GENETIC CHARACTERIZATION OF *Pinus nigra* SUBSPECIES *pallasiana* VARIETIES, NATURAL POPULATIONS (SEED STANDS), SEED ORCHARDS AND PLANTATIONS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY
BURCU (NAZLIER) ÇENGEL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
DEPARTMENT OF BIOLOGY

JULY 2005

Approval of the Graduate School of Natural and Applied Sciences

_____

Prof. Dr. Canan ÖZGEN
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. Semra KOCABIYIK
Head of the Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. Zeki KAYA
Supervisor

Examining Committee Members

Prof. Dr. İnci TOGAN	(METU, BIO)	_____
Prof. Dr. Zeki KAYA	(METU, BIO)	_____
Prof. Dr. Musa DOĞAN	(METU, BIO)	_____
Prof. Dr. Gökhan SÖYLEMEZOĞLU	(Ankara Univ.)	_____
Assist. Prof. Dr. Can BİLGİN	(METU, BIO)	_____

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name: Burcu (NAZLIER) ÇENGEL

Signature:

ABSTRACT

GENETIC CHARACTERIZATION OF *Pinus nigra* SUBSPECIES *pallasiana* VARIETIES, NATURAL POPULATIONS (SEED STANDS), SEED ORCHARDS AND PLANTATIONS

Çengel (Nazlıer) Burcu

Ph. D. Department of Biology

Supervisor: Prof. Dr. Zeki Kaya

June 2005, 115 pages

Pinus nigra subsp. *pallasiana* is one of the most widespread and economically important forest tree species in Turkey. Primary objective of the present study was to to reveal the effects of forestry practices by determining genetic diversity of natural and managed seed sources by means of RAPD markers. Secondly, two varieties were also investigated to reveal their pattern of genetic variation.

Seed stands, seed orchards and plantations were screened against 11 RAPD primers and generated 152 polymorphic DNA loci. Two varieties were compared with a reference seed source and 4 natural seed sources. Seven primers generated 66 polymorphic DNA loci.

An overall average for effective number of alleles was 1.68±0.030; observed heterozygosity was 0.49±0.024; expected heterozygosity was 0.38±0.014 and proportion of polymorphic loci was 93% for all seed sources considered. Results revealed that there was no considerable variation between seed source categories but some degree of variation was observed within seed orchards and plantations.

Mean F_{ST} value estimated for the natural populations revealed that 94% of the total genetic variation was within populations.

Nei's genetic distance values were also estimated for seed source categories (0.03-0.14). Nevertheless, varieties' genetic distance values were considerably higher

than other natural seed sources (0.07-0.19). Their dendrogram also claimed that two varieties are genetically different from natural populations.

The extent of genetic diversity explored by RAPD markers revealed that forestry practices caused no major changes in the managed populations with respect to natural populations. Moreover, further study is needed to illustrate genetic divergence of varieties.

Key Words: *Pinus nigra*, RAPD markers, genetic diversity, seed sources, varieties.

ÖZ

Pinus nigra SUBSPECIES *pallasiana* VARYETELERİNİN, DOĞAL POPULASYONLARININ (TOHUM MEŞCERELERİ), TOHUM BAHÇELERİNİN VE PLANTASYONLARININ GENETİK KARAKTERİZASYONU

Çengel (Nazlıer) Burcu

Doktora, Biyoloji Bölümü

Tez Yöneticisi: Prof. Dr. Zeki Kaya

Temmuz 2005, 115 sayfa

Pinus nigra subsp. *pallasiana*, Türkiye'nin ekonomik açıdan önemli ve en geniş yayılışa sahip türlerinden birisidir Bu çalışmanın öncelikli amacı, doğal ve yönetilen tohum kaynaklarının genetik çeşitlilik parametrelerini belirleyerek ormancılık etkinliklerinin etkisini ortaya koymaktır, İkinci amaç ise Anadolu karaçamının Türkiye'deki 2 varyetesinin genetik yapısını ortaya koymaktır.

Tohum meşcereleri, tohum bahçeleri ve ağaçlandırmalar 11 RAPD primeriyle taranmış ve 152 polimorfik DNA lokusu elde edilmiştir. Varyeteler, referans tohum kaynağı ve tohum meşcereleri 7 primerle taranmış ve 66 polimorfik DNA lokusu elde edilmiştir.

Ortalama etkili allel sayısı 1.68±0.030; gözlenen heterozigotluk 0.49±0.024; beklenen heterozigotluk 0.38±0.014 ve polimorfik lokus oranı %93 olarak tahmin edilmiştir. Tohum kaynağı kategorileri arasında genetik çeşitlilik parametreleri açısından anlamlı fark bulunmamıştır ancak, tohum meşceresi ve ağaçlandırma kategorilerinde gurup içi farklılıklar gözlenmiştir.

Doğal meşcereler için hesaplanan ortalama F_{ST} değeri toplam genetik çeşitliliğin % 94'ünün populasyon içinde olduğunu göstermektedir.

Tüm tohum kaynağı kategorileri için Nei'nin genetik mesafe değerleri 0.03-0.14 arasında bulunmuştur. Diğer yandan, varyeteler için hesaplanan genetik mesafe değerleri doğal tohum kaynaklarından daha yüksektir (0.07-0.19). Varyetelerin doğal tohum kaynaklarıyla kıyaslandığı dendrogramda da, varyeteler doğal meşcerelerden belirgin olarak ayrılmıştır.

RAPD belirteçleriyle elde edilen sonuçlar, doğal meşcereler bazında karşılaştırılan genetik çeşitlilik parametrelerinin tohum meşcereleri, tohum bahçeleri ve plantasyonlar arasında belirgin farklılıklar olmadığını ortaya koymuştur. Ancak varyetelerin genetik ayrışmalarını kesin olarak tesbit edebilmek için yeni çalışmalar gerekmektedir.

Anahtar Kelimeler: *Pinus nigra*, RAPD belirteçleri, genetik çeşitlilik, tohum meşceresi, tohum bahçesi, plantasyon, varyete.

to my mother and daughter

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Prof. Dr. Zeki KAYA for his guidance, supervision and endless patience throughout the study.

I would like to express my thanks to all jury members for their comments and criticism.

I would like to thank Sadi ŞIKLAR and Hikmet ÖZTÜRK, director and vice director of the Forest Tree Seeds and Tree Breeding Research Directorate (FTSTBRD), for their continuous support and advice in the course of the study.

I am grateful to Ercan VELİOĞLU, leader of the project at FTSTBRD, for his continuous help, encouragement and patience throughout this study.

I wish to express my deep gratitude to Yasemin İÇGEN, my colleague, roommate and close friend from FTSTBRD, for his continuous help, advice, collaboration and encouragement in every step of this study.

I am also grateful to my colleagues from FTSTBRD, Murat ALAN, Gaye KANDEMİR and Özlem KÖSE for their support in sample collection and laboratory work of this study.

I would like to thank my colleagues from FTSTBRD, Semra Keskin, for the photographs, Turgay Ezen for his technical support and Belkıs Korkmaz for her valuable friendship.

I would like to thank you my collegues from Department of Biology, Plant Genetics and Tissue Culture Laboratory for their support.

I owe special thanks to my dear family: to my parents, Faruk and Ayten NAZLIER; my brother Bülent NAZLIER; my aunts Gülten NART, Ayfer CANER, Mesude CANER; and also my brother in law, Ömer NART; for their continuous love, support, understanding and encouragement through all my life. Their support is so precious to me.

Last but not least, to my husband Hüseyin Sıtkı ÇENGEL, I bestow sincere thanks for his faith in me and endless love, help, support, encouragement, understanding and patience.

This study was supported and funded by the Ministry of Environment and Forestry (ANK017 1611/1999-2002); Turkish Agricultural Research Project; Turkish Scientific and Technical Research Council (Project No: TOGTAG-2910) and METU AFP (AFP-2002-07-02-00-03).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
BSA	Bovine Serum Albumin
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribose triphosphate
EDTA	Ethylene diamine tetra acetic acid
FTSTBRD	Forest Tree Seeds and Tree Breeding Research Directorate
MOEF	Ministry of Environment and Forestry
NJ	Neighbor Joining
PCR	Polymerase Chain Reaction
PPM	Parts per Million
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SCAR	Sequence Characterized Amplified Regions
SSR	Simple Sequence Repeats
SUBSP	Subspecies
UPGMA	Unweighted Pair Group Method with Aritmetic Means
VAR	Variety
VNTR	Variable Number of Tandem Repeats

CHAPTER I

INTRODUCTION

1.1. Biology of *Pinus nigra* subspecies *pallasiana*

1.1.1. Taxonomy

Pinus nigra Arnold (black pine) belongs to Phylum Pinophyta, Class Pinopsida, Order Pinales, Family Pinaceae, and Genus *Pinus*. Common names associated with the species include black pine, European black pine, Austrian pine, Calabrian pine, Corsican pine, Crimean pine and Pyrenees pine. Although this species has received an excessive number of described names, still there is no general agreement on its nomenclature (Vidakovic, 1991).

Taxonomy of the species started with Miller, who first described black pine as *Pinus maritima* at 1768. From this time, black pine and its many lower taxonomic units have been described by various names, leading to confusion which is still going on. There are opinions however that black pine is not a uniform species.

Several researchers described two or more small geographic species. Such as:

Schwarz (1938) divided black pine into 6 subspecies: subsp. p*allasiana*, subsp. f*enzlii*, subsp. *dalmatica*, subsp. *nigra*, and subsp. *laricio* and subsp. s*alzmanii*.

Villlar (1947) and Svodoba (1953) distinguish the western (*P. clusiana* or *P. laricio*) and eastern species (*P. nigricans* or *P. eunigra*).

Röhrig (1956) considered all forms and varieties of black pine as belonging to one species.

According to Fukarek (1958), black pine is a collective species consisting of four species: *P. clusiana, P. laricio, P. nigricans* and *P. pallasiana*.

Nyman and Gandoger, according to Fukarek (1958), distinguish two groups: *P. laricio* and *P. nigricans*.

According to Vidakovic (1974) the most acceptable classification with some modifications is that of Flora Europeae (Tutin *et al*., 1964). It was stated that *Pinus nigra* is very variable and geographical variants are not clearly discrete. They suggested that following subspecies, which are sometimes regarded as species, worth recognition:

subsp. *nigra* (Austria, Italy, Greece, Macedonia),

subsp. s*alzmannii* (France, northern Pyrenees, central and eastern Spain),

subsp. *laricio* (Corsica, Calabria, Sicily),

subsp. *dalmatica* (central coastal region of Crotia) and

subsp. *pallasiana* (the Balkan peninsula, southern Carpathians, Crimea). However, this classification does not include Northwest Africa and Asia Minor. Turkey, Cyprus and Syrian populations were also included into subsp. *pallasiana* (Davis, 1965; Yaltırık, 1993; Yaltırık and Efe, 1994).

Since black pine have a discontinuous range and significant variation in morphological, anatomical and physiological traits, it is regarded as one species subdivided into several subspecies and varieties (Vidakovic, 1991). This is supported by the fact that geographical subgroups have overlapping distributions, for instance, Austrian and Corsican pine, Calabrian and Austrian pine (Vidakovic, 1974). A considerable amount of information on black pine classification was given by Vidakovic (1974) in his monograph.

In some areas virtually every small clump of trees has been given its own scientific name. Many of them are invalid under the *International Code of Botanical Nomenclature*. Although black pine seems to be an extremely variable species, it shows a level of genetic diversity similar to many other *Pinus* species (Scaltsoyiannes, *et al*. 1994).

Recently, to achieve a better understanding of the phylogenetic relationships or evolution of the genus *Pinus*, various molecular approaches have been employed (e.g. Strauss and Doerksen, 1990; Govindaraju, *et al*. 1992; Wang and Szmidt, 1993; Krupkin *et al*., 1996; Wang *et al.*, 1999).

Anatolian black pine (*Pinus nigra* Arnold subspecies *pallasiana* (Lamb.) Holmboe) is the Turkish subspecies of the European black pine (Alptekin, 1986). In addition to the type variety (var. *pallasiana*), there are also 4 known varieties which are considered as distinct taxa, occurring in Turkey (Boydak, 2001). These are:

1. *Pinus nigra* Arnold subsp. p*allasiana* var. *pyramidata* (Acatay) Yaltırık (It is called pyramidal black pine, "Ehrami karaçam" in Turkish): It was first described by Acatay (1956) as "*Pinus nigra* Arnold var. *pyramidata* (Acatay)" but in 1986 it was moved to subsp. *pallasiana* by Yaltırık.

2. *Pinus nigra* Arnold subsp. *pallasiana* var. *şeneriana* (Saatçioğlu) Yaltırık (In Turkish: Ebe karaçamı): It was first described by Saatçioğlu (1955) as "*Pinus nigra* Arnold var. *şeneriana* Saatçioğlu" but later it was attached to subsp. *pallasiana* by Yaltırık.

3. *Pinus nigra* subsp. *pallasiana* var. *yaltırıkiana* Alptekin. First reported by Alptekin in 1986.

4. *Pinus nigra* subsp. *pallasiana* var. *columnaris-pendula*: First reported by Boydak in 1989.

Fifteen geographical variants were observed by Alptekin in his extensive study on Anatolian black pine (1986). He studied 23 characters (cone, seed and needle characteristics) of Anatolian black pine samples from 92 populations comprising all Turkey; 2 populations from Cyprus and Macedonia. In addition, until this study Anatolian black pine was regarded as var. *caramanica*.

There is no consensus on satisfactory classification of taxonomy for Anatolian black pine. Different publications or different volumes of the same publication for example, 1st volume of the Flora of Turkey and East Aegean islands (Davis, 1965) and 11th volume (Güner *et al*., 2000) do not agree on its taxonomy.

1.1.2. Natural Distribution

Black pine is native to Europe (Figure 1.1). Its range extends from longitude 5º W in Spain and Morocco to about 40º E in eastern Turkey; and from latitude 35º N in Morocco and Cyprus to 48º N in northeastern Austria and to 45º N latitude in the Crimea (Critchfield and Little, 1966). Black pine grows widely throughout southern Europe from the eastern half of Spain, southern France and Italy to Austria; south throughout Macedonia, western Romania, Bulgaria and Greece on the Balkan Peninsula; east to southern Russia in the Crimes and south to Turkey; and on the islands of Cyprus, Sicily and Corsica with outliers in Algeria and Morocco (Mirov, 1967).

Figure 1.1. Natural Distribution of *Pinus nigra*
(Isajev et al., 2004)

Anatolian black pine occurs as a widespread mid-elevation species in the Taurus, western Anatolia and northern Anatolian mountains (Figure 1.1). It covers 3.328.731 million hectares in Turkey (Anonymous, 2001). In the Black sea region, it rarely grows on seaside; but generally on the elevational range of 400 to 1400 m, forms pure stands, but after 1400 m (up to 1700 m) forms mixed stands with *Pinus sylvestris, Abies* spp. and *Quercus* spp. In the northeast, it forms small stands. In the western Black sea region, it forms large pure stands. At Thrace, there are also small stands. In the western Anatolia region, it forms one of the best stands at Bozüyük, Keles, Dursunbey, Bigadiç, Sındırgı, Demirci, Simav, Emet and Tavşanlı. At Ida Mountains (Kazdağı), it grows 200-1400 m high, generally in pure stands. In the Manisa, Akşehir, Bayındır triangle it forms local stands up to Muğla-Denizli line. It forms some of the best stands at Muğla-Yılanlı, Köyceğiz, Fethiye, Gölhisar, Acıpayam and Denizli. Starting from Lakes Region, its distribution is limited up to north (Afyon). However, at Sütçüler, Akseki, Beyşehir triangle there are some of the best stands. In the southern Anatolia region; it occurs at 1200-1400 m with some other species especially with *Juniperus* species. Finally Samandağ is the south margin of the distribution.

"Ebe" black pine occurs between 800-1250 m altitudes; within 38°16'63''– 40°46'03'' north latitudes and 28°29'71''– 31°34'14'' east longitudes; at Bolu (Çaydurt), Manisa (Alaşehir) and Kütahya (Tavşanlı, Domaniç, Aslanapa, Aydıncık) provinces (Yücel, 2000) as individuals or in small groups.

Pyramidal black pine occurs between 980-1350 m elevation; within 39°10' 07''-39°39'50'' north latitudes and 29°20'05''– 29°52'55'' east longitudes; at Kütahya and Tavşanlı (Pullar, Esatlar, Kızık and Vakıf) provinces (Yücel, 2000).

1.1.3. Botany

Anatolian black pine is a tree generally up to 30, rarely 40-50 m high. Trunk is usually straight. Bark is light gray to gray-brown, on older trees deeply and longitudinally furrowed (Figure 1.2). Crown on young trees is broadly conical, and on older trees umbrella-shaped, especially in shallow soil on rocky terrain. Branches with tips are slightly ascending on young trees, while on older trees only branches at the top part of the crown have upturned tips. One-year shoots are glabrous, light

brown to orange-brown. Buds are ovate to oblong-ovate, gray-brown, resinous. Needles are in groups of two (but occasionally three needle genotypes are found in some populations), green to dark green, rather stiff, 12-18 cm long, 1-2 mm in diameter, straight or curved, finely serrate; resin ducts medial; leaf sheath persistent, 10-12 mm long. Flowers appear in May, female inflorescences are reddish and male catkins are yellow.

Cones are sessile, horizontally spreading, 5-12 cm long, 2-4 cm across, yellow –brown or light yellow and glossy; ripening from September to October, and opening in the third year; fertile scales black beneath, apophysis slightly protruding. Seeds are 5-7 mm long; gray, with a 19-26 mm long wing. Six-eight cotyledons can be observed.

The oldest Anatolian black pine individual noted in the literature was found in Göksun-Kaşıkçı forest by Soydinç in 1959. When it was cut in 1959, it was found to be 1.77 m in diameter and 33 m height and 844 years of age. The second oldest individual determined was "Mızıkçamı" in Kütahya-Domaniç. Although, it was dead in 1980, its age was estimated as 743 years. This individual's trunk was kept for protection in its original place until it was destroyed by a storm in 1988. This tree was valued as a monument and played role in many folkloric and historic tails. There are many old individuals of Anatolian black pine noted as monumental trees in Turkey (Asan, 1999).

"Ebe" black pine is a compact tree with multiple branches. It is up to 6-10 m high, branching densely from the base (Figure 1.3). Its crown is rounded and wide. Generally it does not have a main stem but has many sub-stems. Its characteristic shape is obvious even at the first years; lots of ascending stems starting from the 15-50 cm high from the soil level; with 10-20° angles. Needles are in groups of two, bunching at the shoot tips like a rosette, bright green; 5-11 cm long. Cone formation takes 4-5 years and its number is less compared to black pine. Cones are 4-6 cm long, 2-3.5 cm across; seeds are 5-6 mm long. Seed formation ability and fertility is also very low.

a) General appearance **b) Trunk**

c) One-year-old female conelet **d) Male catkins**

Figure 1.2. General appearance of Anatolian black pine and some of its features

(Photo from FTSTBRD archives)

Figure 1.3. General appearance of var. *şeneriana*
(Photo from of FTSTBRD archives)

Figure 1.4. General appearance of var. *pyramidata* from a clonal seed orchard
(Photo from FTSTBRD archives)

Pyramidal black pine is a tree up to 20 m high, and 50-55 cm in diameter (Yücel, 1995). It has a pyramidal shape throughout its lifecycle; crown and branches do not change its structure by age (Figure 1.4). Branches are whorled and ascending with an angle of 10-20°, angle of the branches narrower at older ages. Needles are in groups of two, dark green; 5-13 cm long, 1-1.9 cm in diameter; usually straight or curved. Seed production occurs biannually. During the abundant seed crop year, productivity is high. Cones are 36-73 mm long, 23-38 mm across. Seeds are 5-6 mm long.

1.1.4. Reproductive Biology

Black pine is monoecious, with staminate and ovulate strobili borne separately on the same tree (Vidakovic, 1974). Black pine starts to bloom at age 15 to 20 in its natural habitat. Staminate strobili clustered terminal on the new shoots, mostly on the older lateral branches in the lower crown, are cylindrical, short stalked, bright yellow, about 2 cm long with numerous scales and include pollen in great quantity. One or two ovulate strobili (conelets) appear near the end of the new growth of terminal and lateral branches. They are cylindrical, small, bright red, and short stalked or sessile (Vidakovic, 1974). Pollen dispersal and conelet receptivity take place from May to June. However, ovulate conelets are receptive for the pollen for only about 3 days. Staminate strobili dry and fall within several weeks after pollen dispersal. After a few days of pollination, scales of ovulate strobili close and conelets go through a slow developmental stage. Fertilization occurs 13 months after pollination, in the spring or early summer. Cones turn to green in color and begin to grow rapidly until maturity in the fall.

Seeds mature in autumn of the second year, dispersed from October through November. The average number of sound seeds ranges from 30-40 out of which 15-20 can germinate. Sound seed containing embryo is usually dark in color.

1.1.5. Genetics

Climatically and topographically diverse and fragmented distribution of black pine evolved through natural selection. Significant variations in black pine were recognized by Theophrastus (370-285 B.C.) as early as the 3rd century B.C.

(Van Haverbeke, 1990). The taxonomic records point to a remarkably variable taxon including more than 100 names.

Basic and haploid chromosome numbers are equal (n=12), two of which are heterobrachial and the others mostly isobrachial (Saylor, 1964; Borzan, 1981). Kaya *et al.* (1985) analyzed the karyotypes of black pine and found that chromosomes XI and XII were especially variable.

There are numerous isozyme studies relating to the population genetics of the black pine. The first isozyme study carried out by Bonnet-Masimbert and Bikay-Bikay in 1978. They studied glutamate oxaloacetate transaminase-GOT enzyme polymorphism in 40 origins including the subspecies and compared the allele frequencies. Nicolic and Tucic (1983) also employed isozymes to reveal population differentiation in 28 natural populations and obtained high intra-population diversity. Moreover, Fineschi (1984) investigated population variation in 11 natural populations including 2 subspecies (subsp. *laricio* and subsp. *nigricans*). Then, Silin and Goncharenko (1996), Scaltsoyiannes *et al.* (1994) and Aguinagalde *et al*. also utilized isozyme markers to reveal genetic variation and population differentiation in natural black pine populations across Europe. All these studies indicate that black pine exhibits a pattern of genetic diversity characterized by high intra-population variation.

There are also isozyme variation studies on Anatolian black pine natural populations. Doğan *et al*. (1998) carried out a study on isozyme based linkage analysis in Anatolian black pine populations sampled from Ida Mountains. Tolun *et al*. (2000) and Çengel *et al*. (2000) also studied isozyme variation in natural populations and reported the existence of high genetic diversity localized within populations.

Lastly, utility of RAPD markers in Anatolian black pine for population genetics was investigated by Kaya and Neale (1993). Results of the study have shown that RAPD markers can be used efficiently in population genetics studies of Anatolian black pine.

1.1.6. Ecology

Anatolian black pine is adapted to many soil types and topographic habitats. It mostly occurs on poor, calcareous, sandy and even pure limestone soils; however, it requires a deep soil. It is a light demanding species but grows best in a cool to cold temperate climate. It is resistant to wind, drought, and quite tolerant to urban conditions-perhaps the most pollution-tolerant one among pine species. Excised shoots of black pine and other conifer species are capable of absorbing more SO_2, NO_2, and O_3 than shoots of deciduous species (Elkiey *et al*., 1982). One- to three-year-old European black pine seedlings were found to have no symptoms of ozone damage after exposure to 0.02 ppm of ozone for 5 hour periods by repeated treatments over one growing season (Davis *et al.,* 1981). Anatolian black pine seems to have potential to adapt to climatic extremes and can be grown successfully at steppe lands as long as deep soils are available.

1.1.7. Economic Importance

Anatolian black pine is a widespread and important timber tree for Turkey. Although the wood has a relatively larger proportion of sapwood to heartwood and thus requires a long rotation, it is used extensively throughout the Mediterranean region, where pine timber demand increases every year. Its wood can be used in poles, posts, mines, rail road ties, furniture, veneers and plywood, wood containers, shingles and shakes, fuel wood, pulp and paper, thermal and sound insulation materials and cellulose filament (Göker, 1969). Wood is not heavy, durable and rich in resin, easy to process (Vidakovic, 1991).

It is also valued as a decorative species; planted solitary or in either small or larger groups in parks.

1.2. Genetic Diversity in Forest Tree Species

Genetic diversity is both an element of the biodiversity and is also a necessary element in the safeguarding of all other levels of biodiversity that we value for their subsistence and utility. It is a resource for the survival and future evolution of a species, as well as a potential resource for improving its productivity.

Understanding genetic diversity and changes in diversity are essential for the effective management of a species since genetic variation in natural populations is affected by genetic processes and life history characteristics, such as mutation rate, selection, gene flow, genetic drift and the mating system (Frankel *et al*., 1995).

Mean genetic diversity of woody species is higher than annual species and herbaceous species (Hamrick *et al*., 1979). Mutation is one of the reasons since trees accumulate mutations during their long life spans. Thus, more mutations should accumulate per generation in trees than in herbaceous perennials and more in herbaceous perennials than in annuals (Ledig, 1998a).

Selection is also a cause of genetic variation. Frequency of genes and genotypes may change as a result of selection. It is the major force that keeps some alleles from increasing in frequency in a population. Selection is important in working with quantitative characteristics since tree improvement programs practice artificial selection that is directed and more powerful than natural selection. The environment significantly affects these characters. (Ledig, 1998a).

Genetic drift can cause changes in gene frequencies due to small population numbers. Genetic drift may bring about only a small change in the allelic frequency in a large population. While in a small population; allelic frequency may show large fluctuations in different generations in an unpredictable pattern (Weaver and Hedrick, 1989). Small populations may experience complete loss of some alleles, and decrease in variability. Size of populations may be reduced as a result of deforestation and subsequent fragmentation of widespread forest tree species. Also, if breeding population size is small or care is not taken to keep the population size in effective population size, genetic drift can be expected. (Ledig, 1998a).

Gene flow is the spread of genes as a result of pollen dispersal or seed migration. Gene flow by pollen can be very extensive in coniferous species. Pollen has been deposited 58 km from forests and birds have been observed to carry seeds of pinyon pine up to 22 km from their source (Ledig, 1998a).

