Catkin structure of *Populus nigra*. A. Male catkin, B. Female catkin (www.bgflora.net, Retrieved on 02.01.2013)

Among temperate trees, only poplar and willow have female catkins including seeds with a coma of cottony hairs on parietal placentas in thin-walled capsules (Gaudet , 2006)(Figure 1.3). With the help of the pappus of the seeds promoting wind dispersal over large distances, high rates of migration, gene flow and genetic diversity are obtained (Legionnet and Lefevre, 1996).They reach the reproductive stage when they are 10-15 years old. Braatne *et al*. (1996) reported that a poplar tree will not begin to produce remarkable quantities of seeds until they are more than 20 years old. Leaf initiation starts in the early spring (March-April) (Rae *et al*., 2007).

It is characterized by an efficient dissemination of both seeds and pollen, but also by a good ability to naturally undergo vegetative propagation (Arens *et al.*, 1998). Seeds, disseminated through wind and water, have a short viability and need optimal soil-water conditions and sites where is recent deposits of sand and shingle for germination (Gaudet , 2006). Guilloy-Froget *et al.* (2002) reported that the most favorable condition for germination and seedling survival is a continuously wet substrate. Successful regeneration occurs where the moisture of sediment is suitable for seedling roots to establish (Legionnet *et al.*, 1999). Seedlings germinate on river margins. Although European black poplar is a pioneer species that colonizes raw soil, no seed bank has been constituted (Legionnet *et al.*, 1997) due to low germination capacity declining within 18 days following maturity (Van Splunder *et al*., 1995).

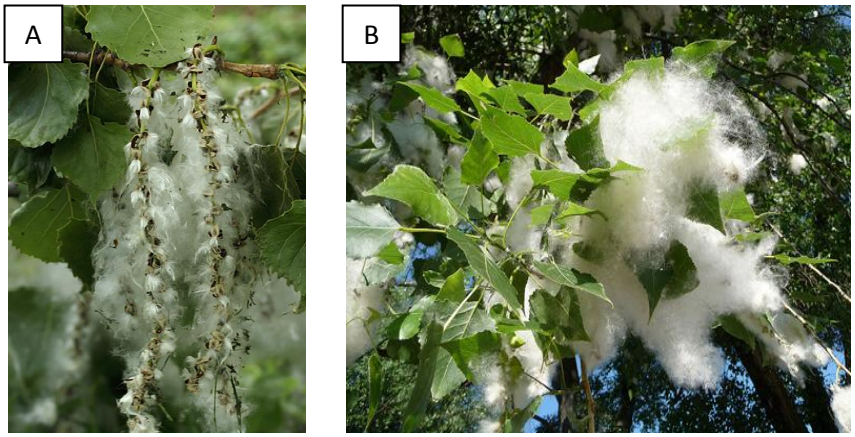

Figure 1.3. Fruit and seed structure of *Populus nigra*. A. Fruit (www.bgflora.net, Retrieved on 02.01.2013) B. Seed (www.fnpsblog.blogspot.com, Retrieved on 02.01.2013)

European black poplar can be hybridized with *P. deltoides*, *P. trichocarpa* and other exotic *Populus* species. Obtained hybrids have desirable characteristics such as wide adaptability to many environments and different kinds of soil, excellent rooting ability of cuttings and fair resistance to common diseases (Cagelli and Lefevre, 1997 and Vanden Broeck, 2003). European black poplar plays significant role as a keystone species for softwood floodplain forest ecosystems (Rathmacher *et al*., 2010). It is considered to be a dominant species in riparian mixed forests together with *P. alba* L., willows, alders, maple, elm, ash on the riverbanks (Arens *et al*., 1998 and Csencsics *et al.*, 2009). Almost 40 percent of black poplars are grown in line plantations along canals and stream banks and around irrigable fields (Toplu, 2005).

European black poplar is the one example of the fastest growing temperate trees which have heterophyllous growth habit (Rae *et al*., 2007). Heliophily and plasticity cause to formation of metapopulation (Cagelli and Lefevre, 1995). There is a great diversity in population types, from isolated trees to huge pure or mixed stands (Lefevre *et al*., 1998). Individual trees may live over 400 years (Popivshchy *et al*., 1997). Naturally grown *P.nigra* stands mainly exist along rivers and streams and their distribution is sometimes very scattered and extended (Guilloy-Froget *et al*., 2002).

1.1.1.2. Taxonomy

Populus nigra, is an angiosperm of the genus *Populus* in the Aigeiros section belonging to the family Salicacea and contain trees with green leaves on both sides and a large petiole (Toplu, 2005). There are three subspecies, but some scientist suggest that there is one more. These are *Populus nigra* subsp. *nigra* spread in Central and Eastern Europe, *Populus nigra* subsp. *betulifolia* (Pursh) W.Wettst distributed in North-west Europe, *Populus nigra* subsp. *caudina* (Ten.) Bugała found in Mediterranean region, also southwest Asia and *Populus*

nigra var. *afghanica* Aitch. & Hemsl. (syn. *P. nigra* var. *thevestina* (Dode) Bean) spread in Southwest Asia. European black poplar has diploid chromosome number of 2n= 38.

Polymorphisms in morphology and physiology vary between geographically distinct populations (Toplu, 2005). The taxonomy of *Populus* is complicated because its members can hybridize with one another (Cagelli and Lefevre, 1997). Due to extensive interspecific hybridization and high morphological diversity in this genus, there are several problems about identifying taxonomic units for comparative evolutionary studies and systematics (Hamzeh and Dayanandan, 2004). In contrast to known, some recent systematic studies have had new results about the placement of the Euroepan black poplar that is not included in Section Aigeiros. *Populus nigra* has a wide range and a clear human intervention. These cause the spread of the species and make the taxonomy of the species particularly complex (Cagelli and Lefevre, 1997).

1.1.1.3. Distribution

Populus is known as tropical in origin and the greatest diversity of the genus lies far South of the boreal region. *P. nigra* is known as an azonal species which is not linked to particular climatic area. Its distribution is linked to the soil moisture (Gaudet , 2006). European black poplar is a geographically widespread tree species (Arens *et al*., 1998) and the natural distribution area of European black poplar ranges from Central, Western (with the exception of Scandinavia, Ireland and Scotland (Toplu, 2005) and Southern Europe to Central and Western Asia and Northern Africa (Rathmacher *et al*., 2010).

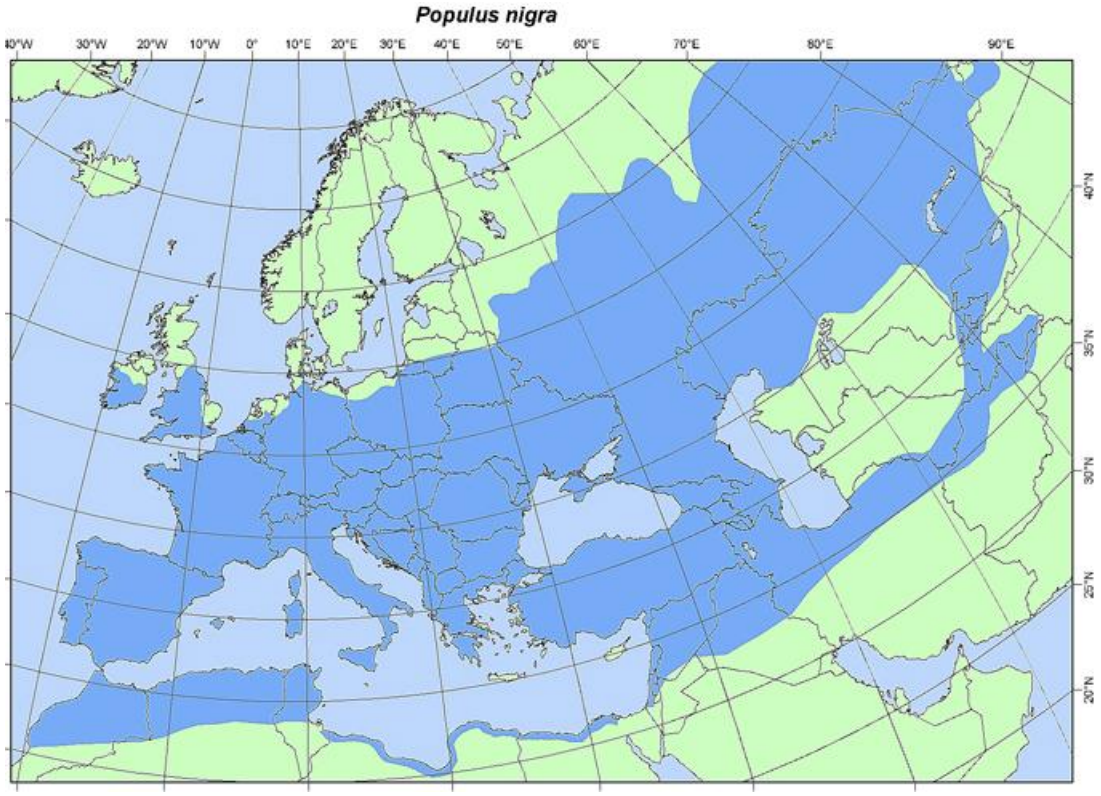

Figure 1.4. The distribution area of *Populus nigra* in the world. The blue area represents the distribution of *P. nigra* (http://www.euforgen.org/fileadmin/www.euforgen.org/Documents/Maps/JPG/populus_nigra.jpg, Retrieved on 02.01.2012)

1.1.1.4. Importance and Use

European black poplars are the most important trees in terms of ecological, economic and social interest in the world. They have ecological importance as indicator species for biodiversity of riparian woodlands. European black poplar forests are accepted as centres for biodiversity, because a large number of threatened and common insects and animals depend on poplars (Vanden Broeck, 2003). In their native ecosystems, they play a major role in the recolonization of sites after disturbances and provide important sites for fish and wildlife. European black poplar is widely used as windbreak in agricultural lands to prevent soil erosion. To stabilize the banks of streams and canals, row or gallery plantations of black poplar have had importance (Stettler *et al.*, 1996).

They are known as unique pioneer species of European riparian ecosystems which contribute to the natural control of flooding and water quality (Gaudet *et al.*, 2008). *Populus* and *Salix* species dominate the early successional stages of floodplain woodlands in many temperate and Mediterranean areas (Vanden Broeck, 2003). European black poplar can be used as a pure species for soil protection, and afforestation in polluted industrial zones by the help of

its plasticity (Popivshchy *et al*., 1997). In order to reduce the carbon dioxide emission levels, poplar species which are fast growing hardwoods are tested for the biomass production (Benetka *et al*., 2002; Laureysens *et al*., 2005). European black poplar is accepted as a parent pool in breeding programmes in many parts of the world. About 63 % of the poplar cultivars descend from it and many successful interspecific hybrids have been produced from them (Crensiscs *et al*., 2009). These hybrids show particularly good stem forms and excellent growth.

European black poplar is widely planted in East European countries for domestic use as round wood for rural construction and for the daily needs of rural people. The wood of European black poplars is used as raw material for the furniture, packaging, particleboard, plywood, and match industries. The wood of European black poplar has no fragrance, so it is used for the manufacture of fruit boxes (Gaudet *et al.*, 2006). In recent years, poplars have received increasing attention as a renewable source of biomass for energy and short fiber furnish for paper making. They have multiple use as a source of fiber, fuel lumber and animal feed, which have been closely associated with agriculture for a long time (Stettler *et al*., 1996).

It has been planted in order to delineate parish and county boundaries. Male trees are largely grown for city poplar plantations and for ornamental purposes in parks, because they do not produce the seed fluff which is considered to be unsightly and have negative effects on human health (Arens *et al*., 1998).

1.1.1.5. Threats

Ninety nine percent of the riparian forests in Europe have disappeared (Lefevre *et al*., 1998; Smulders *et al*., 2008) and the IUCN Red List of Threatened Species includes *Populus nigra* (Vietto, 2008). European black poplar is considered a rare and endangered tree species mainly because of two reasons. The first one is the alteration of riparian ecosystems throughout the species' distribution area and overexploitation of resources. The second one is the introgression from cultivated poplar. Natural habitat of the European black poplar has been lost because of urbanisation, land drainage, canalisation of rivers (Cottrell, 2004), intensive grazing, more frequent felling of trees (Lefevre *et al.* 1998) and wood cutting (Storme *et al.*, 2004). Changes in natural flooding dynamics reduce suitable areas for seedling establishment and European black poplar 's capability for natural regeneration (Pospiskova and Bartakova, 2004; Rathmatcher *et al*., 2010). Van Dam, (2002) points out that habitat change and lack of sexual reproduction have caused a severe decline of European black poplar both in numbers of individuals as well as entire populations.

The potential threat of introgression from cultivated poplars and its hybrids distributed all over continental Europe is a known reason for extinction of black poplar (Vanden Broeck *et al*. 2005). European black poplar can be hybridized with *P. deltoides*, *P. trichocarpa* and other exotic *Populus* species. Many hybrid poplars have replaced many native populations of European black poplar (Rathmatcher *et al*., 2010). Genetic swamping and introgression by

related nonindigenous *Populus* taxa are accepted as a threat for the species' genetic and evolutionary integrity. Introgression has negative effects on the species. It causes inbreeding in further generations and reduces biological fitness. Massive introduction of genes of foreign species into the native European black poplar could lower the effective population size and reduce the overall fitness of seedlings of the native European black poplar (Cagelli and Lefevre, 1995; Vanden Broeck *et al*., 2004). Hybridization and introgression from cultivated trees and *P. nigra* var. *italica* have caused to genetic swamping and change the gene pool of European black poplar (Vietto *et al*.,2008).

1.1.1.6. Genetic studies

Genetic diversity plays important role to allow a population to survive and reproduce under changing environmental conditions. Thus, assessing the genetic diversity of any population has great importance. Also proper identification of poplar cultivars is the necessity for registration, certification and variety control (De-Lucas *et al*., 2008). Accurate identification of poplar trees can be made by the traditional method adopted by the IPC based on a total of 64 morphological, phenological and floral characteristics (UPOV, 1981). This traditional method is not always satisfying because it was described as difficult, ambiguous, time-consuming and subjective (Rajora and Rahman, 2003).

