ASSESSMENT OF GENETIC DIVERSITY IN *PICEA ORIENTALIS* (L.) LINK. IN GENETIC RESOURCES BY MICROSATELLITES

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ABSTRACT

ASSESSMENT OF GENETIC DIVERSITY IN *PICEA ORIENTALIS* (L.) Link. IN GENETIC RESOURCES BY MICROSATELLITES

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Picea comprises about 40 species in the world. One of these species, oriental spruce (*Picea orientalis* (L.) Link.) is naturally distributed in northeastern Turkey, mostly in Artvin Province and in a part of Georgia close to the coast of the Black Sea region. The limited distribution of species and increased anthropogenic threats to its genetic resources signify the importance of studying genetic diversity of the species to have better conservation and management programs. Here, we report the first high throughput genetic diversity analysis of *P.orientalis* using microsatellite markers. In detail, 277 individuals of eight different populations were sampled throughout the geographic range and screened with 15 SSRs (Simple Sequence Repeats) loci to assess the genetic diversity patterns and structure of the species. Oriental spruce populations were evaluated according to the parameters of the basic genetic diversity and the structure. These populations were also grouped and evaluated according to the types of tissues used in the sampling.

According to the descriptive statistic results obtained from the present study in terms of microsatellite markers, the number of polymorphic loci was found as 14 and the percentage of polymorphic loci (PI) was determined as 93%. The highest number of alleles and the highest observed heterozygosity were detected in SS17 locus as 25 and 0.92, respectively. Moreover, the highest effective number of alleles was found in SS15 locus (9.34). Among the populations of oriental spruce, the highest genetic diversity (Ho=0.58±0.07) was detected in C-Trabzon and the highest number of private alleles (13) was found in SE-Artvin population (Southeastern Artvin). Furthermore, SE-Artvin was genetically the most distant population based on the factorial correspondence analysis (FCA). On the other hand, the lowest genetic diversity (Ho=0.45±0.04) was estimated in Giresun and the lowest private alleles (2) were observed in C-Trabzon. The results of genetic structure analysis revealed that the populations of oriental spruce were grouped into four main clusters (K). These were Trabzon (Giresun, W-Trabzon, C-Trabzon, E-Trabzon), Artvin (S-Artvin, N-Artvin), C-Artvin and SE-Artvin. Considering all the data related to this issue, our results comply with a general population genetic pattern where geographically close populations exhibited higher genetic similarity than geographically distant populations.

However, comparing seed and needle samples in respect to geographical location, there was no remarkable differences or similarity in genetic diversity parameters.

In conclusion, this study provided experimental evidence revealing the genetic diversity parameters evaluated by means of SSR markers and structure of oriental spruce populations for the first time. Specifically, SE-Artvin and C-Trabzon populations are recommended to be included in future conservation programs dealing with oriental spruce.

Keywords: *Picea orientalis*, SSR, genetic diversity, conservation, diversity pattern

ÖZ

MİKROSATELLİT BELİRTEÇLER YARDIMYLA *PICEA ORIENTALIS* (L.) Link. GEN KAYNAKLARINDA GENETİK ÇEŞİTLİLİĞİN BELİRLENMESİ

Özdilek, Aslı

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Dünya üzerinde *Picea* cinsi belli başlı 40 türü kapsar ve bu türlerin biri olan doğu ladini (*P.orientalis* (L.) Link.) doğal olarak Türkiye'nin kuzeydoğusunda çoğunlukla Artvin ili çevresinde ve Gürcistan'ın Karadeniz Bölgesi'ne yakın sahil kesimlerinde yayılış gösterir. Türün sınırlı dağılımı ve genetik kaynaklarına karşı artış gösteren insan kaynaklı tehditler, iyi bir koruma stratejisinin geliştirilmesinin ve gen kaynaklarının idaresinin gerekliliğini ve tür içi genetik çeşitliliğin çalışılmasının önemini ortaya koymaktadır. Yapılan bu araştırma mikrosatellit belirteçleri kullanılarak *P.orientalis*' in genetik çeşitlilik analizlerinin geniş kapsamlı olarak yapıldığı ilk çalışmadır. Detaylandırmak gerekirse, sekiz farklı popülasyondan 277 birey coğrafi aralık boyunca örneklendi ve türlerin genetik çeşitlilik durum ve yapısını değerlendirmek için 15 SSRs (Basit Dizi Tekrarları) bölgesi çalışıldı. Doğu ladini popülasyonları temel genetik çeşitlilik ve popülasyon yapısı parametrelerine göre değerlendirildi. Bu popülasyonlar ayrıca toplanan örnek karakterlerine göre gruplandırıldı ve değerlendirildi.

Mikrosatellit belirteçleri açısından bu çalışmada elde edilen tanımlayıcı istatistik sonuçlarına göre, polimorfik lokus sayısı 14 ve polimorfik lokus (PI) oranı ise % 93 olarak tespit edilmiştir. En fazla allel sayısı (25) ve en yüksek gözlenen heterozigotluk (0.92) SS17 lokusunda belirlenmiştir. Ayrıca en yüksek etkili allel (effective allele) sayısı 9.34 olarak SS15 lokusunda bulunmuştur. Doğu ladini popülasyonları arasında, en yüksek genetik çeşitlilik (Ho=0.58±0.07) C-Trabzon (Merkez Trabzon)' da ve en yüksek özgün allel (private allele=13) sayısı SE-Artvin populasyonunda görüldü. Dahası, faktöriyel benzerlik analizine (FCA) göre SE-Artvin popülasyonu en farklı popülasyon olarak belirlendi. Diğer yandan, en düşük genetik çeşitlilik (Ho=0.45±0.04) Giresun' da ve en düşük özgün allel (2) C-Trabzon' da gözlendi. Genetik yapı analizi sonuçları doğu ladin popülasyonlarının dört ana küme (K) halinde gruplandırılması gerektiğini ortaya koydu. Bunlar, Trabzon (Giresun, W-Trabzon, C-Trabzon, E-Trabzon), Artvin (S-Artvin, N-Artvin), C-Artvin ve SE-Artvin'dir. Bu konudaki tüm veriler göz önünüe alındığında coğrafik olarak yakın popülasyonların uzak popülasyonlara göre daha yüksek genetik benzerlik gösterdiği ortaya konmuştur.

Fakat, tohum örnekleri ve iğne yaprak örnekleri genetik çeşitlilik parametreleri açısından karşılaştırıldığında coğrafik konum bağlamında belirgin bir fark ya da benzerlik gözlenmemiştir.

Sonuç olarak, bu çalışma SSR belirteçleri ile değerlendirilen genetik çeşitlilik parametreleri ve doğu ladini popülasyonlarının genetik yapısını ilk kez ortaya koyan deneysel kanıtlar sunmaktadır. Özellikle, SE-Artvin ve C-Trabzon popülasyonlarının gelecekte genetik kaynaklarının koruma altına alınması tavsiye edilmektedir.

Anahtar Kelimeler: *Picea orientalis*, SSR, genetik çeşitlilik, koruma, dağılım paterni

to my lovely mom...

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TABLE OF CONTENTS

LIST OF FIGURES

FIGURES

LIST OF TABLES

TABLES

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
βME	Betamercaptoethanol
cpDNA	Chloroplast DNA
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Tri-Phosphate
DTCS	Dye terminator cycle sequencing
EST	Expressed Sequence Tag
ETOH	Ethanol
GDA	Genetic Data Analysis
GOT	Glutamate-Oxaloacetate Transaminase
H_e	expected Heterozygosity
H_o	observed Heterozygosity
HWE	Hardy–Weinberg Equilibrium
MCMC	Markov Chain Monte Carlo
mtDNA	Mitochondrial DNA
N_e	Effective number of alleles per locus
LAP	Leucine Amino Peptidase
PCR	Polymerase Chain Reaction
PI	Polymorphic locus rate
PVP	Polyvinyl Pyrrolidone
RAPDs	Random Amplified Polymorphic DNA's
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeats
STS	Sequence-Tagged Sites
TBE	Tris Borate EDTA
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean
VNTR	Variable Number Tandem Repeats

CHAPTER I

INTRODUCTION

Evergreen gymnosperms are ecologically and economically important seed plants with almost 1000 species. They are found virtually every continent (except Anctartica) in the world (Wang and Ran, 2014). Pinales, an order of gymnosperms, comprises one of the largest part of the forests in the north temperate zone of the earth. *Pinaceae* is the most abundant and widespread family of modern conifers that is divided into 11 genera with 232 species. In the northern hemisphere, mainly in temperate climate regions, spruces (*Picea*), firs (*Abies*), and pines (*Pinus*) are the predominant genera of *Pinaceae* family (Trapp and Croteau, 2001). Spruce (*Picea* A. Dietrich) species spread in the northern hemisphere, temperate and cold regions in the world with almost 40 different species including *Picea orientalis* (Figure 1). The number of the spruce species is variable depending upon the classification system used (Farjon, 1990, Thomas Ledig et al., 2004). The great majority of them are naturally distributed in Asia with 34 species. North America and Europe follow this old continent with the ratio of one-fourth and one-tenth of the species, respectively (Farjon, 1990).

Picea genus is divided into two main sections. These sections branch out into subsections (which are given below with the representative species):

- Section *Picea*
    - Subsection *Picea*
        - *Picea abies* (L.) H. Karst.

        - *P. asperata* Mast
        - *P. glauca* (Moench) Voss.
        - *P. mariana* (Mill.) Britton et al
        - ***P. orientalis* (L.) Link**
        - *P. wilsonii* Mast.
        - *P. rubens* Sarg.
    - Subsection *Omorikae*
        - *P. brachytyla* (Franch.) E. Pritz.
        - *P. breweriana* S. Watson
        - *P. spinulosa* (Griff.) A. Henry
- Section *Casicta*
    - Subsection *Sitchensis*
        - *P. purpurea* Mast.
        - *P. sitchensis* (Bong.) Carriére
    - Subsection *Pungentes*
        - *P. engelmannii* Parry ex Engelm.
        - *P. pungens* Engelm.

Oriental spruce (*Picea orientalis* L.) is the relict species and native to the major forestry regions of Turkey. The natural distribution of oriental spruce is local and it covers about the area of 350 000 ha in Turkey and 22000 ha in Georgia in the world.

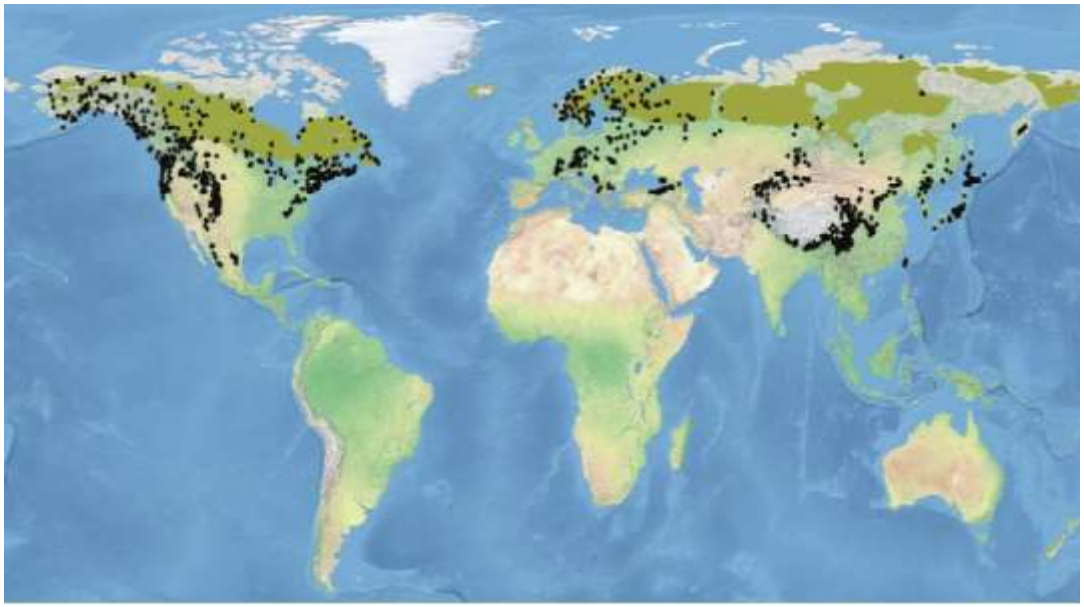

Figure 1 Natural distribution of *Picea* genus in the world (Farjon and Filer, 2013)

1.1 Oriental spruce

Oriental spruce spreads naturally in the northeastern Black Sea region in Turkey and western parts of Georgia. It usually reaches 8 m to 12 m in the landscape, soaring to 60 m in its native habitat (Davis, 1965), growing slowly into a dense pyramidal silhouette that casts dense shade beneath (Figure 2).

Oriental spruce forests are naturally found either as pure stands or mixed with *Fagus orientalis, Abies nordmanniana* and *Pinus sylvestris* and distributed between west of the Melet River (Figure 3) and the southern part of the Caucasian Mountains in Georgia (Turna, 2004, Ercanli et al., 2008). Because of its economical and ecological significance in northeastern Turkey, genetic resources of this species need to be effectively conserved, managed and utilized.