Breeding system affects the pattern of genetic structure of a plant. Out-crossing increases levels of variation, reduces population subdivision and retards differentiation among subdivisions of a population. Inbreeding, on the other hand,

homogenizes genotypes within a family lineage and increases the potential for genetic differentiation among families (inter-population genetic subdivision).

Geographic range also influences genetic structure of a population. Small, isolated populations of endemic species tend to have fewer polymorphic loci and less genetic diversity than more spread species (Hamrick and Godt, 1996).

Ledig (1998a) claimed that genetic patterns may also be affected by climate change and as well as by the migration of species in space and time via seed dispersal. For example in Turkey, geological and climatic history caused conifers to have high levels of genetic diversity. Because in glacial periods, Turkey was likely a glacial refuge for many species since it was never completely covered with ice. During interglacial period, conifer populations expanded. Then due to continual warming and drying, conifer populations were fragmented and conversion of forests to agricultural lands had occurred. This may resultes in differentiation among populations.

As a group, conifers are regarded as one of the most genetically variable groups of species (Hamrick, 1979; Hamrick *et al*., 1979). Several reviews were written in an attempt to define the factors responsible for the maintenance of this high level of genetic variation in conifers (Brown and Moran, 1981; Hamrick, 1982, 1983; Mitton, 1983; Loveless and Hamrick, 1984; Ledig, 1986). Most of these studies attributed this high level of variation to the taxonomic status and the geographic range and distribution of the species in addition to the following life-history features: a) generation length, b) population structure, c) pollination mechanism/mating system, d) stage of succession, e) fecundity, and f) seed dispersal/gene flow. Ledig (1986) has presented an argument demonstrating that most of the above factors do not hold for some cases. Red pine (Fowler and Morris, 1977), western red-cedar (Copes, 1981) and Torrey pine (Ledig and Conkle, 1983) all showed lack of genetic diversity and ranged from localized endemic (Torrey pine) to the wide spread (red pine and western red-cedar).

Human activities also must be considered, especially in Turkey and the Near East, where ancient civilizations have had considerable impact on the landscape. There are many factors, which have caused the loss or decline of forest genetic

diversity as well as resulted in habitat alteration or loss in Turkey. Some of these factors, such as agricultural activities, industrialization and urbanization, touristic developments, unregulated use of plant materials, forest fires and forestry activities, and environmental pollution are still a threat to forest genetic resources.

Agricultural activities fragment the ranges of many forest trees, and domestication and fragmentation have consequences for gene flow, mating system, and genetic diversity (Ledig, 1992). Agriculture related activities such as gaining new agricultural land through conversion of forests, heavy grazing on forest lands, and herbicides use on forests close to agricultural fields are the main factors which have impact on forest genetic resources, both in the past and present (Işık *et al*., 1995; Kaya *et al*., 1997).

In recent years, increased industrialization without development of environment friendly technologies, and rapid urbanization due to increased population growth in cities, have led to loses of plant genetic resources or adversely affected forests by causing habitat loss or alteration (Kaya, 1998). In last years, tourism and related activities have increased considerably in Turkey, and the increased demand for nature tourism has forced the government to open previously untouched habitats and ecosystems for touristic development. In many areas along the Aegean and Mediterranean coasts, increased demand for touristic land development has already caused serious habitat degradation and loss of forest genetic resources; leaving a fragmented forests or habitats behind (Işık *et al*., 1995). Although almost all forest lands are owned and managed by the government, illegal use of forest resources is common. For example, one fifth of the 35 million cubic meters of fuel wood produced annually is obtained by illegal means.

Each year, about 26 300 ha of forest land with the natural habitats are lost due to conversion of forests into agricultural lands and to forest fires (Anonymous, 1993). Because little is known about the genetic constitution of natural populations of forest tree species in Turkey, extra care must be taken in selection of seed sources as well as seed movement when artificial regeneration of forests are considered. Although tree improvement programs in Turkey are still in their infancy, genetically improved clones or varieties may be planted over large areas in future, forming

monocultures, to increase productivity and increase harvest efficiency. Such mono cultural management practices might considerably reduce genetic diversity in the future forests depending on the intensity of methods in tree improvement programs. Thus, genetic implication of forestry practices in a given species should be investigated in advance to prevent drastic changes in gene pool of future forests (Ledig, 1988).

1.3. Determination of Genetic Variation

A wide array of techniques has been used in the studies of forest tree relationships and variation. Initially, descriptive morphology was widely used and is still useful. This was followed by studies of growth, physiological and ecological attributes as well as breeding affinities along with studies of monoterpene, phenolic and flavanoid chemistry. Isozyme chemistry in 1970's and recently DNA technologies have been employed to analyze genetic structure of populations of several forest trees and delineate species (Wang and Szmidt, 2001).

1.3.1. Morphological Markers

The genetic variation of the forest trees is studied traditionally with common garden studies following the approach of progeny tests and provenance tests. Field experiments are set up in different environments and focus on traits of economic value and biological importance such as survival, growth, tolerance to environmental stress, wood characteristics and resistance to pests and pathogens. In these tests, first the plant must be grown to a suitable developmental stage before certain characters can be scored. Classical phenotypic features are mostly quantitative and polygenic in nature, so their expression is influenced by the nature. These tests are still widely used in tree breeding and very effective in identification of families and clones that are specifically adapted to particular environments. These studies are used for assessing the amount of variation and its apportionment among the various classes of effects on phenotypic, genetic, environment and genotype x environment interaction (Mitchell-Olds and Rutledge, 1986). However, field tests are expensive, time consuming, laborious and more importantly based merely on phenotypes. Moreover,

accuracy of the genetic variation assessment and its distribution among and within populations is not certain (Wang and Szmidt, 2001).

1.3.2. Molecular Genetic Markers

Genetic variation studies preceded outstandingly during the 1980s and 1990s, by the introduction of protein electrophoresis techniques such as isozymes (Tanksley 1983; Loveless and Hamrick, 1984; Wendel and Weeden, 1989; Hamrick and Godt, 1990) and the development of various molecular tools (Wang and Szmidt, 2001). A major revolution has came about awareness in microevolution and macroevolution after starch gel electrophoresis was invented in 1955 by Smithies and the histochemical visualization of enzymes on gels by Hunter and Market in 1957. These inventions were proceeded by the classical studies of Harris (1966), Hubby and Lewontin (1966), and Lewontin and Hubby (1966); demonstrating the simple mode of inheritance of several allozymes and gave examples of method's utility in studying genetic variation (Wang and Szmidt, 2001).

The isozymes have contributed to plant population genetics to a great extend, since they are utilized as neutral (or nearly neutral) genetic markers. They are available to characterize patterns of genetic variation within and among populations and to scrutinize the process of dispersal and the patterns of mating that influence levels of genetic differentiation (Brown, 1979; Loveless and Hamrick, 1984, Hamrick and Godt, 1990; Barrett and Kohn, 1991). Although isozymes are useful for forest genetics and tree improvement research, the small number of mapped loci offers only a limited view of the conifer genome (Neale and Williams, 1991).

Isozyme markers widely used in forest genetics for addressing many questions in population biology, yet the development of several molecular markers in last decade may provide complementary approaches to address various questions (Wang and Szmidt, 2001).

Since the DNA itself is potentially the most accurate source of genetic information, molecular or DNA markers are true genetic markers. They are allowed to compare two individual plant's genetic material preventing any environmental effects on gene expression. It is not likely to have any significant contribution to

adaptation, due to DNA variation that existed in the non-coding genomic regions. These are modern genetic markers belonging to so-called anonymous DNA marker type such as microsatellites or simple sequence repeats (SSR), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP) and random amplified polymorphic DNAs (RAPD). Since these marker types generally assess neutral DNA variation, they are not useful for measuring adaptive genetic diversity. On the other hand, they are very convenient to analyze the phylogenetic relationships, population structure, mating system, gene flow, parental assignment, intogressive hybridization, marker-aided selection and genetic linkage.

Markers of all kinds –anonymous or genic, dominat or codominant, highly or less polymorphic, selective or neutral- have several distinct advantages for common applications in forest tree genetics. Different applications, classification, nature and advantage or disadvantages of these markers are reviewed by several researchers (Mandal and Gibson, 1998; Cervera *et al*., 2000; Linhart, 2000; Glaubitz and Moran, 2000; Savolainen and Karhu, 2000). The first and the most important advantage of these markers over isozymes, is that potentially unlimited number of DNA markers can be detected. A second advantage is that DNA markers do not vary among tissue types or developmental stages of the plant because the assays are based on the DNA itself and not the products of genes. There are clear differences in the levels of expression of certain isozymes among tissue types commonly used in isozyme assays (megagametophytes, embryos, buds, needles). A third advantage of DNA markers is that they are not affected by environmental variation. The presence or abundance of isozyme or biochemical marker products can be affected by environmental stimuli (Neale *et al*., 1992).

DNA-based techniques can be placed into different categories according to several criteria such as anonymous or genic, cytoplasmic or nuclear, dominat or codominant, highly or less polymorphic, selective or neutral and each of which has its own particular advantages and disadvantages. The followings are examples of all kinds.

Restriction fragment length polymorphism (RFLP) is widely employed for gene mapping and determination of genetic diversity in plant populations (Bernatsky

and Tanksley, 1986; Helentjaris *et al*., 1986). Hybridization technology is utilized in this analysis; then cloned DNA sequences are labeled and used as probes to identify size differences in specific genomic DNA fragments following digestion by a restriction endonuclease. On the other hand, large size of the conifer genomes and a great deal of repetitive DNA sequences make standard RFLP analysis difficult (Neale and Williams, 1991)

Variable number of tandem repeats (VNTR) is a technique, which utilizes hybridization techniques, but identifies repeated DNA regions of different lengths resulting from variable numbers of several repeats of a core DNA sequence (Dallas, 1988; Nybom and Schaal, 1990). These core sequences are referred to as mini-satellites or micro-satellites. Length variations can be visualized as multi locus fingerprint phenotypes or single locus genotypes.

Amplified Restriction Fragment Polymorphism (AFLP) is a powerful method for detecting polymorphism throughout the genome, based on a two-step amplification strategy that combines restriction enzymes and PCR (Zabeau and Vos, 1993). This highly reproducible technique allows the simultaneous screening of a large number of molecular markers, randomly distributed throughout the genome (Vos *et al*., 1995; Zhu *et al*., 1998).

Random amplified polymorphic DNA (RAPD) is one of the developments that have sprung from the PCR technology (Williams *et al*., 1990). RAPDs may be used to detect DNA variability at different levels, from single base changes to deletions and insertions, but insensitive to sequence differences. Although, identity of the sequence of a particular amplification product is absent; its presence or absence in different samples can serve as an informative character for the evaluation of genetic diversity and relatedness within a species. This method is adaptable to many situations such as DNA fingerprinting, identification of somatic hybrids and population genetic analysis and has been used in forest tree population studies (Russel *et al*., 1993; Kazan *et al*., 1993; Chalmers *et al*., 1994; Nesbitt *et al*., 1995, Isabel *et al*., 1995; Vicario *et al*., 1995; Szmidt *et al*., 1996; Nesbitt *et al*., 1997).

RAPDs are dominant markers and usually reveal variation in nuclear DNA (Carlson *et al*., 1991; Bucci and Menozzi, 1993; Lu *et al*., 1995). Since RAPD

markers are dominant; polymorphism will be detected when a DNA sequence will be amplified from one individual, but may not be amplified from another. Therefore, it is impossible to discern whether an individual is homozygous or heterozygous for any particular RAPD locus. It is the same for mapping studies using segregating F_2 families. Since homozygotes can not be discriminated from heterozygotes valuable information is lost and biased estimates of population diversity parameters may arise (Isabel *et al*., 1995, 1999; Szmidt *et al*., 1996). However the dominant character of RAPD fragments is not problematic in haploid situations. Many conifer species constitute an opportunity to test the inheritance of the dominant RAPD markers. The megagametophytes are haploid and are derived from the same single mother cell after meiosis, which also produces the corresponding egg cell. In this way it is possible to analyze a DNA fragment expressed in diploid tissue for homo- or heterozygosity and to use the segregating loci in megagametophytes as a mapping population.

In RAPD analysis, single primer types are usually added to the reaction mix and a key feature of the RAPD protocol is that the primers used possess a base sequence that is arbitrarily defined; whilst the investigators know what the primer sequence is, they have no idea to which, if any, gene or repeated sequence in plant genome the primer may have homology. Any bands subsequently observed in a gel can be used as raw data for the comparison of plant genotypes. Ethidium bromide stained agarose gels have been used to separate and visualize the amplification products.

Short primers (commonly 10 bases long) are usually employed, in order that the randomly defined primers result in the amplification of some sequences. On average, a 10-mer will hybridize to a strand of DNA about once every million bases. Current PCR technology does not allow the amplification of sequences larger than about 4000 bases, so that DNA sequences will only be amplified if two copies of the single primer used hybridize to opposite strands of a piece of DNA and they are separated by less than 4000 bases. Since the higher plant genome is very large, several amplified fragments are normally observed when one 10-mer is employed.

With reference to the possible uses of RAPD, the essential feature is the identification of polymorphism by the detection of the differences in DNA occurring between individual plants. The most important aspect of this polymorphism is that it can be mapped as the standard genetic marker. Most RAPD markers are rarely inherited as co-dominant alleles. Loss of a priming site results in complete absence of the enclosed amplified segment, not simply a shift in mobility on the gel. In heterozygotes, therefore, differences may appear only as differences in band intensity, which is not usually a reliable phenotype for PCR analysis.

Optimizing RAPD reactions is usually necessary when initiating a RAPD laboratory study. This step is laborious, since many reaction components and many parts of the PCR program can be changed with quite unpredictable effects, although several papers describe how optimization can be achieved (Williams *et al*., 1993; Yu and Paulus, 1992). Instead of finding optimal conditions for each primer, it may be wiser to use the protocol suggested by Williams *et al*. (1990) and to select commercially available primers (Ellsworth and Honeycutt, 1993). It is also common for some primers to fail to amplify DNA (Kazan *et al*., 1993; Lu *et al*., 1995; Pillay and Kenny, 1996; Ronning *et al*., 1995). Once the protocol is established, therefore, it should be kept constant throughout the following analysis.

One of the most important factors that determine the successful application of RAPD markers is the reproducibility (Hedrick, 1992; Riedy *et al*., 1992). RAPD analysis is performed at a low annealing temperature, implying that the binding of the primer to genomic DNA is partly non-specific. Therefore in order to obtain reproducible results the reaction conditions must be kept strictly constant. With a carefully optimized protocol, the reproducibility of RAPD patterns should not pose a major problem. It was demonstrated that highly reproducible RAPDs can be obtained from both haploid megagametophytes and diploid needles (Lu *et al*., 1995). However, Penner *et al*. (1993) studied the reproducibility of RAPD analysis among six different laboratories and found considerable variation. Therefore, researchers may need to complete all RAPD analysis pertaining to a given project at a single laboratory.

As well as ensuring reproducibility, it is equally important to determine whether the RAPD bands are inherited in the Mendelian fashion, as this is a prerequisite for their use as genetic markers (Pillay and Kenny, 1996). Several studies on RAPD inheritance have reported deviations from Mendelian proportions. For instance, up to 40 % of the RAPD bands tested by Reiter *et al*. (1992) revealed such deviations. Therefore, each RAPD locus should be examined individually, using carefully optimized protocols, before being used in population and phylogenetic studies or genomic mapping (Lu *et al*., 1995; Ronning *et al*., 1995). However, most RAPD markers from a wide range of organisms have been demonstrated to be inherited in Mendelian ratios (Brown *et al*., 1992; Bucci and Menozzi, 1993; Carlson *et al*., 1991; Kaya and Neale, 1993; Pillay and Kenny, 1996).

Amplification products corresponding to single copy sequences may be used as RFLP probes and transformed into codominant markers. For instance, RAPD bands can be used as probes to hybridize with genomic DNA to find repetitive sequences that are useful in fingerprinting analysis (Lu *et al*., 1997; Francis *et al*., 1995). By sequencing RAPD fragments, several studies have demonstrated that it is possible to convert RAPDs to codominant markers such as Sequence Characterized Amplified Regions (SCARs) (Garcia *et al*., 1996; Melotto *et al*., 1996). Such markers are more genetically defined and highly reproducible. The finding that most RAPDs from *Pinus* are amplified from single or low copy sequences (Lu *et al*., 1997) suggests that this approach can also be used for gymnosperms.

RAPD fingerprints have been used to estimate genetic and taxonomic relationships. They are widely used for the identification of poplar clones (Castiglione *et al*., 1993; Lin *et al*., 1994; Sanchez *et al*., 1998). The large number of polymorphic bands produced made it possible to determine genetic relationships among the different genomes.

RAPDs are also used in the discrimination and verification of genotypes in *Eucalyptus* (Keil and Griffin, 1994). It was indicated that RAPD profiles that are unique to a genotype can be generated reliably and simply and even closely related genotypes can be distinguished.

Effects of different methods of forest regeneration on the genetic diversity of lodgepole pine (*Pinus contorta* var '*latifolia*)' were studied using RAPD (Thomas *et al*., 1999). Genetic diversity was estimated for naturally regenerated, planted and unharvested stands. RAPD markers were also used to investigate genetic biodiversity impacts of silvicultural practices and phenotypic selection in white spruce (*Picea glauca*) (Rajora, 1999).

1.4. Domestication of Forest Trees and Genetic Consequences

The main purpose of a breeding program is to increase the frequency of desirable alleles found in the breeding population. Despite the fact that breeders know which traits to be improved, they do not have information about which genes impact the traits or their distribution in the population. Therefore, breeding programs must retain sufficient genetic diversity to allow continued genetic gains over multiple generations (Johnson *et al*.2001). In addition, population sizes must be large enough to maintain genes of polygenic traits of current interest and potentially rare traits that may be needed in the future. It is a complicating issue, since, traits of interest changes over time in response to new pests or changes in human needs.

The concept of a breeding program must include both short- and long-term objectives. Short-term objectives enclose both maintaining well-adapted trees and obtaining substantial gains in current traits of interest in the first generation of breeding (Johnson *et al*., 2001). Long-term objectives comprise however, maintenance of low frequency alleles and control of inbreeding. Therefore, short- and long-term objectives are in controversy. Breeding population must be kept large enough to maintain rare alleles; on the other hand selection intensity must also be high to achieve extensive genetic gain.

Unfortunately, it is practically and financially impossible to preserve all genetic diversity in the breeding population for uncertain future needs. In order to maintain low frequency alleles for many generations, thousands of parents are needed (Millar and Libby, 1991; Lynch, 1995; Lande, 1995; Yanchuk, 2001). For that reason, breeders should make well-versed and wise decisions by understanding

which alleles are being influenced by selection and by discovering the genetic variation within a species (Thomas *et al*., 2001).

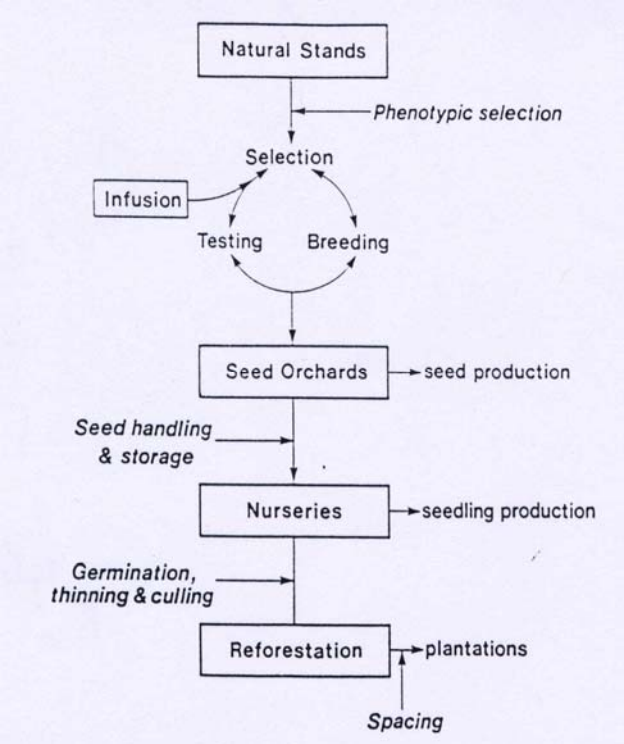


Figure 1.5 Domestication flow chart (El-Kassaby, 1995)

The breeding or domestication programs of wild coniferous species contain several repeated stages. These are: (a) phenotypic selection and breeding programs with its associated activities, (b) seed production, (c) seedling production, and (d) reforestation and establishment of plantations (Figure 1.5).

Phenotypic Selection and Breeding Programs with its associated activities: Selection of seed stands is the first step to get genetic gain. Seed stands are either artificially or naturally (mainly natural) regenerated forests requiring minimum 25 ha area. Special silvicultural practices are carried out to produce high quality seed for regeneration programs. After that, phenotypically superior trees were selected from natural stands. Individuals are selected based on their phenotypic values for some

characters or combination of characters. This is a good approximation of evolutionary significant selection processes and is considered a simple and cost-efficient method in artificial breeding (Cotterill, 1986; Falconer, 1989). However, breeders often select individuals based on predicted breeding values to attain greater genetic gain.

Seed Production: Seed orchard is defined as an area where seeds are mass-produced to obtain greatest genetic gain, as quickly and inexpensively possible (Zobel *et al*., 1958). Seed orchards act as a link between breeding programs and reforestation activities through the delivery of genetically-improved seeds. First generation seed orchards are usually established with 30-100 grafted plus tree clones, selected phenotypically from good stands within a breeding zone. These are artificially established forests which are intensively managed and have limited number of genotypes to produce genetically improved forest tree seeds for various forestry practices.

Achievement of random-mating assumption of the Hardy-Weinberg law is required in order to maintain the same frequency level of desirable genes in the orchard seed crops as in the selected population so that the genetic gain should be maintained (El-Kassaby, 1989). Random mating in seed orchards can be realized only if the clones are in reproductive synchrony and have similar reproductive output (*i.e*. gametic contribution or parental balance). In addition, since coniferous species are mainly wind-pollinated and often display strong inbreeding depression, the potential of pollen contamination from undesirable sources and inbreeding through self-fertilization and/or consanguineous mating are of concern.

Seedling production: During seedling-production phase, biological (seed dormancy, germination rate and speed of gene mutation) and management (thinning and culling) factors play a significant role in affecting the level of genetic variation (El-Kassaby, 1989), if proper handling of materials is not practiced.

Reforestation and establishment of plantations: Seeds collected from seed orchards are used for the production of genetically improved-seedlings for reforestation and establishment of plantations. Industrial plantation or large area

plantations are established and managed intensively for the production of timber to supply several products (El-Kassaby, 1989).

Crop plants experiences pointed out that selection and breeding cause reduction in genetic diversity and altered the genetic structure of these species (Frey, 1981). At present, most coniferous trees are still in their early stages of domestication. The rate of genetic changes in coniferous tree species, due to tree-improvement practices, will be slower than that observed for crop plants. However, the potential for reduction and/or significant alteration in genetic diversity is dependent on gain levels, the breeding strategy adopted, and its method of implementation (El-Kassaby, 1992).

Clearly, there are several steps in the forest-tree domestication process where the genetic diversity could be affected. Consequences of phenotypic selection, breeding, and seed and seedling production have been evaluated in some conifer species by comparing genetic variability in natural and domesticated populations.

El-Kassaby (1992) compared allelic frequencies and levels of heterozygosity between the seed orchards and natural populations for Sitka spruce (*Picea sitchensis*) and western red cedar (*Thuja plicata*) by allozymes. Results indicated that phenotypic selection did not reduce the variability levels observed for Sitka spruce and maintained the known low level of variability of western red cedar. In fact, new alleles observed in the seed orchards, indicating that the sampling of natural population was less efficient than plus tree selection in capturing the various allelic forms present in the species populations.

Gömöry (1992) used allozyme analysis in Norway spruce (*Picea abies* Karst.) to determine whether there were any differences in heterozygosity and gene diversity level related to stand management (virgin vs. managed forests) and/or stand origin (naturally regenerated vs. artificially established stands). He found a 13% reduction in expected heterozygosity for planted (vs. unharvested) stands and an 8% increase in diversity for naturally regenerated stands of Norway spruce, and reported this as a significant impact of artificial regeneration on genetic diversity.

Genetic-variation comparisons were made between natural and production (seed orchard) populations as well as seed and seedling crops produced from the

same breeding zone's seed orchards of British Columbia 'interior' spruce (*Picea glauca* x *engelmanni*) (Stoehr and El-Kassaby, 1997). The comparisons between the seed orchard and the breeding zone produced a similar percentage of polymorphic loci while the expected heterozygosity and average number of alleles per locus were slightly lower in the seed orchard. The proportion of polymorphic loci increased in the seed lot, but decreased to the natural populations' level in the plantation. It was suggested that the reduction in the plantation was caused by an unintentional selection in the nursery.

Rajora (1999) employed 51 random amplified polymorphic loci (RAPD) for the comparison of old growth stands with natural regeneration, phenotypic tree-improvement selections and plantations of white spruce (*Picea glauca*). The study indicated that the plantations and phenotypic tree-improvement selections have significantly reduced diversity as compared to old-growth and natural regeneration, suggesting their narrower genetic base.

Thomas *et al*. (1999) examined the effects of different methods of forest regeneration on genetic diversity of lodgepole pine (*Pinus contorta* var. *latifolia*) using RAPD and SSR markers. Their results suggests that regeneration of lodgepole pine after harvesting, by both planting and natural regeneration results in young populations (20-30 years-old) with similar levels of genetic diversity as mature (100 years-old) unharvested stands.

Genetic diversity within a white spruce (*Picea glauca*) seed orchard (40 clones) and a jack pine (*Pinus banksiana* Lamb.) seed orchard (31 clones) was assessed and compared with genetic diversity in natural populations within the source area for the orchards (Godt *et al*., 2001). Gene diversity maintained within the seed orchards (H_e: 0.157 for white spruce and 0.114 for jack pine) was similar to that found within the source area (H_e: 0.164 and 0.114 for white spruce and jack pine, respectively) for each species. Mean genetic identities between the seed orchards and their natural populations were high (>0.99), indicating that common allele occurrences and frequencies were similar between the orchards and their source area.

