MOLECULAR GENETIC ANALYSIS IN HAZELNUT (*Corylus avellana*)

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ABSTRACT

MOLECULAR GENETIC ANALYSIS IN HAZELNUT (*Corylus avellana*)

European hazelnut (*Corylus avellana* L.), cultivated in several areas of the world including Europe, Anatolia, and the USA, is an economically important nut crop due to its high mineral, oleic acid, amino acid, and phenolic compound content and pleasant flavor. This study examined molecular genetic diversity and population structure of both Slovenian and Turkish hazelnuts. In the first part of the work, genetic diversity of 54 wild accessions and 48 cultivars from the Slovenian national hazelnut collection was determined using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. The accessions were also characterized for ten nut and seven kernel traits and some wild accessions were shown to have breeding potential. An association mapping panel composed of 64 hazelnut cultivars and wild accessions had considerable variation for the nut and kernel quality traits. Morphological and molecular data were associated to identify markers controlling the traits. In all, 49 SSR markers were significantly associated with nut and kernel traits [$P < 0.0001$ and LD value (r^2) = 0.15–0.50]. This work is the first use of association mapping in hazelnut and has identified molecular markers associated with important quality parameters in this important nut crop. In the second part of the work, 402 Turkish hazelnut accessions were screened with 30 SSR markers. The data obtained from this screen allowed selection of a national core collection of hazelnut. This core collection represents a maximum of genetic diversity in a minimum number of individuals. Turkish cultivar ‘Tombul’ was sequenced using next generation sequencing technology and new SSR markers were developed. It was found that seven SSR markers were sufficient to discriminate Turkish hazelnut cultivars from each other. This study provides molecular information for marker-assisted selection in hazelnut and gives new insight to discover the genetic potential of hazelnut germplasm.

ÖZET

FINDIKTA (*Corylus avellana*) MOLEKÜLER GENETİK ANALİZLER

Avrupa, Anadolu ve ABD'yi de içeren dünyanın çeşitli yerlerinde yetişen Avrupa fındığı (*Corylus avellana* L.), yüksek mineral, oleik asit, amino asit ve fenolik bileşik içeriği ve hoş lezzeti nedeniyle ekonomik açıdan önemli bir fındık ürünüdür. Bu çalışmada, hem Sloven hem de Türk fındığının moleküler genetik çeşitliliği ve populasyon yapısı incelenmiştir. Çalışmanın ilk bölümünde Sloven ulusal fındık koleksiyonundan 54 yabani aksesyonun ve 48 çeşidin genetik çeşitliliği ve populasyon yapısı çoğaltılmış fragment uzunluğu polimorfizmi (AFLP) ve basit dizi tekrarı (SSR) işaretleyicileri kullanılarak incelenmiştir. Aksesyonlar ayrıca on meyve ve yedi çekirdek özelliği açısından karakterize edilmiştir ve bazı yabani aksesyonların ıslah potansiyeline sahip olduğu gösterildi. 64 fındık çeşidinden ve yabani aksesyonlardan oluşan bir ilişkilendirme haritası paneli, meyve ve çekirdek kalite özellikleri açısından önemli farklılıklara sahiptir. Morfolojik ve moleküler veriler, özellikleri kontrol eden markörleri tanımlamak için ilişkilendirilmiştir. Toplamda 49 SSR markörü, meyve ve çekirdek özellikleriyle anlamlı derecede bulunmuştur [P <0.0001 ve LD değeri (r2) = 0.15-0.50]. Bu çalışma, fındıkta ilişkilendirme haritalamasının ilk kullanımı olup, bu önemli sert kabuklu bitkide önemli kalite parametreleriyle ilişkili moleküler markörler tespit edilmiştir. Çalışmanın ikinci bölümünde toplam 402 Türk fındığı aksesyonu, 30 SSR markörü ile taranmıştır. Bu çalışmadan elde edilen veriler, fındık için ulusal bir çekirdek koleksiyonunun seçimini sağlamıştır. Bu çekirdek koleksiyon az sayıda bireyde maksimum genetik çeşitliliğin olduğunu göstermiştir. Türk çeşidi 'Tombul', yeni nesil dizileme tekniği kullanılarak dizilendi ve yeni SSR işaretleri geliştirildi ve bunlardan yedi tanesi Türk fındık çeşitlerini birbirinden ayırmak için yeterliydi. Bu çalışma, fındıkta markör yardımlı seçim için moleküler bilgi sağlamaktadır ve fındık germplazmlarının genetik potansiyelini keşfetmek için yeni bilgiler vermektedir.

DOING WHAT YOU LIKE IS FREEDOM,

LIKING WHAT YOU DO IS HAPPINESS!

DEDICATED TO ‘ALL’ FAMILY MEMBERS

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CHAPTER 1

INTRODUCTION

1.1. European Hazelnut (*Corylus avellana*)

Hazelnut is one of the most important edible nut species in the world. *Corylus avellana* L. (European hazelnut) is a diploid (2n=22), monoecious, dichogamous and wind-pollinated species belonging to the Betulaceae family. This species is the source of the commercially important hazelnut cultivars grown in Europe, Anatolia and the USA [1].

European hazelnut (*Corylus avellana* L.) is an economically important nut crop due to its content of minerals, oleic acids, amino acids, phenolic compounds and its nice flavour [2-4]. In addition to high nutritional value, the hazelnut kernel is beneficial to health due to its effect on decreasing LDL (low density lipoprotein) levels in the blood[5]. Hazelnut can be considered as a natural functional food due to these benefits and is consumed worldwide as a table and processed food in the chocolate and confectionery industries [6].