Figure 2 Photos of spruce tree on the left, forest in the middle and a young seedling on the right.

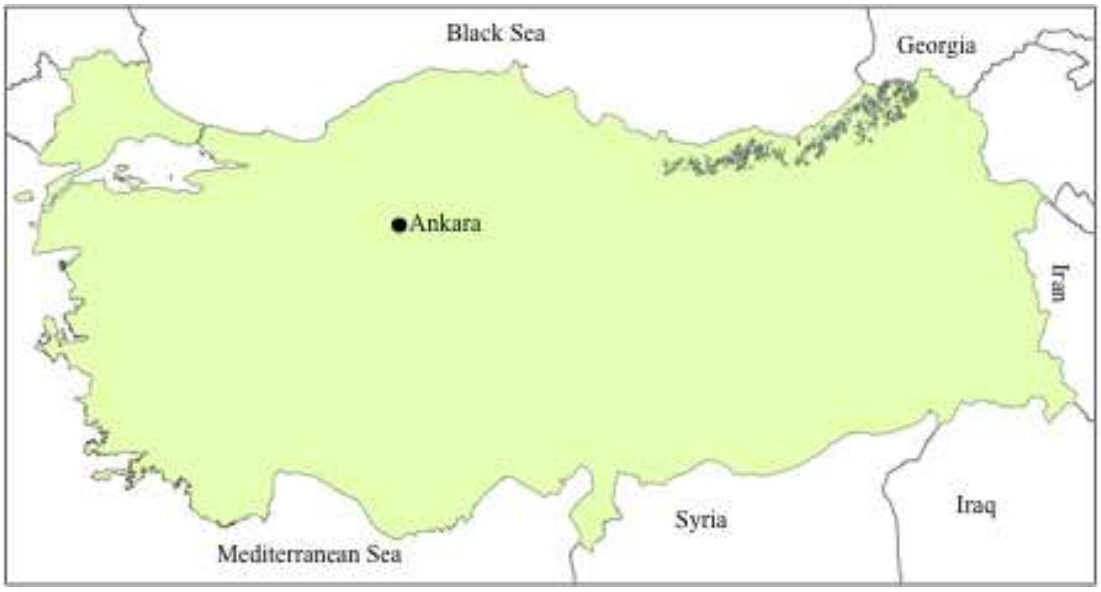

Figure 3 The natural distribution map of *Picea orientalis* in Turkey (Dark gray shaded areas indicate the distribution of the populations).

1.2 Genetic diversity

Population genetics focus on the distribution of genetic variation among species, populations within species, and individuals within populations. Allele and genotype frequencies of a population indicate genetic structure of the population. The genetic structure can be affected by factors such as mutation, migration and genetic drift. Analysis of the population structure can be provided via two important parameters: *F-statistics* (Wright, 1965) and genetic distances (Nei, 1978).

Genetic diversity is characterized by measuring the genetic variability within a species. Forest trees have high rate in respect to genetic diversity, especially within population (White et al., 2007). Populations with high allelic richness have better chance to survive, reproduce and evolve in response to stressful conditions (McNeely et al., 1990). For forest trees, although they have large gene pools and high levels of genetic diversity, both natural and anthropogenic disturbances are of serious concern.

Genetic diversity is essential for the adaptability, continuity and evolution of forest tree populations (Müller-Starck et al., 1992). In general, it is thought that species having high genetic diversity is less susceptible to environmental changes and diseases (Oleksyn et al., 1994). To reveal the genetic diversity of the species, morphological and molecular genetic markers are available. Molecular markers are most widely implemented among the tree species, especially in forest trees, for determination of the genetic structure and pattern of genetic distribution of populations. These markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), isoenzymes (Bartels, 1971, Morgante and Vendramin, 1991, Krutovskii and Bergmann, 1995, Müller-Starck, 1995, Geburek, 1999), randomly amplified polymorphic DNA's (RAPDs) (Collignon and Favre, 2000, Kovačević et al., 2013) and simple sequence repeats (SSRs). Sequence-tagged sites or STS (Scotti et al., 2000b), cpDNA microsatellites (Vendramin et al., 2000) or mtDNA microsatellites (Gugerli et al., 2001, Sperisen et

al., 2001) have been utilized to obtain the genetic diversity of populations. Among these molecular markers, nuclear SSRs or microsatellites are widely used in population genetic studies and for determination of genetic diversity depending on ones interest. Isoenzymes are well known markers and there are numerous studies related with them. Although the polymorphism rate of isoenzymes is quite high, the number of loci is limited. RFLPs, on the other hand, are codominat markers, but it is necessary to use quite high quality and amount of DNA as templates. For RAPDs and AFLPs, it is not necessary to implement the genome sequence information at the begining of the study. Thus, these two markers can be applied to the organisms with unknown genomes. The disadvantages are their dominant characteristics and difficulty of the transfer between different species (Rungis et al., 2004). The SSRs are considered as ideal markers for genetic diversity studies due to their codominant nature, high variability, high reproducibility, high polymorphism rate and ability to cover genomes including organelle genome (Varshney et al., 2005, Kalia et al., 2011, He et al., 2015). Although their high efficiency on genetic studies, it is difficult to identify robust SSR markers from a genome due to the requirements of high amount of primers and genetically non interpretable PCR products that obtained from these primers (Rungis et al., 2004, Squirrell et al., 2003).

1.3 Microsatellites, Minisatellites or Simple sequence Repeats (SSRs)

SSRs are tandem repeats of short nucleic acid motifs (1 to 6 bp) distributed randomly in genomes (Powell et al., 1996, Scotti et al., 2000a). They are located generally in non-coding regions and are barely found in coding regions. SSRs can also be called as short tandem repeats (STR) or variable number tandem repeats (VNTR) in literature. Microsatellites are generally regarded as ‘junk’ DNA because of their non-measurable effect on phenotype (Kashi and King, 2006).

Microsatellites can be typically grouped into three categories according to the source of development. These are:

1. Genomic or nuclear microsatellites (gSSRs) are found in the nuclear genome.

2. Expressed sequence taq microsatellites (EST-SSRs) stem from exploiting EST sequences in databases.

3. Organellar microsatellites are chloroplast SSRs (cpSSRs) and mitochondrial SSRs (mtSSRs) isolated from the chloroplast or mitochondrial genome of an organism.

At present, microsatellites have become one of the most efficient molecular markers in view of the codominant characteristic, random distribution and high variability not only in genetic diversity studies (Varshney et al., 2005), but also fingerprinting, evolution and linkage map construction. Microsatellites have a tendency of high mutation rate because of their high polymorphic nature. Furthermore, multiple alleles are detected in microsatellite locus as homozygote and heterozygote since they reveal codominance.

1.4 Review of *P. orientalis* and its relative species Literature

The research conducted on *P.orientalis* can be classified under two main issues as morphological and genetic based studies. Morphological studies mainly focused on the effect of climate and geographical features on the growth and distribution of the species (Akgül, 1975, Küçük, 1989, Turna, 2004, Ucler et al., 2007). One of these studies revealed that the growth rate of samples descended from Karanlıkmeşe (Artvin), İkizdere (Rize) and Bicik (Giresun) were the best performing origins and have similar morphological features (Ürgenç et al., 1990). Genetic studies on oriental spruce have been carried out via karyotype analysis (Inceer et al., 2009) and by isoenzyme levels so far. An isoenzyme study on seed samples from twenty six different provinces, using LAP and GOT enzyme systems, indicated that the oriental spruce has higher genetic variation than expected (Turna, 1996).

The presence of genetic variation was revealed in the morphological and genetic studies of oriental spruce (Turna, 1996, Turna, 2004). However, this type of information was not sufficient to create a program for understanding the genetic make up of the species and conservation of the genetic variability.

Molecular genetic studies were reported within other spruce species. In a study using nuclear, mitochondrial and choloroplast DNA markers, genetic diversity within *P. abies* populations is found to be higher than that of among populations (Maghuly et al., 2006). In another genetic study, many SSRs have been observed as codominant and dominant markers, according to the presence or absence of bands on agorose gels (Yazdani et al., 2003). Most codominant and dominant SSR loci indicated a simple Mendelian inheritance pattern. Null alleles were detected for many codominant SSR markers. The ratio of detected dominant microsatellites in this study was much higher than that commonly reported in many other studies, close to 50 %. However, in present, it is accepted that microsatellites are codominat feature (Foll and Gaggiotti, 2008, Jelena et al., 2015, Lee et al., 2015).

Seven microsatellite loci were used to determine the genetic diversity and structure of naturally distributed *P.asperata* populations. It was found that the diversity levels vary among *P.asperata* populations due to the differences in environmental conditions. Furthermore, microsattelites were indictaed as powerful tools for determination of genetic diversity (Wang et al., 2005) in this study. In another report, by using deposited expressed sequence tags (ESTs) of *P. glauca* in GenBank, microsatellite markers were developed for *P. sitchensis* (A'hara and Cottrell, 2004).

Microsatellite markers (SSRs) were developed for *P. glauca* and it was proposed that these genetic markers can be used for biotechnology, breeding, tree forensic, genome mapping, protection, restoration and sustainable forestry activities in other spruce species (Rajora et al., 2001). Nevertheless, for at least one of the 15 microsatellite markers developed for *P. glauca* was identified that it could be used

for other six spruce species (*P. mariana, P. engelmannii, P. rubens, P. abies, P. pungens, P. sitchensis*) (Hodgetts et al., 2001). In another study, the maximum similarity coefficient values were identified between *Picea abies* and *P. orientalis* and also between *Pinus wallichiana* and *P. strobus* (Kovačević et al., 2013). In the same study it was indicated that Serbian spruce (*Picea omorika*) is more related to oriental spruce based on hybridization compatibility.

CHAPTER II

JUSTIFICATION OF THE STUDY

In recent years, serious erosion is thought to occur in genetic resources of the *P. orientalis* forests. The threats facing oriental spruce varies from different types of bark beetles, high amount of over-utilization, forest fires and habitat reduction due to global climate changes (Tüfekçioğlu, 2008). Construction of well-organized and planned genetic conservation programs is necessary to secure this relict species existence in the future. In the eastern part of the Black Sea Region of Turkey, *P. orientalis* is a valuable species for timber production and also for establishment of household supplies (Ercanli et al., 2008).

Damaged due to excessive use for years, spruce forests have been subjected to increasing attacks of common coniferous bark beetles (*Dendroctonus micans*, *Ips sexdendatus* and *Ips typographus*) (Yüksel, 1998). According to the Bern Convention that Turkey is one of the signaturers, spruce forests are among the habitats in danger because of increasing insect damage and social pressures. Furthermore, natural structure of forests has been disturbed because of antrophogenic factors.

Therefore, due to deterioration status of the spruce genetic structure, the conservation programs for the genetic diversity in the species should be implemented with a sound genetic data without losing time. With this study, genetic data for in situ and ex situ conservation programs would be available.

CHAPTER III

AIM OF THE STUDY

The objectives of this study were;

1. Determination of genetic structure and diversity of naturally distributed oriental spruce populations by means of SSR markers.
2. Assessment of the differences on the genetic makeup of the open pollinated seedlings and needle sampled populations.
3. Helping to develop a gene conservation program for oriental spruce forests using the genetic data from this study.

In the light of the information obtained from this study, we expected to contribute and help to implement an effective gene conservation program for oriental spruce forests in Turkey.

CHAPTER IV

MATERIALS AND METHODS

In this study, totally 8 populations of oriental spruce were sampled and collected to determine genetic diversity within and between populations and to provide information on genetic relationships among *Picea orientalis* populations. Collection of samples was fulfilled in two steps. The first collection was in 2006 and made up with open pollinated seed samples, whereas the second collection was with needle tissues and it was conducted in 2010. The differences by means of tissue types arised from adaptation study which was previously conducted with the seeds. Needle tissues were preferred for second collection to reduce the time needed for germination of the sample to extract the DNA. In total, 137 seedlings and 140 needle sampled trees of oriental spruce were collected from the natural stands and natural stands set aside as conservation programs. Both sample collections were carried out on different regions of Trabzon, Giresun and Artvin provinces (Figure 4 and Figure 5) (Table 1).

Figure 4 Views of high elevation oriental spruce forests from Trabzon province.