Genetic diversity of Turkish red pine (*Pinus brutia* Ten.) seed sources (seed stands, seed orchards, plantations) were investigated and compared by RAPD

markers (İçgen *et al*., 2005). The mean proportion of polymorphic loci for all seed sources was 77 %, implying high genetic diversity in studied seed sources. Mean F_{ST} value indicated that 87 % of the total variation contained within seed sources. The comparison of genetic diversity parameters between seed orchards and seed stands revealed identical values for *Na, Ne, I* and *He* parameters. However, *Ho* and P (%) were slightly higher in seed orchards than their natural counterparts.

Tree breeding in Turkey was initiated with plus tree selection and establishment of seed orchards in 1972. The first tree breeding organization was established in 1969. Although several seed production plans have been made for Turkey, a systematical long term plan was missing. Then, the first complete plan for Turkey was put into action, in which all aspects of forest tree seed production and breeding are combined and the targets were set. **"**The National Tree Breeding and Seed Production Program for Turkey: 1994-2003" was initiated in 1994 within the framework of Turkish-Finnish Forestry Project. It was prepared by "Enso Forest development Oy Ltd." and Ministry of Environment and Forestry in cooperation with Forest Tree Seeds and Tree Breeding Research Directorate (FTSTBRD) during 1992-1996. The aim of this project was to apply modern seed and plant production techniques and know-how in Turkey (Koski and Antola, 1994).

Pinus nigra subsp. *pallasiana* occupies a large area (more than 3 million ha) covering 16% of the total forest land, so it has a great importance in Turkish forestry. By reforestation volume, it is the second most important tree species in Turkey. About the 464 644 ha of lands was reforested by Anatolian black pine by the end of 2002 (Personal Communication, MOEF, General Directorate of Forestation and Erosion Control). This is the 25.6% of the total reforestation of the country.

Within the framework of Anatolian black pine breeding activities; seven tree breeding zones were designated covering the whole range of the species. Then, seed stand selections were completed from each breeding zone. Plus trees were determined from these stands considering the selection criteria laid out in the National Program. The most important characteristics when selecting phenotypical plus trees are as follows: Growth; stem volume, height and diameter at 1.3 m. and

quality; taper (d 1.3 m – d 6 m), straightness of stem, thin branches, large branch angle (close to 90°), natural pruning, etc.

Up to date, about 80 seed stands were selected, 52 clonal seed orchards were established and 26 gene conservation forests were chosen, covering 10 566 ha, 431 ha, and 3503 ha, respectively (FTSTBRD web site: http://www.ortohum.gov.tr).

1.5. Speciation and Variety Development

Evolution produces diversity in many levels, from genes, races, species, to genera and higher taxa. There are many problems in classifying this diversity, especially with regard to the level in the evolutionary hierarchy in which to place a particular taxon. However, there are several different concepts, ideas and definitions of organic species. Poulton, Dobzhansky and Mayr proposed the biological species concept, in which species are thought of as populations which do not interbreed, and are reproductively isolated from other species (Mallet, 1995). On the other hand, botanists never fully accepted the idea because plants often had high rates of hybridization, local variability, and environmentally-induced plasticity.

In botany, different variations within a species are denominated explicitly as subspecies (subsp.), varieties (var.) or forms (f.); a species may be divided into one or more subspecies, with the subspecies further subdivided into one or more varieties. Subspecies is the taxon immediately subordinate to a species; a group of organisims, which differ from other members of their species by genetically-encoded morphological and physiological characteristics (Sneath and Sokal, 1973). Members of the different subspecies of the same species are potentially capable of breeding with each other, and production of fertile offspring. Variety, which is next below the rank of subspecies, only recognized in botany. One of the taxa always repeats the same name as for the species as a whole; this is referred to as the **type** or **nominate** subspecies and variety, and includes the specimen the species was originally described from.

Plant species are found in populations which are genetically dynamic and constantly changing. Variations observed within the populations are determined by various environmental factors (climate, soil, etc.), breeding system (out-crossing,

selfing, etc.) and biological variables (mutations etc.). However, from time to time populations bearing distinctive characteristics will diverge from their original populations in such a way that gene exchange between the two will be prevented by some barriers. These are called isolation mechanisms and include environmental, reproductive and spatial factors (Woodland, 1997).

When habitats suitable for supporting two separate taxa are limited, gene exchange between populations is restricted and thus environmental isolation occurs. Different soil compositions, light intensity differences, and variation in moisture availability are some examples of environmental factors. The populations can live in the same region and be sympatric geographically, but inhabit different habitats. In regions of low topographic relief and uniform climate, the same species may have a wide distribution. In this case, populations bring into being clines which are gradients of character variation where different populations intergraded. While at the extremes of the range, significant differences may be noticed between these joining populations. In areas of extreme climatic and topographic variation, species become more restricted to specific zones and usually have a more restricted distribution (Woodland, 1997).

When gene exchange is inhibited by reproductive behavior which are genetically controlled differences between individuals of different populations is called reproductive isolation. These could be structural (lack of reproductive organ, etc.) or physiological characteristics (different pollination times, etc.).

Spatial isolation is the final isolation mechanism which is caused by large distance between populations.

1.6. Justification of the Study

Black pine is an economically important tree species in Turkey. Because of its growth characteristics and natural distribution, it is used for most of the afforestation and reforestation lands available. It is one of the most important forest tree species, therefore given high priority in "The National Tree Breeding and Seed production Program for Turkey" (Koski and Antola, 1994) and "National Plan for In situ Conservation of Plant Genetic Resources in Turkey" (Kaya *et al*. 1997). In the last decade, there are increasing number of studies dealing with the species' genetic diversity by means of quantitative traits (Kaya and Temerit, 1994; Şimşek *et al*., 1995; Üçler and Gülcü, 1999; Velioğlu *et al*., 1999); isozymes variation (Doğan *et al*., 1998; Çengel *et al*., 2000; Tolun *et al*., 2000) and RAPD variation (Kaya and Neale, 1993). Since, studies on genetic diversity of Anatolian black pine are limited, there is a need to asses the genetic diversity especially in managed populations.

Moreover, there is an ongoing "National Tree Breeding and Seed production Program for Turkey" covering breeding activity of Anatolian black pine. Therefore, there is a urgent need to assess the impact of forest management activities on genetic structure of newly established forests. The conservation strategy for species' gene resources in forestry practices should be defined within the light of genetic knowledge. Plantations are mainly established with seeds from seed stands. The yield and adaptability of these kinds of plantations are determined by how much of genetic variation existing in natural stands is transferred to the plantations. Thus, it would be valuable to asses the impact of forestry practices such as seed stand selection and management, plus tree selection and establishment of seed orchards, use of seeds from seed stands and seed orchards in establishment of new forests on genetic comparison of future forests (plantations).

CHAPTER II.

OBJECTIVES OF THE STUDY

The general objective of this study was to characterize genetic composition of Anatolian black pine seed sources such as seed stands, seed orchards, plantations and varieties by means of RAPD markers.

Specifically the following objectives were also set for the study:

1. To determine the magnitude and pattern of genetic variation existing in Anatolian black pine seed sources (seed stands; seed orchards, plantations) and varieties by means of RAPD markers.
2. To examine the extent of genetic diversity within and between the seed sources, by employing genetic diversity measures that are; allelic richness, polymorphism, and heterozygosity.
3. To quantify differences among seed sources by estimating genetic distance values which provides a genetic basis for clustering them into meaningful taxonomic groups.
4. To asses the impact of forestry practices such as selection and breeding on genetic composition of future forests by estimating how much genetic diversity was lost or gained during the breeding process.
5. To make implications for sustainable forest management and sound conservation programs for Anatolian black pine seed sources.

CHAPTER III

MATERIALS AND METHODS

3.1. Description of Study Material

Anatolian black pine seed sources were chosen from different categories comprising 4 different breeding zones of Anatolian black pine in Turkey (Figure 3.1). These are seed stands (SS), seed orchards (SO) which were established with grafted seedlings of plus trees from the seed stands and plantations (P) established with seeds from these seed stands (Figure 3.2, Table 3.1). Twenty five trees were selected from each seed source for seed stands, seed orchards and plantations for cone collection.

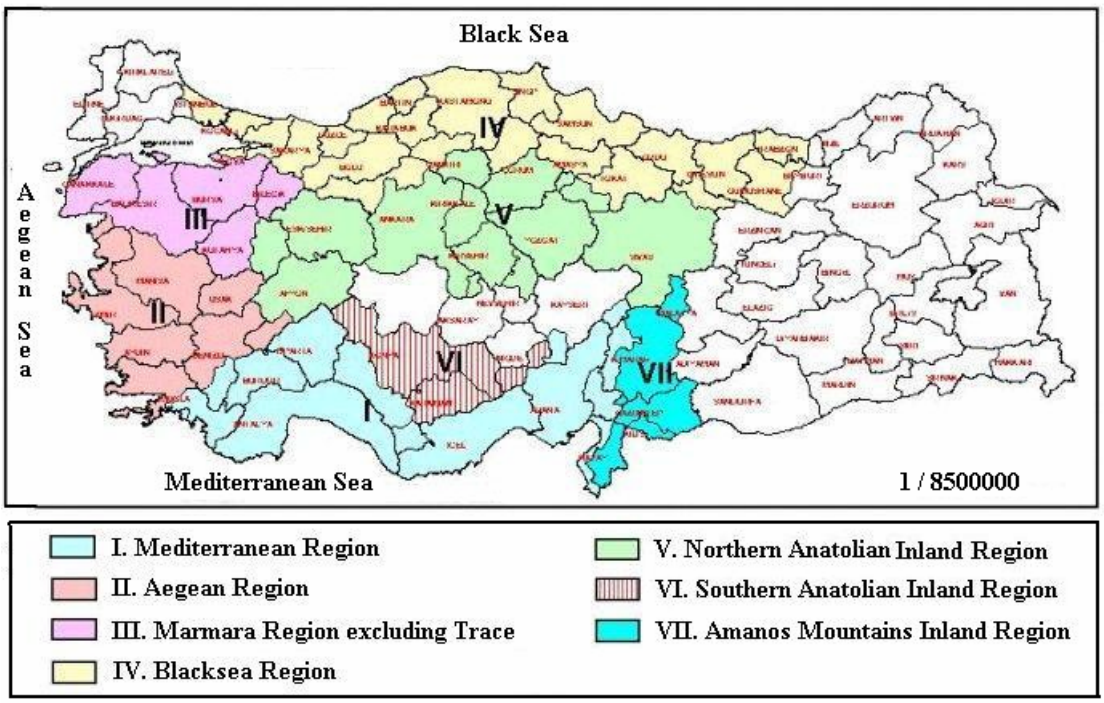


Figure 3.1. Map showing breeding zones of Anatolian black pine (MOEF, Mapping and Photogrametry Department)

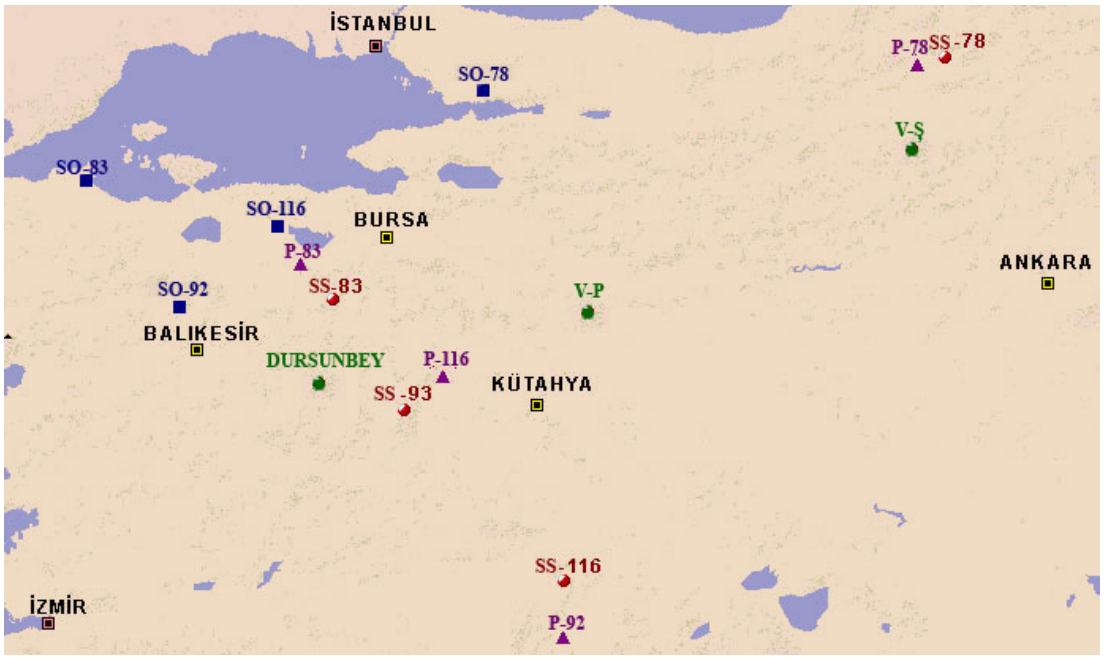


Figure 3.2. Map showing study sites (Codes for SS, SO, P, V-P and V-Ş were given in Tables 3.2, 3.3, 3.4, 3.5)

Two sets of analyses were conducted. The first set of samples included seed stands (SS), seed orchards (SO) which were established with grafted seedlings of plus trees from the seed stands and plantations (P) established with seeds from these seed stands. This set of analyses was used to assess the impacts of forestry practices on genetic composition of future forests. When selecting seed sources for the study, following criteria were considered:

(i) each seed stand should represent a different breeding zone of Anatolian black pine;

(ii) each seed stand selected must have a seed orchard and plantation established with material from the respective seed stand;

(iii) seed orchard and plantation established from a selected seed stand must be mature enough to produce seeds.

The second set of samples contained samples from seed stands, Anatolian black pine varieties (var. *pyramidata* and var. *şeneriana*) and Dursunbey seed stand (Balıkesir Alaçam-Değirmeneğrek) which is used as a reference population to compare with the varieties (Table 3.5). Seeds of pyramidal black pine (var. *pyramidata*) were sampled from a seed orchard in Eskişehir (National Seed Orchard Registration Number: 47). It was established with grafted seedlings with 20 clones from Tavşanlı-İkizoluk seed stand in 1977 (39°36'00'' North latitudes, 29°19'00'' East Longitudes). It covers 687 ha land and desiganted as Nature Conservation Area in 1988 (Anonymous, 2000). All clones of this orchard were sampled for the study. Seeds of "ebe" black pine (var. *şeneriana*) were sampled from a natural stand at Bolu-Çaydurt. This stand is covering 174 ha land, comprising a rich flora and fauna. This stand was designated as "Nature Conservation Area" by the Ministry of Environment and Forestry in 1988. However, only 7 trees could be sampled due to poor seed crop year.

Cones were collected by Turkish Ministry of Environment and Forestry, Forest Tree Seeds and Tree Breeding Research Directorate. In seed orchards, all clones were sampled (25-34). In order to obtain open-pollinated seeds, parent trees in each seed source (seed stands and plantations) were selected by the following criteria:

1. Trees had to be separated by at least 100 m within each population.
2. Elevation range of trees had to be no longer than 300 meters within population.
3. Cones had to be collected from the upper one-third of the crown of trees in order to minimize inbred and close bred seeds.

Detailed description of seed stands, seed orchards, plantations and varieties used in the study are given in Table 3.2, 3.3, 3.4 and 3.5 respectively.

Seeds were extracted from cones at the "Seed Extraction Facilities" of Kızılcahamam Nursery, Ankara and kept in cold storage (+4^{o}C) until they are used.

Table 3.1. The locations of studied seed stands, seed orchards and plantations

Seed Stands	Seed Orchards	Plantations	Breeding Zone	Sample Size
MKP- Burhandağı	Biga- Karabiga	Mustafa Kemal Paşa	3-2	25
Tavşanlı- Balıköy	Balıkesir-Ilıca	Sandıklı	3-3	25
Mengen-Daren	İzmit-Gebze	Mengen	4-2	25
Afyon- Hocalar	MKP- Karacabey	Alabarda	5-3	25

Table 3.2. Description of Studied Seed Stands (SS)

SS No	Region-District	Subdistrict	Longitude	Latitude	Aspect	Altitude
SS 83	Bursa - M.K. Paşa	Burhandağı	28 43 00	39 54 10	W-NW	1000
SS 93	Kütahya-Tavşanli	Baliköy	29 07 45	39 25 00	N	1500
SS 78	Bolu –Mengen	Daren	32 17 00	40 57 20	N-SE	935
SS 116	Eskişehir–Afyon	Hocalar	30 03 21	38 40 47	N-NW	1350

Table 3.3. Description of Studied Seed Orchards (SO)

SO No	Origin	District-Subdistrict	Number of Clones	Longitude	Latitude	Date of Establishment
SO83	SS 83	Biga-Karabiga	30	27 08 54	40 24 15	1977
SO93	SS 93	Balikesir- Ilıca	29	27 47 01	39 52 05	1988
SO78	SS 78	İzmit-Gebze	38	29 28 02	40 50 23	1977
SO116	SS 116	M.K.P-Karacabey	35	28 20 46	40 12 52	1986

Table 3.4. Description of Studied Plantations (P)

No	Origin	District-Subdistrict	Longitude	Latitude	Altitude	Date of Establishment
P 83	SS 83	M. K. P - Burhandağ	28 32 30	40 03 00	400	1971
P 93	SS 93	Sandıklı – Baliköy	29 21 30	39 34 30	1200	1964
P 78	SS 78	Mengen – Daren	32 14 30	40 58 30	1400	1971
P116	SS 116	Hocalar - Alabarda	30 03 40	38 24 00	1130	1974

Table 3.5. Description of Studied Natural Population and Varieties

Code	Seed Source	Type	District-Subdistrict	Longitude	Latitude	Sample Size
D	Dursunbey	Seed Stand	Alaçam-Değirmeneğrek	28 34 10	39 25 50	24
V-P	var. *pyramidata*	Seed Orchard	Eskişehir-İnönü	30 07 35	39 49 20	20
V-Ş	var. *şeneriana*	Conservation Area	Bolu-Çaydurt	31 45 00	40 45 00	7

3.2. Chemicals

The chemicals (their suppliers and code numbers) used in this study were listed in Appendix A.

3.3. Methods

3.3.1. DNA Isolation

DNA extractions were performed with some modifications of the methods described by Kreike (1990) and Dellaporta *et al.* (1983). Seeds were soaked in distilled water at 4^{o}C for 24 hrs. After excising and removing the seed embryo, megagametophytes were homogenized in 400 µl extraction buffer-I (0.1 M Tris HCl pH: 8.0, 0.1 M EDTA, 0.25 M NaCl,) in 1.5 ml Eppendorf tubes. After complete homogenization, 400 µl extraction buffer-II (0.1 M Tris HCl pH: 8.0, 0.1 M EDTA, 0.25 M NaCl, 2% SDS) was added. Homogenized tissues were kept in a 65^{o}C water bath for 30-40 minutes. Then, 250 µl of 5 M potassium acetate solution was added to tubes and incubated on ice in the refrigerator for at least 60 min. Following the centrifugation at 14 000 rpm at 4^{o}C for 15 min, supernatant was transferred to a new tube and mixed with 500 µl chloroform-octanol (24:1) solution. After 10 min. centrifugation (14 000 rpm at 4^{o}C) the top aqueous layer was transferred to a new tube and 700 µl absolute ethanol/0.3 M sodium acetate solution was added. Then, the

tubes were incubated at -80°C for at least 60 min. Following the 10 min centrifugation, supernatant was poured off and precipitation washed twice with cold ethanol (70 %). The pellet was dried and then re-suspended in 50 µl TE buffer (10 mM Tris, 1 mM EDTA pH 8.0). The DNA samples were stored at –80°C.

3.3.2. DNA Quantification

DNA quantification of all samples were performed with Hoefer DyNA Quant™ 200 Fluorometer (Hoefer Pharmacia Biotech, San Francisco, CA) which is a filter fluorescence photometer with a fixed excitation bandpass source (365 nm) and an emission bandpass filter (460 nm). Buffers and solutions used were listed in Appendix B. Bisbenzimide (Hoechst dye) exhibits changes in fluorescence to allow accurate DNA quantification. Determination of DNA concentration of all isolated DNA samples were done by using the fluorometric assay of Cesarone *et al*. (1979). DNA yields per megagametophyte varied from 500-5000 ng. The 6 samples (in each parent tree) with the highest DNA yield were selected and diluted to 3 ng/µl for PCR applications. Diluted DNA samples were stored at –4°C to be able to use throughout the course of the study.

3.3.3. RAPD Primers

Primers which were previously screened by Kaya and Neale (1993) were used in this study. Eleven primers giving the highest number of polymorphic loci were selected and all seed sources were screened to obtain single locus segregation data.

Random 10-base oligonucleotide primer sequences were obtained from University of British Colombia (BC, Canada). The sequence of each primer is arbitrary and generated on a random basis with the requirement that their G+C content will be 60-70% and their ends are not self-complimentary.

In this study, in the first set of samples; 11 RAPD primers were screened against 1800 samples (12 seed sources/25 families/6 samples). In the second set, however, 7 primers were screened against 906 samples (151 families/6 samples) to

obtain single locus segregation data. The sequences of primers used in this study were listed in Table 3.6.

Table 3.6. List of RAPD primers used for Anatolian black pine

Primer	5' to 3' Sequence
UBC-121*	GTG ACG CCG C
UBC-129*	CCG GAC ACG A
UBC-131*	GAG GCG GCG A
UBC-139	AGC GTC GAC T
UBC-144*	CTG CGA CGG T
UBC-149	GCG CGG CAC T
UBC-151	TTG CGC CCG G
UBC-154*	AGA CGC CGA C
UBC-159	GGG AAG AGA G
UBC-162*	GGG TGT GGT T
UBC-190*	GGC CGA TGA T

*: Primers available only for the second set of samples

3.3.4. Optimization of RAPD-PCR Conditions

RAPD-PCR conditions were optimized for the system as described by Kaya and Neale (1993) for Anatolian black pine to our laboratory conditions. Different concentrations of template DNA, primer, $MgCl_2$, dNTP and effects of Tween 20 and bovine serum albumin (BSA) were also tested.

First set of optimization tests included varying DNA and primer concentrations. DNA amounts of 3, 6, 9, 12 ng and primer concentrations of 4.0, 5.0, 6.0, 7.0 and 8.0 picomoles per reaction volume of 25 μl were tested (Table 3.7).

Table 3.7. Tested primer and template concentrations for RAPD-PCR optimization

dNTP (mM)	$MgCl_2$ (mM)	Buffer (μL)	Taq Pol (unit)	Primer (pmol)	DNA (ng)
0.2	2.5	2.4	1.0	4	3
0.2	2.5	2.4	1.0		6
0.2	2.5	2.4	1.0		9
0.2	2.5	2.4	1.0		12
0.2	2.5	2.4	1.0	5	3
0.2	2.5	2.4	1.0		6
0.2	2.5	2.4	1.0		9
0.2	2.5	2.4	1.0		12
0.2	2.5	2.4	1.0	6	3
0.2	2.5	2.4	1.0		6
0.2	2.5	2.4	1.0		9
0.2	2.5	2.4	1.0		12
0.2	2.5	2.4	1.0	7	3
0.2	2.5	2.4	1.0		6
0.2	2.5	2.4	1.0		9
0.2	2.5	2.4	1.0		12
0.2	2.5	2.4	1.0	8	3
0.2	2.5	2.4	1.0		6
0.2	2.5	2.4	1.0		9
0.2	2.5	2.4	1.0		12

The second set of optimization tests included varying DNA and primer concentrations with Tween-20 and Bovine Serum Albumine (BSA). The effects of Tween-20 as a nonionic detergent and BSA as an enhancer on RAPD-PCR reactions were tested (Table 3.8).

Table 3.8. Tested RAPD-PCR conditions in the presence of Tween 20 and BSA

dNTP (mM)	$MgCl_2$ (mM)	Buffer (µL)	Taq Pol (unit)	Primer (pmol)	DNA (ng)	Tween 20 (µL)	BSA (µg/ng)
0.2	2.5	2.4	1.0	6	3	-	-
0.2	2.5	2.4	1.0			0.13	-
0.2	2.5	2.4	1.0			0.13	0.03
0.2	2.5	2.4	1.0			-	0.03
0.2	2.5	2.4	1.0	7	3	-	-
0.2	2.5	2.4	1.0			0.13	-
0.2	2.5	2.4	1.0			0.13	0.03
0.2	2.5	2.4	1.0			-	0.03
0.2	2.5	2.4	1.0		6	-	-
0.2	2.5	2.4	1.0			0.13	-
0.2	2.5	2.4	1.0			0.13	0.03
0.2	2.5	2.4	1.0			-	0.03
0.2	2.5	2.4	1.0	8	6	-	-
0.2	2.5	2.4	1.0			0.13	-
0.2	2.5	2.4	1.0			0.13	0.03
0.2	2.5	2.4	1.0			-	0.03
0.2	2.5	2.4	1.0		9	-	-
0.2	2.5	2.4	1.0			0.13	-
0.2	2.5	2.4	1.0			0.13	0.03
0.2	2.5	2.4	1.0			-	0.03

Optimized PCR conditions contained; 2.0 µl of temple DNA sample (3 ng/µl); 2.4 µl of buffer (750 mM Tris.HCl pH: 8.8, 200 mM $(NH_4)_2SO_4$; MBI Fermentas, Lithuania), 0.2 µl (1 unit) of *Taq* DNA polymerase (MBI Fermentas, Lithuania); 4 µl of deoxynucleoside triphosphate mix (0.2 mM of each nucleotide); 2.5 µl of 25 mM $MgCl_2$; 7.0 µl of 1 pmol primers (BC, Canada); 0.13 µl of Tween 20; 1 µl of 1.8 µg/µl (0.03 µg/ng DNA), BSA (Sigma, USA) and 5.77 µl of doubled distilled sterile water (Table 3.9).