Genetic analysis of *P. nigra* populations have been performed with the use of different molecular markers. Molecular markers are used for differentiation and identification purposes. These are allozyme polymorphism (Rajora, 1989), isoenzymes (Legionnet and Lefevre, 1996), DNA markers such as ribosomal DNA polymorphisms (Rajora and Dancik 1992, Faıvre-Rampant *et al.,* 1992), mitochondrial DNA variation (Barrett *et al*., 1992), chloroplast DNA (Smith and Sytsma 1990), RAPDs (Castiglione *et al*., 1993) and AFLPs (Cervera *et al.,* 2001; Cervera *et al*., 2005; Fossati *et al*., 2005).

Recently, nuclear simple sequence repeats have been used to identify poplar trees and to assess genetic diversity of poplar populations. Microsatellites, or simple sequence repeats (SSR), are abundant polymorphic elements in nuclear genomes and tandemly repeated short DNA sequence motifs. These repeats occur throughout eukaryotic genomes and generally embedded in unique DNA sequences. Different number of repeats provide polymorphism among individuals (Rahman *et al*., 2000). Microsatellite DNA markers are ideal markers for fingerprinting of poplar cultivars and estimation of genetic diversity in a population because of their high level of variability, repeatability and codominance. Also, these markers could be used for genetic diversity assessments, conservation and sustainable management of poplar genetic resources, certification of controlled crosses, cultivar identification during any step of vegetative propagation and plant production and genome mapping (Dayanandan *et al*., 1998, Vietto *et al*., 2008).

Because of containing species-specific alleles, some SSR loci can be used as diagnostic markers to identify hybridisation and introgressive gene flow and to create a tool for obtaining high definition multi-locus genotypes (Fossati *et al*., 2003, Vanden Broeck, 2005).

In Populus, several hundred SSR markers have been identified by Dayanandan *et al.*, (1998) for trembling aspen (*P. tremuloides* Michx), Tuskan *et al.*, (2004) for *Populus trichocarpa* and Van Der Schoot *et al*., (2000) and Smulders *et al*., (2001) for *P. nigra* L. These markers have been used for many different studies from mapping to fingerprinting (Rae *et al*., 2007). Gaudet *et al*., (2008) constructed highly informative genetic maps of *P. nigra* by using amplification fragment length polymorphism (AFLP), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers. The most informative map was obtained by SSR markers. Obtained genetic maps could be useful to characterize *P. nigra* genome structure and provide genetic improvement of this ecologically and economically important European tree species (Gaudet *et al*., 2008).

1.1.2. *Populus nigra* populations in Turkey

Turkey includes two genetic diversity centers of the Vavilov's 8 genetic diversity centers (That are genetic diversity center of the Mediterranean and the Middle East). These two centers are important for the sustainability of agriculture in Turkey and the world at the same time (Kaya *et al*., 1997). These two regions are very rich in genetic diversity of plant genetic resources, so that conservation and sustainable management of economic, social, ecological and environmental research is of great importance. Poplar species are shown as priority species for conservation of plant genetic resources (Çoban 2007, Kaya and Isık, 1997).

Populus nigra is native to the Anatolia and has a wide distribution in our country. European black poplar has been propagated vegetatively in the rural areas. Recent studies indicated that more than 145,000 hectares of poplar plantations exist in Turkey. Approximately 68,000 hectars of these poplar plantations were established with European black poplar, providing 45% of the annual poplar wood production (1.9 million m^3). Remaining consist of various hybrids of poplar (Işık and Toplu, 2004). More than 80% of European black poplar (1.75 million cubic metres) is used for furniture, packaging, particleboard, plywood, and match industries as well as roundwood for rural construction and the daily needs of rural people (Vanden Broeck, 2003; Toplu, 2005). Therefore, poplar plantations play a significant role for rural and the national economy of Turkey.

European black poplar is not only an economically important species but also have an ecological significance because of its distribution in riparian ecosystems, which contribute to the natural control of flooding, water quality and erosion. However, over usage of the species for its wood and mismanagement of natural resources in rural areas, native populations of black poplar are threatened in Turkey.

Poplar propagation needs to be sustainable due to changing environmental conditions and demands. Therefore, breeding and conservation programs of European black poplar in Turkey were initiated under the framework of the European Forest Genetic Resources Program (EUFORGEN). In this framework, Poplar and Fast-Growing Forest Trees Research Institute initiated a countrywide conservation program including *in situ* and *ex situ* studies (Toplu, 2005). *In situ* conservation studies have been carried out on Eastern Anatolia and

five natural populations of European black poplar in the Melet, Kelkit, Munzur, Karasu and Pülümür river basins have been identified (Toplu and Kucukosmanoglu, 2003).

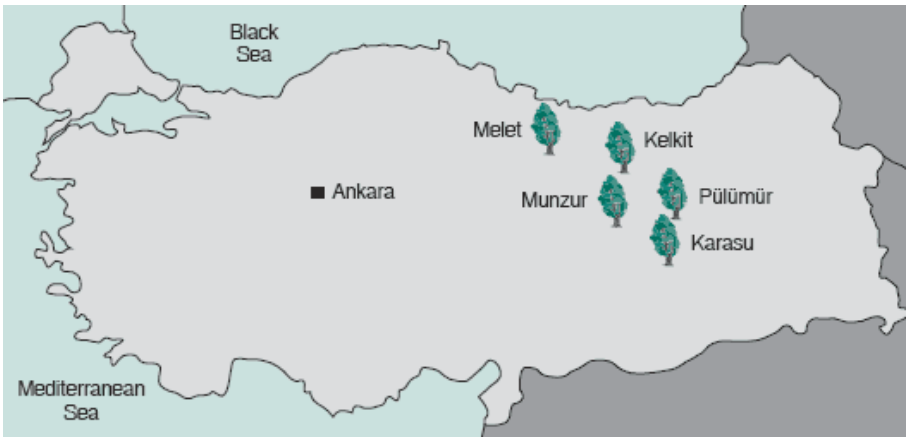

Figure 1.5. The newly found natural populations of *Populus nigra* in Turkey (Toplu and Kucukosmanoglu, 2003).

Ex situ breeding and conservation programs of European black poplar in Turkey were firstly initiated in 1960s, but a systematic selection has only been started and accelerated after 1990s. Up to now, more than 750 European black poplar individuals were selected in natural populations. Some old trial plantations exist in different regions of Turkey. All these selection and breeding efforts have been accomplished with identification of five commercial European black poplar trees (Gazi, Anadolu, Kocabey, Geyve and Ata) which were registered by the International Poplar Commission (IPC).

Approximately 310 European black poplar individuals were chosen from the natural range of the species from different regions of Turkey and transferred to the clone banks established in Ankara, Erzurum and İzmit. To assess growth performance and adaptability of clones to the different ecologic conditions, 297 European black poplar trees including native and foreign commercial trees were selected and transferred to Behiçbey nursery in Ankara. These 297 clones are the important and major genetic resources of European black poplar for future plantations in Turkey. This collection also contains five commercially registered clones (Gazi, Anadolu, Kocabey, Geyve and Ata).

The materials of the commercially registered European black poplar clones as well as the remaining clones in the clone bank in Ankara appear to be mislabelled during the course of clonning and plantation activities. Thus, it is required to characterize the genetic resources of European black poplar and to solve the identity problems of trees in the clone bank. Accurate identification of poplar cultivars and ensuring of their genetic constituents are essential procedures for breeding and management strategies. During the fingerprinting, it will be also

possible to estimate genetic diversity of the European black poplar collection and comparison of this diversity with the natural populations. By this way, efficient conservation and sustainable management of poplar genetic resources would be possible in the future.

CHAPTER 2

OBJECTIVES OF THE STUDY

The aim of this study was to characterize and compare the genetic diversity of both *Populus nigra* clone bank populations and the recently identified natural populations by using polymorphic microsatellite DNA markers.

The specific objectives of the study are :

To characterize the genetic resources of *P. nigra* in Turkey,

To enable a fast, but reliable and transferable identification of *P. nigra* clones,

To compare the genetic diversity of recently identified natural populations with clone bank populations in Turkey,

To solve idendity problems of registered *P. nigra* trees in Turkey,

To use European black poplar genetic resources effectively and to design breeding programmes for *P. nigra* for maximizing the genetic variation in successive generations.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant materials

Two hundreds and ninety-seven *Populus nigra* clones planted in the Behiçbey nursery clone bank, as an *ex situ* conservation program, were sampled in collaboration with the Central Anatolia Forest Research Insititute (Ankara) and the Institute for Poplar and Fast Growing Forest Tree Species (İzmit) of the Ministry of Forestry and Water Affairs. These clones were collected from all over Turkey for the past 60 years by foresters. Additionally, 32 trees were collected from two recently identified natural populations (Tunceli (Munzur, Pülümür) and Melet) in collaboration with the Southeast Anatolia Forest Research Institute (Elazığ). The geographic information on the studied populations are provided in detail in Appendix A. Clones were grouped with respect to their origins. The seven geographical regions of Turkey were taken as the basis of grouping the clones from the clone bank into the populations. Commercially registered five European black poplar trees were originated from Central Anatolia and included in the Central Anatolia population. Number of trees used for sampling was presented in Table 3.1.a and Table 3.1.b.

3.2. DNA isolation

Young leaves of *Populus nigra* individuals were collected in May of 2011 , stored in liquid nitrogen immediately and kept in -80°C until DNA extraction. Nuclear DNA was isolated by using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) method from young leaves of 329 European black poplar trees (Doyle and Doyle, 1987). Firstly, frozen leaves were crushed in liquid nitrogen with the help of sterile mortar and pestle and transferred into 2 ml Eppendorf tubes. After incubation of the CTAB buffer (Cetyl Trimethyl Ammonium Bromide) for 1 hour at 65°C in water bath, 750 µl CTAB buffer, 75 µl βME (Beta Mercaptoethanol) and 5 µl proteinaseK were added on the leaf tissues in 2ml eppendorf tubes. These tubes were vortexed, then incubated in water bath for about 1 hour at 65 °C. All tubes were centrifuged 13000 rpm for 20 min at 4°C. The supernatants in tubes were transferred to sterile 2ml Eppendorf tubes and then, 500 µl Chloroform: Octanol (24:1 V/V) was added and vortexed again. After centrifugation of samples with 13000 rpm for 5 min at 4°C, supernatants were transferred in new Eppendorf tubes. According to double DNA extraction protocol Chloroform: Octanol (24:1) step was repeated. Supernatant were transferred to sterile eppendorf tube and 500 µl cold isopropanol was added. Tubes were inverted gently and incubated at -20°C for 10 minutes. Then, the last centrifugation part was carried with 13000 rpm for 30 min at 4°C. The top aqueuos part was poured off and pellet

was washed with 500 µl of 70% cold ethanol twice. Samples were dried in laminar flow and the pellet was dissolved in 75 ml sterile distilled water.

Table 3.1.a. The number of European black poplar trees in Behiçbey clone bank populations

Behiçbey clone bank populations				
Population	**Number of trees**	**Latitude(range)**	**Longitude(range)**	**Altitude (Meter)**
Central Anatolia	81	37˚52'N-39˚57'N	32˚35'E-32˚54'E	1205
Eastern Anatolia	57	38˚25'N-39˚57'N	38˚20'E-41˚15'E	1829
Aegean	20	37˚42'N-38˚45'N	29˚02'E-30˚33'E	715
Blacksea	34	40˚15'N-40˚40'N	36˚30'E-35˚50'E	1163
Mediterranean	11	37˚05'N-37˚37'N	36˚10'E-36˚53'E	1027
Southeastern Anatolia	10	37˚06'N-37˚46'N	27˚23'E-38˚17'E	748
Marmara	20	37˚47'N-40˚05'N	30˚30'E-30˚05'E	280
Foreign	18	Unknown	Unknown	Unknown
Open pollinated	20	Unknown	Unknown	Unknown
Unknown	24	Unknown	Unknown	Unknown
Total	**297**			

Table 3.1.b. The number of European black poplar trees in natural populations

Natural populations				
Population	**Number of trees**	**Latitude**	**Longitude**	**Altitude (Meter)**
Tunceli	11	39˚ 10'N	39˚ 54'E	979
Melet	21	40˚ 55'N	37˚ 77'E	600
Total	**32**			

3.3. DNA quantification

Concentrations of the isolated DNA samples were measured by using the Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, USA). The quality of the extracted DNA was estimated by calculating the 260 : 280 OD ratios and by checking the suitability of the DNA as a template in the Polymerase Chain Reactions (PCR) with a selected SSR primer.

3.4. Microsatellite Primers

In this study, 12 microsatellite DNA primer pairs were used to study the genetic diversity in *Populus nigra* populations. The primers designated as WPMS03, WPMS04, WPMS05, WPMS09, WPMS10, WPMS12 from Van Der Schoot *et al*. (2000), WPMS14, WPMS15, WPMS18, WPMS20 from Smulders *et al*. (2001) and PMGC14, PMGC2163 from the IPGC (International Populus Genome Consortium) SSR Resource (http://www.ornl.gov/sci/ipgc/ssr_resouce.htm) were selected and used for the screening of clones. The primers were synthesized by (SACEM) with different fluorescent colors (FAM, HEX and TAMR) in order to distinguish their PCR products during the fragment analysis. The list of these primers, their sequences and dyes used for fragment analysis were provided in Table 3.2.

3.5. Optimization of PCR (Polymerase Chain Reaction) conditions

PCR reactions were performed in 20 µl total volume containing 3 µl PCR buffer including $MgCl_2$, 100µM each dNTP, 200nM each primer, 2µl red *Taq* DNA polymerase (SIGMA) and 8µl of a 2ng/µl diluted DNA in 0.2 ml sterile Eppendorf tubes. This setup yielded the best PCR product for 10 primer pairs. For the primers of PMGC2163 and WPMS12, 5U Crimson *taq* polymerase was used (Biolabs^R *ınc*.) The optimized PCR conditions for each primer were provided in Table 3.3.

For amplification of microsatellite regions, two different PCR cycle conditions described by Schoot *et al*. (2000) and Smulders *et al*. (2001) were used with a thermocycler (Eppendorf-Mastercycler, Eppendorf, Canada).

The first PCR condition was used for WPMS04, WPMS20 and PMGC14 primer pairs The second PCR condition was performed for WPMS03, WPMS05, WPMS09, WPMS10, WPMS14, WPMS15 and WPMS18 primer pairs.. For each primer pairs, different annealing temperatures between 50-60^{o}C were used. PCR conditions were given at table 3.4.a and 3.4.b.