Table 1 Studied oriental spruce populations and topographic information on populations

Pop.no.	Populations	Number of Individuals sampled	Latitude (N)	Longitude (E)	Average altitude (range) (m)
1	Giresun (NG+SS)	50	40° 39'	38° 07'	1554 (1400-1700)
2	Western Trabzon (W-Trabzon) (NG+SS+GCF)	30	40° 39'	38° 57'	1516 (1350-1680)
3	Central Trabzon (C-Trabzon) (NG+SS+GCF)	20	40° 50'	39° 18'	1765 (1730-1800)
4	Eastern Trabzon (E-Trabzon) (NG+SS)	40	40° 47'	40° 19'	1348 (1030-1800)
5	South Artvin (S-Artvin) (NG+SS)	30	41° 01'	41° 45'	1663 (1540-1740)

Table 1 Continued

Pop.no.	Populations	Number of Individuals sampled	Latitude (N)	Longitude (E)	Average altitude (range) (m)
6	North Artvin (N-Artvin) (NG+SS)	30	41° 08'	41° 41'	1436 (1150-1830)
7	Central Artvin (C-Artvin) (NG+SS)	30	41° 33'	42° 08'	1140 (770-1560)
8	Southeastern Artvin (SE-Artvin) (NG+SS)	50	41° 44'	42° 44'	1318 (910-1650)

*NG: Natural Growth, SS: Seed Stand, GCF: Gene Conservation Forest. W: Western, C: Central, E: Eastern, S: Southern, N: Northern, SE: Southeastern

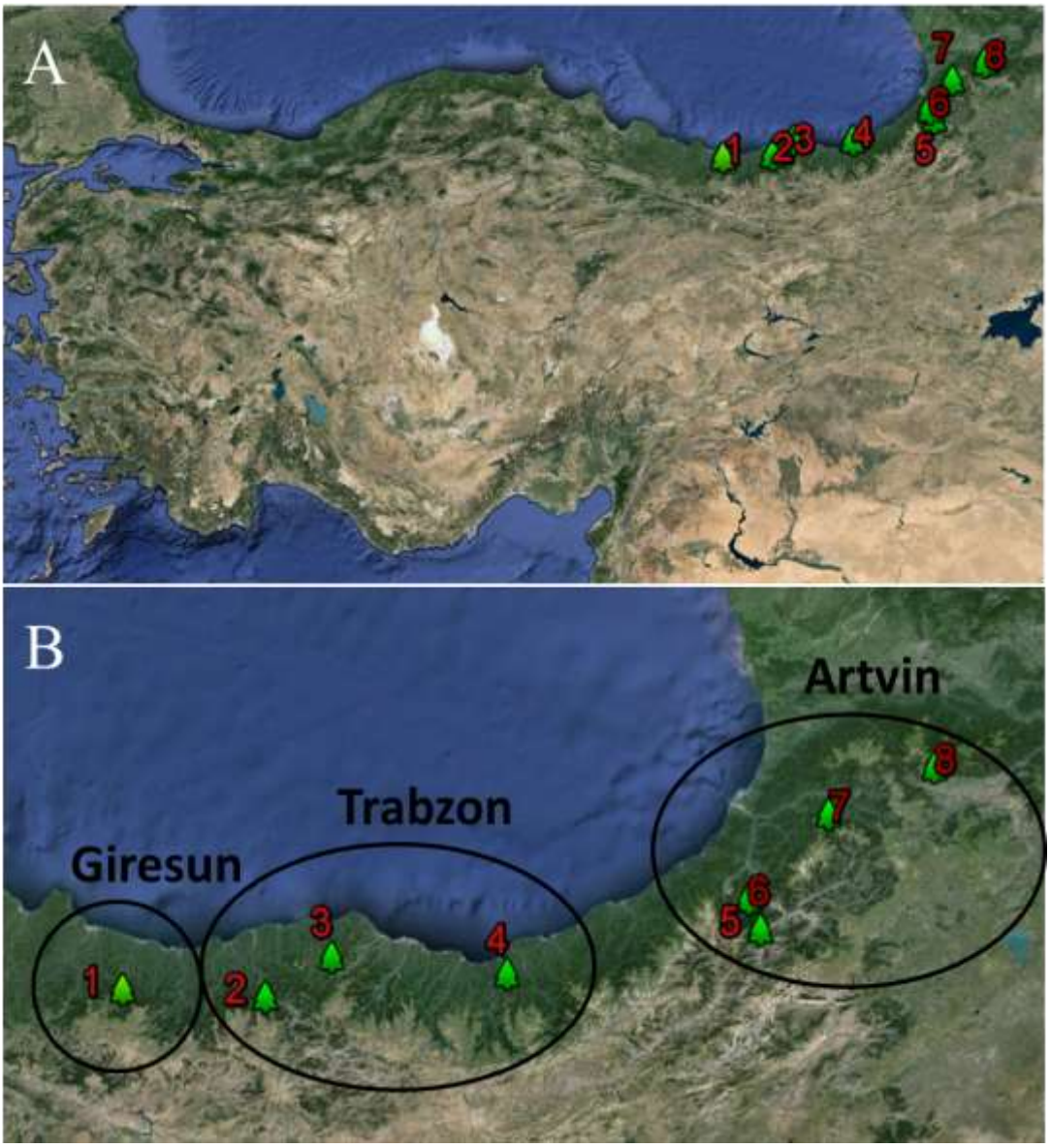

Figure 5 Map showing the locations of sampled populations (Codes for populations were indicated in red colored numbers (A). Black circles are the provincial locations of populations (B)) (Google Earth Pro 7.1.2.2041. (July 10, 2013). Turkey. 38° 36' 44.21"N, 35° 29' 22.74"W, Eye alt 1392.59 km. Borders and labels; places layers. NOAA, DigitalGlobe 2013. <http://www.google.com/earth/index.html> (Accessed March 3, 2015).

Open pollinated seeds of oriental spruce trees obtained from the first collection group were germinated in small pots at approximately 25^{o}C in a growth chamber (Figure 6).

Figure 6 Newly germinated seedlings used for tissue sampling to extract DNA.

Total DNA was extracted from fresh needles of young seedlings in the first collection group and mature needles in the second collection groups of each sample through a slightly modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). For each sample tree in a given population, one seedling was put in an autocklave-sterilized mortar, and crushed by the help of liquid nitrogen and then grounded by adding 1000 µL CTAB extraction buffer (see Appendix A). In case of needles' nature, tissues were ground again by liquid nitrogen, but this time CTAB buffer was prepared by adding polyvinylpyrrolidone (PVP) right before the isolation and heated in water bath at 65^{o}C for at least 30 min until PVP particles dissolved completely. The PVP concentration of the buffer was 4%. After addition of

1000 µL CTAB extraction buffer, 10 µL Beta Mercapto Ethanol (βME) and 4 µL proteinase K were put and vortexed with the samples until the solution mixed well. Then, mixed tubes were incubated in 65^0C water bath for at least 30 min Samples were mixed in every 10 min during they were in water bath. At the end of the incubation, tubes were placed in centrifuge and spinned at 14000 rpm for 10 min All supernatants were taken as carefully as possible without disturbing the pellet. Then, the supernatant was transferred in a clean empty microfuge tube in which 500 µL Chloroform: Octanol (24:1 v/v) was added. In this step, tubes were inverted instead of vortex to avoid damaging the DNA. In the next step, the samples were centrifuged at 14000 rpm for 10 min and the supernatant was transfered to clean microfuge tubes. Afterwards, 500 µl of cold isopropanol was added to the samples and they were stored at –80°C at least for 1 hour to attain proper precipitation of DNA. In the next step, the samples were spinned at 14000 rpm for 10 min and supernatant was removed and the pellets were washed twice with 500 µl, 70 % ethanol (EtOH). Pellets were allowed to dry completely for 15 min and dissolved with 50 µl of PCR grade H_2O. Then, DNA samples were stored at -20°C until further use.

To quantify the concentration of total DNA, Thermo NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc. Nano Drop 2000 Spectrophotometer Version 1.41) was used. 1 µL of DNA sample was dropped onto an optical measurement surface of the device to find out the final DNA concentration of the sample. All sample concentrations were fixed to 10 ng/µL with dilutions.

Initially, 33 primers were selected for the study based on both heterozygosity level of each locus and high allele variation observed in the previous *Picea* studies. These 33 primers were evaluated for segregation, amplification via PCR, and visualizing bands under UV after running maximum 2% agarose gel. Generally, primers with an ability to amplify higher sizes of fragments and having higher number of alleles observed in the previous *Picea* studies were chosen. For this purpose, 2 pairs of EST (PaGB3 and PgGB5) (Besnard et al., 2003) and 13 pairs of nuclear microsatelite (SS12, SS13, SS15, SS16, SS17, SpAGC1, SpAGG3, UAPgCA24, UAPgAG105, UAPgA150A,

UAPgTG87, UAPgCT144 and UAPgCT3) (Hodgetts et al., 2001, Pfeiffer et al., 1997, A'hara and Cottrell, 2004) primers were selected for further processes (Table 2). M13: AGGGTTTTCCCAGTCACGACGTT tail was only added to the 5' end of the forward primers of SS12, SS13, SS15, SS16 and SS17. All forward sequences of each paired primers were labeled fluorescently by Sigma-Proligo (Sigma-Aldrich Company, LTD Irvine, Ayrshire, UK) with different Beckman Coulter WellRED fluorescent dyes named as D2-black, D3-green and D4-blue (Table 3). These dyes were selected according to the sizes of alleles which were determined in earlier studies depicted in Table 2.

Table 2 Information on used microsatellite markers

Microsatellite Locus	Sequences of primer (F/R, 5'-3')	Repeat Unit	Maximum number of alleles observed	Allele size range (bp)	*Picea* species	References
UAPgA150A**	ACCAATGCTTTTACCAAACG TTGATTGCAAGTGATGGTTG	$(AG)_{19}$	4	126-145	*Picea glauca*	Hodgetts et al., 2001
UAPgCA24**	ATGCTCTTCTTAACCACCTG GACAATTCCTACCTCCACAC	$(AC)_{23}$	5	191-231	*Picea glauca*	Hodgetts et al., 2001
UAPgAG105**	CAACTACCTTGAGCCAATCA GTCCGGCATTATTGATCATT	$(AG)_{11}$	1	158	*Picea glauca*	Hodgetts et al., 2001
UAPgTG87**	GCACCAATAATCAAATCATGCC TTTGGAACACTACACATCAACC	$(TG)_{30}$	7	100-169	*Picea glauca*	Hodgetts et al., 2001
UAPgCT144**	CACTCGATCACTTTCTCATC CAAGATAGTAATGGTGAGGC	$(CT)_{18}$	3	136-146	*Picea glauca*	Hodgetts et al., 2001
UAPgCT3**	TTGAAAAAGAGGTTAGGAAGGGA TTCTTAAAGAAGCAGGGCATTG	$(CT)_{15}$	21	179-262	*Picea glauca*	Hodgetts et al., 2001
SpAGC1**	TTCACCTTAGCCGAGAACC CACTGGAGATCTTCGTTCTGA	$(TC)_5TT(TC)_{10}$	2	100-101	*Picea abies*	Pfeiffer et al., 1997
SpAGG3**	CTCCAACATTCCCATGTAGC AGCATGTTGTCCCATATAGACC	$(GA)_{24}$	9	119-158	*Picea abies*	Pfeiffer et al., 1997
SS12**	CTTGATTTTTGGCGATCGTT ACGTGTGAACCGGAGGAGAT	$(T)_{14}ATTTGCG(TGGCG)_4$	17	206-256	*Picea sitchensis*	A'hara and Cottrell, 2004
SS13**	ACTCATAGCGTCACGGGAAC TGAATCTCCACCTCCTCTGG	$(TA)_7$	8	232-267	*Picea sitchensis*	A'hara and Cottrell, 2004

Table 2 Continued

SS15**	GGAATAAAATGGCAGGTGGA GCCTGCAGTAGTTGGCAGA	$(GA)_9A(AG)_8$	19	174-226	*Picea sitchensis*	A'hara and Cottrell, 2004
SS16**	GCAGCACTGGCAACATTCTA ACGGAGACAAATCGCTTGTT	$(TA)_8T(TA)_5$	9	263-326	*Picea sitchensis*	A'hara and Cottrell, 2004
SS17**	CCGCTTTCACGGGTTTAATA GAGGTGGGAGGGTTTTTCTC	$(AT)_{11}$	25	179-231	*Picea sitchensis*	A'hara and Cottrell, 2004
PaGB3*	CCATTGCGGAGAACCCAGAG CGCAGAACAATGAATCTCCAC	$(AT)_{11}$-3'UTR	4	110-127	*P.abies* mRNA for major intrinsic protein (aquaporin)	Besnard et al., 2003
PgGB5*	AGTGATTAAACTCCTGACCAC CACTGAATACACCCATTATCC	$(AT)_9$-5'UTR	6	86-102	*P.glauca* heat shock-like protein *(*hsp 18.2-like) mRNA	Besnard et al., 2003

- stands for EST primers and ** indicates nuclear microsatellites.

Table 3 Grouping the primers for fragment analysis that formed according to their sizes and dyes.

Mix 1 **Primer name / Color of dye**	**Mix 2** **Primer name / Color of dye**	**Mix 3** **Primer name / Color of dye**
UAPgCA24 / D2-Black	SS12 / D2-Black	SS13 / D2-Black
UAPgAG105 / D4-Blue	SS15 / D4-Blue	PaGB3 / D4-Blue
UAPgA150A / D2-Black	SS16 / D3-Green	UAPgTG87 / D3-Green
SpAGG3 / D3-Green	SS17 / D3-Green	UAPgCT144 / D4-Blue
SpAGC1 / D4-Blue	PgGB5 / D4-Blue	UAPgCT3 / D4-Blue

Different PCR conditions and profiles were tried with the different pair of primers. Those with most successful amplification products were chosen as optimized conditions for amplifications of microsatellite markers. The details of conditions and profiles were provided in Table 4 and Table 5.