Table 3.9. Optimized PCR conditions for Anatolian black pine

Component	**Quantity used (µl)**	**Final concentration**
10X buffer	2.4	1x
dNTPs (1.25 mM)	4	0.2 mM
$MgCl_2$ (25 mM)	2.5	2.5 mM
Primer (1 picomole)	7.0	7 picomoles
Taq DNA polymerase (5 u/µl)	0.2	1u
BSA (1.8 µg/µl)	1	1.8 µg
Tween 20	0.13	
Sterile H_2O	5.77	
DNA (3 ng/µl)	2	6 ng
Total reaction mixture	**25**	

PCR amplification cycles were also optimized as reported by Kaya and Neale (1993). The cycling schedule was shown in Table 3.10.

Table 3.10. PCR Cycling Schedule for Anatolian black pine

Step	Temperature	Time	Cycle #	Description
1	85°C	15 sec	1	
2	95°C	5 sec	1	Initial denaturation
3	92°C	1 min. 55 sec	1	
4	95°C	5 sec	45	Denaturation
	92°C	55 sec		
	37°C	1 min		Annealing
	72°C	2 min		Extension
5	72°C	7 min	1	Final extension

3.3.5. Interpretation of the Gels

PCR products were mixed with 2 µl of 25 % formamide loading dye and visualized in 1.7% agarose gels. Gels were run in 1XTAE buffer (0.4 M Tris Acetate) at 80V for 3 hours and stained with 5 µg/ml ethidium bromide for 30 minutes and de-stained with distilled water for 10 minutes.

3.3.6. Data Collection

Amplification products were scored visually. Generuler™ 100 base pair DNA ladder plus (MBI Fermentas, Lithuania) was used to determine the size of RAPD bands. The range of ladder was between 100-3000 base pairs.

With two possible states for a haploid RAPD fragment, presence (marker allele) or absence (null allele), a RAPD locus was defined as a fragment that would segregate or be monomorphic among the haploid megagametophyte samples. Genotypes determined from haploid megagametophytes were converted to diploid genotypes for each family at each locus. For each locus, all dominant homozygotes (AA) and heterozygotes (AB) were scored as individuals possessing the fragment, whereas recessive homozygotes (BB) were scored as individuals with no fragment (Table 3.11).

Table 3.11. Diploid genotype scores for all loci

Sample Size (# of Megagametopytes)	Samples having band	Diploid score
6	6	AA
6	5	AB
6	4	AB
6	3	AB
6	2	AB
6	1	AB
6	0	BB

3.4. Analysis of Data

Once data collection with all primers was completed, the data file was organized as in Appendix C so that it could be analyzed with POPGENE (Version 1.31, Microsoft Windows-Based Freeware for Population Genetics Analysis) (Yeh *et al*., 1997) and BIOSYS Version 1.7 (Swofford and Selander, 1989). The following parameters were estimated: Allele frequencies, allelic richness (number of alleles/locus, number of effective alleles), and proportion of polymorphic loci, observed and expected heterozygosities and Shannon's Information Index.

F-Statistics (F_{IT}, F_{IS} and F_{ST}) was estimated by using GENETIX 4.0 software (Belkhir *et al*., 1996-2001). POPULATIONS 1.0 Software was used to construct neighbor-joining trees between seed sources (Langella, 2000). This program uses the data file in GENEPOP file format and part of the data was given in Appendix D.

3.4.1. Allele Frequencies

The estimation of the allele frequencies were done by the following equation:

$$f(A_i) = \hat{x}_i = \frac{(2N_{ii} + \sum_{j=1}^{m} N_{ij})}{2N}$$

Where $f(A_i)$ is the frequency of any allele, N represents the number of individuals in the population, N_{ii} and N_{ij} represent the number of A_{ii} and A_{ij} genotypes, respectively and m represents the number of alleles in a locus (Nei, 1987).

3.4.2. Measures of Genetic Variation

In order to determine the amount of genetic variation, the following parameters were estimated.

a. Proportion of Polymorphic Loci: To be called polymorphic, the most common allele (x_i) should have a frequency of equal to or less than 0.99 or 0.95. In this study, 0.99 criterion was used. If the sample size and the number of polymorphic loci involved in the study are large enough, genetic variation can be estimated by measuring the proportion of polymorphic loci. The proportion of polymorphic loci was calculated by the following equation:

$\hat{p} = \frac{n_p}{r}$, where n_p is the number of polymorphic loci in r number of loci (Nei, 1987).

b. Heterozygosity: The most widespread measure of genetic diversity in a population is the amount of heterozygosity.

The unbiased estimate of the heterozygosity ($\hat{h}$) at a locus was calculated by the formula;

$$\hat{h} = \frac{2N\left(1 - \sum \hat{x}_i^2\right)}{2N - 1}$$

where N is the number of individuals and x_i, is the frequency of RAPD allele (Nei, 1987).

Whereas, the variance of single locus estimates of ($\hat{h}$) was;

$$V(\hat{h}) = 2\left[\sum \hat{x}_i^3 - \left(\sum \hat{x}_i^2\right)^2\right] / N$$

where $V(\hat{h})$ is the variance of a single locus, N is the number of individuals, x is the frequency of RAPD allele (Nei, 1987).

c. Allelic Richness: Another component of the genetic variation is the mean number of alleles per locus (n_a). It is also called as the allelic richness and is very sensitive to the sample size. The formula used to calculate this value was as follows:

$Mean(n_a) = \frac{\sum_i n_{a_i}}{r}$, where ($n_{a_i}$) is the number of alleles at the i^{th} locus and r is the number of loci (Nei, 1987).

Variance of the single locus estimate of (n_a) is:

$$V(mean\ \hat{n}_a) = \frac{\sum_i (\hat{n}_{a_i\,i} - mean\ \hat{n}_a)^2}{r(n-1)}$$

d. Effective Number of Alleles at a Locus: Mean number of alleles gives an estimate inflated by deleterious genes of which the contribution to genetic variability is small. Kimura and Crow (1978) introduced the concept of effective number of alleles. This number is defined as the reciprocal of homozygosity.

$\hat{n}_e = 1/\sum x_i^2$, where $\hat{n}_e$ is the effective number of alleles and x_i is the frequency of i^{th} allele.

e. Shannon's Information Index: Shannon's information index is the degree of variation within each population, and was calculated from the frequency of the RAPD allele within each population using the formula:

$I = p_i \ln p_i$ (Lewontin, 1972, Yeh *et al*., 1995),

where I is the Shannon's information index, p_i is the frequency of a RAPD allele. It is calculated separately for each putative locus, and the mean value of the index is then produced by averaging over all loci.

3.4.3. F-Statistics

These measures of heterozygosity can be used to define three levels of inbreeding (Nei, 1987). **F_{IS}** measures the fixation index or inbreeding coefficient within subpopulations; the degree to which the actual heterozygosity within sub-populations deviates from Hardy-Weinberg Equilibrium. And F_{IS} was estimated with the following equation:

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

F_{IT} used for measuring the fixation index over the total population (inbreeding coefficient). That is the degree of deviation from Hardy-Weinberg expectations in heterozygosity. It is estimated by the following equation:

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

F_{ST} is the reduction in fixation index due to differences among subpopulations in allele frequencies. It is estimated by the equation:

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

Where,

H_I = observed heterozygosity of an individual in any subpopulation.

H_S = expected heterozygosity of an individual in any subpopulation.

H_T =expected heterozygosity of an individual in the total population.

H_I was estimated by the following equation by Nei, 1987:

$H_I = \frac{\sum_{j=1}^{s} \hat{h}_{o_j}}{s}$, where s is the number of subpopulations and ($\hat{h}_{o_j}$) is the observed heterozygosity in subpopulation j (Nei, 1987).

H_S was calculated by the following equation:

$H_s = \frac{\sum_{j=1}^{s} \hat{h}_j}{s}$, where ($\hat{h}_j$) is the expected heterozygosity in subpopulation j (Nei, 1987).

H_T was also estimated by the following formula:

$H_T = 1 - \sum_i x_{ia}^2$, where (x_{ia}) is the frequency of the i^{th} allele averaged over all subpopulations (Nei, 1987).

The three types of fixation indices are related to each other in the following way, so for example one can estimate one of the indices if other two are known.

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$$

Finally, by using the reduction in fixation index, gene flow between sub-populations (N_m) was estimated by the following formula:

$$\boldsymbol{N_m} = 0.25\ (1 - F_{ST}) / F_{ST}$$

The distribution of genetic variation among populations can be used to estimate amounts of gene flow among populations (Slatkin, 1985; 1987). This is an indirect measure of gene flow.

The movement of one individual per generation is sufficient to prevent substantial differentiation between populations. This result is independent of size because the force of gene flow, which is counteracted by the force of genetic drift, which is proportional to the inverse of the population size denoted N.

Generally, if $Nm < 1$, then local differentiation of populations will result, and if $Nm > 1$, then there will be little differentiation among populations (Wright, 1951). The F indices proposed by Wright (1951) does not consider the unequal finite sample sizes and there is some disagreement on the interpretation of the quantities and on the method of evaluating them. Weir and Cockerham (1984) revised the F coefficients in order to offer some unity to various estimation formulae suggested by different authors. They used the parameters F, θ and f for F_{IT}, F_{ST} and F_{IS}, respectively. These estimators do not make assumptions concerning numbers of populations, sample sizes or heterozygote frequencies and they are suited to small data sets. Parameters, F, θ and f were estimated as follows:

$$\boldsymbol{F = 1 - C/(B + C)}$$

$$\theta = A/(A + B + C)$$

$$f = 1 - C/(A + B + C)$$

Where,

A = inter-population component of allelic frequency variance

B = component of allelic frequencies variance between individuals in each population

C = component of allelic frequencies variance between gametes in each individual

In this study, Weir and Cockerham's approach was used to examine the population structure, but the parameters were donated by F_{IT}, F_{ST} and F_{IS} instead of F, θ and f.

In order to test the significance of estimated F-coefficients, the data were permutated for 1000 times and distribution of the calculated values (F_{IS} and F_{ST}) from the permutated data were generated under the null hypothesis (no population differentiation for F_{ST} and Hardy-Weinberg equilibrium for F_{IS} and F_{IT}). The probability of obtaining original estimated F-coefficients under the null hypothesis was calculated as the proportion of the distribution having values larger than the

original value. For F_{IS}, alleles were permutated within each population whereas for F_{ST}, genotypes were permutated among the samples.

3.4.5. Genetic Distance

Genetic distance is the extent of gene differences between pairs of populations (or species). Distance measures are generally analogous to geometric distances; for example, zero distance is equivalent to no difference between groups. Identity measures are generally the complement to distance measures (Hedrick, 1985).

The most widely used genetic distance measure is that of Nei's Genetic Distance (Nei 1972). I is the identity between two populations x and y, and was measured by using the following equation;

$$I = \frac{J_{xy}}{\left(J_x J_y\right)^{1/2}} \quad , \qquad \text{where;}$$

$$J_{xy} = \sum_i^m x_i y_i \qquad J_x = \sum_i^m x_i^2 \qquad J_y = \sum_i^m y_i^2$$

and x_i and y_i represent the frequencies of the i^{th} allele in the x and y populations.

For multiple loci, J_{xy}, J_x and J_y were calculated by summing over all alleles at all loci studied. Then the average value was calculated by dividing these sums by the number of loci. The average values J'_{xy}, J'_x and J'_y were used to calculate the genetic identity and distance, D'.

$$I' = \frac{J'_{xy}}{\left(J'_x J'_y\right)^{1/2}} \qquad \text{and} \qquad D' = -\ln I'$$

3.4.6. Phylogenetic trees

Phenetic or distance methods are employed to obtain a phylogenetic tree or dendrogram by considering pairwise similarities or distances among seed sources. Therefore, related populations are organized in a biologically meaning way. Where

the genetic basis of the phenotypes studied is unknown, a number of metric and non-metric pairwise indices can be estimated (Sneath and Sokal, 1973); however, the derived trees can not be interpreted in terms of other than of strict phenetic resemblance (Strauss *et al*., 1992).

Phenetic methods of clustering can be distinguished on the basis of whether they assume homogeneity of evolutionary rates. Neighbor-joining (NJ) method (Saitou and Nei, 1987) does not assume homogeneity of substitution rates, therefore the length of branches emerging from particular nodes are free to vary independently from one another. The resulting dendrograms are un-rooted, and are often termed networks. Local topological relationships are first examined and the best tree constructed step by step.

Assessing the statistical significance of a given branching structure as well as the variance of branch lengths is highly desirable, since there are so many possible tree topologies. Quantification of levels of uncertainty is especially crucial in genetic studies for conservation programs, since decisions regarding gene and species extinctions may be guided by knowledge of relationships.

Resampling procedures have been widely used to place confidence limits on phylogenetic topologies. They can be applied to any data set where the observations can be assumed to be independently drawn, because they do not rely on particular statistical distributions. The bootstrap consists of random sampling with replacement. It is usually applied to the character array, but could also be applied to any taxonomic units. For phylogenetic purposes, with each sample of characters a new topology is calculated, and then the sampling process and topology construction is begun a new one. The resampling continues until a reasonable level of confidence is attained (usually 50-100 replicates). The final result is displayed graphically as a number next to each node indicating the percentage of time that cluster is present among the resample trees. If that fraction is high, then one gains confidence that the given cluster actually belongs together.

In this study, dendrograms were constructed by using NJ method to reveal the genetic distance of seed sources. Bootstrap test was repeated for 100 times to show statistical confidence. Dendrograms were constructed for all seed sources such

as seed stands, seed orchards, plantations and varieties with the natural stand. Besides, seed source origin (location) based dendrogram were also formed to reveal whether differences between seed orchards, plantations and their natural counterparts (seed stands) may be caused due to forestry practices and breeding activities.

CHAPTER IV

RESULTS

4.1. Optimization of PCR Conditions for Anatolian Black Pine

In the first set of the optimization studies, primer - template interactions were investigated. DNA concentrations of 3 ng and 6 ng per reaction volume of 25 μl generally yielded good amplification products. Primer concentrations less than 4 picomoles and more than 8 picomoles produced fewer bands or no bands at all. Primer concentrations of 5, 6 and 7 picomoles produced reproducible bands; however, the results were not completely satisfactory (Table 4.1).

In the second set of the optimization studies, effects of Tween-20 (0.13 μl) and BSA (0.03 μg/ng DNA) were tested. The addition of 0.13 μl Tween-20 per reaction volume of 25 μl resulted in bright RAPD bands (Table 4.2). No significant difference was observed when BSA was used alone; however, brighter and clearer bands were obtained with the presence of both Tween-20 and BSA. Best amplification products were observed with 7 picomoles of primer, 6 ng of DNA template, in the presence of 0.13 μl Tween-20 and 0.03 μg/ng DNA of BSA (Table 4.2).

Table 4.1. Optimization of primer and DNA concentrations for RAPD-PCR

dNTP (mM)	$MgCl_2$ (mM)	Buffer (μL)	Taq Pol (unit)	Primer (pmol)	DNA (ng)	Results
0.2	2.5	2.4	1.0	4	3	faint bands
0.2	2.5	2.4	1.0		6	faint bands
0.2	2.5	2.4	1.0		9	faint bands with smear
0.2	2.5	2.4	1.0		12	faint bands with smear
0.2	2.5	2.4	1.0	5	3	good bands
0.2	2.5	2.4	1.0		6	good bands
0.2	2.5	2.4	1.0		9	good bands with smear
0.2	2.5	2.4	1.0		12	faint bands
0.2	2.5	2.4	1.0	6	3	faint bands
0.2	2.5	2.4	1.0		6	faint bands
0.2	2.5	2.4	1.0		9	faint bands
0.2	2.5	2.4	1.0		12	very faint bands
0.2	2.5	2.4	1.0	7	3	very faint bands
0.2	2.5	2.4	1.0		6	good bands
0.2	2.5	2.4	1.0		9	good bands
0.2	2.5	2.4	1.0		12	faint bands
0.2	2.5	2.4	1.0	8	3	faint bands
0.2	2.5	2.4	1.0		6	faint bands
0.2	2.5	2.4	1.0		9	faint bands
0.2	2.5	2.4	1.0		12	very faint bands

Table 4.2. Optimization of RAPD-PCR in the presence of Tween-20 and BSA

dNTP (mM)	$MgCl_2$ (mM)	Buffer (μL)	Taq Pol (unit)	Primer (pmol)	DNA (ng)	Tween 20 (μL)	BSA (μg/ng)	Results
0.2	2.5	2.4	1.0	6	3	-	-	faint bands
0.2	2.5	2.4	1.0			0.13	-	faint bands
0.2	2.5	2.4	1.0			0.13	0.03	faint bands
0.2	2.5	2.4	1.0			-	0.03	faint bands
0.2	2.5	2.4	1.0	7	3	-	-	good amplification
0.2	2.5	2.4	1.0			0.13	-	good amplification
0.2	2.5	2.4	1.0			0.13	0.03	good amplification
0.2	2.5	2.4	1.0			-	0.03	good amplification
0.2	2.5	2.4	1.0		6	-	-	good amplification
0.2	2.5	2.4	1.0			0.13	-	good amplification
0.2	2.5	2.4	1.0			0.13	0.03	best amplification
0.2	2.5	2.4	1.0			-	0.03	good amplification
0.2	2.5	2.4	1.0	8	6	-	-	good amplification
0.2	2.5	2.4	1.0			0.13	-	good amplification
0.2	2.5	2.4	1.0			0.13	0.03	good amplification
0.2	2.5	2.4	1.0			-	0.03	good amplification
0.2	2.5	2.4	1.0		9	-	-	faint bands
0.2	2.5	2.4	1.0			0.13	-	faint bands
0.2	2.5	2.4	1.0			0.13	0.03	blurred backgrounds
0.2	2.5	2.4	1.0			-	0.03	blurred backgrounds

4.2. Genetic Structure of Seed Sources

In the first set of analyses, after elimination of non-segregating and low frequency RAPD bands, a total of 11 primers revealed 152 polymorphic RAPD fragments for studied seed sources (seed stands, seed orchards, plantations). In the second set, a total of 7 primers revealed 66 polymorphic RAPD fragments for studied varieties and natural population, after removal of non-segregating and low frequency RAPD bands. A number of fragments were only observed in some seed sources; however, all of these fragments were rare and their frequencies within any of the seed sources were low (<10%). Therefore, the bands with very low frequencies were removed from the data set and then segregating loci in all seed sources were used in the analysis. As a result, polymorphic markers (segregating at least in one of the seed sources) with 2 alleles were used in the analysis. Monomorphic locus was also dropped out of the data set found. The number of polymorphic fragments scored per primer varied from seven (UBC-190) to thirteen (UBC-154) (Table 4.3).

Table 4.3. Number of RAPD fragments scored per primer

Primer	# of RAPD fragments
UBC-121	12
UBC-129	8
UBC-131	9
UBC-139	9
UBC-144	10
UBC-149	12
UBC-151	8
UBC-154	13
UBC-159	10
UBC-162	10
UBC-190	7
Mean # of fragments/primer	**9**

4.2.1. Genetic Diversity

Genetic diversity parameters were calculated using POPGENE software (Yeh *et al*., 1997). For each seed source, number of observed alleles (*Na*) per locus, number of effective alleles (*Ne*) per locus (Kimura and Crow, 1964), Shannon's Information index (*I*) (Lewontin, 1972), proportion of polymorphic loci (Lewontin, 1972), observed and expected heterozygosity (Levene, 1949) values were estimated. Estimates for those parameters were given at the species level, natural populations versus varieties, seed stands, seed orchards and plantations levels in the following sections.

4.2.1.1. Genetic Diversity between Seed Sources at Species Level

- ***Allelic Richness:*** The number of observed alleles varied between 2±0 in Burhandağ-SO and 1.87±0.03 in Balıköy-P; and the mean number was 1.92±0.02. Averages of the seed source categories, varied between 1.92±0.03 for plantations and 1.95±0.02 for seed stands. Effective number of alleles ranged from 1.65±0.04 in Balıköy-P to 1.75±0.03 in Balıköy-SO; and the mean was 1.68±0.03. Averages of the seed source categories, varied between 1.67±0.03 in plantations and 1.72±0.03 in seed stands. There was no considerable variation in *Na* and *Ne* between seed sources considering standard errors of estimates. Moreover, mean number of effective alleles (*Ne*) was lower than observed number of alleles (*Na*) as it is expected (Table 4.4).
- ***Shannon's Information Index:*** The average Shannon's Information index was estimated as 0.54±0.02 for all studied seed sources. Estimated values ranged from 0.51±0.02 in Balıköy-P to 0.59±0.01 in Burhandağ-SO and Balıköy-SO. Averages of the seed source categories, varied between 0.53±0.02 in plantations and 0.56±0.02 in seed stands and seed orchards. Considering the standard errors of the estimates, there was no difference between seed source categories (Table 4.4).

Table 4.4. Summary of genetic variation statistics (observed (*Na*) and effective number of alleles (*Ne*), Shannon's Information Index (*I*), expected (*He*) and observed heterozygosity (*Ho*), proportion of polymorphic loci (*%P*) and their standard errors for 152 loci considered in Anatolian black pine seed stands, seed orchards and plantations by 11 primers

Categories	Seed Sources	*Na*	*Ne*	*I*	*Ho*	*He*	*P*
Seed Stands	Burhandağ-SS	1.97±.017	1.70±.030	0.56±.017	0.49±.023	0.4±.014	97
	Balıköy-SS	1.94±.024	1.71±.030	0.56±.019	0.48±.025	0.4±.015	94
	Daren-SS	1.96±.020	1.72±.030	0.57±.017	0.48±.024	0.4±.014	96
	Hocalar-SS	1.94±.024	1.73±.031	0.57±.019	0.51±.027	0.4±.015	94
	Average	**1.95±.021**	**1.72±.030**	**0.56±.018**	**0.49±.025**	**0.4±.015**	**95**
Seed Orchards	Burhandağ-SO	2.0±0	1.73±.026	0.59±.013	0.53±.024	0.4±.011	100
	Balıköy-SO	1.97±.017	1.75±.027	0.59±.015	0.57±.023	0.41±.013	97
	Daren-SO	1.91±.029	1.68±.033	0.54±.021	0.5±.027	0.38±.016	91
	Hocalar-SO	1.91±.029	1.65±.036	0.52±.022	0.5±.030	0.37±.017	91
	Average	**1.94±.019**	**1.70±.03**	**0.56±.018**	**0.55±.026**	**0.39±.014**	**94**
Plantations	Burhandağ-P	1.91±.029	1.66±.034	0.53±.022	0.52±.030	0.37±.017	91
	Balıköy-P	1.87±.034	1.65±.038	0.51±.025	0.49±.031	0.36±.017	87
	Daren-P	1.97±.017	1.69±.032	0.55±.019	0.49±.025	0.39±.015	97
	Hocalar-P	1.93±.025	1.69±.034	0.53±.021	0.47±.028	0.38±.016	93
	Average	**1.94±.022**	**1.70±.031**	**0.55±.019**	**0.51±.026**	**0.39±.015**	**92**
	Total Average	**1.92±.020**	**1.68±.030**	**0.54±.017**	**0.49±.024**	**0.38±.014**	**93**

- ***Heterozygosity:*** Observed heterozygosity values varied between 0.47±0.03 in Hocalar-P and 0.57±0.02 in Balıköy-SO. Averages of the seed source categories, varied between 0.49±0.03 in seed stands and plantations to 0.55±0.03 in seed orchards. Expected heterozygosity values were between 0.34±0.02 (Balıköy-P) and 0.41±0.01 (Balıköy-SO). Averages of the seed source categories, varied between 0.37±0.02 in plantations and 0.40±0.01 in seed stands. Mean observed and expected heterozygosities were 0.51±0.03 and 0.39±0.01, respectively. Results revealed that there was no variation between groups in terms of expected and observed heterozygosity values, considering the standard errors of the estimates. In addition, observed heterozygosity values were slightly higher than the expected heterozygosity values (Table 4.4).
- ***Proportion of Polymorphic Loci:*** The proportion of polymorphic loci (0.99 criterion) varied from 87% in Balıköy-P to 100% in Burhandağ-SO. Averages of the seed source categories varied between 92% in plantations and 95% seed stands and seed orchards. Average proportion of polymorphic loci of seed sources was estimated as 94%. There was no major difference between seed source categories (Table 4.4).

4.2.1.2. Genetic Diversity between Natural Populations and Varieties

- ***Allelic Richness:*** The number of observed alleles varied between 1.82±0.04 in var. *şeneriana* and 2±0 in Dursunbey. The mean number of alleles for seed stands and varieties was 1.96±0.02 and 1.85±0.03, respectively. Effective number of alleles ranged from 1.57±0.03 in Dursunbey and 1.73±0.03 in Burhandağ, Daren and Hocalar. The mean number of effective alleles for seed stands and varieties were 1.72±0.03 and 1.61±0.04, respectively. Although *Na* had its highest value (2) in Dursunbey, *Ne* was the lowest (1.57). Considering the standard errors of the estimates, there was a sharp decrease in *Na* in varieties as compared to seed stands and Dursunbey. Moreover, *Ne* of varieties and Dursunbey were much lower than seed stands (Table 4.5).

- ***Shannon's Information Index:*** The average Shannon's Information index was estimated as 0.57±0.01 for seed stands and 0.49±.02 for varieties and Dursunbey. Estimated values ranged from 0.49±0.03 in both varieties and Dursunbey to 0.58±0.01 in Burhandağ and Daren. Considering the standard errors of the estimates, there was a slight decrease in Shannon's Information index for varieties as compared to seed stands and Dursunbey (Table 4.5).
- ***Heterozygosity:*** Expected heterozygosities varied between 0.33±0.02 in Dursunbey to 0.40±0.01 in all seed stands. Observed heterozygosities were between 0.36±0.02 in Dursunbey and 0.51±0.02 in Burhandağ. Mean observed heterozygosities for seed stands (0.49±0.02) and varieties (0.45±0.03) were in close range. Mean expected heterozygosities were also similar to observed heterozygosity and varied between 0.4±0.01 and 0.36±0.02 for seed stands and varieties, respectively. Dursunbey population had the lowest values for both observed and expected heterozygosity. Results revealed that there was no variation between seed stands and varieties in terms of expected and observed heterozygosity values, considering the standard errors of estimates. But, observed heterozygosity values were slightly higher than the expected heterozygosity values in all studied seed sources (Table 4.5).
- ***Proportion of Polymorphic Loci:*** The proportion of polymorphic loci (0.99 criterion) ranged from 82% in var. *şeneriana* to 100% in Dursunbey. Average proportion of polymorphic loci of seed stands and varieties were 96% and 85%, respectively. Therefore, proportion of polymorphic loci of the varieties was much lower than natural seed sources (Dursunbey and seed stands) (Table 4.5).