1.2. Hazelnut Production in Turkey and Slovenia

Turkey and Italy are major hazelnut producers with 77% of world production with the remainder grown by countries such as the USA, Georgia, Azerbaijan, Spain and Slovenia [7]. Turkey is the world's main hazelnut producer with 450,000 tons grown on 701,141 ha, accounting for 61 % of world production [8]. Approximately 163,000 tons of hazelnuts are exported from Turkey each year [8]. The most suitable climatic conditions for hazelnut production in Turkey are in the Black Sea region where Turkish cultivars such as 'Tombul', 'Palaz', 'Mincane', 'Cakıldak' and 'Sivri' are grown [9]. In addition, the area contains many wild hazelnut trees and landraces.

'Tombul' is a Turkish *C. avellana* cultivar which has good characteristics for the human diet such as high oil content, good taste and aroma. In addition, this cultivar has skin that is quickly removable during roasting and its size is suitable for the chocolate industry. This cultivar is also partially self-compatible; thus it can be classified as a good

pollinator [10]. Moreover, it is widely grown in Turkey, especially in Giresun province and other Black Sea provinces. Thus, 'Tombul' has important economic value [11-12] and is a good cultivar for future breeding of new cultivars due to its adaptation in different provinces.

Although Slovenia is a minor hazelnut producing country with less than 1% of the world total, the country has extensive hazelnut genetic resources including wild accessions and cultivars such as 'Istrska dolgoplodna leska' which originated in Croatia but was domesticated in Slovenia. Orchards with mean surface of 2.2 hectares are mainly located in the Stajerska, Dolenjska and Celjska kotlinam regions, producing approximately 200 tons of in-shell nuts per year [13]. On the basis of a long-term investigation, some international cultivars, such as 'Tonda di Giffoni' and 'Daria' from Italy, 'Ennis' from the US and and 'Pauetet' from Spain are recommended for commercial growth in contemporary orchards in Slovenia [4, 14]. In addition, many local wild populations are distributed throughout the country, representing diverse hazelnut genetic resources. These populations are interesting for characterization and further selection and breeding.

1.3. Genome Sequencing in Plants

In 2000, the first sequencing of a plant genome using large-insert bacterial artificial chromosomes (BACs) was completed in the model organism *Arabidopsis thaliana.* This was a key step for the history of genome sequencing [15]. The next genome sequencing study in the plant kingdom was for the crop plant rice again using a BAC strategy [16]. Sequencing of poplar was another key step for genome sequencing because in this study a whole genome shotgun strategy (WGS) was used to obtain a tree genome [17]. In the WGS strategy, the genome is broken into small pieces which are sequenced and assembled. Next generation sequencing (NGS) strategies helped to expedite genome sequencing and reduced its cost. The NGS strategy was introduced by 454 technology. Then Illumina technology was developed and was adopted to sequence the cucumber genome with Sanger sequencing strategy [18]. In recent years, Illumina technology has been the dominant sequencing strategy and was applied to many plant genomes such as Chinese cabbage [19], potato [20], banana [21], pigeonpea [22] chickpea [23], orange [24] and watermelon [25]. Genotyping by Sequencing (GBS) is another high resolution technique for marker-assisted selection

and provides good information about selected accessions after sequencing [26]. However, GBS does not provide sequence for the whole genome. Genome sequencing provides many advantages in scientific studies. One of them is the development of high-density molecular markers for mapping of interesting traits. Such markers can be used to find candidate genes in a genome using QTL analysis [27]. Outcomes of QTL studies can be used in developing new cultivars which have good characteristics for interested traits.

Sequencing studies in hazelnut focused on transcriptome, genome and comparative genomics within species and accessions. In transcriptomic studies, the hazelnut transcriptome was sequenced [28] and 119 polymorphic SSR loci were developed from contigs [29]. In addition, 20 polymorphic EST-SSR from Betulaceae EST sequences [30] and 111 polymorphic SSR from transcriptomic sequences of 'Jefferson' cultivar [31] were developed. Moreover divergence in transcriptomic sequences of *C. mandshurica* and *C. avellana* were compared to find cold resistance genes [32]. Recently the 'Jefferson' cultivar genome was sequenced by Illumina technology. A total of 8,708 tri-nucleotide SSRs were identified and 150 polymorphic SSR markers were developed [33]. Seven cultivars ('Barcelona', 'Ratoli', 'Tonda Gentile delle Langhe', 'Tonda di Giffoni', 'Daviana', 'Hall's Giant' and 'Tombul') were sequenced at lower coverage and aligned to 'Jefferson' [33]. In another study BAC libraries were sequenced by Illumina to find a SSR marker linked to eastern blight resistance gene [34].

1.4. Genetic Analysis with Molecular Markers in Hazelnut

The genetic diversity of hazelnut cultivars was first assessed using random amplified polymorphic DNA (RAPD) [35-37] and amplified fragment length polymorphism (AFLP) markers [37-38]. AFLP was used to fingerprint 57 clones [38]; and, in combination with other markers, to assess diversity in 18 Turkish hazelnut cultivars [37]. In other work, Martins et al. [39] used AFLP and inter simple sequence repeat (ISSR) markers to measure diversity in wild and cultivated hazelnuts from Portugal.

AFLP is a DNA-based marker that does not require knowledge of the DNA sequence of the genotypes of interest [40]. However, this technique requires larger quantities of purer genomic DNA than other methods. Sophisticated machinery and software are also needed to generate and analyze AFLP data. Despite these limitations, AFLP provides

more polymorphic fragments than other techniques and has been frequently used for genetic diversity analyses in trees such as olive [41-42], mulberry [43] and black poplar [44].