Table 3.2. The list of SSR primers used in the study (Van Der Schoot *et al*., (2000), Smulders *et al*., (2001), IPGC SSR Resource)

Microsatellite locus	Primer sequences(Forward,Reverse,5'-3')	Repeat	Dye	Annealing temperature	Reference
WPMS03	TTTACATAGCATTTAGCCTTTAGA TTATGATTTGGGGGTGTTATGGTA	$(GT)_{26-1}$	HEX	60LP	Van Der Schoot *et al*. (2000)
WPMS04	TACACGGGTCTTTTATTCTCT TGCCGACATCCTGCGTTCC	$(GT)_{25}$	TAMR	60NP	Van Der Schoot *et al*. (2000)
WPMS05	TTCTTTTTCAACTGCCTAACTT TGATCCAATAACAGACAGAACA	$(GT)_{27}$	HEX	60LP	Van Der Schoot *et al*. (2000)
WPMS09	CTGCTTGCTACCGTGGAACA AAGCAATTTGGGTCTGAGTATCTG	$(GT)_{21}(GA)_{24}$	TAMR	60LP	Van Der Schoot *et al*. (2000)
WPMS10	GATGAGAAACAGTGAATAGTAAGA GATTCCCAACAAGCCAAGATAAAA	$(GT)_{23}$	FAM	60LP	Van Der Schoot *et al*. (2000)
WPMS12	TTTTTCGTATTCTTATCTATCC CACTACTCTGACAAAACCATC	$(GT)_{19}$	TAMR	55LP	Van Der Schoot *et al*. (2000)
WPMS14	CAGCCGCAGCCACTGAGAAATC GCCTGCTGAGAAGACTGCCTTGAC	$(CGT)_{28-3}$	HEX	60LP	Smulders *et al*. (2001)
WPMS15	CAACAAACCATCAATGAAGAAGAC AGAGGGTGTTGGGGGTGACTA	$(CCT)_{14-3}$	FAM	60LP	Smulders *et al*. (2001)
WPMS18	CTTCACATAGGACATAGCAGCATC CACCAGAGTCATCACCAGTTATTG	$(GTG)_{13}$	TAMR	60LP	Smulders *et al*. (2001)
WPMS20	GTGCGCACATCTATGACTATCG ATCTTGTAATTCTCCGGGCATCT	$(TTCTGG)_8$	FAM	60NP	Smulders *et al*. (2001)
PMGC14	TTCAGAATGTGCATGATGG GTGATGATCTCACCGTTTG	(CTT)	FAM	60NP	IPGC SSR Resource
PMGC2163	CAATCGAAGGTAAGGTTAGTG CGTTGGACATAGATCACACG	(GA)	HEX	55LP	IPGC SSR Resource

Table 3.3. Optimized PCR condition for different SSR primer-pairs

Primer	Reaction mixture components						
	Water	**10XBuffer**	**dNTP(50mM)**	**Primer pairs (10µM)**	**Taq(1U/µl and 5U/µl*)**	**DNA (2µg/µl)**	**Total**
WPMS03	9 µl	3 µl	0.2 µl	0.4 µl	2 µl	5 µl	20 µl
WPMS04	10 µl	3 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
WPMS05	9 µl	3 µl	0.2 µl	0.4 µl	2 µl	5 µl	20 µl
WPMS09	10.3 µl	2.7 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
WPMS10	9 µl	3 µl	0.2 µl	0.4 µl	2 µl	5 µl	20 µl
WPMS12	11,5 µl	3 µl	0.5 µl	0.5 µl	0.5* µl	4 µl	20 µl
WPMS14	10.3 µl	2.7 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
WPMS15	10.3 µl	2.7 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
WPMS18	10 µl	3 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
WPMS20	10 µl	3 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl
PMGC2163	11,5 µl	3 µl	0.5 µl	0.5 µl	0.5* µl	4 µl	20 µl
PMGC14	10 µl	3 µl	0.2 µl	0.4 µl	2 µl	4 µl	20 µl

Amplification products (5µl) were loaded in a 3% agarose gel and stained with 5 µg/ml ethidium bromide. The gels were run in 1XTBE (0.4 M Tris Boric acid EDTA) buffer at 120V for 15 minutes and visualized under UV light. Low molecular weigth DNA ladder (SIGMA) was used to determine the size of the bands. The gels which had desired bands were photographed and digitialized by using a gel imaging system (Vilbor Lourmat, France).

Table 3.4.a. The first PCR cycling steps and conditions for WPMS04, WPMS20, PMGC14 primer pairs

Step	Temperature	Time	Cycle	Events
1	94°C	3 minutes	1	Denaturation
2	94 °C	5 seconds	30	Denaturation
	Annealing Temperature	15 seconds		Annealing
	72°C	60 seconds		Extension
3	72°C	10 minutes	1	Final Extension
4	4°C	-	-	Hold

Table 3.4.b. The second PCR Cycling steps and conditions for WPMS03, WPMS05, WPMS09, WPMS10, WPMS14, WPMS15, WPMS18, WPMS12, PMGC2163 primer pairs

Step	Temperature	Time	Cycle	Events
1	94°C	3 minutes	1	Denaturation
2	94 °C	45 seconds	30	Denaturation
	Annealing Temperature	45 seconds		Annealing
	72°C	105 seconds		Extension
3	72°C	10 minutes	1	Final Extension
4	4°C	-	-	Hold

3.6. Data collection

After evaluating amplification products of the individuals which had a positive result, fragment analysis of PCR products was done by the Refgen Biotechnology, METU Teknokent, Ankara. For each primer pairs, the bands with different size were observed and recorded for each clone. Each microsatellite primer was accepted as a locus which had different allele combination. Number of alleles and their sizes for each primer pairs were listed in Table 3.5.

Table 3.5. Observed number of alleles and size of alleles (base pair) of SSR loci in *Populus nigra* populations

SSR loci	Size of alleles(bp)	Number of alleles
WPMS03	229,259,263,266,268,270,271,272,273,275,279,280	14
WPMS04	236,247,249,252,258,260,263,265,267,274,276,278,282	16
WPMS05	254,267,271,276,278,280,283,285,288	9
WPMS09	246,251,255,257,260,262,274,278,292	13
WPMS10	231,234,237,240,243,245,248,250,252,254,256	12
WPMS12	152,161,163,165,167,168,169,174,177,180,189,212,218,235	14
WPMS14	209,228,231,234,237,243,252,255,257,260,266,269,279	16
WPMS15	194,203,209,212,216,219	9
WPMS18	211,214,226,229,232,235	8
WPMS20	205,211,218,220,226,233,240,246	8
PMGC14	180,184,189,191,195,198,201,204,207,208,211,214,217,220,224,235,248	17
PMGC2163	186,220,222,224,230,232,234,236,238,240,242,244,246,248,254,258,261,263,270	19

3.7. Analysis of data

3.7.1. Estimation of Genetic Variation

The standard parameters of genetic variability were calculated for the *Populus nigra* populations. These are the number of observed alleles (A_p), number of the expected alleles (A_e) for each loci, the proportion of polymorphic loci (P), observed heterozygosity (H_o) and expected heterozygosity (H_e).

All parameters were obtained using the GDA software (Genetic Data Analysis , Lewis and Zaykin, 2002). To describe the distribution of genetic variation within populations, Wright´s fixation index, *F* was used.

3.7.1.1. Proportion of polymorphic loci

Proportion of polymorphic loci indicates the percentage of variable loci in a population. This is calculated by dividing the number of polymorphic loci to total number of loci. When the most common allele has a frequency of equal or smaller than 0.99 or 0.95, one locus is called as polymorphic (Nei, 1987).

$$P = \frac{npj}{ntotal}$$

Where,
npj = Number of polymorphic loci,
ntotal = Total number of loci

3.7.1.2. Observed number of alleles per locus

Observed number of alleles called allelic richness is the total number of alleles at a locus. It is accepted a valuable complementary measure of genetic variation, so it is more sensitive to the loss of genetic variation due to small population size than heterozygosity (Allendorf and Luikart, 2007).

The formula to calculate observed number of alleles is as follows,

$$na = \frac{{}_i\, nai}{r}$$

Where,
r = The number of loci
ni = The number of alleles detected per locus (Nei, 1987)

3.7.1.3. Effective number of alleles

It is the number of alleles that can be present in a population. This measure estimates the number of alleles that would be expected in a locus in each population (Kimura and Crow, 1964).

$$Mean\ A_e\ = \frac{1}{pi^2}$$

Where,
A_e = effective number of allele
pi = frequency of the i^{th} allele in a locus

3.7.1.4. Heterozygosity

The average *H* over all loci is the most widespread measure of genetic diversity in a population. The estimated value may be affected by alleles at higher frequencies.

$$H = \frac{2N\ 1 - \quad Xi^2}{2N - 1}$$

Where,
N = Number of individual
Xi = The frequency of an allele in a SSR loci (Nei, 1987)

3.7.2. F statistics

The variation in gene frequency among subpopulations may be analyzed by the fixation indices or *F*-statistics (Nei, 1973). This parameter is known as the equation for the genetic structure of populations and can be used to define three level of inbreedings (Nei, 1987). *F* statistics measure the deficiency of heterozygotes relative to expected Hardy-Weinberg proportions in the specified base population (Allendorf and Luikart, 2007).

The statistical indexes are:

$F_{IS,}$ is known as a measure of departure from Hardy-Weinberg proportions within subpopulations.

$$F_{IS} = 1 - \frac{H_I}{H_S}$$

F_{IT} is the measure of the total departure from Hardy-Weinberg proportions within subpopulations.

$$F_{IT} = 1 - \frac{H_I}{H_T}$$

F_{ST} is the degree of gene differentiation among subpopulations in terms of allele frequencies.

$$F_{ST} = 1 - \frac{H_S}{H_T}$$

The equation for the genetic structure of populations is estimated by the following equation;

$$1 - F_{IT} \quad = \quad 1 - F_{IS} \quad 1 - F_{ST}$$

H_T = Total gene diversity or expected heterozygosity in the total population as estimated from the pooled allele frequencies

H_I = Average observed heterozygosity in subpopulations

H_S = Average expected heterozygosity estimated from each subpopulation
(Allendorf and Luikart, 2007)

3.7.3. Phylogenetic Trees

In this study, to explore the genetic relationship among populations of European black poplar, two dendogram were constructed by using the UPGMA (Unweighted Pair Group Method Using Arithmetic Averaging) and Neighbor-Joining method with coancestry identity (Nei, 1978 and Reynolds *et al*., 1983).

3.7.4. Solving the identity problems in the clone bank

To solve identity problems of 5 commercially registered trees, tissue materials from these clones which were originally planted and maintained in the clone banks of the Poplar and Fast Growing Research Institute in İzmit and Southeast Anatolia Forest Research Institute in Elazığ were obtained. The fingerprint comparisons of these clones from İzmit, Elazığ and Ankara were made with data generated with WMPS03, WPMS18 and WPMS20 SSR primers.

3.7.5. STRUCTURE Analysis

To determine the number of genetically homogeneous groups, population genetic structure analysis were performed with the STRUCTURE 2.3.4 software (Pritchard *et al*., 2000). In genetic structure analysis of the clone bank populations and natural populations, an admixture model with correlated allele frequencies was used. Sampling location information was utilized as a prior for analysis.

The STRUCTURE 2.3.4 programme is the most widely used Bayesian model to identify subgroups that have distinctive allele frequencies (Evanno *et al.,* 2005). The STRUCTURE algorithm (Pritchard *et al*., 2000) constructs genetic clusters from a collection of individual multilocus genotypes, estimating for each individual coefficients of membership in subpopulations that belong to each cluster. The estimated *log* probability of data does not provide a correct estimation of the number of clusters (K). Using an *ad hoc* statistics, ΔK based on the rate change in *log* probability of data between successive K values, accurate number of clusters could be obtained (Evanno *et al*., 2005).

3.7.5.1. Statistics used to select *K*

To infer true *K* (number of populations), *ad hoc* quantity (ΔK) are calculated. This calculation is based on the second order rate of change of the likelihood (ΔK) (Evanno *et al.*, 2005). When reaching the true value of *K*, ΔK shows a clear peak.

The *log* likelihood for each *K* is Ln P(D) in STRUCTURE output obtained by first computing the *log* likelihood of the data at each step of the Markov Chain Monte Carlo (MCMC). The mean likelihood *L(K)* over 10 runs for each *K* are calculated.

$$L(K) = \text{An average of 10 values of } \ln P\ D$$

The mean difference between successive likelihood values of *K* are plotted.

$$L'\ K\ = L\ K\ - L\ K-1$$

The (absolute value of the) difference between successive values of *L′(K)* are calculated.

$$L''\ K\ = L'\ K+1\ - L'\ K$$

Estimated *ΔK* as the mean of the absolute values of *L″(K)* averaged over 10 runs divided by the standard deviation of *L(K)*.

$$\Delta K = \frac{m\ L''\ K}{s\ L\ K}$$

In this study, the number of clusters varied between 1 to 12. The program ran with a Burn-in period of 20000 iterations to minimize the effect of the starting configuration followed by 100000 MCMC replications to get accurate parameter estimates for each clusters. Each run was replicated 10 times at each of *K*=1 to 12.

The computer program CLUMPP (Jakobsson and Rosenberg, 2007) was used to average the estimated cluster membership coefficient matrices of multiple runs of STRUCTURE clustering program. Output data of the CLUMPP (Jakobsson and Rosenberg, 2007).

CHAPTER 4

RESULTS

4.1. DNA extraction and PCR reactions

The modified CTAB DNA extraction protocol was observed to be very efficient to isolate clean DNA for all *Populus nigra* clones. The optimised amplification protocol for the PCR reactions yielded clearly identifiable peaks for each primer pair. Sometimes unclear peaks were observed for the amplification products of primers, but did not hinder the identification of the prominent peak in each sample.

Following the optimization of the PCR conditions, 12 microsatellite primers were used to assess the genetic diversity of clone bank and natural populations of European black poplar. The representative photos indicating the PCR products of all primers used can be seen at Figures 4.1 and 4.2.

4.2. Population Genetic Structure

4.2.1. Descriptive statistics by loci

When descriptive statistics by loci are examined, all loci were found to be polymorphic (Table 4.1). The observed and expected number of alleles per locus for 12 SSR loci was generally high with an average of 12.92. It ranged from 8 (WPM18 and WPMS20) to 19 (PMGC163) (Table 4.1).

The expected heterozygosities for single loci according to the *Populus nigra* populations ranged from 0.32 to 0.75. Most of the studied loci showed very high gene diversity (H_e) with an average of 0.62. The observed heterozygosities ranged from 0.17 to 0.97 (Average: 0.67). The locus WPMS10 displayed the lowest while the locus WPMS20 had the highest observed heterozygosity values (Table 4.1).