Table 4 Optimized PCR mixtures for all primers

PCR reaction components	**Mixture 1[a]**	**Mixture 2[b]**	**Mixture 3[c]**	**Mixture 4[d]**
Sterile Water	12.92µL	10.82µL	12.82µL	12.32µL
Taq DNA polymerase Buffer (10X)	3µL	4µL	3.5µL	3.6µL
$MgCl_2$ (25mM)	2.4µL	2.5µL	3µL	2.4µL
dNTP (10mM)	1µL	1µL	1µL	1µL
Primers (forward and reverse) (10µM)	2+2µL	2.5+2.5µL	1.5+1.5µL	2+2µL
Taq DNA polymerase	0.18µL	0.18µL	0.18µL	0.18µL
DNA	1.5µL	1.5µL	1.5µL	1.5µL
TOTAL	25µL	25µL	25µL	25µL

Superscript a for SS12, SS13, SS15, SS16 and SS17, b for SpAGC1 and SpAGG3, c for PgGB3 and PaGB5, d for UAPgCA24, UAPgAG105, UAPgA150A, UAPgTG87, UAPgCT144 and UAPgCT3

Table 5 The conditions of PCR amplification cycles for the studied primers

PCR Profiles	Primer group 1[a]	Primer group 2[b]	Primer group 3[c]	Primer group 4[d]
Initial Denaturation	5 min at 95°	3 min at 95°	5 min at 95°	5 min at 94°
Denaturation 1	30 sec at 94°			
Annealing 1	45 sec at 55°			
Extension 1	30 sec at 72°			
	10 cycles			
		30 sec at 94°	60 sec at 94°	30 sec at 94°
		45 sec at 55°	45 sec at 55°	30 sec at 57°
		45 sec at 72°	60 sec at 72°	90 sec at 72°
		28 cycles	36 cycles	30 cycles
Denaturation 2	30 sec at 94°			
Annealing 2	90 sec at 52°			
Extension 2	30 sec at 72°			
	25 cycles			
Final Extension	9 min at 72°	10 min at 72°	6 min at 72°	6 min at 72°

a: SS12, SS13, SS15, SS16 and SS17, b: SpAGC1 and SpAGG3, c: PgGB3 and PaGB5, d: UAPgCA24, UAPgAG105, UAPgA150A, UAPgTG87, UAPgCT144 and UAPgCT3

Amplified SSR fragments were prepared according to the Beckman CEQ DTCS kit. Analysis of the fragments was performed with a CEQ 8000XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA) via AKA Biotechnology Company İstanbul. The allele sizes for each SSR locus were determined with the help of Beckman Coulter CEQ fragment analysis software.

After the collection of data and checking the collected data organized in a format so that it could be analyzed statistically by the help of suitable softwares. Population genetic diversity parameters such as number of alleles per locus (Na), the effective number of alleles per locus (Ne), expected (He), observed (Ho) heterozygosities and gene flow (Nm); the number of private allele, the number of polymorphic loci, the (Lewis and Zaykin, 2001) percentage of polymorphic loci (PI) were calculated using Population Genetic Analysis (POPGENE VERSION 1.31) (Yeh et al., 1997) and Genetic Data Analysis (GDA) softwares (Lewis and Zaykin, 2001). Moreover, F-statistics were calculated using GDA software. Exact p-values were estimated by the Markov Chain method with 1000 dememorization, 100 batches and 1000 iterations as a result of the Hardy Weinberg probability test and null allele frequencies were calculated using GENEPOP version 4.2 (Raymond and Rousset, 1995, Rousset, 2008). Each locus was tested if there was significant deviations from Hardy–Weinberg equilibrium (HWE). The Nei's genetic identity and distance mesasures were utilized to construct a dendrogram using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) cluster analysis (Nei, 1978, Kumar et al., 2009, Zhao et al., 2010).

The STRUCTURE (Pritchard et al., 2000, Falush et al., 2003) software version 2.3.4 (2012) was used to find out the population structure by clustering (Bayesian clustering model) samples into genetically distinguishable groups on the basis of allele frequencies and K (subpopulations) populations assumed by the help of this method. Analyses were performed with a burnin time period of 5,000 and a Markov Chain Monte Carlo (MCMC) replication number set up to 50,000. The programme

was run 20 times and for each ‘k’ ranged from 3 to 8. The ‘‘Structure harvester’’ program was used (http://taylor0.biology.ucla.edu) to determine the final recomended number of populations (Earl, 2012). The distribution of all genotypes was determined by factorial correspondence analysis (FCA) using GENETİX 4.05 (Belkir et al., 2000).

CHAPTER V

RESULTS

5.1 Genetic diversity in oriental spruce

All primers were successfully amplified and allele numbers among the 15 loci varied from 2 to 25. Except for one locus (UAPgAG105), all other loci were detected as polymorphic. In other words, the percentage of polymorphic loci (PI) was 93 % among the studied population. Locus SS17 showed the highest number of observed alleles as 25, while locus SpAGC1 was with the least number of alleles (2 alleles) among the studied populations. The highest effective number of allele was found as 9.34 in the locus SS15. There was a considerable variation between observed and effective number of alleles per locus. Average number of observed alleles per locus (9.3) was almost two times higher than the mean effective number of alleles (4.4) (Table 8). The observed heterozygosities of the loci were varied from 0.28 to 0.92 and the expected heterozygosities were ranged from 0.09 to 0.89. While the lowest observed and expected heterozygosities were recorded in the locus SpAGC1 as 0.02 and 0.09, the highest observed and expected heterozygosities were found as 0.92 in SS17 and 0.89 in SS12, SS15 and UAPgCT3. The observed heterozygosity in almost all loci was lower than the expected, one exception was that SS17 had higher observed heterozygosity than expected heterozygosity (Table 8). Out of the 140 different alleles detected in 15 loci, 43 occurred only once in the investigated locus. These alleles, each found in only one locus, are called private alleles (Hartl et al., 1997) and the private allele numbers ranged from 0 to 11. Positive inbreeding coefficient value within the 12 loci (F_{IS}) was observed, meaning observed heterozygosity (Ho) values for these loci were lower than the expected. Furthermore, negative F_{IS} values were detected in 2 loci (SS17 and PgGB5). This indicated that He values were lower than the observed. The mean F_{IS} for 14 loci was calculated as

0.13. Heterozygosity level was 13 % lower than the expected. The overall fixation index (F_{IT}) ranged from -0.09 to 0.71 with an average of 0.19. The positive values indicated more homozygotes calculated than expected. On the other hand, negative values showed excess of heterozygotes. The mean F_{ST} was 0.07. Exact p-values were found to be significant at $p \leq 0.05$ for 13 loci while two loci (UAPgCA24, UAPgCT144) was found to be non significant (Table 8).

Table 6 Basic population genetic diversity parameters estimated for microsatelite loci of oriental spruce populations (Bold values indicated the highest among all calculated ones)

Locus	N	Na	Ne	Ho	He	Private allele numbers	F_{IS}	F_{IT}	F_{ST}
UAPgA150A	267	4	2.19	0.28	0.54	1	0.4	0.45	0.08***
UAPgCA24	257	5	2.55	0.52	0.61	1	0.06	0.11	0.06
UAPgTG87	228	7	4.45	0.74	0.78	0	0.01	0.05	0.03**
UAPgCT144	277	3	1.72	0.4	0.42	0	0.01	0.03	0.02
UAPgCT3	240	21	8.83	0.76	**0.89**	6	0.07	0.13	0.06***
SpAGC1	277	2	1.10	0.02	0.09	0	**0.67**	**0.71**	**0.14*** **
SpAGG3	256	9	5.21	0.55	0.81	1	0.25	0.30	0.07***
SS12	251	17	9.08	0.66	**0.89**	9	0.15	0.24	0.11***
SS13	254	8	4.59	0.59	0.78	2	0.18	0.23	0.06***
SS15	254	19	**9.34**	0.68	**0.89**	7	0.17	0.22	0.06***

Table 6 Continued

Locus	N	Na	Ne	Ho	He	Private allele numbers	F_{IS}	F_{IT}	F_{ST}
SS16	242	9	5.4	0.34	0.82	3	0.56	0.61	0.12***
SS17	259	**25**	6.52	**0.92**	0.85	**11**	-0.19	-0.09	0.08***
PaGB3	240	4	2.44	0.53	0.6	1	0.02	0.08	0.06***
PgGB5	244	6	1.63	0.41	0.39	1	-0.17	-0.09	0.07**
Mean	**236.4**	**9.3**	**4.4**	**0.49**	**0.62**	**2.86**	**0.13**	**0.19**	**0.07*** **

N= number of samples, Na= number of allele observed Nei (1987), Ne= the effective number of alleles per locus (Kimura and Crow, 1964), Ho= observed heterozygosity, He= expected heterozygosity, F_{IS} = inbreeding coefficient within a subpopulation, F_{IT}= overall fixation index, F_{ST}= fixation index, the relationship between these three is $(1-F_{IT})=(1-F_{IS})(1-F_{ST})$. **p<0.05, ***p<0.001.

5.2 Estimated genetic diversity parameters and genetic structure of oriental spruce populations

Among the 8 populations that were selected based on the geographic range of the species, number of alleles per population ranged from 3.73±0.38 in C-Trabzon to 5.26±0.51 in E-Trabzon. On the other hand, the highest effective number of alleles was detected as 3.79±0.48 in S-Artvin. The mean number of alleles per population was 4.9±0.47 and the average number of effective alleles was found as 3.4±0.18. The overall observed and expected heterozygosities were pretty close to each other as 0.5±0.02 and 0.59±0.02, respectively. However, populations had low and high observed heterozygosities. Especially Giresun, E-Trabzon, SE-Artvin, N-Artvin and C-Artvin populations had high inbreeding coeffients meaning excess of homozygotes. The reason of this may be the Wahlund effect and restricted sample size. The highest number of private allele was observed in SE-Artvin population as 13. Except for C-Trabzon population, the inbreeding coefficients in all populations were found to be positive and ranging from 0.07 to 0.22. It means that observed homozygosity levels of almost all populations were higher than the expected ones. All p-values were found to be highly significant (Table 7). Unrooted UPGMA tree was constructed in order to define genetic similarity of the 8 populations of oriental spruce. According to the tree, three main clades were apparent as Artvin (S-Artvin, N-Artvin and C-Artvin), Trabzon (E-Trabzon, W-Trabzon, C-Trabzon and Girseun) and Southeastern Artvin (Figure 7). The Southeastern Artvin seems to be genetically most distant population to the others.

Table 7 Basic genetic diversity parameters of oriental spruce populations

Population	N	Na	Ne	Ho	He	P	Private allele	F_{IS}	F_{IT}	F_{ST}
Giresun	49.06	5.20±0.44	3.4±0.28	0.45±0.04	0.57±0.04	0.86	7	0.22	0.21***	0***
W-Trabzon	28.86	4.46±0.47	3.06±0.29	0.53±0.05	0.57±0.05	0.86	3	0.07		
C-Trabzon	20	3.73±0.38	2.69±0.25	**0.58**±0.07	0.56±0.06	0.86	2	-0.03	0.11***	0.04***
E- Trabzon	37	5.26±0.51	3.72±0.38	0.47±0.05	0.59±0.05	**0.93**	5	0.21		
S- Artvin	27.13	5.4±0.65	**3.79**±0.48	0.53±0.06	0.61±0.05	**0.93**	5	0.13		
N- Artvin	25.53	4.53±0.53	3.3±0.42	0.48±0.06	0.58±0.05	**0.93**	3	0.18		
C- Artvin	26.26	5.06±0.56	3.63±0.43	0.5±0.06	0.59±0.06	0.86	5	0.17	0.21***	0.06***
SE- Artvin	39.33	**5.6**±0.56	3.72±0.34	0.49±0.05	**0.62**±0.04	**0.93**	**13**	0.21		
Mean	**31.57**	**4.9**±0.47	**3.4**±0.18	**0.5**±0.02	**0.59**±0.02	**0.9**	**5.38**	**0.15**	**0.18*** **	**0.03*** **