1.4.1. Simple Sequence Repeats

SSRs are short nucleotide repeats (1-6) that occur throughout the coding and non-coding regions of the genome [45-47]. SSR markers are effective because they are multi-allelic, easy to score, and reproducible. As a result, they are commonly used in plant genetic diversity and breeding analysis. Genomic SSRs and genic SSRs are derived from DNA (genomic libraries) and RNA (expressed sequence tags, transcriptomic libraries) sequences, respectively. Length polymorphism in these coding and non-coding sequences can be easily detected by polymerase chain reaction. To date, 450 genomic SSRs [33, 48-53]; 20 polymorphic EST-SSRs from the Betulaceae family [30]; and 230 polymorphic SSR loci from transcriptome analysis were developed and used in hazelnut genome analyses [29, 31]. These analyses included determination of genetic diversity [1, 48, 54-57], geographic origin [1, 53, 58], identification of synonymous trees [53, 58-59], and construction of linkage maps [31, 52, 60-62]. In another study, 275 F1 hybrids of 'Tonda Gentile delle Langhe' x 'Merveille de Bollwiller' hazelnut trees were used for quantitative trait locus (QTL) identification for traits such as vigor, sucker habit, and time of bud burst [63]. All of these studies show that SSR markers are effective for hazelnut genomic research and suggest that the development of even more SSR markers will be useful for more comprehensive analyses.

1.4.2. Genetic Diversity of Hazelnut Germplasm

An important aspect of the conservation of genetic resources (germplasm) is the determination of the amount of diversity that characterizes the material. This is an important step in determining if and which germplasm can be beneficial in agriculture. Diversity can be assessed based on phenotype (plant morphological traits) and genotype (traits determined by molecular markers). Good diversity also prevents catastrophic losses due to biotic and abiotic stresses and is necessary for improvement of hazelnut to meet future climate, stress, grower and consumer demands.

Turkish hazelnut germplasm has been systematically collected and grown at the Hazelnut Research Institute in Giresun since its establishment in 1936 with substantial

additions made to the collection from 1969 to 1972 [64] (H.İ. Balik personal communication). The collection currently contains 430 accessions grown at the institute's orchard and includes both selected and bred cultivars, landraces and wild accessions that were found near commercial orchards. Wild accessions and landraces were established in the research institute's orchard by transfer of side-shoots from naturally-occurring trees. Germplasm collections are valuable reservoirs of genetic diversity. In addition to preserving germplasm, the institute has characterized the material, with special emphasis on the cultivars, for morphological and phenological traits [9, 64]. However, it has not yet examined all of the accessions for their molecular genetic diversity. This is necessary to understand the genetic relationships among individuals, information which is especially valuable when selecting parents for hybrid breeding, a relatively recent area of interest to the institute [65]. Both molecular and morphological data are also useful in selecting a core set of germplasm. A core set is a subset of germplasm that encompasses the maximum genetic diversity in a minimum number of accessions from the entire collection [66-67]. Core set selection can help to prioritize preservation and propagation of the collection as well as provide a reasonable number of diverse samples for the measurement of characters and properties that are expensive, time-consuming and/or laborious. Moreover, core sets provide ideal material for association mapping of traits in tree species like hazelnut.

In addition to its contribution to biodiversity, wild germplasm is widely recognized as a potential resource of interesting traits for improved cultivars [68]. The material of the Slovenian national hazelnut collection represents both naturally-occurring and introduced genetic diversity. Thus, the molecular and morphological variation of Slovenian hazelnut genetic resources should be examined for valuable features. In addition, these resources can be used to reveal the molecular bases of agronomic traits by quantitative trait locus (QTL) mapping approaches such as association mapping.

1.4.2.1. Molecular Diversity

Much of the research assessing genetic diversity in hazelnut has been done under the auspices of the SAFENUT European Commission Action which focused on characterization, conservation and use of European hazelnut germplasm (reviewed) [54]. As part of this project, analyses of SSR loci in hazelnut revealed high levels of genetic diversity in accessions from Spain [1, 48, 56] and Southern Europe [55]. In other work, Black

Sea region hazelnuts from Turkey, Georgia, and Azerbaijan were also found to be highly diverse [60]. Most molecular genetic diversity studies in hazelnut have focused on cultivars [54] with only recent interest in wild individuals and landraces [39, 69-73]. Another molecular study in hazelnut about diversity was completed by Solar et al.[74] and identified isozyme polymorphism in leaf tissues using three enzyme systems.

1.4.2.2. Morphological Diversity

Hazelnut descriptors are used to characterize accessions for morphological diversity [75-76]. Until now several morphological diversity studies were performed and they assayed kernel and nut parameters such as: nut weight [69, 77-79], kernel weight [69, 77-78]; nut length [77, 80], kernel length [77, 81], kernel percentage [77, 82], number of nuts per cluster [69, 77], caliber [4], width and thickness [81]. In another study, 14 descriptors were used to analyze involucres, nuts, and kernels [55].

1.4.3. Association Mapping

Association mapping (AM), also called linkage disequilibrium (LD) mapping, was first developed for QTL identification in medical genomics studies and is now frequently implemented in plant genomics studies. Association mapping is more practical than QTL mapping performed in bi-parental mapping population because it does not require the development of experimental populations such as F2 and BC (backcross). The development of such populations is time-consuming especially in tree species like hazelnut [83]. Instead, AM uses an association panel consisting of naturally occurring plant germplasm/populations. AM also has higher resolution than bi-parental QTL mapping because AM uses LD generated by historical recombination and can detect more alleles than are found in bi-parental populations [83-85]. In a recent study, 275 ‘Tonda Gentile delle Langhe’ X ‘Merveille de Bollwiller’ hazelnut F1 hybrids were used for QTL analysis, which was performed for vigor, sucker habit, and time of bud burst characters [63]. However, to date no association mapping has been performed with hazelnut. Nut and kernel traits are important yield and quality parameters for hazelnut. Although these traits have been characterized for a limited number of reference and local cultivars [3-4, 6, 69, 81, 86], to our knowledge, wild hazelnut accessions have not been examined in this way.