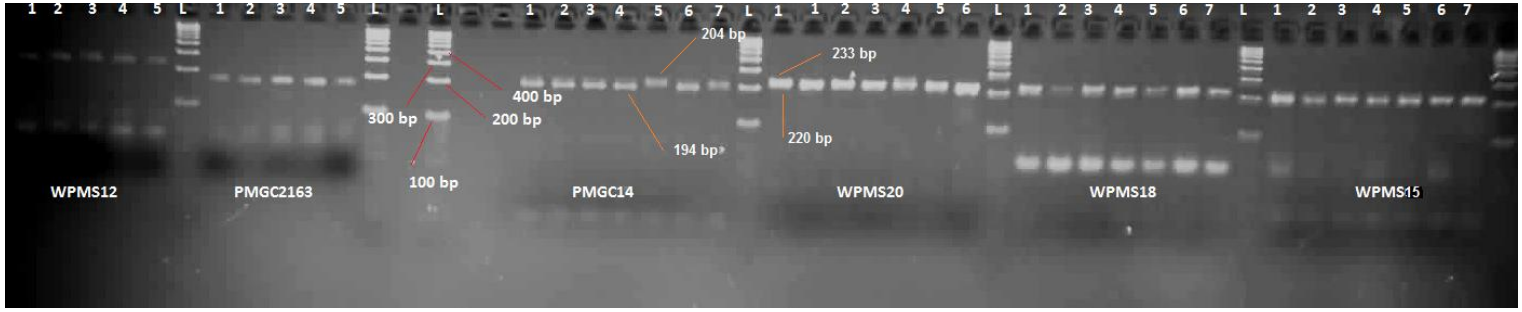

Figure 4.1. Banding pattern of WPMS12, PMGC2163, PMGC14, WPMS20, WPMS18 and WPMS15 primers on agarose gel. L= DNA size ladder

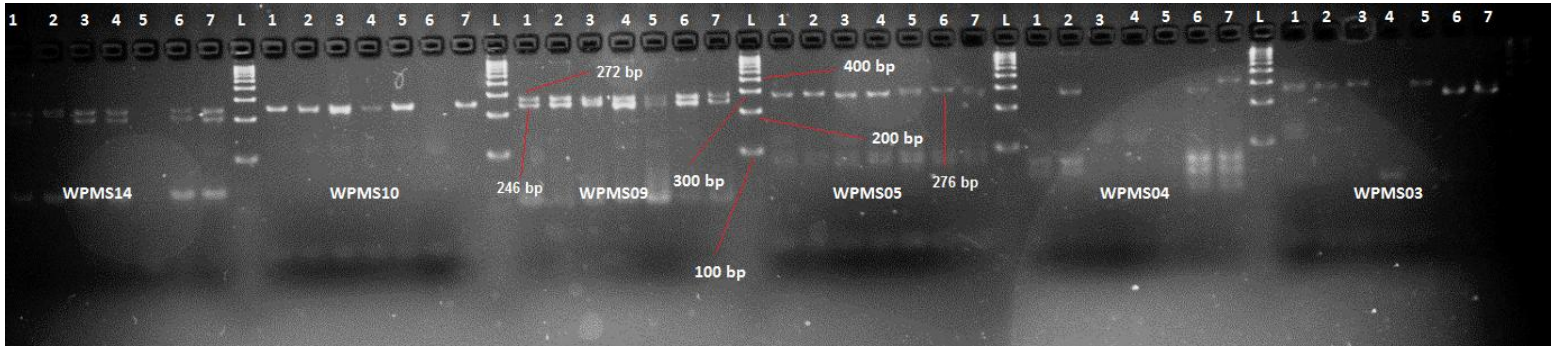

Figure 4.2. Banding pattern of WPMS14, WPMS10, WPMS09, WPMS05, WPMS04 and WPMS03 primers on agarose gel. L= DNA size ladder

Table 4.1. Descriptive statistics of genetic diversity parameters for European black poplar populations in Turkey for 12 loci. N = Sample size, P = Proportion of polymorphic loci, Ap= Observed number of alleles, A = Effective number of alleles, He = Expected heterozygosity, Ho = Observed heterozygosity

Locus	N	P	A	Ap	He	Ho
WPMS03	328	1	14	14	0.70	0.84
WPMS04	325	1	16	16	0.69	0.22
WPMS05	324	1	9	9	0.47	0.25
WPMS09	325	1	13	13	0.48	0.29
WPMS10	329	1	12	12	0.32	0.17
WPMS12	314	1	14	14	0.64	0.89
WPMS14	326	1	16	16	0.75	0.88
WPMS15	328	1	9	9	0.64	0.94
WPMS18	329	1	8	8	0.72	0.70
WPMS20	329	1	8	8	0.62	0.97
PMGC14	323	1	17	17	0.75	0.95
PMGC2163	308	1	19	19	0.67	0.89
Mean	**323.9**	**1**	**12.92**	**12.92**	**0.62**	**0.67**
St.error			**0.00**	**0.01**	**0.00**	**0.00**

4.2.2. Descriptive statistics by population

Diversity estimates were made for 297 European black poplar trees from clone bank and 32 trees from two newly discovered natural populations by using GDA (Genetic Data Analysis) software (Lewis and Zaykin, 2002). Genetic diversity parameters were represented at Table 4.3.

4.2.2.1. Allelic richness

According to the descriptive statistics of populations, the mean number of observed alleles for all populations was 5.57±003 (standard error) and it ranged from 3.36 (Mediterranean population) to 8.08 (Foreign population) (Table 4.3). The mean number of effective alleles for all populations was found to be 5.53±0.011. The Mediterranean population had 3.17 effective alleles, while Foreign population had 8.08. Natural populations had equal number of observed and expected alleles (5.5).

Private alleles were used to determine the genetic diversiy of the populations. In all populations of European black poplars in Turkey, there were 34 private alleles. Eleven of

these private alleles were in the Melet natural population. The Tunceli and Eastern Anatolia populations had six private alleles. South Anatolia and Blacksea populations had no private allele. Private alleles belonging to European black poplar populations were given in Table 4.2.

4.2.2.2. Proportion of polymorphic loci

All populations had high polymorphism rate. Proportion of polymorphic loci were found to be 92% for Mediterranean and Southeastern Anatolia populations. Other populations had the highest values of proportion of polymorphic loci (100%) (Table 4.3).

4.2.2.3. Heterozygosity

The mean expected heterozygosity (gene diversity) was 0.59±0.000 for all populations. The range for expected heterozygosity was from 0.50 to 0.79. Southeastern Anatolia population had the lowest expected heterozygosity value, whereas the foreign population had the highest value. The populations from geographical regions of Turkey had close expected heterozygosity values. On the other hand, natural populations had higher expected heterozygosity values than the rest of other populations. Furthermore, the foreign population and Tunceli natural population differed from other populations with respect to higher expected heterozygosity values (0.79 and 0.69; Table 4.3).

The mean observed heterozygosity was 0.66±0.001 for all populations. The range was from 0.62 in the foreign and open pollinated populations to 0.69 in the Mediterranean population. In general, studied populations didn't differ greatly in the observed heterozygosity values (Table 4.3).

The mean observed heterozygosity (0.66) was higher than the mean expected heterozygosity (0.59), indicating an excess of heterozygotes (F_{is}= -0.12) among studied clones. When all populations were comparatively considered, observed heterozygosity values, except the foreign, open pollinated and Tunceli populations, were higher than expected heterozygosity values (Table 4.3).

Table 4.2. Private (Unique) alleles in *Populus nigra* populations in Turkey

Locus	**Allele**	**Frequency**	**Observed Population**
WPMS15	181	0.047	Melet
WPMS15	199	0.02	Melet
WMPS14	216	0.07	Melet
WMPS14	224	0.125	Tunceli
WMPS14	273	0.06	Tunceli
WMPS14	279	0.055	Foreign
WMPS14	269	0.055	Foreign
WMPS09	274	0.026	Melet
WMPS09	292	0.052	Melet
WMPS09	241	0.026	Melet
WMPS09	278	0.055	Tunceli
WMPS09	236	0.055	Tunceli
WPMS04	241	0.117	Melet
WPMS04	285	0.588	Melet
WPMS04	263	0.025	Marmara
WPMS04	278	0.017	Central Anatolia
WPMS18	223	0.040	Tunceli
WPMS20	211	0.006	Central Anatolia
WMPS03	249	0.023	Melet
WPMS03	236	0.023	Melet
WPMS03	273	0.010	Eastern Anatolia
WPMS12	235	0.021	Unknown
WPMS12	180	0.060	Foreign
WPMS12	189	0.020	Marmara
WPMS12	218	0.008	Eastern Anatolia
WPMS12	212	0.008	Eastern Anatolia
PMGC14	180	0.025	Melet
PMGC14	248	0.008	Eastern Anatolia
PMGC14	235	0.008	Eastern Anatolia
PMGC2163	240	0.050	Tunceli
PMGC2163	186	0.030	Foreign
PMGC2163	238	0.050	Marmara
PMGC2163	270	0.025	Aegean
PMGC2163	263	0.018	Eastern Anatolia

Table 4.3. Estimated population genetic diversity parameters for *Populus nigra* . N = Sample size, P = Proportion of polymorphic loci, Ap= Observed number of alleles, A = Effective number of alleles, He = Expected heterozygosity, Ho = Observed heterozygosity, F_{is} = Inbreeding coefficient, F_{ST}= Genetic differentiation. ** = Statistically significant at P<0.05, *** = Statistically highly significant at P<0.001

Population	**N**	**P**	**A**	**Ap**	**He**	**Ho**	**F_{IS}**	**F_{ST}**	**Mean F_{ST}**
Central Anatolia	80.41	1	7.25±0.38	7.25±0.01	0.58±0.00	0.68±0.00	-0.17***	0.03	0.08***
Eastern Anatolia	57.5	1	6.83±0.01	6.83±0.04	0.53±0.00	0.67±0.01	-0.28***		
Aegean	20	1	4.42±0.03	4.42±0.08	0.52±0.01	0.67±0.02	-0.31***		
Blacksea	34.45	1	5.92±0.02	5.92±0.04	0.56±0.01	0.66±0.01	-0.18***		
Mediterranean	11	0.92	3.17±0.06	3.36±0.09	0.54±0.02	0.69±0.03	-0.30**		
Southeastern Anatolia	10	0.92	3.25±0.07	3.45±0.13	0.50±0.02	0.67±0.04	-0.37**		
Marmara	19	1	5.5±0.03	5.5±0.08	0.56±0.01	0.68±0.02	-0.22***		
Open Pollination	20	1	5.17±0.05	5.12±0.11	0.64±0.01	0.62±0.01	0.02***		
Unknown	23	1	5.83±0.03	5.83±0.07	0.58±0.01	0.66±0.02	-0.15***		
Tunceli	9.92	1	5.5±0.13	5.5±0.19	0.69±0.01	0.64±0.02	0.07***	0.17	
Melet	19.5	1	5.5±0.06	5.5±0.10	0.62±0.01	0.66±0.01	-0.08**		
Foreign	17	1	8.08±0.09	8.08±0.15	0.79±0.01	0.62±0.02	0.21***	-	
Mean	**26.99**	**0.99**	**5.53**	**5.57**	**0.59**	**0.66**	**-0.12*** **	0.18	
St.error.			**0.011**	**0.003**	**0.000**	**0.001**	**0.002**		

4.2.2.4. F statistics

The *F* statistics allow analysis of structures of subdivided populations. It may also be used to measure the genetic distance among subpopulations and detect if any deviation from Hardy - Weinberg expectations in gene frequencies exists.

The mean value of the inbreeding coefficient F_{IS} in populations was -0.12. This value indicated that within the populations, heterozygotes were 12% higher than expected. *Fıs* values for foreign, open pollinated and Tunceli populations were found as 0.21, 0.02 and 0.07, respectively (Table 4.4). Positive value of inbreeding coefficient F_{IS} showed heterozygote deficiency, that is, observed heterozygosity values for these populations was lower than expected heterozygosity. F_{IS} values for each populations were found highly significant by considering Hardy-Weinberg exact tests in GENEPOP programme (Rousset, 2008). Estimation of exact P-Values was performed by the Markov chain method. Markov chain parameters for all tests are dememorization number:1000, the number of batches:100 and the number of ıterations per batch:1000.

When performing Hardy-Weinberg exact tests for 12 loci, F_{IS}, F_{IT} and F_{ST} values were found highly significant. The mean F_{IS} value was calculated as -0.15. WPMS04, WMPS05, WPMS09, WMPS10 had positive F_{IS} value. These values indicated that these loci had more homozygotes than heterozygotes. The WPMS20 locus had the highest rate of heterozygotes (-0.58) within all populations (Table 4.4).

F_{IT} indicating total inbreeding coefficient of individuals within sub populations has a mean value of -0.06. This value showed that 0.6% excess of heterozygotes were observed within populations. The loci WPMS04, WPMS05, WPMS09, WMPS10, WPMS18 yielded positive F_{IT} value showing more homozygotes than expected (Table 4.4)

The mean F_{ST} values for all populations including natural populations was found to be 0.08 (Table 4.3). There was 8 % of total genetic variation among populations. Great portion (92%) of total genetic variation was within populations. According to this value, it is understood that low level of differentiation has occurred among these populations. The WPMS04 and WPMS20 loci had the highest contribution to differentiation between European black poplar populations (F_{ST} = 0.22; Table 4.4). When accepting Turkish populations as one group, overall genetic differentiation among Turkish European black poplar population, foreign population and natural populations was found to be 0.18. The genetic differentation among European black poplar populations from seven region of the Turkey was low (0.03). Also, the newly found two natural populations (Melet and Tunceli) had quite high F_{ST} value (0.17) (Table 4.3).