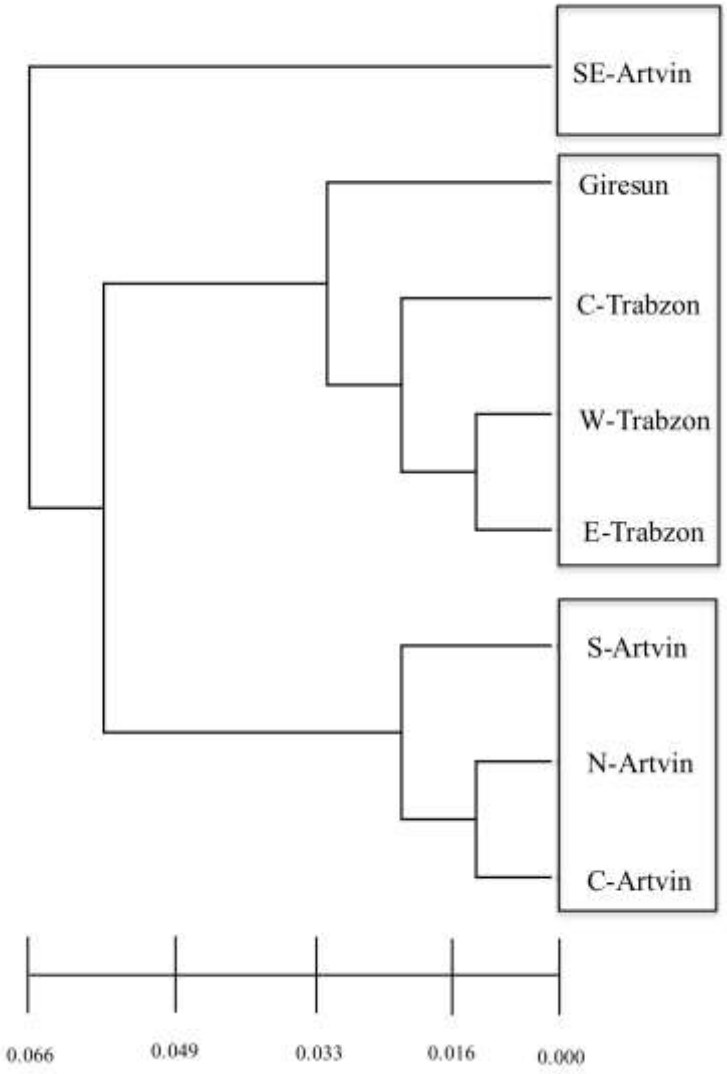

Figure 7 The Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) dendogram showing the groupings of oriental spruce populations based on genetic distance (Nei 1978)**.**

Bayesian inference of the structure of the populations was generated via the software STRUCTURE (Pritchard et al., 2000, Falush et al., 2003). By this method, a model of K clusters under the admixture model, in our case 4 (Table 8), is assumed and samples are grouped in order to minimize linkage disequilibrium (LD) and to maximize conformity to Hardy-Weinberg equilibrium across all analyzed loci. The ratio of estimated membership value ranged from 0.04 to 0.669 and the highest assumption membership value was observed in C-Trabzon population in first subpopulation (cluster). The highest value in the second and third clusters were 0.664 in N-Artvin and 0.986 in C-Trabzon, respectively. The last estimated membership values varied between 0.02 as in C-Trabzon and 0.762 as in SE-Artvin. Moreover, obtained results were displayed graphically in a bar chart as well identified as three main groups (the red, blue, yellow and green components). These four colors represent four different gene pools as Trabzon (Giresun, W-Trabzon, C-Trabzon, E-Trabzon), Artvin (S-Artvin, N-Artvin), C-Artvin and SE-Artvin. (Figure 8). The C-Trabzon population was appeared to have poor membership in the cluster from the bar plot graph (blue color).

Table 8 Membership estimations of four genetically inferred groups

Oriental spruce populations	**Number of samples**	**Ratio of membership of every predefined population in four clusters**			
		1	2	3	4
Giresun	50	0.008	0.406	**0.583**	0.003
W-Trabzon	30	0.004	0.314	**0.679**	0.003
C-Trabzon	20	0.01	0.002	**0.986**	0.002
E- Trabzon	39	0.032	0.495	**0.469**	0.004
S- Artvin	31	0.324	**0.654**	0.019	0.004
N- Artvin	30	0.328	**0.664**	0.004	0.004
C- Artvin	29	**0.669**	0.310	0.005	0.016
SE- Artvin	48	0.221	0.005	0.012	**0.762**

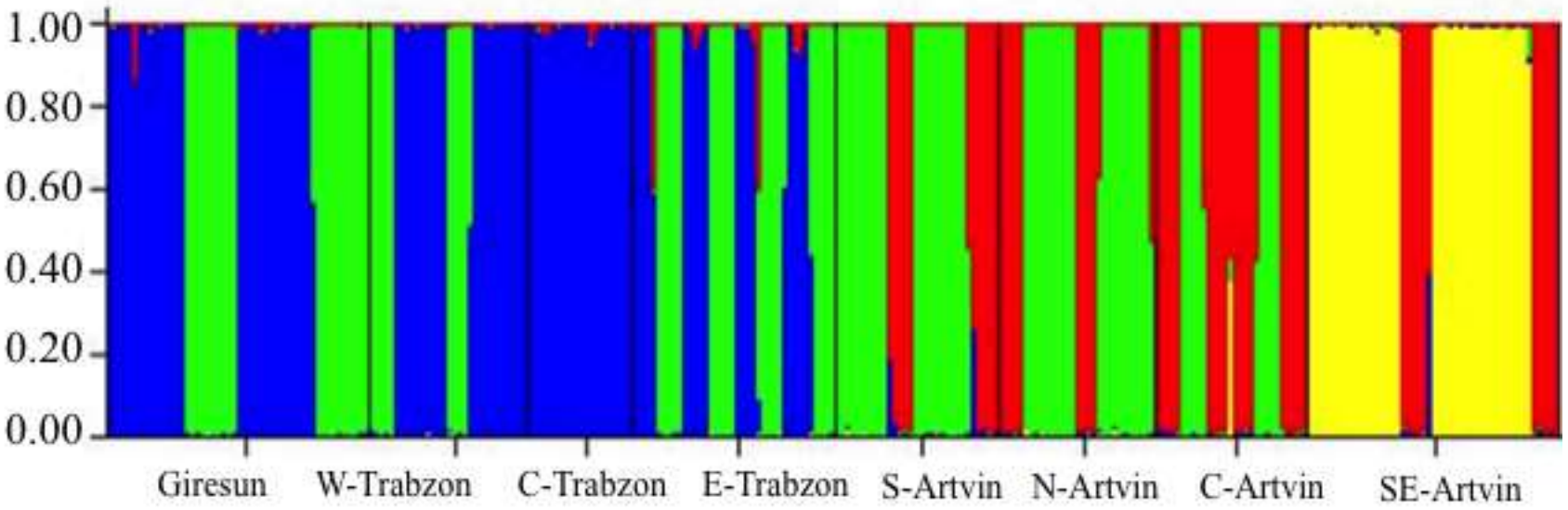

Figure 8 The bar plot of subpopulations of oriental spruce according to the best K value (4) result.

The factorial correspondence analysis (FCA) distributed all accessions into three main groups (Figure 9). These were Trabzon (Giresun, W-Trabzon, C-Trabzon, E-Trabzon), Artvin (S-Artvin, N-Artvin C-Artvin) and SE-Artvin. Seperation of these three main groups confirmed the findings obtained from the dendogram (Figure 7). Especially, SE-Artvin was clearly separated and located in different part of the graph.

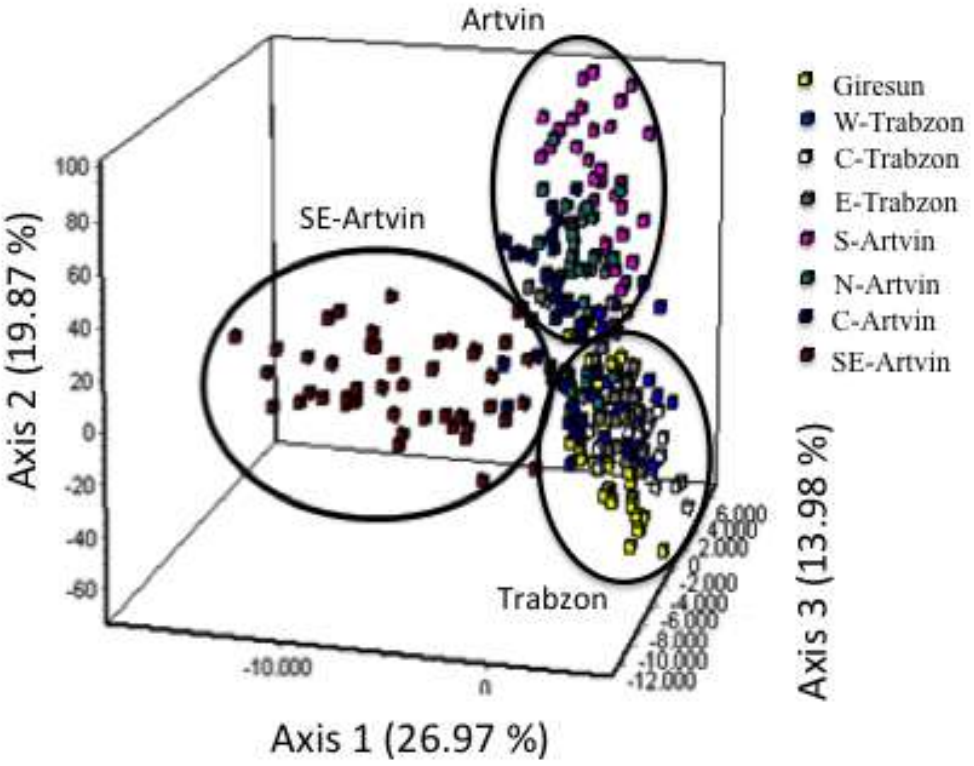

Figure 9 Three dimentional-factorial correspondence analysis (3D-FCA) of oriental spruce populations. Symbols with the same color represent the indivduals of a population, each axis representing a portion of the total variation in the data.

Alele frequency distribution of three randomly choosen loci of 8 naturally distributed oriental spruce populations were shown on the map and it was clearly indicated that allele frequency level is increasing from west to east.

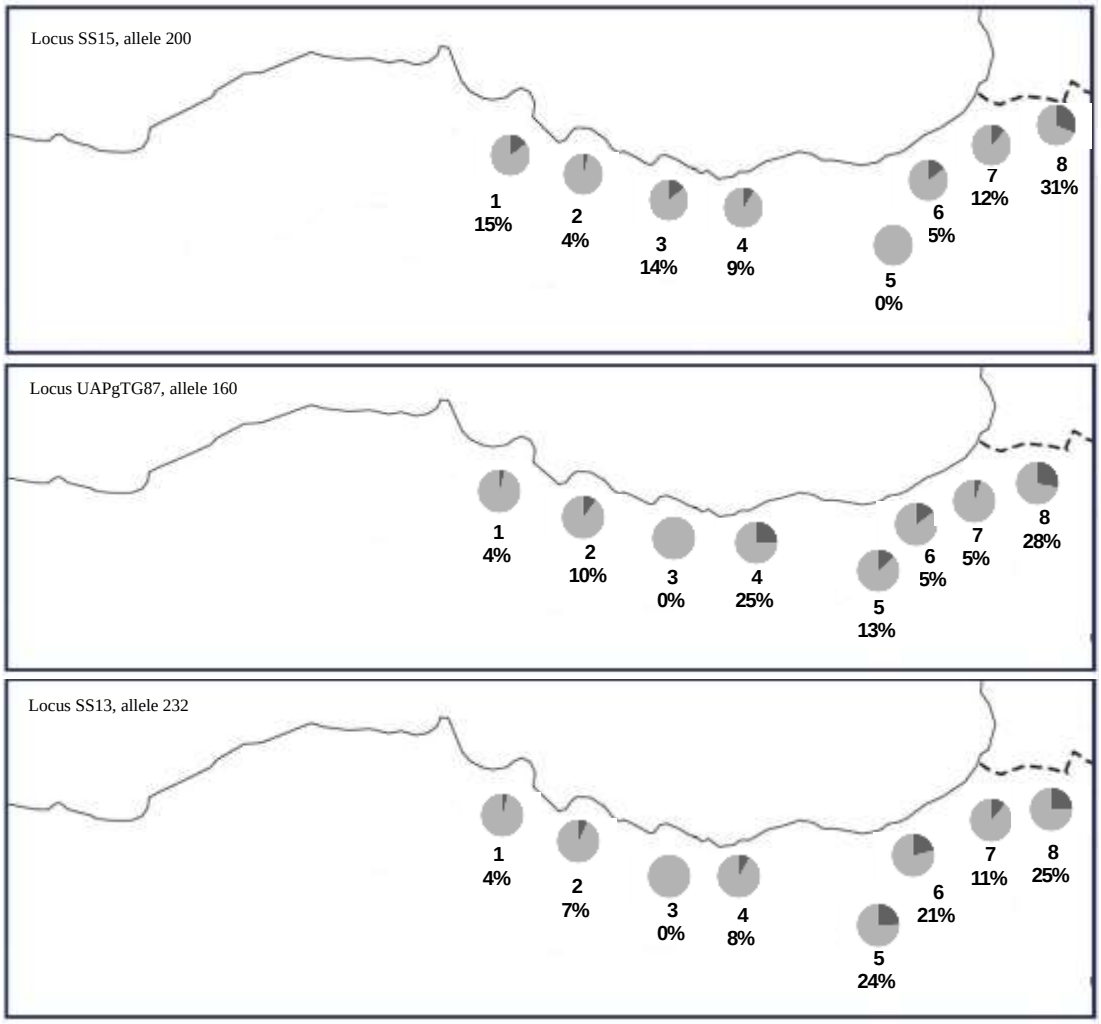

Figure 10 Frequency distribution of randomly selected three common allele.