Morphological and molecular characterization of wild accessions for nut and kernel traits is important to assess their breeding potential. In addition, identification of molecular markers linked to QTLs for quality traits is essential for the implementation of marker-assisted selection in hazelnut for targeted breeding of nut and kernel traits.

1.4.4. Discrimination Analysis for Hazelnut

Hazelnut is a tree so it is important to know what you are growing because hazelnut does not reach maturity for five to ten years, therefore, nut and kernel traits cannot be used to distinguish and verify cultivars when an orchard is established. Turkish hazelnut cultivars are classified depending on their nut shape and kernel quality and cultivar names refer to a group of trees which have the same agro-morphological traits [37]. In addition, as with other cultivars such as 'Tonda Gentile delle Langhe' [59] 'Longue d'Espagne', 'Daviana', and 'Merveille de Bollwiller' [87], Turkish hazelnut cultivars can have many variants at the molecular level which results in problems with certification [88-90]. This problem can be solved using genetic discrimination analysis.

Starting in the 1990s, molecular analyses were done to discriminate cultivars and find true-type (clonal) accessions. For example, Solar et al.[74] showed isozyme polymorphism for three enzyme systems in 15 hazelnut cultivars. Later research used DNA-level polymorphism. In early work, five randomly amplified polymorphic DNA (RAPD) markers were used to discriminate six cultivars and their variants from the Campania region of Italy [35]. In another study, 10 of 18 Turkish cultivars were distinguished using five random amplified polymorphic DNA (RAPD), four inter-simple sequence repeat (ISSR), and eight amplified fragment length polymorphism (AFLP) primers which yielded 34 cultivar-specific markers [37]. Chloroplast DNA was also used to find the origins of 75 cultivars from Spain, Italy, Turkey (10 cultivars) and Iran using four polymorphic simple sequence repeat (SSR) loci [91]. In a more recent study, 14 SSRs were developed for fingerprinting 102 worldwide cultivars [92]. This was the first time that SSR markers were used for discrimination in hazelnut despite the fact that they have been previously shown to be convenient for fingerprinting in many other tree species such as apple [93], apricot [94], peach [95], pear[96] and olive [97].

1.5. Goals

The present thesis is composed of several goals to develop molecular breeding methods in hazelnut. The first aim of this study was to analyze the genetic diversity and population structure of 102 wild and cultivated hazelnut accessions grown in Slovenia. The clonal accessions included 54 wild accessions collected in five regions in Slovenia and 48 cultivars originating from Europe and the USA. These accessions were evaluated with molecular marker data from 11 AFLP primer combinations and 49 SSR markers. The germplasm was also evaluated for nut and kernel traits and these data were used to identify QTLs for these parameters via association mapping. Thus, this study is the first AM QTL report for nut and kernel quality traits in hazelnut.

The other aim of the research was to analyze the molecular genetic diversity and population structure of 402 hazelnut accessions (143 wild accessions, 239 landraces and 20 cultivars) in the Turkish national collection using SSR markers. We also selected a core set of the most diverse material for further morphological and biochemical profiling and association mapping analyses. The core collection will be an efficient and economical resource for future hazelnut preservation, characterization and improvement.

The last aim of the research was to identify hazelnut specific SSR markers using next generation sequencing technologies. To achieve this aim, genomic DNA of a popular Turkish hazelnut cultivar (*C. avellana* cv. 'Tombul') was sequenced by Illumina Next Generation Sequencing (NGS) technology for identification of SSRs. Finally, a set of 50 SSR markers were validated in 47 hazelnut accessions to demonstrate their usefulness for examination of genetic diversity and population structure. Seven of the 50 SSR markers were chosen to discriminate 19 Turkish cultivars from each other.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Slovenian Hazelnut Germplasm Plant Materials

For genetic diversity analysis, 48 individuals of *C. avellana* were sampled from the national hazelnut collection in Maribor, NE, Slovenia. These accessions represent cultivars that have been introduced into Slovenia from other countries including Italy (12 genotypes), the USA (11 genotypes), France (5 genotypes), Spain, the UK and Germany (4 genotypes each) with one or two cultivars of Croatian, Hungarian, Romanian and unknown origin. Leaves and catkins were taken from one single, true-to-type plant of the three replicates that were planted per cultivar. An additional 54 samples were obtained by in situ collection of wild accessions from five hazelnut growing regions in Slovenia (Figure 2.1, Table 2.1). The Koroska region is characterized by a humid continental climate (Dwb) and is one of the coldest areas in Slovenia beside the Alps. Maribor and Dolenjska, two regions with extensive vineyard production, have temperate climate with dry winters (Cwb). The Vipava-Razdrto and Bovec regions have a similar climate but without a dry season (Cfwb) and are areas where a Mediterranean influence can be felt.

A panel composed of 24 cultivars and 40 wild accessions was randomly chosen from the germplasm described above for morphological characterization and association mapping of nut and kernel traits.

Figure 2.1 Map of Slovenia showing the regions where hazelnut genotypes were collected. Red cross marks Bovec Region black marks Maribor Region, purple marks Koroska Region, green marks Vipava-Razdrto Region and orange marks Dolenjska Region.

Table 2.1. Slovenian hazelnut germplasm and origins.