Table 4.4. Summary of *F* statistics calculated separately for the 12 SSR loci in *Populus nigra* populations. *** = Statistically highly significant at P<0.001

Locus	F_{IS}	F_{IT}	F_{ST}
WPMS15	-0.51***	-0.46***	0.03***
WPMS14	-0.29***	-0.15***	0.03***
WPMS09	0.31***	0.42***	0.16***
WPMS10	0.37***	0.46***	0.15***
WPMS05	0.34***	0.49***	0.22***
WPMS04	0.64***	0.68***	0.12***
WPMS18	-0.03***	0.04***	0.07***
WPMS20	-0.58***	-0.54***	0.22***
WPMS03	-0.25***	-0.19***	0.05***
WMPS12	-0.46***	-0.40***	0.04***
PMGC14	-0.31***	-0.26***	0.04***
PMGC2163	-0.37***	-0.32***	0.04***
Mean	**-0.15*** **	**-0.06*** **	**0.08*** **

4.3. Genetic distance of *Populus nigra* populations in Behiçbey clone bank and natural populations

For estimation of the genetic relationship between Turkish clone bank populations and natural populations, coancestry identity values were calculated among these populations (Nei, 1978) by using GDA software (Lewis and Zaykin, 2002). Distance matrix constructed distances/identity measures based on 12 loci for 12 European black poplar populations. Coancestry distance (Genetic distance) values range from 0 to 1. 0 means that there is no difference between populations. Coancestry identity is equivalent to the Weir and Cockerham (1984) pairwise *F*. With these values, the UPGMA (Unweighted pair group method using arithmethic averaging) and Neighbor- Joining method were used to construct the dendogram.

According to the UPGMA method, two major clusters on the dendogram were detected. The first cluster was composed of two sub clusters. The first sub cluster included the Central Anatolia, Mediterranean, unknown, Eastern Anatolia, Blacksea, Southeastern Anatolia, Aegean and Marmara populations which were the most closely related populations with little genetic distance among them. The other sub cluster included the foreign and open pollinated populations. There was a little differentation between these two sub clusters. The natural populations took part in the second major cluster. There was found many genetic distance between these two major clusters (Figure 4.3).

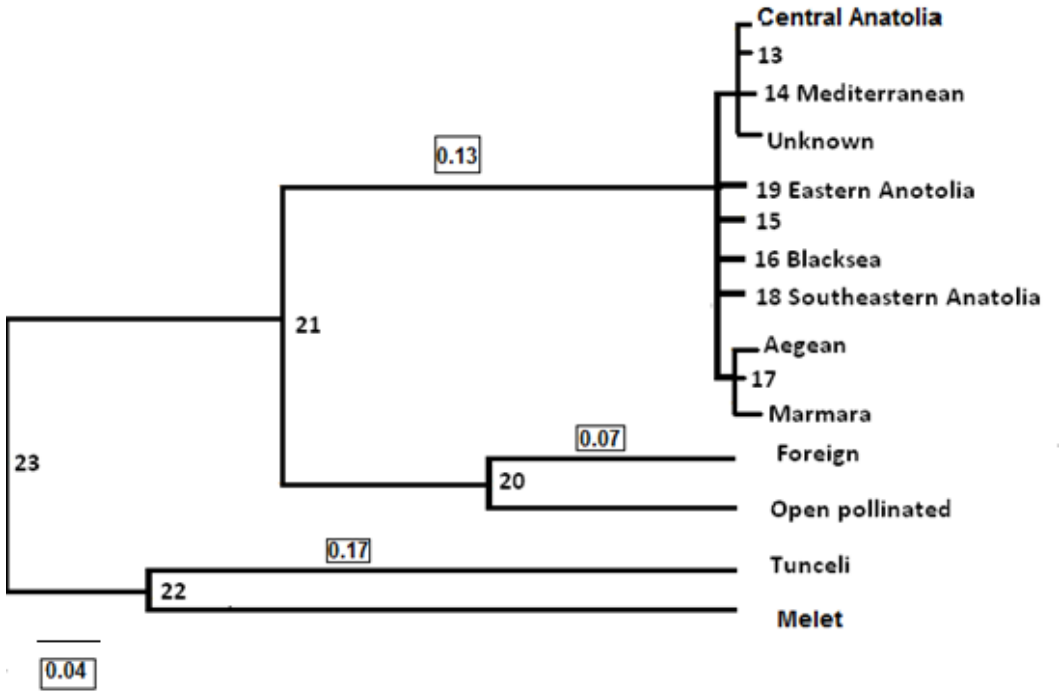

Figure 4.3. Dendogram based on coancestry identity for 12 *Populus nigra* populations with UPGMA method. Boostrap values are shown on the branches.

When looking Neighbor- Joining tree, natural, foreign and open pollinated populations were found to have high level of genetic distance with other European black poplar populations sampled from seven region of Turkey. Although all Turkish populations of *P. nigra* had no genetic distance with each others on UPGMA dendogram, Central Anatolia and Mediterranean populations displayed a low level of genetic distance with other Turkish populations on Neighbor- Joining dendogram (Figure 4.4). Foreign and natural populations shaped a major cluster as two sub clusters, while open pollinated population constituted other major cluster.

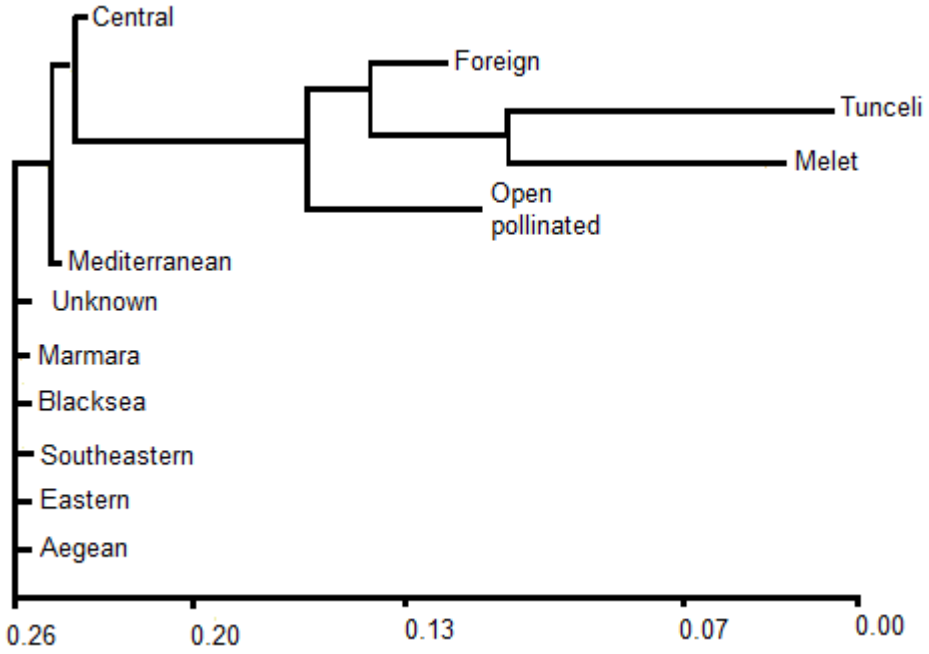

Figure 4.4. Dendogram based on coancestry identity for 12 *Populus nigra* populations with Neighbor- Joining Tree method.

4.4. Clonal propagation

After analyzing microsatellite loci, it was found that 100 individuals from the European black poplar populations have the same allelic genotype for the 12 loci. This allelic combination for all loci can be seen in Table 4.5. All populations collected from the seven regions of the Turkey have varying number of clones with the same genetic make up. The numbers varied from 1 in Mediterranean to 27 in the Eastern Anatolia populations. Obviously, the clones with same allelic genotypes belongs to the same genetic resource. This situation could be explained by the propagation and distribution of the same genotype widely within a given geographic region. The Central Anatolia and Eastern Anatolia populations included high number of trees originating from a single clone while the foreign, open pollinated and natural populations had no clones which were repeated during their propagation and distribution (Table 4.6). The ramets of the same genet were displayed in the figure occurring in Appendix E. The figure was obtained from STRUCTURE programme.

Table 4.5. Allele combination of 12 microsatellite loci that is same for 100 individuals in *Populus nigra* populations in Turkey

SSR loci	**Allele combination**
WPMS03	268, 279
WPMS04	274
WPMS05	276
WPMS09	246
WPMS10	248
WPMS12	165, 174
WPMS14	209, 243
WPMS15	203, 209
WPMS18	211, 226
WPMS20	220, 233
PMGC14	191, 198
PMGC2163	224, 242

Table 4.6. Number of individuals which have the same genotype for 12 microsatellite loci in clone bank and natural populations

Population name	**Number of individuals in each population**	**Number of individuals which have the same genotype for 12 microsatellite loci**	**Number of unique genotypes**
Central Anatolia	81	25	56
Eastern Anatolia	57	27	30
Aegean	20	13	7
Blacksea	34	14	20
Mediterranean	11	1	10
Southeastern Anatolia	10	4	6
Marmara	20	8	12
Open Pollination	21	0	21
Unknown	24	8	16
Tunceli	11	0	11
Melet	21	0	21
Foreign	19	0	19
Mean	**329**	**100**	**229**

After excluding repeated genotype, Mediterranean population demonstrated the lowest gene diversity in all European black poplar populations (He= 0.54), whereas foreign population had the highest value of He (0.79). The highest observed heterozygosity value was found in the Mediterranean, Central Anatolia and Marmara populations (Ho= 0.69) (Table 4.7).

The mean values of the inbreeding coefficient F_{IS} in populations was found as -0.03 after excluding the ramets of the same genotype from populations. *Fıs* value for each population was reduced without duplibated tree. For instance *Fıs* value for Eastern Anatolia population changed from -0.28 to -0.04 (Table 4.7). Blacksea population possessed positive *Fıs* value (0.15) after excluding duplicated tree. This tree was found heterozygote for eight loci and homozygote for four loci (Table 4.5). This caused to excess of heterozygotes in clone bank populations

Table 4.7. Estimated population genetic diversity parameters for *Populus nigra* after excluding repeated genotype. N = Sample size, P = Proportion of polymorphic loci, Ap= Observed number of alleles, A = Effective number of alleles, He = Expected heterozygosity, Ho = Observed heterozygosity, F_{is} = Inbreeding coefficient, F_{ST}= Genetic differentiation. ** = Statistically significant at P<0.05, *** = Statistically highly significant at P<0.001

Population	**N**	**P**	**A_e**	**A_p**	**H_e**	**H_o**	**F_{is}**	**F_{ST}**
Central Anatolia	52.41	1	7±0.08	7±0.25	0.65±0.02	0.69±0.01	-0.06***	
Eastern Anatolia	29.5	1	6.8±0.08	6.8±0.19	0.65±0.02	0.67±0.01	-0.04***	
Aegean	10	1	4.4±0.06	4.4±0.13	0.63±0.03	0.68±0.01	-0.08**	
Blacksea	19.75	1	5.9±0.08	5.9±0.12	0.66±0.02	0.65±0.01	0.15***	
Mediterranean	11	0.92	3.1±0.06	3.36±0.09	0.54±0.02	0.69±0.03	-0.30**	**0.07**
Southeastern Anatolia	7	0.92	3.25±0.07	3.45±0.11	0.55±0.04	0.67±0.02	-0.22**	
Marmara	12.9	1	5.5±0.08	5.5±0.14	0.64±0.03	0.69±0.01	-0.08***	
Open pollination	20.3	1	5.16±0.05	5.16±0.11	0.64±0.01	0.62±0.01	0.02***	
Unknown	15.9	1	5.8±0.09	5.8±0.14	0.65±0.03	0.66±0.01	-0.01***	
Tunceli	9.92	1	5.5±0.13	5.5±0.19	0.69±0.01	0.65±0.02	0.07***	
Melet	19.5	1	5.5±0.06	5.5±0.10	0.62±0.01	0.66±0.01	-0.08***	
Foreign	17.6	1	8.08±0.09	8.08±0.15	0.79±0.00	0.62±0.02	0.21***	
Mean	**18.82**	**0.98**	**5.51±0.07**	**5.54±0.15**	**0.64±0.02**	**0.66±0.01**	**-0.03*** **	

4.5. Fingerprinting analysis of commercially registered clones

By using three SSR primers (WPMS03, WPMS18, WPMS20), identity problems of commercial registered clones was resolved. The reasons for choosing these primers are the higher polymorphism rate in terms of allele number and heterozygosity values and more identifiable peaks in fragment analysis. When comparing the commercially registered clones sampled from Behiçbey, Elazığ and İzmit clone banks, with the exception of Gazi trees other commercial trees were identical in terms of alleles at three loci (Table 4.8).

The comparisons of clones from three clone banks have indicated that Gazi clone in the Elazığ clone bank was mislabelled based on the presence of different alleles. Gazi tree had 262 / 280 bp allele for Behiçbey and İzmit clone bank, whereas Elazığ clone bank's tree showed 262 / 268 bp alleles at WPMS03 locus. Also except Gazi trees, all commercial trees in three clone banks had the 226 bp allele at WPMS18 locus (Table 4.8). All commercial trees had the same alleles at WPMS20 locus (220, 233).

Table 4.8. Comparison of the commercially registered European black poplar clones from three clone banks in Turkey

Clone banks									
	Behiçbey Clone Bank (Allele bp for three loci)			**Elazığ Clone Bank (Allele bp for three loci)**			**İzmit Clone Bank (Allele bp for three loci)**		
Name of commercially registered clones	**The SSR loci used in comparison**								
	WPMS03	**WPMS18**	**WPMS20**	**WPMS03**	**WPMS18**	**WPMS20**	**WPMS03**	**WPMS18**	**WPMS20**
Anadolu	268/279	226	220/233	268/279	226	220/233	No trees obtained	No trees obtained	No trees obtained
Kocabey	262/280	226	220/233	262/280	226	220/233	262/280	226	220/233
Gazi	262/280	232	220/233	262/268	226/232	220/233	262/280	232	220/233
Ata	262/280	226	220/233	262/280	226	220/233	No trees obtained	No trees obtained	No trees obtained
Geyve	262/280	226	220/233	262/280	226	220/233	262/280	266	220/233

4.6. Population Structure of European Black Poplar

All subsequent analysis of nuclear SSRs were carried out on 329 genotypes representing 12 European black poplar populations. The STRUCTURE (Pritchard *et al*., 2000; Falush *et al*., 2003, Falush *et al*., 2007) analysis suggested the existence of four groups as inferred from ΔK statistics (Figure 4.5).

4.6.1. Regional population structure

The analysis of log likelihood values across STRUCTURE runs using ΔK supported the presence of four groups (Figure 4.5). Pritchard *et al*. (2000) stated that *We may not always be able to know the TRUE value of K, but we should aim for the smallest value of K that captures the major structure in the data*. Our result is suitable for this description.

Table 4.9. The proportion of estimated membership to each of the four inferred genetic groups of a given category of origin.