5.3 Genetic diversity parameters and the structure of open pollinated seed and needle samples

Seed and needle samples of oriental spruce populations were collected from different part of Giresun, Trabzon and Artvin. Artvin populations were grouped as Northern, Southern and Eastern due to their locations in seed samples. In needle samples, Trabzon popluations were evaluated as Eastern and Western.

Among populations with open pollinated seed samples, the mean number of alleles, the mean number of effective alleles and the mean number of private alleles were found as 4.04±0.43, 2.67±0.17 and 7.4, respectively. All observed heterozygosities were lower than the expected and inbreeding coefficient value (F_{IS}) was 0.15. High inbreeding coefficient or excess in homozygotes indicates seedlings are more likely to include genetically related progenies in the analysis.

According to the basic genetic diversity parameters results obtained from the needle sample populations, the mean number of alleles, the mean number of effective alleles and the mean number of private alleles were found as 3.98±0.42, 2.72±0.14 and 6, respectively. Mean observed and expected heterozygosities were almost equal (Ho=0.53±0.02 and He=0.55±0.02) and the average F_{IS} value was vey low (F_{IS}=0.04) in needle samples. This notifies that the population of needle samples is almost in Hardy-Weindberg (HW) equilibrium. Also, each population F_{IS} was positive except W-Trabzon (Table 9).

Table 9 Genetic diversity parameters estimated for populations in which open-pollinated newly germinated seed tissues used (A), Genetic diversity parameters estimated for populations from which needle tissues used sampled from mature tree (B).

Population	**N**	**Na**	**Ne**	**Ho**	**He**	**P**	**Private allele**	F_{IS}	F_{IT}	F_{ST}
(A)										
Giresun	27.93	4±0.45	2.53±0.23	0.42±0.06	0.5±0.05	0.87	11	0.168		
Trabzon	17.06	3.6±0.49	2.48±0.34	0.4±0.08	0.48±0.07	0.87	2	0.166		
N-Artvin	28.4	4.26±0.39	2.77±0.25	0.42±0.06	0.55±0.05	0.93	5	0.229		
S-Artvin	18.35	3.86±0.51	2.68±0.33	0.51±0.08	0.54±0.06	0.93	3	0.049	0.25	0.09
E-Artvin	29.33	4.47±0.51	2.89±0.29	0.47±0.05	0.55±0.05	0.93	16	0.141		
Mean	**24.2**	**4.04**±0.43	**2.67**±0.17	**0.45**±0.03	**0.52**±0.03	**0.91**	**7.4**	**0.15**		
(B)										
Artvin	33.4	5±0.6	3.06±0.29	0.55±0.05	0.57±0.05	0.86	16	0.04		

Table 9 Continued

Population	N	Na	Ne	Ho	He	P	Private allele	F_{IS}	F_{IT}	F_{ST}
E-Trabzon	19.93	3.73±0.41	2.68±0.26	0.52±0.06	0.56±0.06	0.86	4	0.06		
W-Trabzon	30	4.2±0.44	2.66±0.21	0.57±0.05	0.55±0.05	0.86	5	-0.04		
Giresun	30	3.86±0.39	2.72±0.23	0.48±0.05	0.54±0.05	0.86	4	0.12	0.11	0.08
Borjomi	10	3.13±0.45	2.46±0.34	0.51±0.11	0.52±0.09	0.86	1	0.02		
Mean	**24.6**	**3.98**±0.42	**2.72**±0.14	**0.53**±0.02	**0.55**±0.02	**0.86**	**6**	**0.04**		

CHAPTER 6

DISCUSSION

In recent years, especially the last few decades, genetic diversity studies based on microsatelite data have become very popular among the population geneticists due to the high variability characters of these markers in each locus and its codominant feature. Because of this reason, microsatelite markers were preferred in the present study to find out the genetic diversity patterns and structures of relict oriental spruce populations.

6.1 Analysis of descriptive population genetic parameters

In present study, both genomic and EST-SSR markers were utilized to determine genetic diversity in *Picea orientalis*. At the begining of the current study, different groups of microsatellite primers were selected from the previously reported genetic diversity studies in *Picea* species (Hodgetts et al., 2001, Pfeiffer et al., 1997, A'hara and Cottrell, 2004, Besnard et al., 2003). It should be noted that some of the selected primers had been specifically designed for *Picea* species. Two chosen loci namely paGB3 and pgGB5 were created for *Picea glauca* and *Picea abies*, respectively (Besnard et al., 2003). These primers were tested on oriental spruce for the first time and revealed successful results in context of PCR. Also, they were the only EST-SSR primers used in the current study. The same primers were employed in a study across Norway spruce populations in Sweden (Androsiuk et al., 2013). When the results from the previous studies (Besnard et al., 2003, Androsiuk et al., 2013) are compared with the current study, the lowest number of alleles per locus, the lowest observed and expected heterozygosities were detected for both primer PaGB3 and PgGB5. On the other hand, the highest F_{ST} value was detected in current study in terms of these

two primers (Table 12). While the F_{ST} range <0.05 indicates little genetic differentiation as observed in previous studies (Besnard et al., 2003, Androsiuk et al., 2013), in present study this range was greater than 0.05 though it was in the range reported for conifers. For example, the overall mean F_{ST} of *P. orientalis* was (0.07) almost similar with *P. glehnii* (0.083) (Aizawa et al., 2014), and endemic *P. alcoquiana* (0.073) (Aizawa et al., 2008). The distribution areas of these two species are also very limited and local like oriental spruce. However, the F_{ST} value estimated for oriental spruce was lower than other relict spruce species such as in *P. asperata* (0.168) (Wang et al., 2005), *P. omorika* (0.165) (Aleksić and Geburek, 2014) and *P. koyamae* (0.209) (Katsuki et al., 2011). On the other hand, the mean F_{ST} of *P. orientalis* was higher than that of *P. abies* (0.05) (Meloni et al., 2007). According to the nuclear microsatellite study among *P. asperata*, it was suggested that high rate of inbreeding and habitat fragmentation increase the F_{ST} (Wang et al., 2005).

Table 10 The comparison of genetic diversity parameters of current study with the previous studies

Study	Loci	Na	Ho	He	F_{ST}
Besnard et al., 2003	PaGB3	9	0.65	0.76	0
	PgGB5	10	0.75	0.77	0
Androsiuk et al., 2013	PaGB3	11	0.73	0.76	0.005
	PgGB5	9	0.75	0.75	0.014
Current study	PaGB3	4	0.53	0.6	0.06
	PgGB5	6	0.41	0.39	0.07

When loci, namely SS12 (17), SS13 (8), SS15 (19), SS16 (9) and SS17 (25) were taken into account, number of variable alleles was higher (indicated in the

parenthesis with the relevant locus) than other group of loci. These primers were specifically developed for *Picea sitchensis* (A'hara and Cottrell, 2004). According to the results mentioned in the study, it was clearly exhibited that the most variable loci were SS17, SS12 and SS15. These loci showed the highest numbers of private allele (the allele specific to a subpopulation) across all loci. Higher number of private alleles indicates the higher allelic variation based upon studied oriental spruce populations. Private alleles are also useful markers to characterize the genetic materials if they are specific to particular populations. Moreover, the highest observed heterozygosity value was detected in SS17 locus to be 0.92. Also, negative F_{IS} value of SS17 locus demonstrates the excess of heterozygotes. Our findings consistently revealed parallel outcomes with the results of A'hara and Cottrell (2004) based on loci. In general, each locus has its own power to determine the genetic differences. Among these group of primers, SS12, SS15 and SS17 demonstrated more powerful discrimination ability to detect genetic diversity in *Picea orientalis*.

Among the UAPgCA24, UAPgAG105, UAPgA150A, UAPgTG87, UAPgCT144 and UAPgCT3 primers, only the UAPgAG105 had monomorphic feature. However, the study conducted by Hodgetts et al (2001) demonstrated that this locus was polymorphic with seven alleles scored for 15 white spruce trees. Moreover, UAPgTG87 was one of the most diverse primers in that study. While in current study, the UAPgCT3 was the most diverse locus.

SpAGC1 and SpAGG3 that were developed for Norway spruce by Pfeiffer et al (1997) were the most utilized primers by researchers. The number of observed alleles and expected heterozygosity were found 8 and 0.427 for SpAGC1, 17 and 0.924 for SpAGG3, respectively (Pfeiffer et al., 1997). In present study, these values were found as 2 and 0.09 for SpAGC1 and 9 and 0.81 for SpAGG3. These results showed that both alleles number and expected heterozygosity levels were lower in oriental spruce populations than Norway spruce populations via these two loci.

Overall, genetic diversity parameters of SSRs are almost similar among spruce species. This emphasizes less variation by means of these markers among spruces although each of them divert to different geographic and climatic conditions in the world.

6.2 Genetic diversity of oriental spruce

Eight natural populations of oriental spruce were analysed using 15 SSR markers in order to determine genetic diversity and structure of populations. The results of the present study revealed that the most diverse population was SE-Artvin. The average He values was found in close magnitute in all 8 of populations (0.56–0.62) and exhibited a tendency for declining from east to west (from Artvin to Giresun). This result may be associated with the effect of climatic changes arising from the geographic characteristics of the Black Sea region. During the field study, it was observed that although the geographic distances among some locations were very close, there was an observable difference by means of climate and geographic features of the region. Atalay (1984) indicated that natural distribution area of oriental spruce forests were completely different from each other according to the climate and vegetation conditions. Additionally, there was close relationship between the natural occurance of oriental spruce and orography (topographic relief of mountains) and altitude (Atalay, 1984). However, the studies on conifers depicted that there was no significant correlation between genetic diversity and geographic distance. The conflict of this issue was discussed in literature in numerous studies. It was reported that there was no significant relation between genetic diversity and geographic distances in *Pinus rzedowskii* and *Pinus nelsonii* (Delgado et al., 1999, Cuenca et al., 2003). In contrast, it was demonstrated the significant association between genetic diversity and geographic distance in *Pinus pinceana*, *Pinus nelsonii* and *Picea sitchensis* (Ledig et al., 2001, Cuenca et al., 2003, Mimura and Aitken, 2007). Furthermore, global climate factors (i.e temperature and moisture) and local geographical factors (i.e mountains) might change the distribution and cause local extinction of spruce species (Aizawa et al., 2008).

When F_{IS} value deviate from zero and becomes negative, it means presence of excess heterozygotes (which occurs frequently in conifers and may be ascribed to a negative assortative mating system or to a selection favouring heterozygotes). However if it becomes positive it means the presence of excess homozygotes. All F_{IS} values, except the value of C-Trabzon population were positive in our study. In other words, our oriental spruce populations have excess homozygotes. In general, populations demonstrate decline in heterozygosity represent lowered evolutionary potential because of their insufficient genetic variation to respond to environmental changes. This may cause lower reproductive fitness and potential risk of maladaptation. In the light of these information, oriental spruce populations have more potential of reduction in population size due to its relict feature in addition of excess homozygotes in populations. However, it should be kept in mind that sample size in a study is important.

Comparison of the F_{IS} of subpopulations with that of total population gives F_{ST} estimation. The overall F_{ST} value and genetic variation among the populations of oriental spruce were detected as 0.03 and 3%, respectively. This data resulted in 97% of the genetic variation among individuals within populations. In conclusion, genetic variation data depicted a low genetic differentiation among the populations of oriental spruce. Similar results (*i.e* high genetic diversity within populations and low genetic differentiation among the populations) were observed in earlier reports in conifers (Godt et al., 2001, Rajora et al., 2005). Specifically, among the populations of oriental spruce, Artvin populations showed the highest F_{ST} value. In terms of expected heterozygosity findings, the highest He was also estimated for SE-Artvin. Artvin populations, especially the SE-Artvin should be taken into consideration for conservation program due to possessing the highest private alleles, highest allelic richness and high genetic distance from others.

The constructed UPGMA tree showed that there is no remarkable genetic distance among the populations collected from closer regions. However, genetic distance

among the populations, which were geographically distant, displayed significant genetic distances. The constructed dendrogram revealed that the 8 populations were grouped into three main clusters. First cluster consisted of the geographically closer populations (Giresun, C-Trabzon, W-Trabzon and E-Trabzon). The second cluster had the populations of S-Artvin, N-Artvin and C-Artvin. In this cluster, populations N-Artvin and C-Artvin grouped together while population S-Artvin was more distant to them. The SE-Artvin population differed considerably from other populations and was clustered alone as a third group.