Table 4.5. Summary of genetic variation statistics (Observed number of alleles (*Na*) and effective number of alleles (*Ne*), Shannon's Information Index (*I*), Expected (*He*) and Observed heterozygosity (*Ho*), Proportion of polymorphic loci (*%P*) and their standard errors for 66 loci considered in Anatolian black pine seed stands and varieties by 7 primers

Categories	Seed Sources	*Na*	*Ne*	*I*	*Ho*	*He*	*P*
Natural Populations (Seed Stands)	Burhandağ-SS	1.98±.012	1.73±.030	0.58±.015	0.51±.022	0.40±.013	98
	Balıköy-SS	1.95±.020	1.69±.030	0.55±.018	0.48±.024	0.40±.015	95
	Daren-SS	1.96±.020	1.73±.030	0.58±.016	0.48±.025	0.40±.013	97
	Hocalar-SS	1.95±.020	1.73±.030	0.57±.018	0.49±.026	0.40±.015	95
	Average-SS	1.96±.020	1.72±.030	0.57±.015	0.49±.020	0.40±.014	96
Varieties	Var. *şeneriana*	1.82±.038	1.62±.037	0.49±.025	0.48±.032	0.37±.019	82
	Var. *pyramidata*	1.88±.033	1.60±.037	0.49±.024	0.42±.029	0.34±.018	88
	Average-V	1.85±.030	1.61±.040	0.49±.023	0.45±.030	0.36±.017	85
Reference Population	Dursunbey	2.0±0	1.57±.030	0.49±.020	0.36±.024	0.33±.016	100

4.2.1.3. Genetic Diversity between Seed Stands

- ***Allelic Richness:*** The number of observed alleles varied between 1.94±0.02 (Balıköy-SS) and 1.97±0.02 (Burhandağ-SS); and the mean number was 1.95±0.02. Effective number of alleles ranged from 1.70 in Burhandağ to 1.73 in Hocalar; and the mean was 1.72. There was no variation among seed stands for *Na* and *Ne* considering standard errors of estimates (Table 4.4).
- ***Shannon's Information Index:*** The average Shannon's Information index (genetic diversity) was estimated as 0.56±0.02 for seed stands. Considering the standard errors of estimates, there was no difference between seed stands for this parameter (Table 4.4).
- ***Heterozygosity*:** Expected heterozygosity values for all seed stands were the same (0.4±0.01). Observed heterozygosity varied between 0.48±0.02 in Balıköy- and Daren to 0.51±0.03 in Hocalar. From the results, it appeared that there was no variation between seed stands for expected and observed heterozygosity values. In addition, observed heterozygosities were higher than the expected heterozygosity values (Table 4.4).
- ***Proportion of Polymorphic Loci:*** Average proportion of polymorphic loci of seed stands was found to be 95%. The proportion of polymorphic loci (0.99 criterion) did not vary greatly and ranged from 94% in Balıköy and Hocalar to 97% in Burhandağ (Table 4.4).

4.2.1.4. Genetic Diversity between Seed Orchards

- ***Allelic Richness:*** Considering the standart errors of the estimates, the number of observed alleles varied considerably among seed orchards. Values ranged from 1.91±0.03 in Daren and Hocalar and 2±0 in Burhandağ. Effective number of alleles was slightly different among seed orchards and varied from 1.65±0.04 in

Hocalar to 1.75±0.03 in Balıköy. Moreover, mean number of effective alleles (*Ne*) was lower than observed number of alleles as it is expected (Table 4.4).

- ***Shannon's Information Index:*** The average Shannon's Information index was estimated as 0.56±0.02 for seed orchards. Considering the standart errors of the estimates, there was a slight difference among seed orchards (0.52±0.02 in Hocalar and 0.59±0.01 in Burhandağ and Balıköy) (Table 4.4).
- ***Heterozygosity:*** Expected heterozygosity values did not vary between seed orchards and ranged from 0.37±0.02 in Hocalar and 0.41±0.01 in Balıköy. Observed heterozygosity values, also, was not different between seed orchards and ranged from 0.50±0.03 in Hocalar and Daren and 0.57±0.02 in Balıköy. Average observed and expected heterozygosities were 0.55±0.03 and 0.39±0.01 respectively. Results revealed that there was no considerable variation between seed orchards in terms of observed heterozygosity values, considering the standart errors of the estimates. In addition, observed heterozygosity values were higher than expected ones (Table 4.4).
- ***Proportion of Polymorphic Loci:*** The proportion of polymorphic loci (0.99 criterion) varied from 91% in Daren and Hocalar to 100% in Burhandağ. Average proportion of polymorphic loci of seed orchards was found to be 95%. Therefore, proportion of polymorphic loci values varied notably within the category (Table 4.4).

4.2.1.5. Genetic Diversity between Plantations

- ***Allelic Richness:*** Considering the standart deviations of the estimates, number of observed alleles was considerably different among plantations and varied from 1.87±0.03 in Balıköy to 1.97±0.02 in Daren. The mean value of *Na* was found to be 1.92±0.03. Effective number of alleles did not vary much and ranged from 1.65±0.04 in Balıköy to 1.69±0.03 in Daren and Hocalar; and the mean was 1.67±0.03. Moreover, mean number of effective alleles (*Ne*) was lower than observed number of alleles (*Na*) as it is expected (Table 4.4).

- ***Shannon's Information Index:*** Estimated Shannon's Information index values were in close range and varied from 0.51±0.02 in Balıköy to 0.55±0.02 in Daren. Considering the standart deviations, estimated values did not vary among plantations (Table 4.4).
- ***Heterozygosity:*** Expected heterozygosity values for plantations were in close range and varied from 0.36±0.02 in Balıköy and 0.39±0.01 in Daren. Observed heterozygosity values were between 0.47±0.03 in Hocalar and 0.52±0.03 in Burhandağ. Average observed and expected heterozygosities are 0.49±0.03 and 0.37±0.02 respectively. Considering the standart errors of the estimates, there was no major variation among plantations. In addition, observed heterozygosity values were much higher than expected ones (Table 4.4).
- ***Proportion of Polymorphic Loci:*** Average proportion of polymorphic loci (0.99 criterion) of plantations was found to be 92%. The proportion of polymorphic loci varied from 87% in Balıköy to 97% in Daren. Therefore, proportion of polymorphic loci values varied considerably within the category (Table 4.4).

4.2.1.6. Genetic Diversity between Seed Sources Considering Locations

Burhandağ: In general, genetic diversity parameters had its lowest values (except for *Ho*) in plantation but highest values in seed orchard for this location. Considering the standard errors of the estimates, there was no considerable variation in effective number of alleles, Shannon's Information index, observed and expected heterozygosities values. However, there was a considerable reduction in observed number of alleles and proportion of polymorphic loci in plantation with respect to seed orchard considering the standart errors of the estimates. Although there were differences in seed orchard and plantation, seed stand's genetic diversity parameters were in close range with its seed orchard and plantation (Table 4.4).

Balıköy: Same pattern was observed for Balıköy location i.e. genetic diversity parameters had its lowest values (except for *Ho*) in plantation but highest values

in seed orchard. Considering the standard errors of the estimates, there were major differences in allelic richness, observed heterozygosity, Shannon's Information index and proportion of polymorphic loci values between plantation and seed orchard. Yet, seed stand and seed orchard were notably different with respect to observed heterozygosity (Table 4.4).

Daren: Genetic diversity parameters (allelic richness, Shannon's Information index, proportion of polymorphic loci and expected heterozygosity) had its lowest values in seed orchard but highest values in seed stand. However, observed heterozygosity was lowest in seed stand and highest in seed orchard. However, there was no considerable difference between categories except for a slight decrease of proportion of polymorphic loci in seed orchard than seed stand and plantation (Table 4.4).

Hocalar: All genetic diversity parameters (except for *Ho*) had its lowest values in seed orchard but highest values in seed stand. Considering the standard errors of the estimates, there was no variation in genetic diversity parameters among categories (Table 4.4).

In order to define genetic resemblance of seed sources, location based dendrograms were also constructed to compare seed sources from the same location (seed orchards and plantations originated from the respective seed stands) (Figure 4.1).

For Hocalar and Daren locations, seed stands seemed to be at the same distance to seed orchard and plantation.

On the other hand, for Balıköy location seed orchard was genetically closer to plantation than seed stand.

For Burhandağ, plantation and seed orchard was closer to each other, but seed stand seemed to be distant to both seed sources (SO and P).

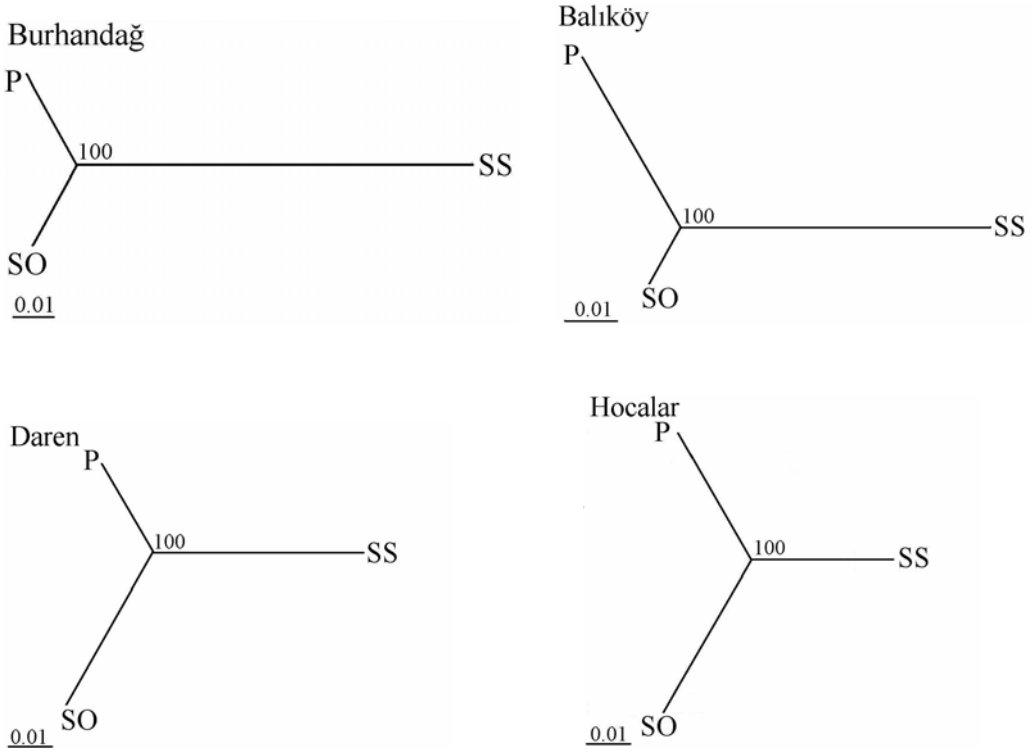


Figure 4.1. Dendrograms based on Nei's genetic distance values for seed sources considering locations

4.2.2. F-Statistics

Wright's F-Statistics were also employed to investigate the pattern of genetic variation in each population and if any deviation from Hardy-Weinberg expectations in gene frequencies may occur (Wright, 1969; Nei, 1987) (Table 4.6).

Fixation index values within sub-populations (F_{IS}) for seed stands, seed orchards, plantations and varieties were estimated as to be 0.25, 0.36, 0.34 and 0.33, respectively ($P<0.001$). All estimated F_{IS} values were negative indicating that within subpopulations among categories; heterozygotes were 25 to 36% higher than expected. Since all estimated F_{IS} values were statistically significant, it could be infered that there are significant deviations from Hardy-Weinberg expectations within subpopulations.

Table 4.6. Summary of F-statistics

	Sample Size	F_{IS}	F_{IT}	F_{ST}	Nm
Seed stands	200	- 0.25***	- 0.18***	0.06***	3.9
Seed orchards	200	- 0.36***	- 0.28***	0.05***	4.15
Plantations	200	- 0.34***	- 0.25***	0.06***	3.7
Varieties	54	- 0.33***	- 0.25***	0.06***	3.5

(***: $p<0.001$)
F_{IS}: Fixation index within subpopulations
F_{IT}: Total fixation index
F_{ST}: Genetic differentiation
Nm: Gene flow

Total fixation index values (F_{IT}) were estimated to be 0.18, 0.28, 0.25 and 0.25 for seed stands, seed orchards, plantations and varieties, respectively. All of the F_{IT} values were also negative and highly significant, claiming that there are significant deviations from Hardy-Weinberg expectations.

The reduction in fixation index due to genetic differentiation among subpopulations (F_{ST}) was also estimated. All of the F_{ST} values were similar for seed source categories and highly significant ($P<0.001$), indicating that at least one of the populations in each group has been differentiated significantly from others. For instance, genetic differentiation of seed stands was 0.06 showing that 6% of the genetic variation is between seed stands and 94 % of the variation is within seed stands.

Gene flow values were also estimated by means of F_{ST} values. Gene flow estimates were in close range among seed source categories and ranged from 3.5 for the varieties to 4.15 for seed orchards. Since all Nm values are larger than 1, it is expected that gene flow among populations is sufficient to prevent genetic drift.

4.2.3. Genetic Distance

Nei's unbiased genetic distance values were calculated for seed source pairs in each category to reveal the degrees of relatedness among seed sources (Nei, 1972). This value ranges between "0 and 1" and 0 distance is equivalent to no difference. Genetic distance values were calculated for seed stands, seed orchards, plantations and seed stands vs. varieties and natural population seperately (Table 4.7, 4.8, 4.9, 4.10, 4.11). Dendrograms were constructed by using Nei's genetic distance values by Neighbor Joining method.

Seed Stands: Genetic distance values of seed stands infer that Burhandağ and Balıköy, and Daren and Hocalar are the most closely related seed stands (D=0.04). On the other hand, Burhandağ and Hocalar are the most distant seed stands (D=0.07) (Table 4.7).

Table 4.7. Nei's Unbiased Genetic Distance Values for Seed Stands

	Burhandağ	**Balıköy**	**Daren**	**Hocalar**
Burhandağ	****			
Balıköy	0.04	****		
Daren	0.06	0.05	****	
Hocalar	0.07	0.06	0.04	****

Seed Orchards: Estimated genetic distance values for SOs showed parallel results to the magnitude observed in seed stands. But the most close and distant seed sources were different. Balıköy and Hocalar are the most closely related seed orchards (D=0.03), but Hocalar and Daren are the most distant seed orchards (D=0.08) (Table 4.8).

Table 4.8. Nei's Unbiased Genetic Distance Values for Seed Orchards

	Burhandağ	Balıköy	Daren	Hocalar
Burhandağ	****			
Balıköy	0.04	****		
Daren	0.07	0.06	****	
Hocalar	0.05	0.03	0.08	****

Plantations: Genetic distances among seed sources were in similar magnitude with those observed in SSs and SOs. But the most close and distant seed sources among plantations followed a different pattern. The most closely related plantations are Balıköy and Daren; Burhandağ and Balıköy; Burhandağ and Daren (D=0.04); on the other hand Balıköy and Hocalar are the most distant plantations (D=0.08) (Table 4.9).

Table 4.9. Nei's Unbiased Genetic Distance Values for Plantations

	Burhandağ	Balıköy	Daren	Hocalar
Burhandağ	****			
Balıköy	0.04	****		
Daren	0.04	0.04	****	
Hocalar	0.07	0.08	0.06	****

In addition, seed sources were grouped on the basis of origins of the seed sources and genetic distances among them were estimated again (Table 4.10). For Burhandağ, D ranges from 0.04 to 0.14; for Balıköy 0.05-0.10; for Daren 0.06-0.08 and for Hocalar 0.07-0.09.

The most genetically identical seed sources were Burhandağ-SO and Burhandağ-P (D=0.04) and the most distant ones are Burhandağ-SS and Burhandağ-P (D=0.14). Similar pattern was also observed in Balıköy seed sources that SS and P were genetically more distant than SO and P.

Table 4.10. Nei's Unbiased Genetic Distance Values for 4 origins

LOCATION	SEED SOURCE	SEED STAND	SEED ORCHARD	PLANTATION
Burhandağ	**Seed Stand**	***		
	Seed Orchard	0.09	***	
	Plantation	0.14	0.04	***
Balıköy	**Seed Stand**	***		
	Seed Orchard	0.07	***	
	Plantation	0.10	0.05	***
Daren	**Seed Stand**	***		
	Seed Orchard	0.08	***	
	Plantation	0.07	0.06	***
Hocalar	**Seed Stand**	***		
	Seed Orchard	0.08	***	
	Plantation	0.07	0.09	***

For the second set of experiments (natural populations versus varieties) genetic distance values were also estimated (Table 4.11). Burhandağ and Balıköy was the most closely related seed sources (D=0.04). On the other hand, Dursunbey and Daren seemed to be the most distant seed sources (D=0.26). Genetic distance between varieties and other seed sources were consistently high. Alos, Dursunbey population seem to be genetically distant to all seed sources.

Table 4.11. Nei's Unbaised genetic Distance Values for Natural Populations (Seed Stands) versus Varieties

	Burhandağ	**Baliköy**	**Daren**	**Hocalar**	**Dursunbey**	**Var. *Şeneriana***	**Var. *Pyramidata***
Burhandağ	****						
Balıköy	0.04	****					
Daren	0.06	0.04	****				
Hocalar	0.08	0.06	0.06	****			
Dursunbey	0.23	0.24	0.26	0.24	****		
Var. *şeneriana*	0.14	0.13	0.15	0.13	0.19	****	
Var. *pyramidata*	0.15	0.14	0.14	0.11	0.15	0.07	****

4.2.4. Phylogenetic Trees

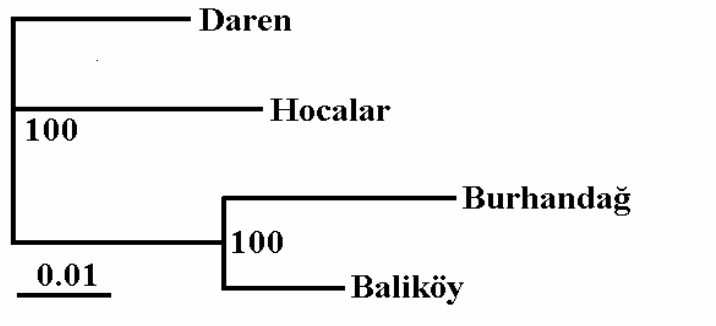
Dendrograms were constructed using Nei's genetic distance values by the Neighbor-Joining method.

Seed sources were grouped as seed stands, seed orchards and plantations to investigate the pattern of genetic resemblance among seed sources within the seed source category (Figure 4.2). All bootstrap values in dendrograms were high, claiming that groupings are highly significant. Seed stands' dendrogram had 3 groups: first one contained Burhandağ and Balıköy seed stands; second and third ones had Daren and Hocalar seed stands (Figure 4.2a).

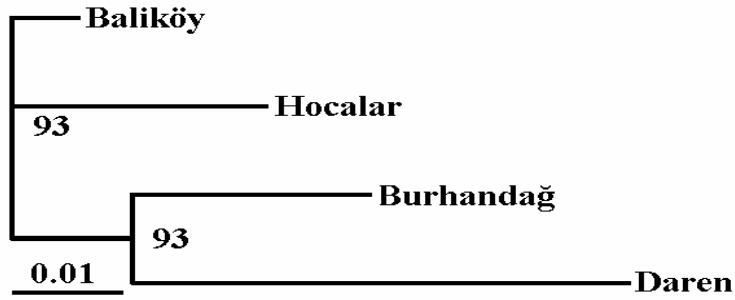
Seed orchards' dendrogram had also 3 arms: first containing Burhandağ and Daren seed orchards; the second and the third arms were Balıköy and Hocalar. The pattern of genetic resemblance among seed sources within the SO group was diffrent than that of SSs (Figure 4.2b).

The pattern of genetic resemblance within plantation group was similar to the seed stand group (Figure 4.2c).

a) Seed Stands



b) Seed Orchards



c) Plantations

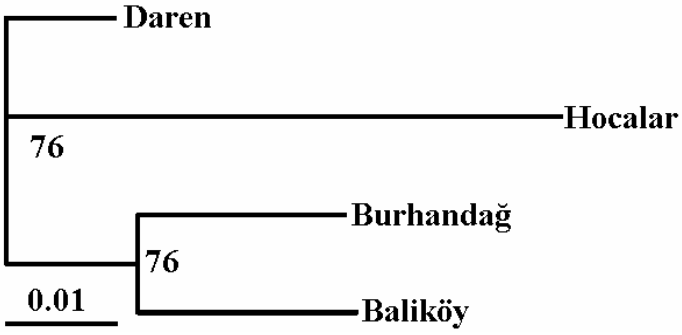


Figure 4.2. Dendrograms based on Nei's genetic distance values for seed stands, seed orchards, plantations

Another dendrogram was constructed for natural populations versus varieties (Figure 4.3). In this dendrogram, two varieties formed a distinct group from other natural stands and reference population (Dursunbey) was also diverged from other natural populations.

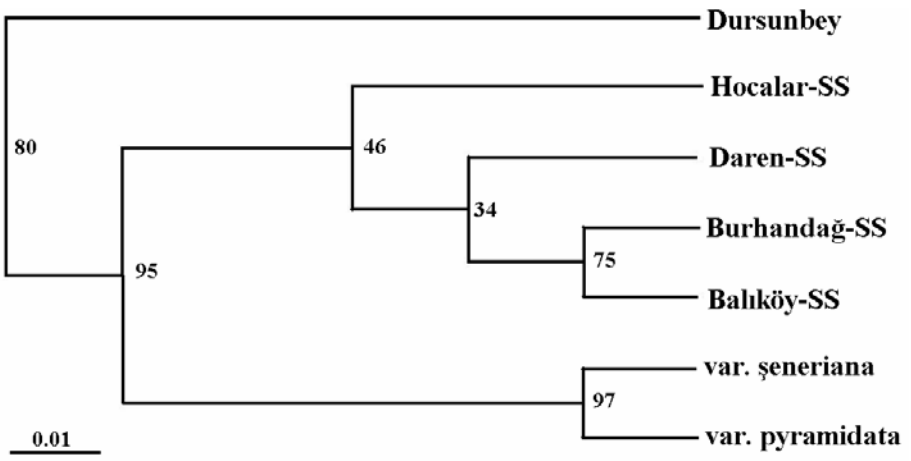


Figure 4.3. Dendrogram based on Nei's genetic distance values for seed stands and varieties

CHAPTER V

DISCUSSION

5.1. Genetic Structure of Seed Sources

Genetic characterization of Anatolian black pine seed stands, seed orchards, plantations and varieties was accomplished by using RAPD markers. Allelic richness, Shannon's Information Index, observed and expected heterozygosity and proportion of polymorphic loci values were estimated for all seed sources.

5.1.1. Genetic diversity

- Species Level:

Observed number of alleles is one of the components of the genetic variation. The present study revealed that allelic richness values estimated was high (1.87-2) and consistent with findings of other studies obtained with RAPD markers. *Na* was estimated as 1.86 for *Pinus brutia* (Lise, 2001); 1.9 for *Picea mariana* (Isabel *et al*., 1995); 1.67-1.89 for *Picea glauca* (Rajora, 1999); for *Pinus brutia* (İçgen *et al.*, 2005).

Effective number of alleles estimated for all seed sources (1.65-1.75) were high and in close range with findings of other studies. *Ne* was estimated as 1.47 and 1.48 for *Pinus brutia* (İçgen *et al.*, 2005; Lise, 2001); 1.46-1.69 for *Picea glauca* (Rajora, 1999) and 1.9 for *Picea mariana* (Isabel *et al*., 1995). The actual or observed number of alleles is equal to effective number of alleles when all alleles have the same frequency (Kimura and Crow, 1964). Otherwise effective number of alleles is always smaller than observed number of alleles since deleterious genes are not included in effective number of alleles. In this study, mean effective number of alleles was lower than observed number of

alleles as it was expected. Therefore, difference between seed source categories for allelic richness values was not significant and results were consistent with findings of other studies with RAPD markers in other conifer species.

Shannon's Information Index values, was also high for the studied seed sources (0.51-0.59). Similarly high results were reported by RAPD markers for *Pinus brutia* as 0.40 and 0.45 (İçgen *et al.,* 2005; Lise, 2001); for *Picea glauca* 0.38-0.55 (Rajora, 1999).

Estimated *expected heterozygosity* values (0.47-0.55) were consistent with findings of other RAPD marker studies. *He* was estimated as 0.34-0.44 for *Pinus sylvestris* (Szmidt *et al*., 1996), 0.28 for *Pinus brutia* (İçgen *et al*., 2005) and 0.26-0.38 for *Picea glauca* (Rajora, 1999). *Observed heterozygosity* values (0.36-0.41) was higher than findings of other RAPD marker studies. For example, estimated *Ho* values were 0.23 and 0.25 for *Pinus brutia* (Lise, 2001; İçgen *et al*. 2005), and 0.34 for *Picea mariana* (Isabel *et al*., 1995). This difference may be caused by different sample size and number of loci detected with different studies. These parameters may also result in different genetic diversity values even with the same marker type (Szmidt *et al*., 1996; Aagaard *et al*.1998). In this study, sample size and polymorphic loci number were considerably high when compared to previous studies (Szmidt *et al*., 1996; Rajora, 1999; Isabel *et al*., 1995). In addition, observed heterozygosity values were slightly higher than the expected heterozygosities. It was also reported in a study with isozyme variation of Anatolian black pine populations from Bolkar mountains (Tolun *et al*. 2000).

Proportion of polymorphic loci, which is another measure of genetic variation, calculated as 93% on the average. This value was high when compared to other *Pinus* species. *P* is calculated as 88% for *Pinus sylvestris*; 77 % for *Pinus brutia*; 82.78% for *Pinus oocarpa* (Szmidt *et al*., 1996; İçgen *et al.*, 2005; Diaz *et al*., 2001).