***Populus nigra* populations**	**The estimated membership values for genetically homogeneous groups**				**Number of individuals**
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	
Central Anatolia	0.50	0.19	0.29	0.02	81
Eastern Anatolia	0.65	0.17	0.17	0.01	58
Aegean	0.74	0.13	0.12	0.01	20
Blacksea	0.59	0.27	0.13	0.01	34
Mediterranean	0.56	0.05	0.38	0.01	11
Southeastern Anatolia	0.81	0.14	0.03	0.02	10
Marmara	0.57	0.38	0.04	0.01	20
Foreign	0.04	0.87	0.08	0.01	18
Open pollinated	0.04	0.11	0.84	0.01	21
Unknown	0.63	0.21	0.12	0.05	24
Tunceli	0.01	0.01	0.01	0.97	11
Melet	0.04	0.01	0.01	0.94	21

The clusters were determined by the STRUCTURE run of highest estimated probability among those performed in the unsupervised population structure. The CLUMPP 1.1.1 software (Jakobsson and Rosenberg, 2007) was used to plot the average membership across STRUCTURE runs at the number of clusters (*K*) that was best supported by ΔK approach. The first cluster included populations from seven region of Turkey and the "unknown population". The Southeastern Anatolia population had the highest estimated membership values (0.81), whereas the Central Anatolia population had the low estimated membership values (0.50) in their cluster. Foreign and open pollinated populations occurred in the second and third clusters with 0.84 and 0.87 membership values, respectively, while, Melet and Tunceli natural populations were placed in the fourth cluster with a high membership probabilities (>0.90). The estimated membership values were represented in Table 4.9.

Figure 4.5 shows the four inferred clusters with the associated populations. Each colour represents a different cluster and black segments separate the preassumed populations. The preassumed populations are partitioned into 4 coloured segments that correspond to the membership coefficients in the sub groups.There was a distinct differentiation between four groups. In addition, the proportion of membership in the first group was mosaic and did not correspond to the respective geographic origin (Figure 4.5). There was no association between genetic and geographic distances for 12 populations. This means that geographically distant populations don't always exhibit different genetic structure. For instance, Marmara and Eastern Anatolia populations are placed in to the same genetic group, although they are sampled from remote regions of Turkey. Natural populations is seen as genetically more homogenous than other populations.

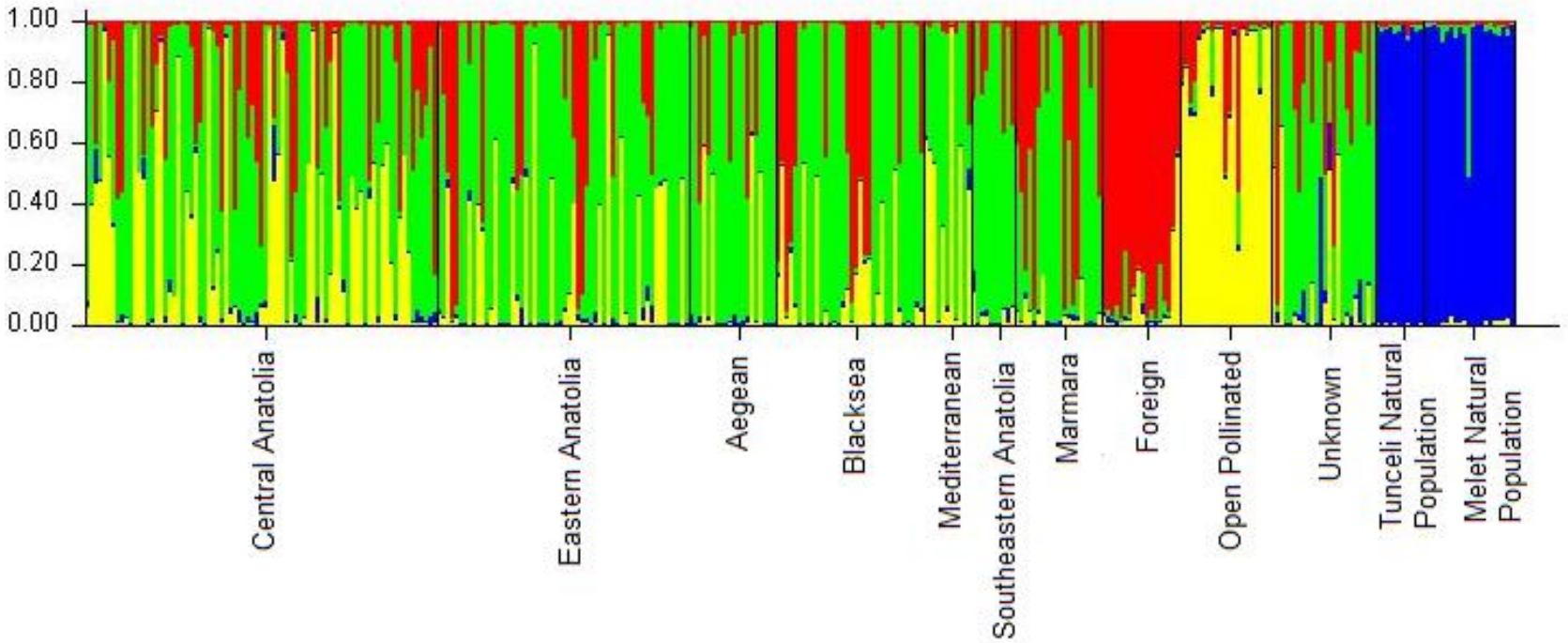

Figure 4.5. Nuclear SSRs-based STRUCTURE analyses for 329 *P. nigra* trees from 12 population in Turkey. Distribution of STRUCTURE defined groups among 12 populations for $K = 4$, membership coefficients for each population were averaged to generate drainages *Q*-matrix. Green, red, yellow and blue colours represent cluster 1, cluster 2, cluster 3 and cluster 4, respectively.

CHAPTER 5

DISCUSSION

5.1. Genetic Diversity of *Populus nigra* Populations in Turkey

5.1.1. Allelic diversity and private alleles in the studied populations

Genetic diversity assessment generally depends on allelic diversity. All genetic diversity parameters are calculated according to alleles. The more the allelic diversity, the more genetic diversity is seen in populations. ElMousadik and Petit (1996) emphasised that allelic richness was more important than heterozygosity for measuring diversity, especially in the context of genetic conservation.

The Melet,Tunceli and Eastern Anatolia populations had high number of private alleles. The number of private alleles present in populations is a good indicator of the genetic diversity. Kalinowski (2004) stated the importance of the allelic richness (the number of allele and private alleles) for identifying populations that deserve special management. Populations that have many private alleles could be protected to conduct new cultivation programmes. In the present study natural populations and Eastern Anatolia population could be used to diversify clonal banks and breeding population of European black poplar in Turkey.

In this study, there was little difference between mean observed and effective number of alleles per locus between clone bank and natural populations. The highest effective (8.08) and observed (8.08) number of alleles were noted to be in the foreign population. The Mediterranean population had the lowest effective allele (3.17) and observed allele number (3.36). This result demonstrates that *P. nigra* populations in Europe have more genetic diversity than Turkish populations. Among the Turkish populations, Central Anatolia population had the highest effective and observed allele number (7.25).

When polymorphism in microsatellite loci is considered, the loci WPMS18 and WPMS20 were found to be having the lowest number of effective and observed alleles (8), while the locus PMGC2163 displayed the highest number of alleles (19). Previously, Ratcmatcher (2009) reported that the locus WPMS14 had the highest number of alleles in a study containing 18 European black poplar populations from Germany. Similarly, Smulders *et al*. (2008) and Liesebach (2010) found the highest allele number for WPMS14 and WPMS09 loci in European black poplar populations from European river basins.

5.1.2. Proportion of polymorphic loci

This parameter explains the fact that a locus is displaying variation. All studied populations and loci were highly polymorphic (100%), except for the Mediterranean and Southeastern Anatolia populations (92%).

5.1.3. Heterozygosity

The mean observed and expected heterozygosity values didn't differ greatly. Observed heterozygosity values were ranged from 0.62 for open pollinated and foreign populations to 0.69 in the Mediterranean. These values indicated that all studied populations have close observed heterozygosity values at multiple loci.

Expected heterozygosity values for clone bank and natural populations ranged between 0.50 to 0.79. The foreign population had the highest value of expected heterozygosity (0.79). Only the foreign, open pollinated and Tunceli populations had observed heterozygosities which were lower than expected. The high rate of homozygosity found in these populations could be explained by their small effective population size. Furthermore, Pemberton *et al*. (1995) and Dakin and Avise (2004) stated that the observed heterozygosity deficiency is in part due to the occurrence of null alleles and/or allele drop-out. These events are common at microsatellite loci. Smulders *et al*. (2008) used seven microsatellite loci to assess genetic diversity of 16 black poplar populations across European river systems. They found gene diversity (Expected heterozygosity) between 0.70 and 0.82. Smulder's result was higher than our result because they used natural populations which naturally occur along the river basin and have more genetic diversity.

According to the Hardy-Weinberg rule, when the observed heterozygosity rate is higher than the expected heterozygosity, inbreeding is not expected and genetic diversity in populations is at the desired level. When mean observed and expected heterozygosity values (0.59-0.66) were considered, European black poplar populations in Turkey appear to maintain a desired level of expected heterozygosity rate.

In general, the mean observed heterozygosity value (0.59) for European black poplar trees in the clone bank was higher than mean expected heterozygosity (0.66). Similarly, Van Dam (2002) reported mean observed and expected heterozygosity values as 0.77 and 0.79 for 23 black poplar populations with 12 microsatellite markers. Also, Pospiskova (2004) stated that their observed and expected heterozygosity values were between 0.74-0.84 and 0.77-0.81 in four populations from Morava river basin in Czech Republic. The very high value of genetic diversity within these poplar populations can be explained by the high degree of polymorphism of analysed microsatellite loci. Difference between our populations and other populations could be caused by different sample size of populations and different microsatellite markers which were used.

5.2. Genetic Differentation of *Populus nigra* Populations in Turkey

F statistics

The average F_{is} was -0.12. This value indicates that observed heterozygosity was higher than expected heterozygosity for Euroepan black poplar populations in Turkey, that is, there was excess of heterozygotes all over the populations. Allendorf and Luikart (2007) stated that excess of heterozygotes can be obtained in small randomly mating populations. Also, natural selection may cause excess of heterozygotes, when heterozygotes have a high probability of surviving than homozygotes.

Although the F_{is} values were generally found as negative for the studied European black poplar populations, the foreign, open pollinated and Tunceli populations demonstrated an overall departure from Hardy-Weinberg genotypic proportions with positive fixation coefficients F_{is} = 0.21, 0.02 and 0.07, respectively. The observed positive value of the mean fixation coefficient indicates an excess of homozygotes in populations. The excess of homozygotes relative to Hardy-Weinberg proportions could result from inbreeding within local populations and Wahlund effect due to subdivison of a population in to separate demes. The presence of null alleles also causes excess of homozygotes (Allendorf and Luikart, 2007).

The average F_{IS} of -0.15 for 12 loci was the indicator of the excess of heterozygosity. F_{IS} values for single loci in the European black poplar populations reflected an excess of homozygotes for four loci (WPMS04, WPMS05, WPMS09 and WPMS10). These loci showed significant deviations from zero, especially WMPS04. Ratcmatcher (2010) also found similar results. In their study, WPMS05, WPMS09, WPMS20 and PMGC2163 showed significant deviations from zero.

In contrast to Ratmatcher's result (2010), there was a significant excess of heterozygotes for locus WPMS20. The significant positive F_{IS} of the European black poplar populations indicated a loss of genetic diversity. This yielded heterozygote deficits, which reflect deviations from Hardy–Weinberg equilibrium.

According to *F* statistics, genetic differentation among clone bank and natural populations is very low. F_{ST} value for 12 populations was found to be 0.08. According to this value, it is understood that a minimum level of differentiation occurs among clone bank and two new natural populations. Low variation among these populations could be caused by efficient gene flow (Pospiskova, 2004). Due to seed dispersal mechanisms and effective cross pollination in European black poplar, the level of genetic differentation is low. Such low values were also reported for other European black poplar populations from relatively small regions. For example, Gebhardt *e*t *al. (*2002) found F_{ST} = 0.053 for two European black poplar populations in Germany.

Van Dam (2002) reported the F_{ST} value as 0.314 for 23 European black poplar populations from all over Europe by using seven microsatellites markers which were used in this study. There was a high level of genetic differentation between these 23 populations. Very high

value of genetic diversity within the poplar populations were explained by the high degree of polymorphism present in the analysed microsatellite loci. The F_{ST} value obtained for their study is larger than ours (Van Dam, 2002). Difference of F_{ST} values between Turkish and Europe European black poplar populations may be caused by sampling locations and population size. Turkish populations were sampled from all over Turkey not considering their location and growth habitat.

Genetic differentation between Turkish European black poplar populations collected from seven region of the Turkey was found as 0.03. This value indicates that there is very little level of genetic differentation among Turkish European black poplar populations. However, the F_{ST} value for two natural populations was found to be 0.17 which is quite high. Trees of these populations were collected from two river basins. These two river basins include natural stands of European black poplar. More genetic diversity is expected along river basins. Also, the high genetic differentiation among two study sites indicates that polen and seed flow from other poplar populations may be prevented.

Genetic distance

According to the constructed dendogram, two major clusters were formed. The clone bank populations constituted one of the major group with two sub clusters. The first major cluster included populations from seven region of Turkey and unknown. The Central Anatolia, Mediterranean and unknown population occurred in the same sub cluster. The Central Anatolia and Mediterranean populations were close geographic regions. It appears that the clones of the unknown population is likely to come from either the Central Anatolia or from the Mediterranean populations. The Eastern Anatolia, Blacksea, Southeastern Anatolia, Marmara and Aegean populations were found within the subclusters of the first major cluster. The Eastern Anatolia, Southeastern Anatolia and Blacksea regions are close to each other as well as Aegean and Marmara regions. Geographically close populations were found also genetically close according to our dendogram. The second sub-cluster of the first major group composed of the foreign and open pollinated populations. The open pollinated population includes hybrid trees.

The other major group included only natural populations. There are no clones from these populations in the clone bank. The genetic distance between Melet and Tunceli populations could not be able to calculated. When comparing the natural populations with clone bank populations, foreign and open pollinated populations are closer to natural population than Turkish black poplar populations. Seven populations collected from all over Turkey are composed of generally vegetatively propagated trees and there seemed to be a high level of gene flow between them. Natural, foreign and open pollinated populations have their distinct genetic structures because of their location and propagation type.