Name (Genotype)	Type of Material	Origin	Genetic Background
101 (s1)	Cultivar	Italy	s54 x s13
119 (s2)	Cultivar	Italy	s54 x s13
Apolda (s4)	Cultivar	Italy	
Arutela (s5)	Cultivar	Romania	Merveille de Bollwiller x s54
Bandnuss (s6)	Cultivar	United Kingdom	
Bearn (s7)	Cultivar	France	
Brixnut (s8)	Cultivar	USA	
Corabel = N-473 (s12)	Cultivar	France	s21 seedling
Cosford (s13)	Cultivar	United Kingdom	
Daviana (s15)	Cultivar	United Kingdom	
E-104 = Daria (s16)	Cultivar	Italy	s54 x s13
Ennis (s17)	Cultivar	USA	
F-104 (s18)	Cultivar	Italy	s54 x s13
Feriale (s20)	Cultivar	France	s28 x Butler
Fertile de Coutard = Barcelona (s21)	Cultivar	USA	

(Cont. on the next page)

Table 2.1. (cont.)

Ferwiller (s22)	Cultivar	France	Merveille de Bollwiller x Tonda G. Romana
Frutto Grosso (s23)	Cultivar	Italy	
G1 (s24)	Cultivar	Italy	Payrone x Tonda Gentile Romana
Gem (s25)	Cultivar	USA	
Gunslebert (s26)	Cultivar	Germany	
Heynich's Zellernuss (s27)	Cultivar	Germany	
Imperiale de Trebizonde (s28)	Cultivar	Turkey	
Istrska dolgoplodna leska (s29)	Cultivar	Croatia	
Istrska okrogloplodna leska (s30)	Cultivar	Croatia	
Lambertskibeli (s31)	Cultivar	Germany	
Landsberg (s32)	Cultivar	Germany	
Lansing (s33)	Cultivar	USA	
Lewis = OSU 243.002 (s34)	Cultivar	USA	(s21 x Tombul Ghiaghli) x s58
Mogul (s37)	Cultivar	United Kingdom	
Morell (s38)	Cultivar	Spain	
Mortarella (s39)	Cultivar	Italy	
N-650 = H368-22 (s40)	Cultivar	France	Tonda Gentile Romana x s54
Negret (s41)	Cultivar	Spain	
Nocchione = Montebello (s42)	Cultivar	Italy	
OSU 166.034 (s43)	Cultivar	USA	Casina x Butler
OSU 167.002 (s44)	Cultivar	USA	
OSU 238.125 (s45)	Cultivar	USA	
OSU 244.001 (s46)	Cultivar	USA	(s21 x Tombul Ghiaghli) x s58
Pauetet (s47)	Cultivar	Spain	
Riccia di Talanico (s48)	Cultivar	Italy	
Romische Zellernuss (s49)	Cultivar	unknown (Germany?)	
Romai (s50)	Cultivar	Hungary	
Segorbe (s51)	Cultivar	Spain	
Sodlinger (s52)	Cultivar	unknown (Germany?)	
Tonda di Giffoni (s53)	Cultivar	Italy	

(Cont. on the next page)

Table 2.1. (cont.)

Tonda Gentile delle Langhe (s54)	Cultivar	Italy	
Valcea (s57)	Cultivar	Romania	clonal selection of Furfulak
Willamette (s58)	Cultivar	USA	s42 x Compton
d1	Wild	Dolenjska	
d2	Wild	Dolenjska	
d4	Wild	Dolenjska	
d5	Wild	Dolenjska	
d6	Wild	Dolenjska	
d7	Wild	Dolenjska	
d8	Wild	Dolenjska	
d9	Wild	Dolenjska	
d10	Wild	Dolenjska	
d11	Wild	Dolenjska	
d12	Wild	Dolenjska	
kor1	Wild	Koroska	
kor2	Wild	Koroska	
kor4	Wild	Koroska	
kor5	Wild	Koroska	
kor6	Wild	Koroska	
kor7	Wild	Koroska	
kor8	Wild	Koroska	
kor9	Wild	Koroska	
kor10	Wild	Koroska	
kor11	Wild	Koroska	
kor12	Wild	Koroska	
mb1	Wild	Maribor	
mb2	Wild	Maribor	
mb4	Wild	Maribor	
mb5	Wild	Maribor	
mb6	Wild	Maribor	
mb7	Wild	Maribor	

(Cont. on the next page)

Table 2.1. (cont.)

mb8	Wild	Maribor
mb9	Wild	Maribor
mb10	Wild	Maribor
v1	Wild	Vipava-Razdrto
v2	Wild	Vipava-Razdrto
v3	Wild	Vipava-Razdrto
v4	Wild	Vipava-Razdrto
v5	Wild	Vipava-Razdrto
v6	Wild	Vipava-Razdrto
v7	Wild	Vipava-Razdrto
v8	Wild	Vipava-Razdrto
v9	Wild	Vipava-Razdrto
v10	Wild	Vipava-Razdrto
v11	Wild	Vipava-Razdrto
v12	Wild	Vipava-Razdrto
b1	Wild	Bovec
b2	Wild	Bovec
b4	Wild	Bovec
b5	Wild	Bovec
b6	Wild	Bovec
b7	Wild	Bovec
b8	Wild	Bovec
b9	Wild	Bovec
b10	Wild	Bovec
b11	Wild	Bovec
b12	Wild	Bovec

2.1.2. Turkish Hazelnut Germplasm Plant Materials

A total of 402 hazelnut accessions which represent the diversity of material present in Turkey's Black Sea region was used from the Hazelnut Research Institute, Giresun. This collection contains all 20 Turkish cultivars [98] as well as landraces and wild accessions collected by the research institute from Giresun (240 accessions), Ordu (49 accessions), Trabzon (49 accessions), Samsun (4 accessions), Rize (3 accessions), Sinop (2 accessions), Artvin, Duzce, Kastamonu and Erzurum (1 accession each) (Figure 2.2, Table 2.2) [64]. The remaining 31 accessions were of unknown origin but collected from the Black Sea region.