Population structure is an important factor to decide the correlation between genes drawn at different subdivided populations. Migration, mutation, selection and genetic drift may have significant roles on structure of populations. The results for structure of populations showed that K value (subpopulations number) was smaller than expected and equals to 4. It means that the best group number of the populations should be 4 according to microsatellite data. Reduction of population size to 4 instead of 8 implied that some of these populations were genetically grouped together. Trabzon population, for instance, clustered with Giresun population. Additionally, S-Artvin and N-Artvin populations were genetically a single group. This was an acceptable situation due to the geographical closeness of these populations. SE-Artvin population was the most genetically different population. These findings were also confirmed by factorial correspondence analyses (FCA).

6.3 Genetic diversity of seed and needle samples of the populations

Oriental spruce populations were evaluated according to the structure of the samples. Both seed and needle samples were collected and analyzed with respect to the genetic diversity parameters. Although seed and needle samples were originated from different sources in the same region, no remarkable difference was observed between these specimens. This may be attributed to lower genetic variation within individuals of the populations than the variation among populations.

At the beginning of the study, these samples were collected to find out the genetic differences between naturally distributed and conserved seed stands of the populations. Because of the differences in collection time and purpose, structures of samples were different in open pollinated seeds and needles. Evaluation of the results within and among samples of populations showed that there were no distinctive differences within samples, but there were differences among samples.

CHAPTER 7

CONCLUSION

This study has shed light on genetic diversity and the population structure of *Picea orientalis* species which have very limited local distribution area in the world according to microsatelite markers (SSRs) data. The main objective of this study was to obtain genetic data to help identifying the genetic structure and diversity pattern of naturally distributed oriental spruce populations in Turkey.

With respect to the results based on loci analysis; the most variable locus was found to be SS17. UAPgCT3, SS15 and SS12 followed in decreasing order. These primers are suggested to be used in further studies to understand the genetic diversity of other spruce species.

SSR marker-based molecular characterization of oriental spruce revealed that large genotypic variation exists in Southeastern Artvin (SE-Artvin) population among all studied populations, resulting in excess of heterozygotes.

According to the result obtained from the evaluation of the data based on the seed and needle samples, Northern-Artvin (N-Artvin) population was observed to possess slightly variable characters with respect to needle samples. Furthermore, seed samples from E-Artvin populations had higher genetic diversity than Giresun and Trabzon populations.

To sum up, this is the first report on molecular analysis of genetic diversity and population structure of *Picea orientalis* from its native distributional range in Turkey. The current study revealed that among all groups of studied populations, the most variable ones were Artvin populations. The information basically indicates that eastern populations of oriental spruce have different genetic makeup than the populations distributed western part of the coastal region of Eastern Black Sea. Moreover, Artvin population, more specifically Southestern Artvin population was found to be possessly the highest private allele number (13), highest expected heterozygosity (0.62) and highest observed allele number (5.6). Furthermore, this population was the most divergent one according to the genetic distance (UPGMA dendogram), genetic structure (bar plot graph) and FCA analysis. According to this findings, SE-Artvin population should be urgently taken into consideration for management and conservation programs.

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APPENDICES

A. Buffer for DNA extraction

2X CTAB:

10 g CTAB (Cetyl Trimethyl Ammonium Bromide),

50 mL (pH: 8.0) Tris HCl,

40 mL (pH: 8.0) 0.5M EDTA,

41 g NaCl is completed with 500 mL with dH_2O

Chloroform-Octanol: (24:1)

β-Mercaptoethanol: Pure β-Mercaptoethanol, room temperature

Isopropanol, (FLUKA)**:** Pure isopropanol, ice cold

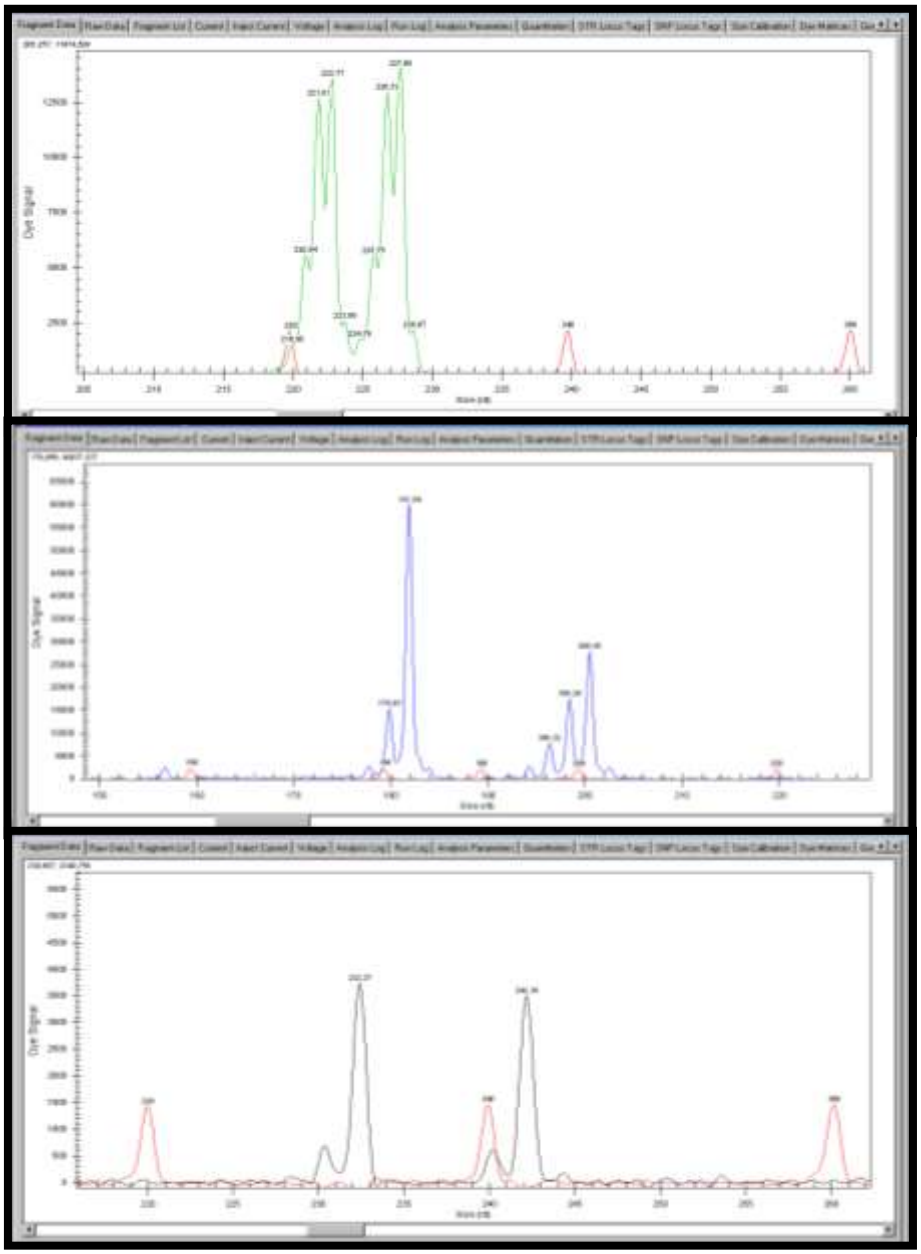
Ethanol: 70% in dH2O

B. Specific population number, location, individual numbers in the population, latitude and longitude information of oriental spruce populations before group them into 8 main populations

Pop.no.	Location	Individual numbers	Latitude (N)	Longitude (E)	Altitude (m)
1	Giresun-Ordu-*Çambaşı* (NG)	10	40° 41'	37° 57'	1400
2	Giresun-Ordu-*Gölköy* (NG)	10	40° 39'	37° 42'	1600
3	Giresun-Tirebolu-*Akılbaba* (NG)	10	40° 41'	38° 52’	1350
4	Trabzon-Maçka-*Hamsiköy* (NG)	10	40° 41'	39° 27'	1510
5	Trabzon-Pazar-*Çamlıhemşin* (NG)	9	40° 57'	41° 04'	1050
6	Artvin-Merkez-Tütüncüler, Taşlıca (TM), *Hatila* (NG)	10	41° 07'	41° 37'	1330
7	Artvin-Saçinka-*Artvin* (NG)	10	41° 09'	41° 47'	1150
8	Artvin-*Borçka*-Balcı (NG)	9	41° 21'	41° 47'	770
9	Artvin-*Yusufeli*-Altıparmak (NG)	10	40° 57'	41° 24'	1740
10	Artvin-*Ardanuç*, Tepedüzü ve Ovacık (NG)	10	41° 04'	42° 06'	1710
11	Artvin-*Şavşat*, Yayla (NG)	10	41° 14'	42° 22'	1280
12	Artvin-Şavşat-*Meydancık* (NG)	10	41° 26'	42° 12'	1370
13	Artvin-Şavşat-*Veliköy* (SS)	10	41° 18'	42° 29'	1650
14	Erzurum-*Ardahan*-Posof (NG)	8	41° 29'	42° 43'	1380
15	Trabzon-*Sürmene* (SS)	10	40° 34'	40° 24'	1800
16	*Borjomi* (Geo) (NG)	10	41° 50'	43° 23'	910
17	*Khulo* (Geo) (NG)	10	41° 39'	42° 28'	1560
18	*Tonya* (GCF)	10	40° 44'	39° 12'	1730
19	*Kumbet* (SS)	10	40° 34'	38° 25'	1700
20	*İkisu* (SS)	10	40° 35'	38° 24'	1700
21	*Bicik* (SS)	10	40° 42'	38° 11'	1400
22	*Maçka* (SS)	10	40° 48'	39° 38'	1030
23	*Göktaş* (SS)	10	40° 39'	39° 01'	1090
24	*Vakfikebir* (SS)	10	40° 55'	39° 23'	1800
25	*Öğdem* (SS)	10	40° 59'	41° 40'	1540
26	*Örümcek* (GCF)	10	40° 39'	39° 01'	1520
27	*Günyüzü* (SS)	10	40° 40'	38° 57'	1680
28	*Taşlıca* (SS)	10	41° 07'	41° 36'	1830

*NG: Natural growth, SS: Seed stand, GCF: Gene conservation forest.

C. Example peak profiles of heterozygotes samples



D. A partial data file of POPGENE software

/* Data Set: Picea orientalis */

Number of populations = 8

Number of loci = 15

Locus name :

150A CA24 AGG3 AGC1 G105 TG87 T144 SS12 SS15 SS16 SS17 AGB3 GGB5 ACT3 SS13

name = Giresun

DC AA EG AA AA DD BB UU PD HH FU BB BB IM DG

DC DA EE AA AA FE BB UU PD FF FU BB BB IM DG

DC DD FF AA AA FE BB UU EP FH FU BB BB IP GG

DD DD DF AA AA DD BB UU PD EE UJ BB GB IP GG

DC DA EE AA AA DF BB UU RN EE UJ AB GB HD GG

CC DA FF AA AA DD BB GU PD EE FY BB BB IM DG

CC DD GF AA AA BE BC GU RN EE UJ AB BB HP DG

DC CD DD AA AA DD BB GU ED FF FY AB BB HP DG

name = Westerntrabzon

CC CD EB AA AA BG BB TZ SH CC AB BB .. JS CC

DD CA FE AA AA BB BB KZ SH CC AF AA .. .. ..

CC DD EG AA AA BC BB TZ KS CF BK AB .. CM CC

DD AA EE AA AA BB BB KK KS CE AK BB .. .. AC

CC DA FF AA AA BB BC KZ DS CE AF BB .. JS CC

DD AC EC AA AA BB BB ZZ SS CE AK AA .. CJ ..

DD AA EB AA AA DF BB KT KS EG .. AA .. .. ..