5.3. Clonal propagation

According to the allelic combination of 12 microsatellite loci, about a third of 329 European black poplar trees have the same allelic genotype. Single genotype duplicated many times in some of the studied populations. It is estimated that this genotype might have excellent growth habit and resistance to cold and drought conditions. In the Behiçbey clone bank, the open pollinated and foreign populations had no clonal duplications. Also, as expected, the natural populations included no duplicated clones. These populations have unique genotypes. It was estimated that foreign, open pollinated and natural populations were systematically sampled. However, other populations contained groups of identical ramets which were vegetatively produced and widely distributed among local farmers as well as foresters. This result shows that populations of Turkey were not sampled properly.

Similar inproper samplings were observed in other studies. Smulders *et al*., (2008) studied vegetatively propagated European black poplar populations in Austria, Germany, Britain, and Netherlands. For example, Britain population consisted of 72 trees, 70 of the 72 trees came from one clone. Also, Storme (2004) stated that in Britain more than half of the trees and in Netherlands almost half of the trees in gene banks were duplicated. In these gene banks, single genotypes were duplicated many times.

These duplicated trees might be the result of human assisted migration and selection activities. The branches of trees might be cut and planted in different regions by natives. In the past, many rural people carried plant materials of poplar trees that grow in their farms with them to new areas where they moved in. The river flow could also help to the distribution of clones with help of twigs long distances. In addition to flow, floods could cause the migration of branches of black poplar trees from natural distribution area to different region. Also within a population, root suckers of mature trees produce dublicated nearest neighbour clones close to each other (Smulders, 2008).

The occurrence of the same clone across Turkey suggest that this clone was not naturally distributed and established. All duplicated clones come from one original genotype. The ramets of the same genotype were sampled 100 times from seven region of Turkey and established in Behiçbey nursery. Actually, all populations which have duplicated clones of the same genotype include closer duplicated clone numbers relative to their sample size. Eastern Anatolia population had 27 duplicated clone numbers as the highest. It is concluded that propagation by man is one of the most important reason for the widespread occurrence of this clone in Turkey.

5.4. Fingerprinting Analysis of Commercially Registered Clones

Identity problems of commercially registered clones was solved by comparing five commercial trees from three clone banks with help of three SSR primers. Except Gazi trees other trees were found identical for Behiçbey, Elazığ and İzmit clone banks. Gazi trees in Elazığ clone bank had different allele composition than other clone banks. To obtain more trustable result, more primers could be used and all trees could be analyzed.

5.5. Population structure and landscape genetics

The amalgamation of genetic and spatial data possibly explains how landscape features as well as spatial information have important roles in shaping the genetic structure of populations. Population structure analysis with nuclear SSRs data provided a support for the presence of four genetically separated groups. One unknown and seven Turkish European black poplar populations occurred in the first cluster. These populations were genetically close to each other. This was explained with high level of gene flow caused by effective pollen and seed dispersal over large distance by wind and water. Also human integrated migration of poplar trees among these regions could cause the closeness of these populations. The clustering also showed a high level of admixture within the first group (Figure 4.5). It could be caused by inappropriate sampling of trees from Turkey Individuals can have membership in one or more of the groups. Additionally, Bayesian analysis showed that the studied populations are not well separated spatially. The predefined clusters do not show clear spatial separation.The second and third cluster included foreign and open pollinated populations. Foreign population includes trees from different European countries. Occurrence of this population in a separate cluster could be explained different genetic structure. The last cluster composed of Tunceli and Melet natural populations. The Tunceli and Melet populations are close to Eastern and Southeastern Anatolia populations geographically. However, they constitutied a different cluster due to their different genetic composition. A clear spatial separation was observed for natural, foreign and open pollinated populations.

Smulders et al. (2008) found nine inferred clusters for 17 European black poplar populations sampled from seven European river catchments. They found Danube and Inn populations in Austria were genetically more similar to the Vltava population in Czech Rebublic. This showed that gene flow and dispersal takes place across larger distances than it is expected.

Ismail (2010) performed a study with 38 *P. trichocarpa* natural populations including 369 trees from British Columbia. These populations were classified in to three groups identified by using STRUCTURE clustering analysis, and the, populations were well separated spatially and formed three distinct groups corresponded to northern, interior, and southern regions. Each population in his study contained less individual compared with present study and trees could be sampled systematically.

Eight Turkish European black poplar populations were found genetically close in both STRUCTURE analysis and UPGMA method. These eight populations include totally 100 ramets of the same genotype. The genetic closeness of these populations are caused by 100 ramets of the same genotype in addition to gene flow. To increase the unrelatedness in clone bank, new additional clones should be sampled and introduced in to the clone bank.

CHAPTER 6

CONCLUSION

The results of the this study indicated that 12 nuclear microsatellite markers provided sufficient resolution to study the demographic history and population genetic structure of *Populus nigra* populations in Turkey.

Obtained genetic diversity parameters in the present study are 3.17- 8.08 for effective allele number, 3.36- 8.08 for observed allele number, 92- 100 % of proportion of polymorphic loci, 0.50- 0.79 for expected heterozygosity and 0.62- 0.69 for observed heterozygosity. Foreign population had the highest values for expected and observed allele number and proportion of polymorphic loci. Although, Mediterranean population had the highest value of observed heterozygosity, expected and observed allele numbers for this population was lowest.

Fixation index within populations for 12 loci was found as -0.12, indicating that the heterozygotes were 12% higher than expected. Tunceli, Foreign and Open pollinated populations had deviations from expected heterozygosity values. Eight percent of total genetic variation of clone bank and natural populations was between population and 92% of total genetic variation was within populations. As expected, within population variation was high compared to among-population variation.

According to population genetic structure study, four major spatially and genetically distinct groups were obtained. The first cluster include *P. nigra* populations from seven regions of Turkey. The second and third clusters were composed of foreign and open pollinated populations. Natural populations shaped thefourth cluster. A high admixture proportion was found at the first cluster which is likely due to an excessive gene flow among closely related populations.

The results given in this study showed the level of genetic diversity for 12 European black poplar populations in Turkey. In addition, the results demonstrated that these populations are genetically grouped into three groups not related with geographic origin. When all the populations were compared (Behiçbey clone bank and natural populations) in terms of expected and observed heterezoygosity, number of alleles, polymorphic loci ratio among populations, significant differences were not observed. Genetic diversity of all populations is generally in the desired ratio. With help of this information, breeding studies for later generations of black poplar populations would be performed to keep the desired level of genetic diversity and adaptability to different ecological environments.

Using SSR markers, identity confusion of commercially registered clones were resolved and cultivar identification of all trees in the Behiçbey clone bank were carried out. Microsatellite analysis revealed the occurrence of duplicated clones in clone bank populations. These duplicated trees have affected the genetic diversity, heterozygosity, and genetic structure of existing populations. With the help of microsatellite markers, it will be possible to identify duplicated clones and exclude these from clone bank. In further studies, new *P. nigra* trees should be collected systematically from different regions of Turkey, especially from natural stands of trees (river boundaries) and should be analyzed to characterize the genetic structure of them accurately. To conserve and develop populations in Turkey, many studies with different molecular markers should be performed.

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APPENDIX A

The Location of European Black Poplar Trees in Behiçbey Clone Bank in Turkey

Table A.1

Clone Identity	Region	City
N.03.375.1	Central Anatolia	Kirsehir
N.02.05.09.5	Open pollination	Open pollination
N.02.02.06.5	Open pollination	Open pollination
N.03.378.5	Central Anatolia	Ankara
N.02.338.5	Unknown	Unknown
KELKİT 11.5	Blacksea	Gumushane
KELKİT 4.5	Blacksea	Gumushane
N.03.364.5	Eastern Anatolia	Erzurum
N.02.07.03.5	Open pollination	Open pollination
LVUBAKA.5	Foreign	Foreign
N.02.05.03.5	Open pollination	Open pollination
N.03.324.5	Unknown	Unknown
N.02.05.08.5	Open pollination	Open pollination
N.03.367.5	Eastern Anatolia	Erzurum
N.03.333.5	Unknown	Unknown
N.90.010.5	Eastern Anatolia	Van
KELKİT 8.5	Blacksea	Gumushane
N.02.05.07.5	Open pollination	Open pollination
KELKİT 7.1	Blacksea	Gumushane
N.02.02.01.5	Open pollination	Open pollination
N.03.357.1	Eastern Anatolia	Erzincan
N.03.368.1.5	Eastern Anatolia	Erzurum
N.03.376.1	Central Anatolia	Kirsehir
N.03.365.5.5	Eastern Anatolia	Erzurum
N.02.07.02.1	Open pollination	Open pollination
64/14.10.1	Central Anatolia	Ankara
N.03.372.5	Eastern Anatolia	Elazig
EFN.2.1	Foreign	Foreign
N.02.01.02.5	Open pollination	Open pollination
N.02.02.03.1	Open pollination	Open pollination
N.02.05.12.5	Open pollination	Open pollination
N.02.08.01.1	Open pollination	Open pollination
N.02.05.02.5	Open pollination	Open pollination

Table A.1 (Continued)

Clone Identity	Region	City
N.02.05.01.1	Open pollination	Open pollination
N.02.01.05	Open pollination	Open pollination
N.92.217.1	Central Anatolia	Konya
N.92.142.1	Central Anatolia	cankiri
N.92.156.5	Blacksea	Kastamonu
N.91.092.1	Central Anatolia	Aksaray
N.92.302.1	Aegean	Afyon
N.91.118.5	Mediterranean	Antalya
N.91.119.1	Southeastern	Gaziantep
N.90.102.5	Unknown	Unknown
N.90.036.1	Eastern Anatolia	Erzurum
N.92.258.5	Central Anatolia	Eskisehir
N.90.024.1	Eastern Anatolia	Sivas
N.92.179.5	Blacksea	Tokat
N.91.075.1	Mediterranean	Kahramanmaras
N.92.140.5	Marmara	Kocaeli
KELKİT 6.1	Blacksea	Gumushane
KAE N.92.5	Unknown	Unknown
N.02.07.04.1	Open pollination	Open pollination
N.03.371.5	Eastern Anatolia	Elazig
N.02.05.06.5	Open pollination	Open pollination
KELKİT 3.1	Blacksea	Gumushane
N.03.377.5	Central Anatolia	Ankara
KELKİT 1.1	Blacksea	Gumushane
N.02.05.013.1	Open pollination	Open pollination
64/14.06.1.5	Central Anatolia	Ankara
N.02.07.05.1	Open pollination	Open pollination
N.02.01.04.5	Open pollination	Open pollination
64/14.12.5	Central Anatolia	Ankara
KELKİT 9.1	Blacksea	Gumushane
N.03.366.5	Eastern Anatolia	Erzurum
N.90.030.1	Eastern Anatolia	Sivas
N.03.373.5	Central Anatolia	Kirsehir
N.91.078.1	Mediterranean	Kahramanmaras
N.92.144.5	Central Anatolia	cankiri
64/13.1	Central Anatolia	Ankara
N.92.298.5	Marmara	İsparta
N.92.219.1	Central Anatolia	Konya
N.96.321.5	Eastern Anatolia	Van

Table A.1 (Continued)

Clone Identity	Region	City
N.91.094.1	Eastern Anatolia	Malatya
N.96.316.5	Eastern Anatolia	Malatya
N.93.304.1	Southeastern	Gaziantep
N.92.187.5	Blacksea	Amasya
N.82.008.1	Unknown	Unknown
N.92.260.5	Mediterranean	Adana
N.92.164.1	Blacksea	Amasya
N.92.286.5	Aegean	Denizli
N.91.080.1	Mediterranean	Kahramanmaras
N.90.013.5	Eastern Anatolia	Mus
N.91.074.5	Central Anatolia	Ankara
N.92.297.1	Marmara	isparta
N.91.120.5	Southeastern	Gaziantep
N.91.059.1	Central Anatolia	Yozgat
N.92.165.5	Blacksea	Amasya
N.96.320.1	Eastern Anatolia	Bitlis
N.91.089.5	Central Anatolia	Nigde
N.92.247.1	Central Anatolia	Karaman
N.92.295.5	Marmara	isparta
N.92.289.1	Aegean	Denizli
N.90.014.5	Eastern Anatolia	Bingol
N.96.325.1	Eastern Anatolia	Van
N.92.132.5	Marmara	Bilecik
N.92.130.1	Marmara	Bilecik
N.92.204.5	Central Anatolia	cankiri
N.93.306.1	Marmara	Bursa
N.91.122.5	Marmara	Sakarya
N.96.310.1	Eastern Anatolia	Malatya
N.91.108.5	Southeastern	Gaziantep
77/40.1	Central Anatolia	Ankara
N.92.137.5	Marmara	Yalova
N.96.319.1	Eastern Anatolia	Elazig
N.92.299.5	Marmara	isparta
85/16.1	Eastern Anatolia	Agri
85/11.5	Eastern Anatolia	Erzurum
64/14.1	Central Anatolia	Ankara
GAZİ .4	Central Anatolia	Ankara
88/5.5	Aegean	Denizli
N.90.038.1	Eastern Anatolia	Erzincan
N.92.176.5	Blacksea	Tokat

Table A.1 (Continued)

Clone Identity	Region	City
N.92.276.1	Aegean	Usak
N.92.256.5	Central Anatolia	Eskisehir
N.91.021.1	Unknown	Unknown
N.91.077.5	Mediterranean	Kahramanmaras
N.92.073.1	Unknown	Unknown
N.91.111.5	Mediterranean	Osmaniye
N.91.105.1	Southeastern	Gaziantep
88/4.5	Aegean	Aydin
N.92.215.1	Central Anatolia	Konya
N.91.102.5	Southeastern	Gaziantep
N.91.212.1	Unknown	Unknown
ATA 1.5	Central Anatolia	Ankara
N.90.050.5	Central Anatolia	Ankara
N.92.224.1	Central Anatolia	Konya
N.95.045.5	Unknown	Unknown
N.92.133.1	Central Anatolia	Eskisehir
N.92.131.5	Marmara	Bilecik
N.92.282.1	Aegean	Afyon
N.90.020.5	Eastern Anatolia	Sivas
N.92.250.1	Central Anatolia	Konya
N.92.245.5	Marmara	İcel
N.96.315.1	Eastern Anatolia	Malatya
N.92.252.5	Central Anatolia	Konya
N.92.171.1	Blacksea	Amasya
88/1.5	Central Anatolia	Ankara
85/1.1	Eastern Anatolia	Agri
62/172.5	Central Anatolia	Ankara
82/2.1	Central Anatolia	Kirsehir
83/1.5	Central Anatolia	Yozgat
83/10.1	Central Anatolia	Kayseri
ANADOLU .5	Central Anatolia	Ankara
83/9.1	Central Anatolia	Kayseri
83/6.5	Central Anatolia	Kirsehir
88/6.1	Aegean	Denizli
82/4.5	Central Anatolia	Kirsehir
82/1.1	Central Anatolia	Kirsehir
KOCABEY .5	Mediterranean	Adana