Genetic diversity measures estimated in this study reveals that genetic variation is considerably high for studied Anatolian black pine seed sources. Since this is the first population genetics study for black pine with RAPDs, results could only be compared with other *Pinus* species and conifers. Observation of high genetic diversity based on

RAPD markers in the current study should not be a surprise since previous studies also revealed that black pine show evidence of substantial variation not only in the molecular level, but also in morphological, anatomic, physiologic and genetic traits (Kaya *et al*., 1985; Alptekin, 1986; Matziris, 1989; Portfaix, 1989; Economou, 1990; Işık, 1990; Kaya ve Temerit; 1994; Şimşek *et al*., 1995; Üçler and Gülcü, 1999).

In addition to that, several studies carried out with isozyme variation also support the existence of high level of genetic diversity in black pine in general and Anatolian black pine populations (Table 5.1). Results of those studies revealed that black pine exibits a pattern of genetic diversity characterized by high intra-population and moderate degree of inter-population diversity, which results in a high total diversity. The considerable intra-population diversity of the species is consistent with the findings of Hamrick and Godt (1989) that gymnosperms, long-lived trees, outcrossing species, and species with high fecundity are associated with high intra-population genetic diversity. All of these are also life history characteristics of of Anatolian black pine.

However, the effects of life history characteristics are usually surpassed by historical factors operating during the last glacial and inter-glacial periods i.e. during the Pleistocene and Holocene (Ledig, 1998a). Genetic variation patterns are influenced by the effects of climate change on population size and the migration of species in space and time via seed dispersal. Turkey's geologic and climatic history and its role as a glacial refuge for many species during the Pleistocene, could explain high levels of genetic variation in conifers occurring there. A continued warming and drying trend in the early Holocene caused expansion and then fragmentation of conifer populations but then conversion of forests to agriculture. Nevertheless, long distance colonizations (founder effects) did not come with these events; and surviving populations' diversity probably has not been reduced since these events are recent enough.

Table. 5.1. Genetic diversity parameters estimated by isozyme markers for black pine

REFERENCE	Sample Size	Locus No	*Na*	*%P*	*Ho*	*He*	F_{IT}	F_{ST}	*Nm*
Black Pine									
Nicolic and Tucic (1983)	Bulk	4	3.02	66		0.27		.13	
Scaltsoyiannes *et al.* (1994)	Bulk	16	2.02	70		0.20		0.06*	
Silin and Goncharenko (1996)	203	24	2.9	66	0.24	0.25	-0.004	0.13	19
Anatolian black pine									
Cengel *et al.* (2000)	315	29	1.67	56.6	0.15	0.26	0.41	0.06	3.7
Tolun *et al.* (2000)	190	24	1.55	48	0.25	0.21	-0.22	0.06	7.5

*** : G_{ST}**

Therefore, possible explanations of high genetic diversity for Anatolian black pine seed sources are life history characteristics and adaptation mechanisims to the micro-environments which are prevailing in natural range of species (Kaya and Temerit, 1994).

- Varieties versus natural populations

Genetic diversity parameters estimated for natural populations (seed stands) versus varieties were considerably different. Considering the standard errors of the estimates, except for expected and observed heterozygosity, all estimated genetic diversity parameters were considerably higher in seed stands than varieties. Although, varieties had lower levels of variation when compared to seed stands, it was expected to observe much lower values; because, these populations are considered as varieties which undergo speciation process. Since the sample size was low, reasons for reduction in allele number and polymorphism can not be distinguished. Further analyses based on larger samples are needed to confirm results. There may be some other reasons to cause

differentiation of those varieties such as mutation coupled with strong natural selection. There appear to be still gene exchange occurring between varieties and natural stands nearby, since there is still considerable amount of genetic diversity existing in small populations of these varieties. Another explanation may be that mutation occurred only in the genes controlling the stem and branching form and so speciation process is still operating.

Further investigations are needed to throw light on the connection between genetic divergence, geographical isolation and genetic diversity of Anatolian black pine varieties. In many cases, RAPDs allow discrimination between species, subspecies, varieties, cultivars, clones and are useful for the establishment of genetic relationships at these taxonomic levels (Adams and Demeke, 1993; Morell *et al*., 1995; Millan *et al*., 1996; Nesbitt *et al*. 1997; Hsiang and Huang, 2000; Gallois *et al*., 1998). In addition, different marker systems could also be used such as chloroplast markers.

- Seed Stands, Seed Orchards and Plantations

Allelic richness, Shannon's Information Index, proportion of polymorphic loci and *heterozygosity* estimates were almost the same for all seed source categories, so there was no considerable variation observed between seed source categories. However, there was considerable variation within seed source categories. *Number of observed alleles* was significantly different within seed orchards and within plantations.

In terms of observed heterozygosity, there was no major variation between seed source categories and within seed sources. For all seed sources, observed heterozygosity estimtes were larger than expected heterozygosity values.

Although there was no variation between seed source categories, p*roportion of polymorphic loci* was considerably different within seed source categories. Large deviations were observed within seed orchards (91-100) and plantations (87-97). Proportion of polymorphic loci was higher in seed stands and seed orchards than plantations. Higher proportion of polymorphic loci values were expected for seed orchards and reduction in plantations was expected and can be explained with sampling

and nursery practices. The studied plantations have been established from nursery raised seedlings originating from bulk seeds from respective seed stand. The details of the seed collections, and the nursery and seedling-handling conditions are not always well known. It may be possible that the seeds for those plantations have come from a relatively small number of seed trees. Seeds could be collected in a poor seed crop year or seed stands were not sampled adequately; which would narrow their genetic base. Nursery raised seedlings are subject to a number of selective forces that may also affect genetic diversity. Daren and Hocalar plantations represent best their natural counterparts. In fact, genetic diversity residing in all natural populations was successfully captured all in seed orchards and plantations, since these values are considerably high compared to other species. Proportion of polymorphic loci was estimated as 88% for *Pinus sylvestris* (Szmidt *et al*., 1996), 77 % for *Pinus brutia* (İçgen *et al.*, 2005) and 82.78% for *Pinus oocarpa* (Diaz *et al*., 2001).

- Location based comparisons

In Burhandağ location, genetic diversity parameters had its lowest values (except for *Ho*) in plantation but highest values in seed orchard. Although there was no considerable difference between seed stand, seed orchard and plantation of the same origin, there was a considerable reduction in observed number of alleles and proportion of polymorphic loci in plantation with respect to seed orchard.

In Balıköy, all genetic diversity parameters had its lowest values (except for *Ho*) in plantation but highest values in seed orchard and all these differences were important. In addition, seed stand and seed orchard was considerably different with respect to observed heterozygosity.

In Daren location, there were no major difference between genetic parameter estimates for seed stand, seed orchard and plantation except for a slight decrease in proportion of polymorphic loci in seed orchard.

In Hocalar location, all genetic diversity parameters (except for *Ho*) had its lowest values in seed orchard but highest values in seed stand, however, these differences were not meaningful.

Therefore, on the basis of location based comparisons, there were considerable differences between natural (seed stands) and managed seed sources (plantations) of Burhandağ and Balıköy origins.

In Daren and Hocalar origins, there were no variation between seed source categories. Therefore, there was no sign of any effect of forest management activities on managed seed sources such as seed orchards and plantations.

5.1.2. F-Statistics

The inbreeding coefficients (F_{IS}, F_{IT}) or fixation indices are direct measures of the increase in homozygosity due to inbreeding (Wright, 1969). F_{IS} measures the degree of inbreeding within a subpopulation; F_{IT} measures the degree of inbreeding when the subpopulations are lumped into a single population. These measures also demonstrate any deviation from the Hardy-Weinberg expectations in heterozygosity.

Estimated F_{IS} which is the degree of inbreeding within a subpopulation, were all negative, stating that in all seed source categories heterozygosity was more than expected. Moreover, all F_{IS} values were statistically significant at the $P<0.001$ level. While seed orchards, plantations and varieties exhibit similar level of inbreeding coefficient though seed stands had a much lower value (-0.25). These results revealed that within each seed stand heterozygotes are 25% higher than expected. Similar negative F_{IS} values were obtained for *Pinus sylvestris* (Szmidt *et al*., 1996)*, Pinus brutia* (Özel, 2001)*, Picea mariana* (Isabel *et al*., 1995) as -0.26, -0.30, -0.13 respectively.

F_{IT} measures estimated in this study infer that seed stands as a group have 18% excess heterozygosity and other categories also have the excess of heterozygosity with larger magnitudes 26 % for seed orchards and 25% for plantations.

Excess heterozygosity can be caused by negative assortative mating and selection against homozygotes (El-Kassaby *et al*., 1987; Fady and Conkle, 1993). In

conifers, inbred individuals commonly express inbreeding depression and are removed from the population during the early stages of life (Eriksson *et al*., 1973). Strong selection against the more homozygous, relatively inbred individuals at the early stages is the likely cause of the negative fixation index observed in the current study as well as in previous studies (Szmidt and Muona, 1985; Plessas and Strauss, 1986; Szmidt *et al*., 1996; Tolun et al., 2000).

F_{ST} values were at the similar magnitude between seed source categories that can be used to partition variation among and within populations. For example, seed stands' F_{ST} value indicated that 6% of the total variation is between the seed stands and 94% of the variation is within the seed stands. This is similar to other studies' findings that conifers exhibit high level of genetic variation within populations (Szmidt *et al*., 1996; Isabel *et al*., 1995; Thomas *et al*., 1999). Our results indicate that the majority of genetic diversity is contained within populations, but there is moderate levels of differentiation among populations, as indicated by Wright (1978) for F_{ST} values between 0.05-0.15 (Hartl and Clark, 1997).

The distribution of genetic variation among populations can be used to estimate amounts of gene flow (*Nm*) among populations that is the number of immigrants per generation. When $Nm < 1$, populations begin to differentiate due to genetic drift; and $Nm < 0.5$, populations will diverge extensively as a result of drift (Wright, 1969). It can not be ignored, since low levels of gene flow allow higher levels of differentiation among populations. In this study, all categories of populations had significant amount of gene flow to prevent genetic drift. This is also consistent with the other forest tree species. Almost all estimates by both population genetic structure methods and paternity methods suggest that conifers have moderate to high levels of gene flow sufficient to prevent drift (Govindaraju, 1989; Adams and Birkes, 1990; Ledig 1998b). Since forest trees are highly outcrossed, forest geneticist should operate under the assumption that gene flow is probably present and should be monitored if it is expected to have an impact on a management program (Ellstrand, 1992).

Interestingly, estimated gene flow (*Nm*) for varieties was found to be 3.5 which is high enough to slow down speciation process of varieties.

5.1.3. Genetic Distance and Dendrograms

Estimates of Nei's genetic distance values (1972) among seed sources as well as seed source categories were determined and genetic relationships among them are depicted in dendrograms.

The genetic distance values of seed stands were higher than the previous studies findings such as 0.026-0.1 (İçgen *et al*., 2005) and 0.025-0.057 for *P. brutia* (Lise, 2001).

Dendrograms constructed for seed stands revealed that Burhandağ and Balıköy are the most closely related seed stands. These seed stands are not only genetically, but also geographically closest seed sources when compared to others. Although plantations' dendrogram also reflects the same situation, seed orchards' dendrogram interestingly showed a different pattern. Plus tree selection practices may have better captured the genetic diversity in natural stands than seed collections which are made periodically for plantations.

Location (origin) based dendrograms revealed that, in terms of maintaining genetic diversity at seed stands, seed orchards and plantations, the best forestry practices have been carried out at Hocalar location. Since each seed source category was in same proximity to each other for this locality. In Burhandağ, Balıköy and Daren locations; seed orchards seem to be closer to plantations than seed stands. Therefore, the present results suggest that nursery, clonal selection and seed collection practices may cause some genetic alteration in seed orchards and plantations using seeds from such seed sources.

5.2. Genetic Consequences of Forestry Practices

This study aimed to test whether some forestry practices such as selection of seed stands, establishment of seed orchards and plantations could alter the genetic

composition of Anatolian black pine forests. Estimated genetic diversity parameters appeared to be similar between seed source categories studied inferring that forestry practices did not cause major negative impact on natural and breeding populations of Anatolian black pine.

Forest management activities relying on natural or artificial regeneration systems, including tree breeding can extensively impact the genetic diversity of forest populations in the future (Rajora, 1999). However, effects of forest management and domestication on genetic makeup of forest trees are largely unknown. However, properly applied silvicultural treatments need not cause genetic degradation. Several studies confirmed this statement (Neale, 1985; Gömöry, 1992; Savolainen and Karkkainen, 1992).

Seed orchards are actually tree-improvement selections which are made for the desired characteristics such as: height, diameter, stem form, taper, crown shape etc. Thus their genetic base expected to be narrower. However, genetic diversity parameters estimated for seed orchards were in close range with seed stands and plantations. Only variation was observed for observed heterozygosity, in which, there was a slight increase in seed orchards than seed stands and plantations. In other conifers, seed orchard clones or advanced-generation breeding stocks have been found to have a genetic diversity comparable to or even higher than that of the natural counterparts (reviewed in Savolainen and Karkkainen, 1992; El-Kassaby, 1995).

For sustainable management of species, genetic base of the tree improvement selections needed to be broadened. Either clone numbers must be increased or multiple breeding populations should be maintained (Namkoong, 1984). Another concern over seed orchards is the loss of alleles due to small sample sizes. When seed orchards are established with a limited number of parents, genetic drift depletes genetic variation very slowly, at a rate of $1/2Ne$ per generation (where Ne is the effective population size) (Savolainen and Karkkainen, 1992). It is evident that seed orchards with even moderate number of clones or genotypes will lose very little of the expected heterozygosity in one generation, however, regenerated forests or plantations are not going to continue with

small population size. Additive genetic variation behaves as expected heterozygosity, and it is likely to be preserved to a high degree. However, some of the genetic variation may be in the form of rare alleles; e.g. disease resistance. Seed orchards typically contain low numbers of clones that even fairly common alleles are lost (Hattemer *et al*., 1982). However, empirical results on variation in natural stands and seed orchards confirm that expected heterozygosity is similar in natural and managed populations (Muona and Harju, 1989; Yazdani *et al*., 1985; Shaw and Allard, 1982; Neale, 1985).

Second stage of loss of gene diversity in seed orchards occur when there is unequal contribution of gametes by the orchard parents (Harju, 1995). Studies indicate that truly random mating does not happen in the seed orchard, because 80 % of the seed is typically produced by 20% of the clones (Johnson and Lipow, 2002). Although various factors contribute to the unequal reproductive contribution of parents, they all reduce genetic diversity in the resulting seed orchard.

Our results suggests that, number of plus tree clones (25) used in the establishment of Anatolian black pine seed orchards was enough to maintain the high level of diversity in seed orchards since Anatolian black pine maintains high levels of genetic diversity within populations, if these numbers could be maintained in the future breeding and plantation program.

Although the genetic diversity of the plantations studied was comparable, our results suggests that their genetic base may be different because dendrograms constructed with genetic distance values based on origins produced conflicting results for 2 origins (Burhandağ and Balıköy). In order to maintain genetic diversity in plantations their genetic base needs to be broadened and seed collections need to be monitored so that the seed is a mixture from a large number of trees. Therefore, seeds must be collected in a good crop year, from large number of trees as much as possible. In addition, generally, these plantations are established with nursery raised seedlings as in our case. Nursery raised seedlings are subject to a number of selective forces that may also affect genetic diversity, so they should be monitored carefully.

There are several studies, examining the impact of forestry practices such as selection of seed stands, silviculture, and breeding on genetic diversity, produced diverse results. Johnson and Lipow (2002) summarized results of the 14 allozyme studies comparing genetic variation between seed orchards and natural populations. In general, seed orchards retain most of the genetic variation present in their natural counterparts. In most studies allelic richness and proportion of polymorphic loci values did not differ significantly between groups.

Genetic diversity within a white spruce (*Picea glauca*) seed orchard (40 clones) and a jack pine (*Pinus banksiana* Lamb.) seed orchard (31 clones) was assessed and compared with genetic diversity in natural populations within the source area for the orchards (Godt *et al*., 2001). Gene diversity maintained within the seed orchards (H_e=0.157 for white spruce and 0.114 for jack pine) was similar to that found within the source area (H_e=0.164 and 0.114 for white spruce and jack pine). Mean genetic identities between the seed orchards and their natural populations were high (>0.99), indicating that common allele occurrences and frequencies were similar between the orchards and their source area.

As in the case of our study, other studies involving the direct comparison of genetic variation within seed orchards and natural populations generally confirm similar genetic variation (Knowles, 1985; Chaisurisri and El-Kassaby; 1994; El-Kassaby and Ritland, 1996; Schmidtling and Hipkins, 1998; Godt *et al*., 2001).

CHAPTER VI

CONCLUSION

The results of this study demonstrated that RAPD markers have considerable practical utility in monitoring changes in genetic diversity of Anatolian black pine natural seed sources as well as managed populations due to forestry practices.

One clear result to emerge from this study is that; estimated genetic diversity parameters; which are allelic richness, Shannon's information index, proportion of polymorphic loci and heterozygosity values, were found to be generally high in studied Anatolian black pine seed sources.

Current study has shown that the loss of genetic variation through inadequate sampling does not appear to have taken place in any of the managed seed sources. Therefore, in general, forestry practices seem to be capturing the existing genetic diversity in natural populations (seed stands) for seed orchards and plantations. Nevertheless, seed sources at different categories for some localities had altered genetic structure that requires specific monitoring during different stages of forestry practices.

Our results indicate that the majority of genetic diversity is contained within Anatolian black pine seed sources but there is still appreciable differentiation among seed sources, that is, studied seed sources are characterized by high intra-population variation.

The present data demonstrate that number of plus tree clones (25) used in the establishment of seed orchards was adequate to capture the high level of diversity in seed orchards at least at the early stages of the breeding program of Anatolian black pine. But in the future, selection and advance breeding activities will cause reduction of number of clones in seed orchards; therefore number of clones should be increased.

Genetic diversity parameters estimated for two varieties of Anatolian black pine also imply considerable genetic variation in those seed sources. They formed a distinct cluster in dendrogram, claiming that these two seed sources are appreciably different from natural stands. However, further study is needed to illustrate these differences with larger samples or different genetic markers.

REFERENCES

Aagaard, J. E., Krutovskii, K. V. and Strauss, S., H. 1998. RAPD markers of mitochondrial origin exhibit lower population diversity and higher differentiation than RAPDs of nuclear origin in Douglas fir. Mol. Ecol. 7: 801-812.

Acatay, A. 1956. Ehrami Karaçam (*Pinus nigra* var. *pyramidata*). İ.Ü. Orman Fakültesi Dergisi. Seri A, 6/2: 92-99.

Adams, W.T. and Birker, D.S. 1991. Estimating mating patterns in forest tree populations. In Fineschi, S., Malvotti, M.E., Cannata, F. and Hattemer, H.H. (Eds.) Proceedings of International Workshop on Plant Biology, Biochemical Markers in Population Genetics of Forest Trees. Institute for Agro forestry of the national Research Council of Italy.

Adams R.P. and Demeke, T. 1993. Systematic relationships in *Juniperus* based on RAPDs. Taxon 42:553-571.

Alptekin, Ü. 1986. Anadolu karaçamı (*Pinus nigra* Arn. subsp. *pallasiana Lamb.*)'nın coğrafik varyasyonları. İ.Ü. Orman Fakültesi, Ph. D. Thesis. 170 pp.

Anonymous, 1993. Birinci Ormancılık Şurası. T.C. Orman Bakanlığı Yayınları, Yayın No:13/6. Ankara.

Anonymous, 2000. Türkiye'nin Tabiatı Koruma Alanları. United Nations Development Programme (GEF/SGP). Kırsal Çevre ve Ormancılık Sorunları Araştırma Derneği Yayın No: 9. Dönmez Ofset, Ankara. 166 pp.

Anonymous, 2001. Sekizinci beş yıllık lakkınma planı. Ormancılık Özel İhtisas Komisyonu Raporu. Devlet Planlama Teşkilatı Yayın No: 2531, ÖİK: 547. Ankara

Asan, Ü. 1999. Anıtsal Karaçamlar. In: 1. Uluslararası Ehrami Karaçam Sempozyumu. 23-25 Eylül 1999. Kütahya/Türkiye.

Barrett, S.C.H. and J. R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: Implications for conservation. In: Falk, D.A. and Holsinger, K.E. (Eds.) Genetics and Conservation of Rare Plants. Oxford University Press, New York.

Belkhir, K., Borsa, P., Chikhi, L., Goudet, J. and Bonhomme F. 1996-2000. Genetix 4.00 Windows^{TM} software for population genetics. Laboratoire Génome, Populations, Interactions, University of Montpellier, France. http://www.univ-montp2.fr/~genetix/genetix/genetix.htm. Last Accessed Date: December 2004.

Bernatsky, R. and Tanksley, S.D. 1986. Towards a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics. 112: 887-898.

Bonnet-Masimbert, M., and Bikay-Bikay, V. 1978. Intraspecific variability of isozymes of glutamate-oxaloacetate-transaminase in *Pinus nigra* Arnold of value in the taxonomy of the species. Silvae Genetica. 27:71-79.

Brown, A.H.D. 1979. Enzyme polymorphism in plant populations. Theor. Pop. Biol. 15: 1-42.

Brown, A.H.D. and Moran G.F. 1981. Isozymes and the genetic resources of forest trees. In: Conkle, M.T., Tech. Coord., Proc. Symp. Isozymes of North American Forest Trees and Forest Insects, pp. 1-10. USDA Forest Service Technical Report PSW-48.

Brown, J.R., Beckenbach, A.T. and Smith, M.J. 1992. Mitochondrial DNA length variation and heteroplasmy in populations of white sturgeon (*Acipenser transmontanus*). Genetics. 132: 221-228.

Borzan, Z. 1981. Karyotype analysis from the endosperm of European black pine and Scots pine. Ann. For. 10/1: 1-42.

Boydak, M. 1989. Türkiye'de Anadolu Karaçamının Yeni Bir Varyetesi. İ.Ü. Orman Fakültesi Dergisi A39: 119-129.

Boydak, M. 2001. A new variety of *Pinus nigra* Arn. Subsp. *pallasiana* from Anatolia. The Karaca Arboretum Magazin. 6(1): 15-23.

Bucci, G. and Menozzi, P. 1993. Segregation analysis of random amplified polymorphic DNA (RAPD) markers in *Picea abies* Karst. Molecular Ecology. 2: 227-232.

Carlson, J.E., Tulsiearam, L.K., Glaubita, J.C., Luk, V.W.K., Kauffeldt, C. and Rutledge, R. 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl. Genet. 83: 194-200.

Castiglione, S., Wang, G., Damiani, G., Bandi, C., Bisoffi, S. and Sala, F. 1993. RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor. Appl. Genet. 87: 54-59.

Cervera, M.T., Plomion, C. and Malpica, C. 2000. Molecular markes and genome mapping in woody plants. In: Jain, S.M. and Minocha, S.C. (Eds.). Molecular biology of woody plants. Forestry Sciences, Volume 64. Kluwer Academic Publishers, The Netherlands.

Cesarone, C., Bolognesi, C. and Santi, L.1979. Improved microfluorometric DNA determination in biological material using 33258 Hoechst. Anal Biochem. 100: 188-197.

Chaisurisri, K. and El-Kassaby, Y.A. 1994. Genetic diversity in a seed production population vs. natural population of Sitka spruce. Biodiv. Conserv. 3: 512-523.

Chalmers, K.J., Newton, A.C., Waugh, R., Wilson, J. and Powell, W. 1994. Evaluation of the extent of genetic variation in mahoganies (*Meliaceae*) using RAPD markers. Theor. Appl. Genet. 89: 504-508.

Copes, D.L. 1981. Isoenzyme uniformity in western red cedar seedlings from Oregon and Washington. Can. J. For. Res. 11: 451-453.

Cotterill, P.P. (1986). Genetic gains expected from alternative breeding strategies including simple low cost options. Silvae Genetica 34: 64-68.

Çengel, B., Velioğlu, E., Tolun, A.A., and Kaya, Z. 2000. Pattern and Magnitude of Genetic Diversity In *Pinus nigra* Arnold Subspecies p*allasiana* Populations from Kazdağı: Implications For In Situ Conservation. Silvae Genetica: 49 (6): 249-256.

Dallas, J.F. 1988. Detection of DNA 'fingerprints' of cultivated rice by hybridization with a human mini-satellite probe. Proc. Natl. Acad. Sci. (USA). 85: 6831-6835.

Davis, P.H. 1965. Flora of Turkey and East Aegean Islands. Volume 1, pp. 74. University of Edinburgh Press, Edinburgh.

Davis, D.D., Umbach, D.M. Coppolino, J.B. 1981. Susceptibility of tree and shrub species and response of black cherry foliage to ozone. Plant Disease 65(11): 904-907.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA minipreparation: version II. Plant Mol. Biol Rep. 1:19-21.

Diaz, V., Muniz, M. and Ferrer, E. 2001. Random Amplified Polymorphic DNA and Amplified Fragment Length Polymorphism assessment of genetic variation in Nicaraguan populations of *Pinus Oocarpa*. Molecular Ecology. 10: 2593-2603.

Doğan, B., Özer, S., Gülbaba, G., Velioğlu, E., Doerksen, A.H., Adams, W.T. 1998. Inheritance and linkage of allozymes in black pine (*Pinus nigra* Arnold) pp.249-257. In: Zencirci, N., Kaya, Z., Anikster, Y. and Adams, W.T. (Eds.). The Proceedings of International Symposium on *In Situ* Conservation of Plant Genetic Diversity. Central Research Institute for Field Crops, Ankara-Turkey.

Economou, A. 1990. Growth intercept as an indicator of site quality for planted and natural stands of *Pinus nigra* var. *pallasiana* in Greece. Forest Ecol. Manag. 32:103-115.

El-Kassaby, Y.A. 1989. Genetics of seed orchards: expectations and realities. In: Proc. 20th South Forest Tree Improvement Conference, Charleston, South Carolina.

El-Kassaby, Y.A. 1992. Domestication and genetic diversity-should we be concerned? For. Chron., 68: 687-700.