DD AA EB AA AA BD BB KZ SS EG AK AA .. CM AC

E. A partial data file of GDA software

```
#NEXUS
[! Sample data P.orientalis]
begin gdadata;
        dimensions nloci=15 npops=8;
        format tokens labels missing=?;
        locusallelelabels
          1  'UAPgA150' [/ 1 2 3 4],
        2  'UAPgCA24' [/ 1 2 3 4 5 6],
        3  'SpAgg3'   [/ 1 2 3 4 5 6 7 8],
        4  'SpAgC1'   [/ 1 2],
        5  'UAPgAg105'[/ 1],
          6  'UAPgTg87' [/ 1 2 3 4 5 6 7 8],
          7  'UAPgCT144'[/ 1 2 3],
          8  'SS12'     [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23],
          9  'SS15'     [/ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20],
         10  'SS16'     [/ 1 2 3 4 5 6 7 8 9];
        MATRIX
        giresun:
      -1- 4/4  1/4  5/7   1/1   1/1  5/6  2/3  11/22  18/18   7/8  ?/?   1/1   2/2   3/3    ?/?
      -2- 4/4  1/4  6/6   1/1   1/1  5/4  2/3  11/11  18/5    7/8  1/2   1/1   1/2   3/9    ?/?
      -3- 4/4  3/4  7/6   1/1   1/1  6/2  2/2  22/18  18/5    7/8  2/4   1/1   1/1   3/9    3/3

        wt:
       -1- 3/3  3/4  5/2   1/1   1/1  2/7  2/2  19/23  18/8    3/3  1/2   2/2   ?/?   10/18  3/3
      -2- 4/4  3/1  6/5   1/1   1/1  2/2  2/2  11/23  18/8    3/3  1/6   1/1   ?/?   ?/?    ?/?
     -3- 3/3  4/4  5/7   1/1   1/1  2/3  2/2  19/23  11/18   3/6  2/11  1/2   ?/?   3/13   3/3
     -6- 4/4  1/3  5/2   1/1   1/1  2/2  2/2  23/23  18/18   3/5  1/11  1/1   ?/?   3/10   ?/?
     -7- 4/4  1/1  5/2   1/1   1/1  4/6  2/2  11/19  11/18   5/7  ?/?   1/1   ?/?   ?/?    ?/?
```

F. A partial data file of STRUCTURE software

150A CA24 AGG3 AGC1 G105 TG87 T144 SS12 SS15 SS16 SS17 AGB3 GGB5 ACT3 SS13

1 1 43 11 57 11 11 44 22 2020 164 88 620 22 22 913 47

5 1 43 41 55 11 11 65 22 2020 164 66 620 22 22 913 47

7 1 43 44 66 11 11 65 22 2020 516 68 620 22 22 916 77

9 1 44 44 46 11 11 44 22 2020 164 55 2010 22 72 916 77

10 1 43 41 55 11 11 46 22 2020 1714 55 2010 12 72 84 77

11 1 33 41 66 11 11 44 22 720 164 55 622 22 22 913 47

17 1 33 44 76 11 11 25 23 720 1714 55 2010 12 22 816 47

19 1 43 34 44 11 11 44 22 720 54 66 622 12 22 816 47

23 1 43 44 52 11 11 46 22 720 54 68 622 22 72 913 47

25 1 43 13 42 11 11 25 22 720 164 68 2010 12 72 84 47

4 1 44 11 67 11 11 46 22 1914 164 64 610 12 22 913 77

8 1 44 41 66 11 11 66 33 1914 1714 66 610 11 22 88 47

9 1 44 13 66 11 11 46 23 1919 1714 64 613 11 22 99 77

12 1 44 41 76 11 11 66 33 1919 1414 66 613 12 22 137 77

14 1 44 11 66 11 11 66 22 1919 1616 68 613 12 22 913 87

16 1 44 41 55 11 11 44 23 1414 164 84 1013 22 12 1913 47

18 1 44 41 62 11 11 45 22 1914 55 66 1013 12 12 94 47

25 1 43 31 66 11 11 46 23 1414 164 68 613 12 12 99 77

26 1 43 33 66 11 11 65 22 1919 1414 68 613 12 22 1913 84

30 1 44 44 66 11 11 55 22 1414 1616 64 1013 11 12 134 84

G. A partial data file of GENEPOP software

Microsat on p.orientalis,

Loc1

Loc2

Loc3

Loc4

Loc5

Loc6

Loc7

Loc8

Loc9

Loc10

Loc11

Loc12

Loc13

Loc14

Loc15

Pop

g, DC AA EG AA AA DD BB UU PD HH FU BB BB IM DG

g, DC DA EE AA AA FE BB UU PD FF FU BB BB IM DG

g, DC DD FF AA AA FE BB UU EP FH FU BB BB IP GG

g, DD DD DF AA AA DD BB UU PD EE UJ BB GB IP GG

pop

wt, CC CD EB AA AA BG BB TZ SH CC AB BB .. JS CC

wt, DD CA FE AA AA BB BB KZ SH CC AF AA .. .. ..

wt, CC DD EG AA AA BC BB TZ KS CF BK AB .. CM CC

wt, DD AA EE AA AA BB BB KK KS CE AK BB .. .. AC

H. Detailed microsatelite loci information

Locus	Species tested first	Allele size range observed in early study (number of alleles)	Allele size range observed in current study (number of alleles)
UAPgA150A	*Picea glauca*	150-164 (8)	126-147 (4)
UAPgCA24	*Picea glauca*	201-285 (11)	192-263 (5)
UAPgAG105	*Picea glauca*	167-175 (7)	158 (1)
UAPgTG87	*Picea glauca*	110-200 (18)	100-169 (7)
UAPgCT144	*Picea glauca*	134-180 (10)	136-151 (3)
UAPgCT3	*Picea glauca*	238-304 (11)	201-262 (21)
SpAGC1	*Picea abies*	79-117 (8)	98-111 (2)
SpAGG3	*Picea abies*	110-148 (17)	115-158 (9)
SS12	*Picea sitchensis*	200-250 (19)	206-256 (17)
SS13	*Picea sitchensis*	250-260 (11)	232-267 (8)
SS15	*Picea sitchensis*	202-224 (13)	174-230 (19)
SS16	*Picea sitchensis*	306-322 (9)	263-326 (9)
SS17	*Picea sitchensis*	170-244 (18)	180-242 (25)
PaGB3	*P.abies mRNA for major intrinsic protein (aquaporin)*	117-153 (9)	111-138 (4)
PgGB5	*P.glauca heat shock-like protein (hsp 18.2-like) mRNA*	88-106 (10)	86-102 (6)

İ. Sum of the best K value based on the delta K method

K	Mean LnP (K)	Stdev LnP (K)	Ln' (K)	Ln" (K)	Delta K
3	-14073.9450	208.7140	NA	NA	NA
4	-13470.4700	131.2288	603.475000	240.235000	**1.830657**
5	-13107.2300	149.1623	363.240000	229.100000	1.535911
6	-12973.0900	516.0615	134.140000	150.195000	0.291041
7	-12688.7550	638.0091	284.335000	232.830000	0.364932
8	-12637.2500	1093.0012	51.505000	NA	NA

J. Allele ferquency divergence among populations (net nucleotide distance), computed using point estimates

	1	**2**	**3**	**4**
1	-	0.0892	0.0421	0.0949
2	0.0892	-	0.0938	0.0626
3	0.0421	0.0938	-	0.1024
4	0.0949	0.0626	0.1024	-

K. Average Distances (expected heterozygosity) between individuals in the same cluster and mean F_{ST} values

	Average distances	Mean F_{ST}
Cluster 1	0.7059	0.1133
Cluster 2	0.6721	0.0895
Cluster 3	0.6919	0.1050
Cluster 4	0.6618	0.1708

L. Summary of F-Statistics and Gene Flow for All Loci based on Nei (1987)

Locus	Sample size	Nm
UAPgA150A	276	2.8
UAPgCA24	266	4.01
UAPgTG87	286	7.01
UAPgCT144	286	11.5
UAPgCT3	246	4.12
SpAGC1	286	1.53
SpAGG3	265	3.47
SS12	260	1.94
SS13	233	3.99
SS15	263	3.75
SS16	251	1.88
SS17	267	2.99
PaGB3	249	3.71
PgGB5	249	3.59
Mean	**261**	**3.28**

Nm: Gene flow estimated from $F_{ST} = 0.25 (1- F_{ST})/ F_{ST.}$

M. Nei's Unbiased Measures of Genetic Identity and Genetic Distance (1987)

Pop ID	1	2	3	4	5	6	7	8
1	****	0.9296	0.8986	0.9217	0.8760	0.8617	0.8873	0.8581
2	0.0730	****	**0.9499**	0.9419	0.8833	0.8775	0.9050	0.8675
3	0.1069	0.0514	****	0.9036	0.8421	0.8119	0.9002	0.8149
4	0.0816	0.0599	0.1014	****	0.9190	0.9355	0.9244	0.8963
5	0.1324	0.1241	0.1718	0.0845	****	0.9086	0.9152	0.8704
6	0.1489	0.1307	**0.2084**	0.0667	0.0959	****	0.9165	0.8790
7	0.1195	0.0998	0.1052	0.0786	0.0886	0.0872	****	0.8844
8	0.1531	0.1421	0.2046	0.1094	0.1388	0.1289	0.1229	****

Above diagonal represents Nei's genetic identity, below diagnoal represents genetic distance

N. Estimated null allele frequencies

POPULATIONS

LOCUS	Giresun	W-Trabzon	C-Trabzon	E-Trabzon	S-Artvin	N-Artvin	C-Artvin	SE-Artvin
UAPgA150A	0.7243	No inf	0.2682	0.6808	0.0000	No inf	0.3235	0.5454
UAPgCA24	0.0366	0.0395	0.0000	0.0601	0.0000	0.0026	0.0000	0.0887
SpAGG3	0.2014	0.1258	0.0441	0.1869	0.0905	0.0353	0.1234	0.0457
SpAGC1	No inf	No inf	No inf	No inf	No inf	No inf	No inf	No inf
UAPgAG105	No inf	No inf	No inf	No inf	No inf	No inf	No inf	No inf
UAPgTG87	0.0048	0.0459	0.1006	0.0000	0.0113	0.0000	0.0668	0.0000
UAPgCT144	No inf	No inf	No inf	No inf	0.1864	0.0000	0.3437	0.0728
SS12	0.2170	0.2046	0.0755	0.3285	0.0542	0.3849	0.2187	0.1233
SS15	0.1482	0.0261	0.0932	0.0662	0.1435	0.0396	0.0000	0.1625
SS16	0.2369	0.1350	0.1440	0.3064	0.2056	0.3216	0.3143	0.2121
SS17	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PaGB3	No inf	0.0593	0.0000	0.0976	0.0000	0.1980	0.0000	0.0000
PgGB5	0.0753	0.0000	0.0000	No inf	0.0631	0.0000	0.0729	No inf
UAPgCT3	0.0161	0.0000	0.0255	0.0053	0.0000	0.1077	0.0851	0.1618
SS13	0.1731	0.2039	0.0000	0.0639	0.2324	0.2109	0.2782	0.1012

CURRICULUM VITAE

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EDUCATION

Degree	**Institution**	**Year of Graduation**
MS	METU, Biology	2007
BS	Ankara University, Biology	2003
High school	Çankaya YDAL, Ankara	1999

WORK EXPERIENCE

Year	**Place**	**Position**
2004-present	METU, Biological Sciences	Research and Teaching Assistant
2011 -2012 (1 year)	Mississippi State University (MSU), Department of Forestry	Visiting Scholar

2012 (3 months)	Mississippi State University (MSU), Department of Forestry	Visiting Scholar

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

Özdilek, A., Çengel, B., Kandemir, G., Tayanç, Y., Velioğlu, E.,and Kaya, Z.2012.Molecular phylogeny of relict endemic Liquidambar orientalis Mill based on sequence diversity of the chloroplast-encoded matK gene. Plant Sytematics and Evolution 298:237-349.

Özgen M., **Özdilek A.**, Birsin M.A., Önde S., Sahin D., Açikgöz E. and Kaya Z.2012.Analysis of ancient DNA from in vitro grown tissues of 1600-year-old seeds revealed the species as Anagyris foetida. Seed Science Research 22, 279286.

ORAL and POSTER PRESENTATIONS

Hayta Sukru, **Özdilek Aslı**, Doğan Gülden, Bağcı Eyüp and Zeki Kaya. A Molecular Phylogeny of the genera of Apiaceae (Umbelliferae) inferred from nuclear ribosomal internal transcribed spacer (ITS), 3rd International Molecular Biology and Biotechnology Congress2014, Sarajevo, Bosnia-Herzigovina (Oral Presentation).

Dogan Gulden, Bağcı Eyüp, **Özdilek Aslı**, and Zeki Kaya. An approach to the phylogeny of genus *Centaurea* L. whereby cpDNA sequences of non-coding region of trn L (UAA) intron, In: International Symposium on Biology of Rare and Endemic

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Özdilek A, Kaya Z, İçgen Y, Çengel B, Kandemir G, and Velioğlu E. Genetic structure of Turkish Sweetgum (Liquidambar orientalis) populations and identification of varieties with the study of matK region of cpDNA. *"Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breed. & Conser. of For. Gen. Res."*, , (2006), p.167.

Özdilek A, Kaya Z, İçgen Y, Çengel B , Kandemir G, and Velioğlu E. Molecular Diversity in *Liquidambar orientalis* Mill. Assessed by Sequence Analysis of matK Region of Chloroplast Genome. *"Physiologia Plantarum"*, 133, (2008), p. P07-070.

Özdilek Aslı, Hsu Chuan-Yu, Kaya Zeki, Yuceer Cetin. Allelic variation in the flowering locus FT2 gene in populus deltoides, In: American Society of Plant Biology 2012, Austin Texas, P16076

Özdilek Asli, Yuceer Cetin, and Kaya Zeki. Allelic Differences in the Flowering Locus T2 Gene Among Poplar species, In: International Symposium on Biology of Rare and Endemic Plant Species (BIORARE-2014), Agust 19-23 2014, Antalya Turkey, PP-32, Page 99.

AWARDS and HONORS

YÖK-Abroad research scholarship for PhD students for 1 year-Mississippi State University, Department of Forestry, Genetic Lab.- (December 2011-December 2012).

TUBITAK-TOVAG 107O684 scholarship for 1 year.

HOBBIES

Swimming, walking and running, cooking