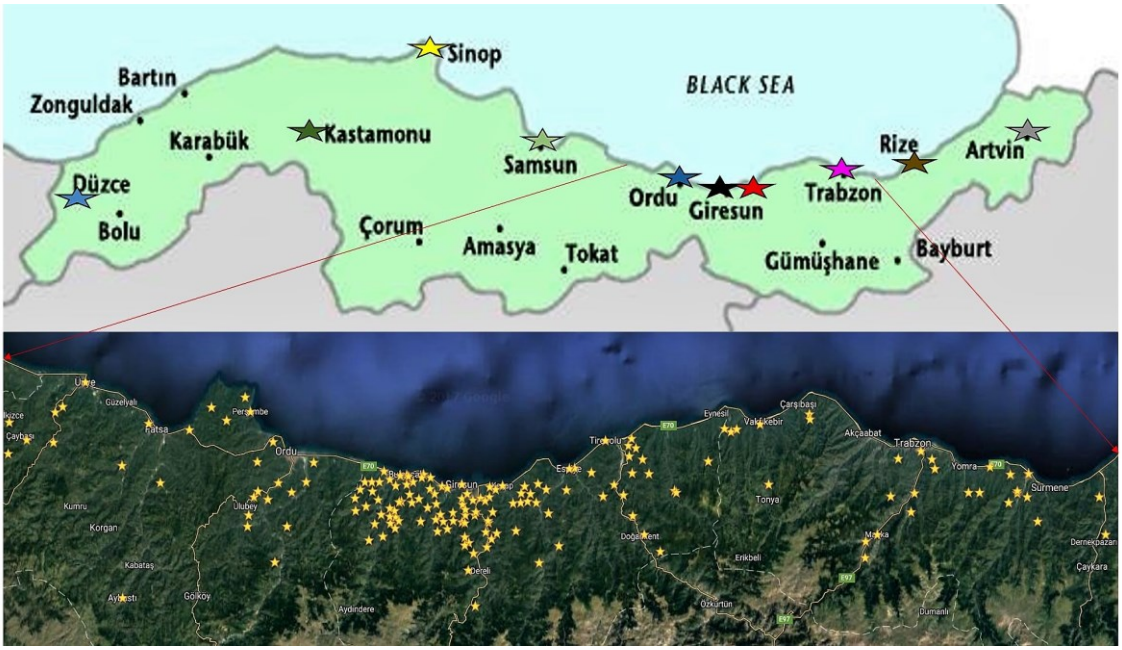

Figure 2.2 Map of Turkey's Black Sea region where hazelnut accessions were collected. Light blue star: Duzce; dark green star: Kastamonu; yellow star: Sinop; green star: Samsun; blue star: Ordu; black star: Giresun; red star: cultivars; fuchsia star: Trabzon; brown star: Rize and gray star: Artvin. Erzurum (not shown) is located in eastern Anatolia region and south of Rize and Artvin. Red arrows show the area expanded in the lower map with yellow stars indicating original collection locations of accessions.

Table 2.2. Turkish hazelnut accessions and origins.

Accession Name	Type of Material	Province	District
Aci	Cultivar	Giresun	Hazelnut Research Institute
Allahverdi	Cultivar	Giresun	Hazelnut Research Institute
Cakildak	Cultivar	Giresun	Hazelnut Research Institute
Cavcava	Cultivar	Giresun	Hazelnut Research Institute
Fosa	Cultivar	Giresun	Hazelnut Research Institute
Giresun Melezi	Cultivar	Giresun	Hazelnut Research Institute
Incekara	Cultivar	Giresun	Hazelnut Research Institute
Kalinkara	Cultivar	Giresun	Hazelnut Research Institute
Kan	Cultivar	Giresun	Hazelnut Research Institute
Kara	Cultivar	Giresun	Hazelnut Research Institute
Kargalak	Cultivar	Giresun	Hazelnut Research Institute
Kus	Cultivar	Giresun	Hazelnut Research Institute
Mincane	Cultivar	Giresun	Hazelnut Research Institute
Okay28	Cultivar	Giresun	Hazelnut Research Institute
Palaz	Cultivar	Giresun	Hazelnut Research Institute
Sivri	Cultivar	Giresun	Hazelnut Research Institute
Tombul	Cultivar	Giresun	Hazelnut Research Institute
Uzun Musa	Cultivar	Giresun	Hazelnut Research Institute
Yassibadem	Cultivar	Giresun	Hazelnut Research Institute
Yuvarlakbadem	Cultivar	Giresun	Hazelnut Research Institute
FAI001	Wild	?	
FAI002	Wild	Giresun	Bulancak; Bostanli
FAI003	Landraces	Giresun	Bulancak;Icilli
FAI004	Landraces	Giresun	Tekke
FAI005	Landraces	Giresun	Darikoy
FAI006	Wild	Giresun	Dereli;Kuknarli
FAI008	Landraces	Giresun	Konacik

(Cont. on the next page)

Table 2.2. (cont.)