El-Kassaby, Y.A. 1995. Evaluation of the tree improvement delivery system: factors affecting genetic potential. Tree Physiology 15: 545-550.

El-Kassaby, Y. A. and Ritland, K. 1996. Impact of selection and breeding on the genetic diversity in Douglas fir. Biodiversity Conservation. 5: 795-813.

El-Kassaby, Y. A., Meagher, M. D., Parkinson, J. and Portlock, F. T. 1987. Allozyme inheritance, heterozygosity and out crossing rate among *Pinus monticola* near Ladysmith, British Columbia. *Heredity* 58: 173-173

Elkiey, T., Ormrod, D. P. and Marie, B. 1982. Foliar sorption of sulfur dioxide, nitrogen dioxide and ozone by ornamental woody plants. Hortscience, 17(3): 358-360.

Ellstrand, H. 1992. Gene flow by pollen: implications for plant conservation genetics. Oikos. 63: 77-86.

Ellsworth, D.L. and Honeycutt, R.L. 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. BioTechniques. 14: 214-218.

Eriksson, G., Schelander, B. and Akebrand, V. 1973. Inbreeding depression in and old experimental plantation of *Picea abies*. Hereditas 73:160-166

Fady, B. and Conkle, M.T. 1993. Allozyme variation and possible phylogenetic implications in *Abies cephalonica* and some related eastern Mediterranean firs. Silvae Genetica 42: 351-359.

Falconer, D. S. 1989. Introduction to quantitative genetics. 3rd Edition. Longman Scientific and Technical, Essex, England.

Fineschi, S. 1984. Determination of the origin of an isolated group of trees of *Pinus nigra* through enzyme gene markers. Silvae Genetica 33:169-172.

Fowler, D.P. and Morris, R.W. 1977. Genetic diversity in red pine: evidence for genetic heterozygosity. Can. J. For. Res. 7: 343-347.

Francis, H.A., Leitch, A.R. and Koebner, R.M.D. 1995. Conversion of a RAPD-generated PCR product, containing a novel dispersed repetitive element, into a fast and robust assay for the presence of rye chromatin in wheat. Theor. Appl. Genet. 90: 636-642.

Frankel, O.H., Brown, A.H. and Burdon, J.J. 1995. The conservation of plant diversity. Cambridge University Press.

Frey, K. 1991. Plant Breeding II. The Iowa State University Press. Ames, Iowa.

Fukarek, P. 1958. Standortrassen der Schwarzföhre (*Pinus nigra* Arn.sen.lat.) Centralblatt f.d. gesamate Forstwesen 75: 203-207.

Gallois, A., Audran, J. C. and Burrus, M. 1998. Assessment of genetic relationships and population discrimination among *Fagus sylvatica* L. by RAPD. Theor. Appl. Genet. 97: 211-219.

Garcia, G.M., Stalker, H.T., Shroeder, E. And Kochert, G. 1996. Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. Genome. 39: 836-845.

Glaubitz, J. and Moran, G.F. 2000. Genetic tools: the use of molecular markers. In: Young, A., Boshier, D. and Boyle, T. (Eds.) forest Conservation genetics. CABI Publishing, UK.

Godt, M.J.W., Hamrick, J.L., Edwards-Burke, M.A. and Williams, J.H. 2001. Comparisons of genetic diversity in white spruce (*Picea glauca*) and jack pine (*Pinus banksiana*) seed orchards with natural populations. Can. J. For: Res. 31: 943-949.

Govindaraju, D., Dancik, B.P. and Wagner, D.B. 1989. Novel choloroplast DNA polymorphisims in a sympatric region of two pines. Journal of Evolutionary Biology 2: 49-59.

Govindaraju, D., Lewis, P. and Cullis, C. 1992. Phylogenetic analysis of pines using ribosomal DNA restriction fragment length polymorphisms. Plant Systematics and Evolution.

Göker, Y. 1969. Dursunbey ve Elekdağ karaçamları (*Pinus nigra* subsp. *pallasiana*)'nın Fiziksel, Mekanik Özellikleri ve Kullanılış Yerleri hakkında Araştırmalar. İ.Ü. Orman fakültesi Dergisi, Seri A, Cilt XIX, Sayı 2.

Gömöry, D. 1992. Effect of stand origin on the genetic diversity of Norway spruce (*Picea abies* Karst.) populations. Forest Ecology and Management 54: 215-223.

Güner, A., Özhatay, N., Ekim, T. and Başer, K.H.C. 2000. Flora of Turkey and East Aegean Islands (Supplement 2), Volume 11. University of Edinburgh Press, Edinburgh.

Hamrick, J.L. 1979. Genetic variation and longevity. In: Solbrig, O.T., Jain, S. and Raven, P.H. (Eds), Plant Population Biology, pp. 84-113. Columbia University Press, N.Y.

Hamrick, J.L. 1982. Plant population genetics and evolution. Amer. J. Bot. 69: 1685-1693.

Hamrick, J.L. and Godt M.J.W. 1989. Allozyme diversity in plant species. In: Urbanski, K. (ed). Differentiation patterns in higher plants. pp. 53-67. Academic Press, N.Y.

Hamrick, J.L. and Godt, M.J.W. 1990. Allozyme diversity in plant species. In: Plant population genetics, breeding and genetic resources. Brown A.H.D., Clegg, M.T., Kahler, A.L. and Weir B.S. (eds.) pp. 43-63. Sinauer Associates. Sunderland, USA.

Hamrick, J.L. and Godt, M.J.W. 1996. Conservation genetics of endemic plant species. In: Avise, J.C and Hamrick, J.L. (Eds), pp. 281-301. Conservation Genetics, Chapman and Hall, New York.

Hamrick, J.L., Linhart, Y.B. and Mitton, J.B. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10: 173-200.

Harju, A. 1995. Genetic functioning of Scots pine seed orchards. Ph.D. thesis. University of Oulu, Finland. Acta Universitatis Ouluensis 271.

Harris, H. 1996. Enzyme polymorphism in man. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 164: 298-310.

Hartl, D.L. and Clark, A.G. 1997. principles of populatipn genetics. Sinauer Associates, Sunderland, MA.

Hattemer, H.H., H.R. Gregorius, M. Ziehe and G. Müller-Starck. 1982. Klonanzahl forstlicher Samenplantagen und genetische Vielfalt. Allg. Forst- u. J.-Ztg. 153:183-191

Hedrick, P. 1992. Shooting the RAPDs. Nature. 335: 679-680.

Hedrick, P.W. 1985. Genetics of populations. Jones and Barlett Publications Inc., Boston.

Helentjaris, T. Slocum, M., Wright, S., Schaefer, A. and Nienhuis, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72: 761-769.

Hsiang, T. and Huang, J. 2000. The use of RAPD markers to distinguish juniper and cedar cultivars. Can. J. of Bot. 78:655-658.

Hubby, J. L. and Lewontin, R.. C. 1966. A molceular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics. 54: 577-594.

Hunter, R. L. and Market, C. L. 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science 125: 1294-1295.

Isabel, N., Beaulieu, J. and Bousquet, J. 1995. Complete congruence between gene diversity estimates derived from genetypic data at enzyme and random amplified polymorphic DNA loci in black spruce. Proc. Natl. Acad. Sci. USA. 92: 6369-6373.

Isabel, N., Beaulieu, J., Theriault, P. and Bousquet, J. 1999. Direct evidence for biased gene diversity estimates from dominant RAPD fingerprints. Mol. Ecol. 8: 477-483.

Isajev, V., Fady, B., Semerci, H. and Andonovski, V. 2004. EUFORGEN Technical guidelines for genetic conservation and use for European black pine (*Pinus nigra*). International Plant Genetic Resources Institute, Rome, Italy, 6 pages.

Işık, K. 1990. Seasonal course of height and needle growth in *Pinus nigra* grown in summer-dry central Anatolia. Forest Ecol Manag. 32:103-115.

Işık, K., Kaya, Z. and Atalay, İ. 1995. Biodiversity action plan for Turkey: Forest Ecosystems. The Report Submitted to the World Bank. Ankara, 117 p.

İçgen, Y., Kaya, Z., Çengel, B., Velioğlu, E., Öztürk, H. and Önde, S. 2005. Potential impact of forest management and breeding practices on established *Pinus brutia* plantations. Forest Ecology and Management. Submitted.

Johnson, R. and Lipow, S. 2002. Compatibility of Breeding for Increased Wood Production and Long-term Sustainability: The Genetic Variation of Seed Orchard Seed and Associated Risks. Proc. From the Wood Comp. Initiative Workshop 18:169-179.

Johnson, R., St Clair, B. and Lipow S. 2001. Genetic Conservation in Applied Tree Breeding Programs. In: Thielges, B. (Ed). Proceedings, international conference on *ex-situ* and *in-situ* conservation of commercial tropical trees. Yogykarta, Indonesia: Faculty of Forestry, Gadjah Mada University: 215-230.

Kaya, Z. 1998. Current status of forest genetic resources in Turkey. In: Zencirci, N., Kaya, Z., Anikster, Y. And Adams, W.T. (eds), Proceedings of the International Symposium on *in situ* Conservation of Plant Genetic Diversity, pp. 17-31. Central Research Institute for Field Crops, Ankara, Turkey.

Kaya, Z. and Neale, D.B. 1993. Random Amplified Polymorphic DNA (RAPD) Polymorphisms in *Pinus nigra* var. *pallasiana* and *Pinus brutia*. Doğa Turkish Journal of Agriculture and Forestry. 17: 295-306.

Kaya, Z. and Temerit, A. 1994. genetic structure of marginally located *Pinus nigra* var. *pallasiana* populations in central Turkey. Silvae Genetica 34:148-156.

Kaya, Z., Ching, K.K., Stafford, S.G. 1985. A statistical analysis of karyotypes of European black pine (P*inus nigra* Arn.) from different sources. Silvae Genetica. 34(4-5): 148-156.

Kaya, Z., Kün, E. and Güner, A. 1997. National plan for *in situ* conservation of genetic diversity in Turkey. Submitted to the Republic of Turkey, The Ministry of Environment, Ankara. 125p.

Kazan, K., Manners, J.M. and Cameron, D.F. 1993. Inheritance of random amplified polymorphic DNA markers in an interspecific cross in the genus *Stylosanthes*. Genome, 36: 50.56.

Keil, M and Griffin, A.R. 1994. Use of randomly amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theor. Appl. Genet. 89: 442-450.

Kimura, M. and Crow, J.M. 1964. The number of alleles that can be maintained in a finite population. Genetics 49, pp. 725-738.

Kimura, M. and Crow, J.M. 1978. Effect of overall phenotypic selection on genetic change at individual loci. Proc. Natl. Acad. Sci., Washington. 75: 6168-6171.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and plantations: jack pine and black spruce. Can. J. For. Res. 15: 902-908.

Koski, V. and Antola, J. 1994. National Tree Breeding and Seed Production Programme for Turkey: 1994-2003. Prepared in Cooperation with The Research Directorate of Forest Tree Seeds and Tree Breeding. ENSO Forest Development OY LTD.

Karkkainen and Savolainen, 1993. The degree of early inbreeding depression determines the selfing rate at the seed stage: model and results from *Pinus sylvestris* (Scots pine). Heredity 71:160-166.

Kreike, J. 1990. Genetic analysis of forest tree populations: isolation of DNA from spruce and fir apices. Plant Molecular Biology, 14: 877-879.

Krupkin, A.B., Liston, A., and Strauss, S.H. 1996. Phylogenetic analysis of the hard pines (*Pinus* subgenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. American Journal of Botany 83: 489-498.

Lande, R. 1995. Mutation and conservation. Conservation Biology. 9: 782-791.

Langella, O. 2000. POPULATIONS: Population genetics software (Individuals or populations distances, phylogenetic trees). CNRS, France. http://pge.cnrs-gif.fr/bio-info/populations. Last accessed date: June 2005.

Ledig, F.T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. In: Soule, E. (Ed), Conservation Biology: The Science of Scarcity and Diversity, pp. 74-104. Sinauer Association, Sunderland Massachusetts.

Ledig, F.T. 1988. The conservation of diversity in forest trees. BioScience. 38: 471-479.

Ledig, F.T. 1992. Human impacts on genetic diversity in forest ecosystems. Oikos. 63: 87-108.

Ledig, F.T. 1998a. Genetic diversity in tree species: with special preference to conservation in Turkey. In: Zencirci, N., Kaya, Z., Anikster, Y. And Adams, W.T. (eds), Proceedings of the International Symposium on *in situ* Conservation of Plant Genetic Diversity, pp. 231-248. Central Research Institute for Field Crops, Ankara, Turkey.

Ledig, F.T. 1998b. Genetic variation in *Pinus*. In: Richardson (Ed). Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge.

Ledig, F.T. and Conkle, M.T. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry *ex* Carr.). Evolution. 37: 79-85.

Leven, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91-94.

Lewontin, R.C. 1972. The apportionment of human diversity. Evolutionary Biology. 6: 381-398.

Lewontin, R., C. and Hubby, J. 1966. A molceular approach to the study of genic heterozygosity in natural populations II. Amounts of variation and degree of heterozygosity in natural populations of *Drospophila pseudoobscura*. Genetics. 54: 595-609.

Lin, D., Hubbies, M. and Zsuffa, L. 1994. Differentiation of poplar and willow clones using RAPD fingerprinting. Tree Physiol. 14: 1097-1105.

Linhart, Y.B. 2000. Variation in woody plants: molecular markers, evolutionary processes and conservation biology. In: Jain, S.M. and Minocha, S.C. (Eds.) Molecular biology of woody plants. Forestry Sciences, Volume 64. Kluwer Academic Publishers, The Netherlands.

Lise, Y. 2001. The impact of anthropogenic factors on the composition of genetic variation on *Pinus brutia* Ten. Populations determined by DNA markers. Master Thesis. Middle East Technical University, Ankara.

Loveless, M.D. and Hamrick, J.L. 1984. Ecological determinants of genetic genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15: 65-95.

Lu, M.-Z., Szmidt, A.E. and Wang, X.-R. 1995. Inheritance of RAPD fragments in haploid and diploid tissues of *Pinus sylveristis* (L.). Heredity. 74: 582-589.

Lu, M.-Z., Wang, X.-R. and Szmidt, A.E. 1997. Molecular properties of RAPDs in *Pinus sylveristis* (L.) and their implications for genetic analysis. Forest Genetics.

Lynch, M. 1995. A quantitative genetic perspective on conservation issues. In: Avise, J.C. and Hamrick, J.L. (Eds.) Conservation Genetics: Case histories form nature. Chapman and Hall, New York.

Mallet, J. 1995. A species definition for the modern synthesis. Trends Ecol. Evol. 10: 294-299.

Mandal, A.K. and Gibson, G.L. (Eds) 1998. Forest Genetics and Tree Breeding. CBS Publishers, New Delhi, India.

Matziris, D. I. 1989. Variation in growth and branching characters in black pine (*Pinus nigra*) of Peloponnesos. Silvae Genetica 38:77-81.

Melotto, M., Afandor, L. and Kelly, J.D. 1996. Development of a SCAR marker linked to the I gene in common bean. Genome. 39: 1216-1219.

Millan, T., F. Osuna, S. Cobos, A.M. Torres and J.I. Cubero. 1996. Using RAPDs to study phylogenetic relationships in Rosa. Theor. Appl. Genet. 92:273-278.

Millar, C. J. and Libby, W.J. 1991. Strategies for conserving clinal, ecotypic and disjunct populations diversity in widespread species. In: Falk, D.A. and Holsinger, K.E (Eds.) Genetics and Conservation of Rare Plants. Oxford University Press. New York.

Mirov, N.T. 1967. The Genus *Pinus*. New York: The Ronald Press. 602.

Mitchell-Olds, T. and Rutledge, J.J. 1986. Quantitative Genetics in Natural Plant Populations: A Review Of The Theory. Am. Nat. 127:379-402.

Mitton, J.B. 1983. Conifers. In: Tanksley, S.D. and Orton T.J. (eds.). Isozymes in Plant Genetics and Breeding. Pp. 443-472. Elsevier Science Publishers B.V., Amsterdam.

Morell, M.K., Peakall, R., Appels, R., Preston, L.R. and Lloyd H.L. 1995. DNA profiling techniques for plant variety identification. Australian Journal of Experimental Agriculture. 35:807-819.

Muona, O. and Harju, A. 1989. Effective population sizes, genetic variability, and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genet. 38: 221-228.

Namkoong, G. 1984. Strategies for gene conservation in tree breeding. In: Yeatman, D., Kafton, D. and Wilkes, G. (Eds.) Plant Genetic resources: A Conservation Imperative. Westview Press, Boulder, Colo., Am. Assoc. Adv. Sci. Ser. Symp. 87.

Neale, D. 1985. Genetic implications of shelterwood regeneration of Douglas-fir in south-west Oregon. For. Sci. 31:995-1000.

Neale, D. and Williams, C. 1991. Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can. J. For. Res. 21: 545-554.

Neale, D.B., Devey, M.E., Jermstad, K.D., Ahuja, M.R., Alosi, M.C. and Marshall, K.A. 1992. Use of DNA markers in forest tree improvement research. New Forests. 6: 391-407.

Nei, M. 1972. Genetic distance between populations. American Naturalist. 106: 283–292.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89: 583-590.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia Uni. Press, Newyork.

Nesbitt, K.A., Potts, B.M., Vaillencourt, R.E., West, A.K. and Reid J.B. 1995. Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtacea). Heredity. 74: 628-637.

Nesbitt, K. A., Potts, B. M., Vaillancourt, R. E. And Reid, J. B. 1997. Fingerprinting and pedigree analysis in *Eucalyptus globulus* using RAPDs . Silvae Genetica 46(1): 6-11.

Nikolic, D. and Tucic, N. 1983. Isoenzyme variation within and among populations of European black pine (*Pinus nigra* Arn.). Silvae Genetica. 32 (3-4): 80-89.

Nybom, H. and Schaal, B.A. 1990. DNA 'fingerprints' applied to paternity analysis in apples (*Malus* x *domestica*). Theor. Appl. Genet. 79: 763-768.

Özel, E. 2001. The pattern of genetic variation of *Pinus brutia* Ten. Populations from southern Turkey, determined by nuclear SSR markers. Master Thesis. Middle East Technical University, Ankara.

Penner, G.A., Bush, A., Wiese, R., Kim, W.D., Kasha, K., Laroche, A., Scoles, G., Molnar, S.L. and Fedak, G. 1993. Reproducibility of random amplified DNA, RAPD analysis among laboratories. PCR Methods Applications. 2: 341-345.

Pillay, M. and Kenny, S.T. 1996. Random amplified polymorphic DNA (RAPD) markers in hop, *Humulus lupulus*: Level of genetic variability and segregation in F-1 progeny. Theoretical and Applied Genetics. 92: 334-339.

Plessas, M.E. and Strauss, S.H. 1986. Allozyme differentiation among populations, stands and cohorts in Monterey pine. Can. J. For Res. 16: 1155-1164.

Portfaix, C. 1989. exploration of genetic variability of 5 natural stands of Corsican pine (*Pinus nigra* ssp. *laricio* var c*orsicana*). Ann. Sci. For. 46:217-232.

Rajora, O.P. 1999. Genetic biodiversity impacts of silvicultural practices and phenotypic selection in white spruce. Theor. Appl. Genet. 99: 954-961.

Reiter, R.S., Williams, J.G.K., Feldmann, K.A. and Rafalski, J.A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA. 89: 1477-1481.

Riedy, M., Hamilton, W.I., and Aquadro, C. 1992. Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR. Nucleic Acid Research. 20: 918.

Ronning, C.M., Schnell, R. J. and Kuhn, D.N. 1995. Inheritance of random amplified polymorphic DNA (RAPD) markers in *Theobroma cacao* L. Journal of the American Society for Horticultural Science. 120: 681-686.

Röhrig, E. 1956. Über die Schwarzkiefer und ihre formen. Silvae genetica. Vol.6.

Russel, J.R., Hosein, F., Johnson, E., Waugh, R. And Powell, W. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. Molecular Ecology. 2: 89-97.

Saitou, N. and Nei, M. 1987. The neighbor joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sanchez, N., Grau, J.M., Manzenera, J.A. and Bueno, M.A. (1998). RAPD markers for the identification of *Populus* species. Silvae Genetica. 47: 67-71.

Savolainen, O. and Karkkainen, K. 1992. Effect of forest management on gene pools. New For., 6: 329-345.

Savolainen, O. and Karhu, A. 2000. Assessment of biodiversity with molecular tools in forest trees. In: Jain, S.M. and Minocha, S.C. (Eds.) Molecular biology of woody plants. Forestry Sciences, Volume 64. Kluwer Academic Publishers, The Netherlands.

Saylor, L.G. 1964. Karyotypic analysis of *Pinus* – group *Lariciones*. Silvae Genetica 13: 167-170.

Scaltsoyiannes, A., Rohr, R., Panetsos, K.P., and Tsaktsira, M. 1994. Allozyme frequency distributions in five European populations of black pine (*Pinus nigra* Arn.). I) Estimation of genetic variation within and among populations. II) Contribution of isozyme analysis to the taxonomic status of the species. Silvae Genetica. 43(1): 20-30.

Schmidtling, R.C and Hipkins, V. 1998. Genetic diversity in long-leaf pine (*Pinus palustris*): influence of historical and prehistorical events. Can. J. For. Res. 28: 1135-1145.

Schwarz, O. 1938. Uber die systematik und nomenklatur der europaischen Schwarzkiefern. Notizblatt des Bot. Garten zu Berlin Dahlem XIII. 117: 226-243.

Shaw, D.V. and Allard, R.W. 1982. Estimation of outcrossing rates in Douglas fir using isozyme markers. Theor. Appl. Genet. 62: 113-120.

Silin, A.E and Goncharenko, G.G. 1996. Allozyme variation in natural populations of Eurasian pines. IV. Population structure and genetic variation in geographically related and isolated populations of Pinus nigra Arnold on the Crimean peninsula. Silvae Genetica. 45 (2-3):67-75.

Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787-792.

Sneath, P.H.A. and Sokal, R. R. 1973. Numerical Taxonomy. Freeman. San Francisco.

Soydinç, M. 1959. Türkiye'nin en büyük karaçamı. Yeşil Ufuk Dergisi, Sayı:4.

Stoehr, M.U. and El-Kassaby, Y.A. 1997. Levels of genetic diversity at different stages of the domestication cycle of interior spruce in British Columbia. Theor. Appl. Genet. 94: 83-90.

Strauss, S.H. and Doerksen, A.H. 1990. Restriction fragment analysis of pine phylogeny. Evolution 44: 1081-1096.

Strauss, S.H., Bousquet, J., Hipkins, V. and Hong, Y.-P. 1992. Biochemical and molecular genetic markers in biosystematic studies of forest trees. New Forests 6: 125-158.

Swofford, D. and Selander, R. B. 1989. BIOSYS-1, A computer program for the analysis for allelic variation in population genetics and biochemical systematics. Release 1.7.

Szmidt, A.E., and O. Muona. 1985. Genetic effects of Scots pine (*Pinus sylvestris* L.) domestication. pp. 241-252. In: H.-R. Gregorius (Ed.), Population Genetics in Forestry. Lecture Notes in Biomathematics No. 60. Springer-Verlag, Berlin.

Szmidt, A.E., Wang, X-R. and Lu, M.-Z. 1996. Emprical assessment of allozyme and RAPD variation in *Pinus sylvestris* (L.) using haploid tissue analysis. Heredity, 76: 412-420.

Şimşek, Y., Erkuloğlu, Ö.S., Tosun, S. 1995. Türkiye'de karaçam (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) orijin denemelerinin ilk sonuçları. OAE Teknik Bülten. No: 247. Ankara-Türkiye.

Tanksley, S.D. 1983 Molecular markers in plant breeding, Plant. Mol. Biol. Rep. 1:3-8

Thomas, B.R., Macdonald, S.E., Hicks, M., Adams, D.L. and Hodgetts, R.B. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: an assesment using microsatellite and randomly amplified polymorphic DNA markers. Theor. Appl. Genet. 98: 793-801.

Tolun, A.A., Velioğlu, E., Çengel, B., and Kaya, Z. 2000. Genetic structure of black pine (*Pinus nigra* Arn. subsp. *pallasiana*) populations sampled from the Bolkar Mountains.

Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D.H., Walters, S.M. And Webb, D.A. (Eds.) 1964. Flora Europaea Vol 1. Cambridge University Press, 464 P.

Üçler, A.Ö. and Gülcü, S. 1999. Isparta göller yöresi doğal Anadolu karaçamı (*Pinus nigra* Arnold subspecies *pallasiana* Lamb. Holmboe) alanlarından örneklenen bazı populasyonlarda kozalak ve tohum morfolojisi varyasyonları. 1. Uluslararası Ehrami Karaçam Sempozyumu. Kütahya.

Van Haverbeke, D. F. 1990. *Pinus nigra* Arnold, European Black Pine. In: Burns, R. M. and Honkala, B. H. Technical Coordinators. Silvics of North America: 1. Conifers. Agriculture Handbook 654. U.S. Deptartment of Agriculture, Forest Service, Washington D.C.

Velioğlu, E., Çengel, B., Kaya, Z. 1999. Kazdağları doğal karaçam (*Pinus nigra* Arnold subspecies *pallasiana*) populasyonlarında genetik çeşitliliğin yapılanması. OATIAM. Teknik Bülten No:1. Ankara.

Vicario, F., Vendramin, G. G., Rossi, P., Leo., P. and Giannini, R.1995. Allozyme, chloroplast DNA and RAPD markers for determining genetic relationships between

Abies alba and the relic population of *Abies nebrodensis*. Theor. Appl. Genet. 90:1012-1018.

Vidakovic, M. 1974. Genetics of European Black pine (*Pinus nigra* Arn.) Ann. Forest. 6/3: 57-86.

Vidakovic, M. 1991. Conifers: morphology and variation. Graficki Zavod Hrvatske, Zagreb, Crotia, 755 pp.

Villar, E., H. 1947. Quel est le nom valable du *Pinus laricio* Poir. Bericht der Schweiz. Botan. Gesellsch. LVII, 152.

Vos, P., Hogers, R., Bleeker, M., Reijans, M, Vandelee, T., Hornes, M, Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research. 23:4407-4414.