Table A.1 (Continued)

Clone Identity	Region	City
83/12.1	Central Anatolia	Kayseri
83/3.5	Central Anatolia	Kayseri
ÇUBUK 1.1	Central Anatolia	Ankara
83/8.5	Central Anatolia	Kirsehir
82/3.1	Central Anatolia	Kirsehir
85/15.5	Eastern Anatolia	Erzurum
88/8.1	Aegean	Denizli
N.90.032.5	Eastern Anatolia	Erzurum
85/14.1	Eastern Anatolia	Erzurum
62/160.5	Central Anatolia	Ankara
62/191.1	Central Anatolia	Ankara
85/4.5	Eastern Anatolia	Agri
ÇUBUK 2.1	Central Anatolia	Ankara
88/3.1	Central Anatolia	Ankara
85/6.5	Eastern Anatolia	Agri
83/13.1	Central Anatolia	Kayseri
83/5.5	Central Anatolia	Kirsehir
GEYVE .1	Central Anatolia	Ankara
85/7.5	Eastern Anatolia	Agri
N.92.271.1	Aegean	Kutahya
87/1.5	Central Anatolia	Kirsehir
N.91.095.1	Southeastern	Adiyaman
N.92.058.5	Unknown	Unknown
N.92.149.1	Blacksea	Kastamonu
N.91.085.5	Central Anatolia	Kayseri
N.92.185.1	Blacksea	Amasya
N.90.045.5	Eastern Anatolia	Erzurum
N.92.169.1	Blacksea	Tokat
N.91.081.5	Central Anatolia	Kayseri
EFN.1.1	Foreign	Foreign
N.92.206.5	Aegean	Kutahya
N.96.317.1	Eastern Anatolia	Malatya
N.91.058.5	Central Anatolia	Yozgat
N.03.358.1	Eastern Anatolia	Erzincan
N.90.008.5	Eastern Anatolia	Kars
N.90.016.1	Eastern Anatolia	Malatya
N.90.028.5	Eastern Anatolia	Sivas
N.03.365.1	Eastern Anatolia	Erzurum
N.03.399.5	Unknown	Unknown

Table A.1 (Continued)

Clone Identity	Region	City
FARCFLHHZ 35.1	Foreign	Foreign
URIFFMH.5	Foreign	Foreign
N.03.368.A.1	Eastern Anatolia	Erzurum
N.03.368.1	Eastern Anatolia	Erzurum
N.03.355.5	Eastern Anatolia	Erzincan
N.92.230.5	Central Anatolia	Konya
N.91.101.1	Southeastern	Gaziantep
N.02.05.10.5	Open pollination	Open pollination
N.03.356.1	Eastern Anatolia	Erzincan
N.90.011.1	Eastern Anatolia	Van
N.92.211.5	Aegean	Afyon
ALTERRA .1	Foreign	Foreign
N.92.223.5	Central Anatolia	Ankara
N.92.154.1	Blacksea	Kastamonu
N.92.240.5	Central Anatolia	Nigde
N.92.124.5	Marmara	Sakarya
N.85.018.1	Unknown	Unknown
N.92.208.5	Aegean	Kutahya
N.91.052.1	Blacksea	corum
N.90.012.5	Eastern Anatolia	Van
N.92.152.1	Blacksea	Kastamonu
N.91.112.5	Mediterranean	Hatay
N.91.109.1	Mediterranean	Osmaniye
N.90.039.5	Eastern Anatolia	Erzincan
N.92.254.1	Aegean	Afyon
N.92.097.5	Unknown	Unknown
N.92.236.1	Central Anatolia	Konya
N.92.293.5	Marmara	isparta
N.93.309.1	Central Anatolia	Sivas
N.92.160.5	Blacksea	Samsun
N.90.065.1	Unknown	Unknown
N.91.076.5	Mediterranean	Kahramanmaras
N.91.091.1	Central Anatolia	Aksaray
N.92.114.5	Unknown	Unknown
N.92.167.1	Blacksea	Tokat
N.92.138.5	Marmara	Yalova
N.92.239.1	Central Anatolia	Nigde
N.91.088.5	Central Anatolia	Nigde

Table A.1 (Continued)

Clone Identity	Region	City
N.92.153.1	Blacksea	Kastamonu
N.92.128.5	Marmara	Bilecik
N.91.063.1	Central Anatolia	Yozgat
N.90.046.5	Eastern Anatolia	Erzurum
N.91.002.1	Unknown	Unknown
N.92.301.5	Aegean	Afyon
N.91.067.1	Eastern Anatolia	Sivas
N.92.123.5	Marmara	Sakarya
N.92.200.1	Blacksea	corum
N.92.134.5	Central Anatolia	Eskisehir
N.85.010.1	Unknown	Unknown
N.92.269.5	Blacksea	Zonguldak
N.91.083.1	Central Anatolia	Kayseri
N.96.322.5	Eastern Anatolia	Van
N.92.232.1	Central Anatolia	Konya
N.90.027.5	Eastern Anatolia	Sivas
N.91.103.1	Southeastern	Gaziantep
N.91.068.5	Eastern Anatolia	Malatya
N.62.164.1	Unknown	Unknown
N.92.162.1	Blacksea	Samsun
N.90.062.5	Unknown	Unknown
N.91.110.1	Mediterranean	Osmaniye
N.92.237.5	Central Anatolia	Konya
N.82.166.1	Unknown	Unknown
N.92.060.5	Unknown	Unknown
N.92.168.1	Blacksea	Tokat
N.92.159.5	Blacksea	Sinop
N.90.034.1	Eastern Anatolia	Erzurum
N.92.195.5	Blacksea	Amasya
N.90.035.1	Eastern Anatolia	Erzurum
N.92.218.5	Central Anatolia	Konya
N.92.233.1	Central Anatolia	Karaman
N.92.209.5	Aegean	Afyon
N.92.243.1	Mediterranean	Adana
N.92.292.5	Marmara	İsparta
N.96.323.1	Eastern Anatolia	Van
N.91.073.5	Eastern Anatolia	Malatya
N.92.278.1	Aegean	Usak
N.91.090.5	Central Anatolia	Nigde
N.91.071.1	Eastern Anatolia	Malatya

Table A.1 (Continued)

Clone Identity	Region	City
N.92.126.5	Marmara	Bilecik
N.92.166.1	Blacksea	Tokat
N.92.255.5	Aegean	Afyon
N.91.054.1	Unknown	Unknown
N.92.213.5	Aegean	Afyon
N.92.148.1	Central Anatolia	cankiri
N.91.084.5	Central Anatolia	Kayseri
N.92.831.1	Unknown	Unknown
N.92.202.5	Central Anatolia	cankiri
N.92.182.1	Blacksea	Tokat
N.92.170.5	Blacksea	Amasya
N.92.214.1	Central Anatolia	Konya
N.92.284.5	Aegean	Denizli

APPENDIX B

Buffers Chemicals and Equipments

Buffers and solutions for DNA isolation

2X CTAB: 2 gr CTAB (Cetyl Trimethyl Ammonium Bromide), (SIGMA)
4 ml (pH:8) 0.5 M EDTA, (FLUKA)
10 ml (pH:8) Tris HCL, (SIGMA)
28 ml NaCl is completed with 100 mL distilled water

Isopropanol, (FLUKA) : Pure Isopropanol, ice cold

Chloroform isoamil alcohol, (FLUKA) : (24/1)

Ethanol: 70% in distilled water

β mercapto ethanol, (SIGMA) : 17,5 ml β mercapto ethanol is completed with 250 ml with distilled water

TE buffer: 10mm Tris HCL (pH:7) 10mm ethylene diamine tetra acetic acid disodium salt (EDTA)

Buffers and solutions for PCR

Sterile water
10X PCR buffer including MgCl2 (SIGMA)
dNTPs (SIGMA): 10mM
Primer Pairs: 10μM
Taq DNA Polymerase (SIGMA Red Taq): 1U/μl
DNA: 10ng/ μl

Agarose Gel Electrophoresis Buffers and Gel System

10X TBE Buffer: 108 gr Trizma Base, (SIGMA), 55 gr Boric Acid, (SIGMA)
40 ml EDTA, (FLUKA) (0.5 M, pH:8) completed with 1000 ml with distilled water
Running Buffers: X TBE prepared in distilled water
Agarose, (SIGMA): 3 % Agorose Gel
Ethyidium Bromide,(SIGMA):4 mg/ ml
Low molecular weight DNA Ladder (SIGMA)

Equipments

Autoclave: Yamato
Centrifuge: Nüve- NF048
Deepfreezer: UĞUR- Freezer
Electrophoresis System: Thermo Scientific
Thermocyclers: Eppendorf- Mastercycler
Magnetic Stirrer: Labor Brand – Hotplate L-81
Oven: Dedeoğlu
pHmeter: Hanna Inst.
Refrigerator: Siemens
UV Transilluminator: Vilbor Lourmant
Vortex: Nüve- NM110
Water Bath: Memmert
Micropipettes: Gılson

APPENDIX C

A part of the GDA Data File

Figure C.1

```
#nexus

[!Data from Quercus
]

    begin gdadata;
    dimensions nloci=12 npops=12;
    format tokens missing=? datapoint=standard;
                    locusallelelabels
                    1 'WPMS15'   [/ 1 2 3 4 5 6 7 8 9],
                    2 'WPMS14'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16],
                    3 'WPMS09'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13],
                    4 'WPMS10'   [/ 1 2 3 4 5 6 7 8 9 10 11 12],
                    5 'WPMS05'   [/ 1 2 3 4 5 6 7 8 9],
                    6 'WPMS04'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16],
                    7 'WPMS18'   [/ 1 2 3 4 5 6 7 8 9],
                    8 'WPMS20'   [/ 1 2 3 4 5 6 7 8],
                    9 'WPMS03'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14],
                   10 'WPMS12'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14],
                   11 'PMGC14'   [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17],
                   12 'PMGC2163' [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19];
matrix
Central:
1     2/3   1/6   1/1   7/7   4/4   10/10   1/3   4/6   5/11   4/8   4/6   4/11
17    2/3   1/6   1/1   7/7   4/4   10/10   3/3   6/6   5/11   4/8   4/6   4/11
112   2/3   1/6   1/1   7/7   4/4   10/10   1/3   4/6   5/11   4/8   4/6   4/11
131   2/3   6/7   1/1   7/7   4/4   2/2     5/5   3/6   3/5    4/8   6/11  3/7
181   2/4   3/6   1/5   7/7   4/6   3/11    2/4   3/6   3/5    5/8   6/13  4/5
191   3/4   3/3   2/2   3/7   6/6   11/11   3/4   4/6   5/11   4/5   8/12  6/17
201   2/3   3/6   1/5   7/7   4/6   3/11    5/5   3/6   3/5    5/8   6/13  4/11
236   2/3   1/6   1/1   7/7   4/4   10/10   1/3   4/6   5/11   4/8   4/6   4/11
296   2/4   3/6   1/5   7/7   4/6   2/10    5/5   3/6   6/11   5/8   6/13  4/5
316   2/3   3/7   1/3   7/7   2/2   3/11    3/3   4/6   5/10   4/4   4/8   11/15
336   3/5   3/7   1/3   7/7   2/4   3/11    5/5   3/6   5/10   4/8   6/15  4/5
356   2/3   1/6   1/1   7/7   4/4   11/11   1/3   4/6   5/11   4/5   6/7   14/16
```

APPENDIX D

A Part of the STRUCTURE Data File

Figure D.1

		WPMS15	WPMS14	WPMS09	WPMS10	WPMS05	WPMS04	WPMS20	WPMS18	WPMS03	WPMS12	PMGC14	PMGC
1	1	23	16	11	77	44	1010	13	46	511	48	46	411
17	1	23	16	11	77	44	1010	33	66	511	48	46	411
112	1	23	16	11	77	44	1010	13	46	511	48	46	411
131	1	23	67	11	77	44	22	55	36	35	48	611	37
181	1	24	36	15	77	46	311	24	36	35	58	613	45
191	1	34	33	22	37	66	1111	34	46	511	45	812	617
201	1	23	36	15	77	46	311	55	36	35	58	613	411
236	1	23	16	11	77	44	1010	13	46	511	48	46	411
296	1	24	36	15	77	46	210	55	36	611	58	613	45
316	1	23	37	13	77	22	311	33	46	510	44	48	1115
336	1	35	37	13	77	24	311	55	36	510	48	615	45
356	1	23	16	11	77	44	1111	13	46	511	45	67	1416
366	1	23	16	11	77	44	1010	13	46	511	48	46	411
371	1	23	67	13	77	44	511	66	36	312	-1	-1	-1
381	1	45	23	15	29	33	33	24	56	55	44	810	56
416	1	23	16	11	77	55	1010	13	46	511	48	46	411
441	1	35	13	15	39	66	310	24	56	55	??	812	513
446	1	23	16	11	77	44	1010	13	46	511	48	46	411
464	1	23	16	11	77	44	1010	13	46	511	48	46	411
476	1	23	16	11	77	44	1010	13	46	511	48	46	45
481	1	23	16	11	77	44	1010	13	46	511	48	46	411
516	1	23	16	11	77	44	1010	13	46	511	48	46	411
541	1	23	36	15	77	46	210	55	66	35	58	613	45
571	1	34	37	35	77	24	1010	66	36	510	44	611	45
576	1	24	67	13	77	44	1212	55	36	312	58	613	-1
601	1	23	16	11	77	44	1010	13	46	511	48	46	411
636	1	24	23	45	33	66	33	24	55	55	44	812	-1
651	1	23	67	13	77	44	510	33	36	312	44	615	44
662	1	23	16	11	77	44	1010	13	46	511	48	46	411
666	1	23	16	11	77	44	1010	13	46	511	48	46	411
676	1	44	33	44	22	36	22	13	46	55	48	46	411

APPENDIX E

SSR Based STRUCTURE Analysis for 329 *P. nigra* Trees

Figure E.1. * shows the ramets of the same *Populus nigra* tree in Behiçbey clone bank.