FAI009	Landraces	Giresun	Gurkoy
FAI010	Landraces	Giresun	Dereli; Calca
FAI011	Wild	Giresun	Kesap; Karabulduk
FAI012	Landraces	Giresun	Incegeris
FAI013	Wild	Giresun	Dereli; Iklikci
FAI015	Wild	Giresun	Mesudiye
FAI016	Landraces	Giresun	Gurkoy
FAI017	Wild	Giresun	Yagmurca
FAI018	Landraces	Giresun	Ulper
FAI019	Wild	Giresun	Ulper
FAI020	Landraces	Giresun	Piraziz; Gokceali
FAI021	Landraces	Giresun	Akcali
FAI022	Wild	Giresun	Mesudiye
FAI023	Wild	Giresun	Konacik
FAI024	Landraces	Giresun	Piraziz; Bulbullu
FAI025	Wild	Giresun	Piraziz; Kilicli
FAI027	Landraces	Giresun	Akcali
FAI029	Landraces	Giresun	
FAI031	Wild	Giresun	Akcali
FAI032	Landraces	?	
FAI033	Wild	Giresun	Darikoy
FAI034	Landraces	Giresun	Boztekke
FAI035	Wild	Giresun	Darikoy
FAI039	Landraces	Giresun	Piraziz; Kilicli
FAI041	Wild	Ordu	Eyuplu
FAI042	Landraces	Giresun	Espiye; Orman Kirani
FAI043	Landraces	Giresun	Erikliman
FAI044	Wild	Giresun	Alinca

(Cont. on the next page)

Table 2.2. (cont.)

FAI046	Wild	Giresun	Darikoy
FAI047	Wild	?	
FAI049	Wild	Giresun	Hisargeris
FAI052	Landraces	Ordu	Eyuplu
FAI053	Wild	Ordu	Aydinlar
FAI055	Landraces	Giresun	Kesap; Karabulduk
FAI056	Wild	Sinop	Ayancik; Agacli
FAI057	Wild	Giresun	Piraziz; Bulbullu
FAI058	Landraces	Giresun	Piraziz; Bulbullu
FAI059	Wild	Giresun	Piraziz; Bulbullu
FAI061	Wild	Giresun	Bulancak; Şeyhmusa
FAI063	Wild	Giresun	Piraziz; Bulbullu
FAI064	Wild	Giresun	Piraziz; Bulbullu
FAI065	Wild	Giresun	Piraziz; Bulbullu
FAI066	Wild	Giresun	Akkoy; Madenyani
FAI067	Landraces	Ordu	Eyuplu
FAI068	Landraces	Giresun	Bulancak; Şeyhmusa
FAI070	Wild	Giresun	
FAI072	Wild	Ordu	Persembe
FAI073	Landraces	Giresun	Bulancak
FAI074	Wild	Giresun	Bulancak; Pazarsuyu
FAI076	Wild	Giresun	Ortakoy
FAI077	Wild	Giresun	Yazlik
FAI078	Wild	Giresun	Candir
FAI079	Landraces	Bolu	Akcakoca
FAI080	Wild	Giresun	Yazlik
FAI081	Wild	?	
FAI082	Landraces	Giresun	Pinarcukuru

(Cont. on the next page)

Table 2.2. (cont.)

FAI084	Wild	Giresun	Hazelnut Research Institute
FAI086	Landraces	Giresun	Bulancak
FAI088	Landraces	Giresun	Bulancak
FAI089	Landraces	Giresun	
FAI091	Landraces	Giresun	
FAI092	Landraces	Ordu	Bayadi
FAI093	Wild	?	
FAI094	Landraces	Kastamonu	Inebolu; Culurye
FAI095	Wild	Giresun	Konacik
FAI096	Landraces	Giresun	Konacik
FAI097	Landraces	Giresun	Sarvan
FAI098	Landraces	Giresun	Darikoy
FAI099	Landraces	Giresun	Barca
FAI101	Landraces	Giresun	Burhaniye
FAI103	Landraces	Giresun	Barca
FAI104	Landraces	Giresun	Guveckoy
FAI105	Landraces	Giresun	Hazelnut Research Institute
FAI106	Landraces	Giresun	Sarvan
FAI107	Landraces	Giresun	Barca
FAI108	Landraces	Giresun	Piraziz; Kilicli
FAI109	Wild	Giresun	Bulancak; Bozat
FAI112	Landraces	Ordu	Uzunisa
FAI114	Wild	Giresun	Bulancak; Yalikoy
FAI116	Landraces	Ordu	Aydinlar
FAI117	Wild	Giresun	Hazelnut Research Institute
FAI118	Wild	Ordu	Persembe; Yumrutas
FAI119	Landraces	Ordu	Persembe; Yumrutas
FAI120	Landraces	Ordu	Persembe; Dogankoy

(Cont. on the next page)

Table 2.2. (cont.)

FAI121	Wild	Ordu	Ulubey; Kirazli
FAI122	Landraces	Ordu	Ulubey; Findikli
FAI123	Landraces	Ordu	Ulubey; Akpinar
FAI125	Landraces	Giresun	Kesap; Yolagzi
FAI126	Landraces	Ordu	Ulubey; Karakoca
FAI128	Wild	Giresun	Guneykoy
FAI129	Landraces	Giresun	Guneykoy
FAI130	Wild	Giresun	Guneykoy
FAI131	Landraces	Giresun	Guneykoy
FAI133	Wild	Giresun	Kesap; Gurpinar
FAI135	Landraces	Giresun	Kesap; Saraycik
FAI136	Landraces	Ordu	Unye; Kalekoy
FAI137	Landraces	Ordu	Unye; Kurna Mengen
FAI138	Landraces	Ordu	Caybasi; Haciali
FAI140	Landraces	Giresun	Kesap; Saraycik
FAI141	Landraces	Giresun	Kesap; Saraycik
FAI142	Landraces	Ordu	Unye; Kalekoy
FAI143	Landraces	Giresun	Hazelnut Research Institute
FAI144	Landraces	Ordu	Unye; Cinarcik
FAI145	Landraces	Trabzon	Vakfikebir; Cumhuriyet mahallesi
FAI147	Landraces	Trabzon	Besikduzu; Korkuthan
FAI148	Landraces	Ordu	Unye
FAI149	Landraces	Trabzon	Besikduzu; Turkelli
FAI150	Wild	Giresun	Kesap; Karabedir
FAI152	Landraces	Giresun	Kesap; Guneykoy
FAI154	Landraces	Giresun	Ergence
FAI155	Landraces	?	