Wang, X.-R, and Szmidt, A.E. 1993. Chloroplast DNA-based phylogeny of Asian *Pinus* species (Pinaceae). Plant Systematics and Evolution. 188: 197-211.

Wang, X-R and Szmidt, A.E. 2001. Molecular markers in population genetics of forest trees. Scand. J. For. Res. 16: 199-220

Wang, X.-R, Tsumura, Y., Yoshimaru, H., Nagasaka, K., Szmidt, A.E. 1999. Phylogenetic relationships of Eurasian pines (*Pinus*, Pinaceae) based on chloroplast RBCL, MATK, RPL20-RPS18 spacer and TRNV intron sequences. Amarican Journal of Botany 86(12): 1742-1753.

Weaver, R.F. and Hedrick, P.W. 1989. Genetics, Wm. C. Brown Publishers, Iowa.

Weir B.S. and Cockerham C.C. 1984. Estimating F statistics for the analysis of population structure. Evolution, 38: 1358-1370.

Wendel, J. F. and Weeden, N. F. 1989. Visualization and interpretation of plant isozymes. In: Soltis, D. E. and Soltis, P. S. (Eds). Isozymes in Plant Biology, pp. 5-45. Chapman and Hall, London.

Williams, J.G.K., Hanafey, M.K., Rafalski, J.A. and Tingey, S.V. 1993. Genetic analysis using random amplified polymorphic markers. Methods in Enzymology. 218:704-740.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18: 6531-6535.

Woodland, D.W. 1997. Contemporary Plant Systematics. 2nd Edition. Berrien Springs, Michigan, Andrews University Press. USA.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics. 15: 323-354.

Wright, S. 1969. Evolution and Genetics of Populations: The Theory of Gene Frequencies. Chicago. University of Chicago Press.

Wright, S. 1978. Evolution and Genetic Populations. Vol 4, Variability Within and Among Populations. University of Chicago Press, Chicago, USA.

Yaltırık, F. 1986. Ülkemizin az tanınan iki çam varyetesi: Ebe karaçamı ve Ehrami karaçam. Çevre Koruma Dergisi. Sayı 28: 19-25.

Yaltırık, F. 1993. Dendroloji 1. Gymnospermae İ. Ü. Orman Fakültesi Yayınları. No: 3443/386, İstanbul, Türkiye. 320 pp.

Yaltırık, F. and Efe, A. 1994. Dendroloji Ders Kitabı, Gymnospermae, Angiospermae. İ.Ü. Orman Fakültesi Yayınları. No: 3836/431. İstanbul, Türkiye. 382 p.

Yanchuk, A.D. 2001. The role and implications of biotechnological tools in forestry. *Unasylva* 204(52): 53–61.

Yazdani, R., Yeh, F.C. and Rimsha, J. 1985. Genetic structure of a *Pinus sylvestris* L. seed tree stand and naturally regenerated understory. For. Sci. 31:430-436.

Yeh, C.F., Yang, R-C, Boyle, T., Ye, Z., Mao, J.X. 1997. POPGENE: User Friendly Shareware for Population Genetics Analysis. Molecular Biology and Biotechnology

Centre, University of Alberta, Canada. http://www.ualberta.ca/~fyeh/download.htm. Last accessed date: June 2005.

Yeh, F.C., Chong, D.K.X., Yang, R-C. 1995. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides* Michx.) from Alberta. Journal of Heredity. 86: 454-460.

Yu, K., and Paulus, K.P. 1992. Optimization of the PCR program for RAPD analysis. Nucleic Acids Research. 20: 2606.

Yücel, E. 1995. Ehrami Karaçam (*Pinus nigra* ssp. *pallasiana* var. *pyramidata*)'ın doğal yayılışı ve ekolojik özellikleri. Anadolu Üniversitesi Yayınları; No:847, Fen Fakültesi Yayınları; No:2. Anadolu Üniversitesi Basımevi, Eskişehir, Türkiye. 153 p.

Yücel, E. 2000. Ecological properties of *Pinus nigra* ssp. *pallasiana* var. *şeneriana*. Silvae Genetica. 49(6): 264-277.

Zabeau, M. and Vos, P. (Inventors). 1993. Selective Restriction Fragment Amplification: a general method for DNA fingerprinting. European Patent Application No. 0534858.

Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T. and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. Theor. Appl. Genet. 96: 602-611.

Zobel, B. and Talbert, J. 1984. Applied forest tree improvement. John Wiley & Sons, New York, USA. 505pp.

Zobel, B.J., Barber, J., Brown, C.L. and Perry, T.O. 1958. Seed orchards; their concept and management. J. For. 56: 815-825.

APPENDIX A

CHEMICALS AND SUPPLIERS

2-mercapto ethanol	Sigma M6250
Acetic acid (glacial)	Merck 1.00056
Agarose	Prona / Basica le
BSA (Bovine Serum Albumin)	Sigma B6917
Bromophenol blue	Sigma B-8026
Chloroform	Merck 1.02431
dNTP Set (Molecular Biology Grade)	MBI Fermentas R0185
EDTA	Sigma E4884
Ethidium bromide	Sigma E-8751
Formamide	Sigma F-7503
Gene Ruler^{TM} 100bp DNA Ladder Plus	MBI Fermentas SM0321
Hoechst 33258 Dye	Pharmacia-Biotech 80-6226-87
1-Octanol	Sigma O-4500
Potassium acetate	Merck 1.04820
Sodium acetate	Merck 1.06265
Sodium chloride	Baker 0278
SDS (Lauryl sulfate)	Sigma L-5750
Taq DNA polymerase (Recombinant)	MBI Fermentas EP0406
Trisma Base	T 1503
Tween 20	Baker 7374

APPENDIX B

SOLUTIONS FOR DNA QUANTIFICATION

10 X TNE Buffer

(1000 ml, buffer stock solution)

100 mM Tris	12.11 g
10 mM EDTA $Na_2.2H_2O$	3.72 g
2 M NaCl	116.89 g

- Dissolve in 800 ml distilled water.
- Adjust pH to 7.4 with concentrated HCl.
- Add distilled water to 1000 ml.
- Filter before use (0.45 μm)
- Store at 4°C for up to 3 months.

Hoechst Dye Stock Solution (10 mg/ml Hoechst H 33258)

- Add 10 ml distilled water to 10 mg H33258.
- Do not filter
- Store at 4°C in an amber bottle up to 6 months.

Assay Solution: Low range (A)

(10 to 500 ng/ml final DNA concentration)

0.1 μg/ml H 33258 in 1XTNE (0.2M NaCl, 10mM Tris-Cl, 1mM EDTA, pH 7.4)

H33258 stock solution	10 μl
10 X TNE buffer	10 ml
Distilled filtered water	90 ml

DNA Standard for Low Range

1:10 dilution (100 μg/ml) of 1 mg/ml DNA standard stock solution.

Mix:

1 mg/ml DNA standard stock	100 μl
10 X TNE buffer	100 μl
Distilled water	800 μl

APPENDIX C

A PART OF THE POPGENE DATA FILE

/* Diploid RAPD data Set */
Number of populations = 2
Number of loci = 25
Locus name :
121-1 121-2 121-3 121-4 121-5 121-6 121-7 121-8 121-9 121-10 121-11 121-12
131-1 131-2 131-3 131-4 131-5 131-6 131-7 131-8 131-9
144-1 144-2 144-3

POP BurM
BurM, AA BA BA BA AA BA BA BA AA BA BA BA BA AA BA AA BA BB BA BA BA BA BA BA BA
BurM, BA BA AA BA BA BA BA BA AA BA BB BA BA AA BA AA BA BA BA BB BA BA BA BA BA
BurM, BA BA AA BA BA BA BA BA AA BA AA AA BA AA BA AA AA BA BA BA BA BA BA BA BA
BurM, BA BA AA BB BA BA BA BA AA BB BA BA AA AA AA AA AA BA AA BA AA BA BA BB AA
BurM, BA BA BA BA BB BB BA AA BA BA AA AA BA AA AA AA BA BA AA BB AA BA BA BA BA
BurM, BB BB AA AA BB AA AA BA AA BB BA AA AA AA AA AA BA BA BA BA AA AA BA BA BA
BurM, BA BA AA BA BA BA BA BA AA BA BA BA BB AA AA BA BB BB BA BB BA BA BA BA BA
BurM, BA BA AA BA BB BA BA BA AA BA BA AA BA AA AA BA BA BA BA BB BA BB BA BB BA
BurM, BA BA BA BB BA BA BA BA AA BA BA AA BA AA AA AA AA BA BA BB BA BA BA BA BA
BurM, AA BA AA BB BA BB AA BA AA BA BA AA BA AA AA BA BA BA BA BB BA BA AA BB AA
BurM, BA BB AA BB AA BA AA BA AA BB AA AA AA AA AA AA BA BA BA BB BA BA AA BB AA
BurM, AA BA BA BA AA BA BA BB AA BA BA AA AA AA AA AA BA BA BA BB BA BA AA AA AA
BurM, BA BA BA BA BA AA BB BA AA BB AA AA BA AA BA AA BA BA AA BA AA BA AA BA BA
BurM, AA BA AA BA BB AA BB BA AA BB AA AA AA AA BA AA AA BA AA BA AA BA AA AA AA
BurM, AA BA AA BB BA AA BB BA AA BA BA AA BA AA BA AA BA BA BA BB BA BB AA BA AA
BurM, AA AA AA BB BA BA BB BA AA BA BA BA BA AA BA AA BA BA BA BB BA BB AA BB AA
BurM, AA BA AA BB BA BA BB BA AA BA BA BA BA AA AA AA BA BA BA BB BA BA AA BA AA
BurM, BA BB AA BB BB BA BB BA AA BA BB AA BA AA AA AA AA AA BA BA BB AA BA BA
BurM, BA BA BA BA BA BA BA BA BA BA BA AA AA AA BA BB BA BA BA BA AA BA BA
BurM, BA BB AA BA BA BA BA BA AA BB BA BA AA AA AA AA BA BA AA BA BA BA AA BA BA
POP BalM
BalM, AA AA AA BA BA BA BA BA AA BA AA BA AA AA AA AA AA BB AA BB BB BA AA BA AA
BalM, BA BA BA BA BA BB BA BA BA BA BA BA AA AA AA AA BA BB BA BA BB BB AA BA BA
BalM, AA BA AA BA BB BB AA BA AA BA BA BA BA AA AA AA BA BB BA BB BB BA AA BA AA
BalM, BA BA BA BB BA BB BA BB AA BA BA BA AA AA AA AA BA BB AA BA BB BA AA AA AA
BalM, AA BB AA BB AA BB BA BA AA BA BB AA BA AA AA AA BA BB BA BA AA BA AA BA AA
BalM, AA BA AA BB BA BB BA BA AA BB BA BA BA AA BA AA BA BA BA BA AA AA AA BA AA
BalM, AA BA AA BA BA BB AA AA AA BB BB AA BA AA AA AA AA BB BA BA AA BB AA BB AA
BalM, BA BA BA BB BA BB BA AA AA BB BA AA BB AA AA AA BA BA BA BA BA BA AA BA AA
BalM, AA BA AA BB AA BA AA BB AA BA BB BA BA AA BA AA BA BA AA BA BA BA AA BA AA
BalM, BA BA BA BA BA BB BA BA BA BA BB BA BA AA BA AA BA BA BA BA BA BA AA AA AA
BalM, AA BA AA BA BA BB BA BA AA BA BA AA BB AA AA AA BA BA AA BA BA BB AA BA AA
BalM, BA BB AA AA BA BA BA BA AA BA AA BA BA AA AA AA AA BA BA BA AA BB AA BB AA
BalM, BA BA AA BA BB BA BA AA AA BA BA BA BA AA AA AA AA BA BA BA BA BA AA BB AA
BalM, BA BA AA BA BA BB BA BA AA BB BA BB BA AA AA AA BA BA BA BA BA BB AA BB AA
BalM, BA BA BA BA BA BA BA AA AA BB BA BA AA AA AA AA AA AA BA BA BA AA BB AA
BalM, BA BA BA BA BB BA BB BA AA BA BA BA AA AA AA AA BA BA BA BB BA BA AA BA AA
BalM, AA AA AA BA BA BA BA AA AA BA BB BA AA AA AA AA BA BA BA BA BB BA AA BB AA
BalM, AA AA AA BA BB BA BA BA AA BA BB BB AA AA AA AA AA BA AA BA BA BA AA BA AA
BalM, BA BA BA BA BA BA BA BA AA BB AA BA AA AA AA AA BA BA BA BA BB BA AA BB AA
BalM, AA AA AA BA BA BA BA AA AA BB AA BB AA AA AA AA BA BA BA BA BA AA AA AA

APPENDIX D

A PART OF THE POPULATIONS DATA FILE

/* Populations data Set */
121-1
121-2
121-3
121-4
121-5
121-6
121-7
121-8
121-9
121-10
121-11
121-12
131-1
131-2
POP BurM
BurM, 01:01 01:02 01:02 01:02 01:01 01:02 01:02 01:02 01:01 01:02 01:02 01:02 01:02 01:01
BurM, 01:02 01:02 01:01 01:02 01:02 01:02 01:02 01:02 01:01 01:02 02:02 01:02 01:02 01:01
BurM, 01:02 01:02 01:01 01:02 01:02 01:02 01:02 01:02 01:01 01:02 01:01 01:01 01:02 01:01
BurM, 01:02 01:02 01:01 02:02 01:02 01:02 01:02 01:02 01:01 02:02 01:02 01:02 01:01 01:01
BurM, 01:02 01:02 01:02 01:02 02:02 02:02 01:02 01:01 01:02 01:02 01:01 01:01 01:02 01:01
BurM, 02:02 02:02 01:01 01:01 02:02 01:01 01:01 01:02 01:01 02:02 01:02 01:01 01:01 01:01
BurM, 01:02 01:02 01:01 01:02 01:02 01:02 01:02 01:02 01:01 01:02 01:02 01:02 02:02 01:01
BurM, 01:02 01:02 01:01 01:02 02:02 01:02 01:02 01:02 01:01 01:02 01:02 01:01 01:02 01:01
BurM, 01:02 01:02 01:02 02:02 01:02 01:02 01:02 01:02 01:01 01:02 01:02 01:01 01:02 01:01
BurM, 01:01 01:02 01:01 02:02 01:02 02:02 01:01 01:02 01:01 01:02 01:02 01:01 01:02 01:01
BurM, 01:02 02:02 01:01 02:02 01:01 01:02 01:01 01:02 01:01 02:02 01:01 01:01 01:01 01:01
BurM, 01:01 01:02 01:02 01:02 01:01 01:02 01:02 02:02 01:01 01:02 01:02 01:01 01:01 01:01
BurM, 01:02 01:02 01:02 01:02 01:02 01:01 02:02 01:02 01:01 02:02 01:01 01:01 01:02 01:01
BurM, 01:01 01:02 01:01 01:02 02:02 01:01 02:02 01:02 01:01 02:02 01:01 01:01 01:01 01:01
BurM, 01:01 01:02 01:01 02:02 01:02 01:01 02:02 01:02 01:01 01:02 01:02 01:01 01:02 01:01
BurM, 01:01 01:01 01:01 02:02 01:02 01:02 02:02 01:02 01:01 01:02 01:02 01:02 01:02 01:01
BurM, 01:01 01:02 01:01 02:02 01:02 01:02 02:02 01:02 01:01 01:02 01:02 01:02 01:02 01:01
BurM, 01:02 02:02 01:01 02:02 02:02 01:02 02:02 01:02 01:01 01:02 02:02 01:01 01:02 01:01
BurM, 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01
BurM, 01:02 02:02 01:01 01:02 01:02 01:02 01:02 01:01 02:02 01:02 01:02 01:01 01:01
BurM, 01:02 01:02 01:02 02:02 01:02 01:02 01:02 01:01 01:01 02:02 01:02 01:01 01:01 01:01
BurM, 01:01 02:02 01:01 01:02 01:02 02:02 01:02 01:01 01:01 01:02 02:02 01:02 01:01 01:01
BurM, 01:02 02:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01 01:02 02:02 01:02 01:01 01:01
BurM, 01:02 02:02 01:01 01:02 01:02 01:02 02:02 01:02 01:01 02:02 02:02 01:02 01:01 01:01
BurM, 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01
POP BalM
BalM, 01:01 01:01 01:01 01:02 01:02 01:02 01:02 01:02 01:01 01:02 01:01 01:02 01:01 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 02:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01 01:01
BalM, 01:01 01:02 01:01 01:02 02:02 02:02 01:01 01:02 01:01 01:02 01:02 01:02 01:02 01:01

BalM, 01:02 01:02 01:02 02:02 01:02 02:02 01:02 02:02 01:01 01:02 01:02 01:02 01:01 01:01
BalM, 01:01 02:02 01:01 02:02 01:01 02:02 01:02 01:02 01:01 01:02 02:02 01:01 01:02 01:01
BalM, 01:01 01:02 01:01 02:02 01:02 02:02 01:02 01:02 01:01 02:02 01:02 01:02 01:02 01:01
BalM, 01:01 01:02 01:01 01:02 01:02 02:02 01:01 01:01 01:01 02:02 02:02 01:01 01:02 01:01
BalM, 01:02 01:02 01:02 02:02 01:02 02:02 01:02 01:01 01:01 02:02 01:02 01:01 02:02 01:01
BalM, 01:01 01:02 01:01 02:02 01:01 01:02 01:01 02:02 01:01 01:02 02:02 01:02 01:02 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 02:02 01:02 01:02 01:02 01:02 02:02 01:02 01:02 01:01
BalM, 01:01 01:02 01:01 01:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:01 02:02 01:01
BalM, 01:02 02:02 01:01 01:01 01:02 01:02 01:02 01:02 01:01 01:02 01:01 01:02 01:02 01:01
BalM, 01:02 01:02 01:01 01:02 02:02 01:02 01:02 01:01 01:01 01:02 01:02 01:02 01:02 01:01
BalM, 01:02 01:02 01:01 01:02 01:02 02:02 01:02 01:02 01:01 02:02 01:02 02:02 01:02 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01 01:01 02:02 01:02 01:02 01:01 01:01
BalM, 01:02 01:02 01:02 01:02 02:02 01:02 02:02 01:02 01:01 01:02 01:02 01:02 01:01 01:01
BalM, 01:01 01:01 01:01 01:02 01:02 01:02 01:02 01:01 01:01 01:02 02:02 01:02 01:01 01:01
BalM, 01:01 01:01 01:01 01:02 02:02 01:02 01:02 01:02 01:01 01:02 02:02 02:02 01:01 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:02 01:01 02:02 01:01 01:02 01:01 01:01
BalM, 01:01 01:01 01:01 01:02 01:02 01:02 01:02 01:01 01:01 02:02 01:01 02:02 01:01 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 01:02 01:01
BalM, 01:02 01:02 01:02 01:02 01:02 02:02 01:02 01:01 01:01 01:01 01:02 01:01 01:01 01:01
BalM, 01:02 01:02 01:02 01:02 01:01 02:02 01:02 01:02 01:01 01:02 01:02 01:01 01:02 01:01
BalM, 01:02 01:01 02:02 02:02 01:01 01:02 02:02 01:01 01:01 02:02 02:02 01:01 01:01 01:01
BalM, 01:02 01:01 01:02 02:02 01:01 01:02 01:02 01:01 01:01 02:02 01:02 01:01 01:01 01:01
POP Durs
Durs, 02:02 02:02 01:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:01 01:01 01:02 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:01 01:01 01:01 01:02 01:02 02:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 01:02 01:02 01:01 02:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:01 02:02 01:01 01:02 01:01 02:02 01:01 01:01 01:02 02:02
Durs, 02:02 02:02 01:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 02:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 02:02 01:02 01:02 02:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 01:02 01:01 01:01 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 01:02 02:02 01:02 02:02 01:02 01:01 01:01 02:02 01:01 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 02:02 02:02 01:02 01:02 01:01 01:02 01:01 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 02:02 02:02 01:01 01:01 01:01 02:02 01:01 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:01 02:02 01:01 01:02 01:01 02:02 01:01 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 02:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:01 01:01 02:02 01:02 02:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 02:02 02:02 01:01 01:02 01:01 01:02 01:02 01:02 02:02
Durs, 02:02 01:02 02:02 02:02 01:02 02:02 01:01 01:02 01:01 01:02 01:02 01:02 02:02 02:02
Durs, 02:02 01:01 02:02 02:02 01:01 02:02 01:01 01:01 01:01 01:02 01:02 01:01 02:02 02:02
Durs, 02:02 01:02 02:02 02:02 01:01 02:02 01:01 01:01 01:01 02:02 01:02 01:01 02:02 02:02
Durs, 02:02 01:02 02:02 02:02 01:01 02:02 01:01 01:01 01:01 01:02 01:02 01:02 01:02 02:02
Durs, 02:02 02:02 02:02 02:02 02:02 01:01 02:02 01:02 01:01 01:01 01:01 01:01 01:02 01:02 02:02
POP Vars
Vars, 02:02 02:02 02:02 02:02 02:02 01:02 02:02 01:02 01:02 01:01 01:02 01:02 02:02 01:01 01:01
Vars, 01:02 01:02 01:02 01:02 01:02 02:02 01:01 01:02 02:02 02:02 01:02 01:02 01:01 01:01
Vars, 01:02 01:02 01:02 01:02 01:02 02:02 01:01 01:02 02:02 02:02 01:02 01:02 01:01 01:01
Vars, 01:02 01:02 01:02 01:02 01:02 02:02 01:01 01:01 02:02 02:02 01:02 01:01 01:01 01:01
Vars, 02:02 01:02 01:02 02:02 01:02 02:02 01:01 01:01 02:02 02:02 01:02 01:01 01:01 01:01
Vars, 02:02 01:02 01:01 02:02 01:02 02:02 01:01 01:02 02:02 02:02 01:02 02:02 01:01 01:01
Vars, 02:02 01:02 01:02 01:02 01:01 02:02 01:02 01:02 02:02 02:02 01:02 02:02 01:01 01:01

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name : Çengel, Burcu

Nationality : T.C.

Date and Place of Birth : 05.04.1972, Ankara

Marital Status : Married

Phone : +90 312 212 6519

Fax : +90 312 212 3960

Email : nazlier@yahoo.com

EDUCATION

Degree	Institiuon	Year of Graduation
MS	METU, Biology	1998
BS	METU, Biology	1995
High School	Ayrancı Lisesi, Ankara	1989

WORK EXPERIENCE

Year	Place	Enrollment
1995-present	Ministry of Environment and Forestry, Forest Tree Seeds and Tree Breeding Research Directorate	Researcher

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

Çengel, B. 1998. Pattern and Magnitude of Genetic Diversity in *Pinus nigra* subsp. *pallasiana* Populations from Kazdağı. Master Thesis.

Kaya, Z., Zeydanlı, U., **Çengel, B.**, Yılmaz, T. 1999. ODTÜ Kampüsü Kırçiçekleri Rehberi. Dönmez Ofset, Ankara.

Velioğlu, E., **Çengel, B.**, Kaya, Z. 1999. Kazdağları'ndaki doğal karaçam (Pinus *nigra* subspecies *pallasiana*) populasyonlarında genetik çeşitliliğin yapılanması. OATIAM Teknik Bülten No: 1.

Velioğlu, E., Tolun, A.A., **Çengel, B.**, Kaya, Z. 1999. Bolkar dağları'ndaki doğal karaçam (*Pinus nigra* subspecies *pallasiana*) populasyonlarının izoenzim çeşitliliği. OATIAM Teknik Bülten No: 2.

Velioğlu, E., Çiçek F., **Çengel, B.**, Kaya, Z. 1999. Kazdağları'ndaki doğal Kazdağı göknarı (*Abies equitrojani*) populasyonlarında genetik çeşitliliğin yapılanması. OATIAM Teknik Bülten No: 3.

Velioğlu, E., **Çengel, B.**, Kaya, Z. 1999. Kazdağları'ndaki doğal karaçam (Pinus *nigra* subspecies *pallasiana*) populasyonlarında izoenzim çeşitliliği. OATIAM Teknik Bülten No: 4.

Çengel, B., Velioğlu E., Tolun A. A., Kaya Z. 2000. Pattern and Magnitude of Genetic Diversity in *Pinus nigra* Arnold subspecies *pallasiana* Populations from Kazdağı. Silvae Genetica 49, 6: 249-256.

Kaya, Z., Tolun A. A., **Çengel, B.**, Velioğlu E., Tolun, G. 2000. The Pattern of Genetic Variation in P*inus nigra* subspecies *pallasiana* Natural Populations from the Kazdağı and Bolkar Mountains, Turkey: Implications *for in situ* Gene Conservation. In:

Genetic Response of Forest Sysyems to Changing Environmental Conditions. Müller-Starck, Schubert, R. (Eds). Kluwer Academic Publishers.

Tolun A. A., Velioğlu E., **Çengel, B.**, Kaya Z. 2000. Genetic Structure of Black Pine (*Pinus nigra* subspp. *pallasiana*) Populations Sampled from Bolkar Mountains. Silvae Genetica 49, 3: 113-119.

Velioğlu, E., **Çengel, B.**, İçgen, Y., Kandemir, G., Alan, M., Kaya Z. 2003. Moleküler belirteçler Yardımıyla Karaçam(*Pinus nigra*) Tohum Meşcerelerinde, Tohum Bahçelerinde ve Ağaçlandırmalarında Bulunan Genetik Çeşitliliğin Karşılaştırılması. OATIAM Teknik Bülten No:11, ANKARA.

Velioğlu, E., İçgen, Y., **Çengel, B.**, Öztürk, H., Kaya Z. 2003. Moleküler Belirteçler Yardımıyla Kızılçam (*Pinus brutia* Ten.) Tohum Meşcerelerinde, Tohum Bahçelerinde ve Ağaçlandırmalarında Bulunan Genetik Çeşitliliğin Karşılaştırılması. OATIAM. Teknik Bülten No:11, ANKARA.

İçgen, Y., Kaya, Z., **Çengel, B.**, Velioğlu, E., Öztürk, H., Önde, S. 2005. Potential impact of forest management and breeding practices on established *Pinus brutia* plantations. Forest Ecology and Management. Submitted.