(Cont. on the next page)

Table 2.2. (cont.)

FAI157	Landraces	Giresun	Bulancak; Pazarsuyu
FAI158	Landraces	Giresun	Seyitkoy
FAI161	Landraces	Giresun	Yukarialinli
FAI163	Landraces	Giresun	Kemaliye
FAI164	Landraces	Giresun	Bulancak; Erdogan
FAI165	Wild	Giresun	Kesap; Surmenli
FAI166	Wild	Giresun	Kesap; Surmenli
FAI167	Landraces	Giresun	Sivaci
FAI168	Landraces	Ordu	Unye; Baskoy
FAI169	Landraces	Giresun	Camili
FAI170	Landraces	Giresun	Hazelnut Research Institute
FAI171	Landraces	Giresun	Kemaliye
FAI172	Landraces	Giresun	Kemaliye
FAI173	Landraces	Giresun	Seyitkoy
FAI174	Landraces	Ordu	Caybasi; Egribucak
FAI175	Landraces	Giresun	Kayadibi
FAI176	Landraces	Ordu	Caybasi; Saricaerik
FAI177	Landraces	Ordu	Caybasi; Saricaerik
FAI178	Landraces	Ordu	Caybasi
FAI179	Wild	Giresun	Kesap; Karadere
FAI180	Landraces	Ordu	Caybasi
FAI181	Wild	Giresun	Kesap; Cakirli
FAI182	Landraces	Giresun	Kayadibi
FAI183	Wild	Ordu	Unye;Kalekoyu
FAI184	Landraces	Giresun	Bulancak; Ahmetli
FAI185	Landraces	Giresun	Bulancak; Kayhan
FAI186	Wild	Giresun	Bulancak; Ahmetli
FAI187	Landraces	Giresun	Bulancak;Saracli

(Cont. on the next page)

Table 2.2. (cont.)

FAI188	Landraces	Giresun	Bulancak; Kayhan
FAI189	Landraces	Ordu	Kizilhisar
FAI190	Wild	Giresun	Bulancak; Tepecik
FAI191	Landraces	Giresun	Bulancak; Erdogan
FAI192	Landraces	Giresun	Bulancak; Seyhmusa
FAI194	Wild	Giresun	Bulancak; Icilli
FAI195	Landraces	Giresun	Bulancak; Hacet
FAI196	Landraces	Giresun	Bulancak; Tepecik
FAI197	Landraces	Giresun	Bulancak; Erdogan
FAI198	Landraces	Giresun	Bulancak; Kuzkoy
FAI199	Wild	Giresun	Bulancak; Ahmetli
FAI200	Landraces	Giresun	Bulancak; Kuzkoy
FAI202	Landraces	Ordu	Unye; Kalekoyu
FAI203	Wild	?	
FAI204	Landraces	Ordu	Fatsa; Oluklu
FAI205	Landraces	Samsun	Terme; Bazlamac
FAI206	Landraces	Ordu	Fatsa; Korucuk
FAI207	Wild	Ordu	Fatsa; Evkaf
FAI209	Wild	Samsun	Terme; Bazlamac
FAI210	Landraces	Samsun	Carsamba; Kocalar
FAI211	Wild	Ordu	Fatsa; Oluklu
FAI212	Wild	Samsun	Terme; Kocamanbasi
FAI213	Landraces	Ordu	Akcatepe
FAI215	Wild	Ordu	Boztepe
FAI216	Landraces	Giresun	Bulancak; Pazarsuyu
FAI217	Landraces	Ordu	Boztepe
FAI218	Landraces	Giresun	Bulancak; Pazarsuyu
FAI219	Landraces	Giresun	Bulancak; Inece

(Cont. on the next page)

Table 2.2. (cont.)

FAI220	Landraces	Giresun	
FAI221	Wild	Giresun	Bulancak; Eriklik
FAI222	Landraces	Trabzon	Bahcekaya
FAI224	Wild	Trabzon	Macka; Yukarikoy
FAI225	Landraces	Trabzon	Macka; Yukarikoy
FAI226	Landraces	Trabzon	Carsibasi;Kavakli
FAI227	Landraces	Giresun	Hazelnut Research Institute
FAI228	Landraces	Trabzon	Macka; Kaynarca
FAI230	Wild	Trabzon	Ortahisar; Caglayan
FAI231	Wild	Trabzon	Kavala
FAI232	Landraces	?	
FAI233	Wild	Trabzon	Cilekli
FAI234	Landraces	Trabzon	Kavala
FAI235	Landraces	Trabzon	Yomra; Komurcu
FAI236	Landraces	Trabzon	Yomra; Komurcu
FAI237	Landraces	Trabzon	Ortahisar; Cukurcayir
FAI238	Wild	?	
FAI239	Landraces	?	
FAI240	Landraces	?	
FAI241	Landraces	?	
FAI243	Wild	?	
FAI244	Landraces	Trabzon	Macka; Catak
FAI245	Landraces	Trabzon	Kisarna
FAI246	Landraces	Trabzon	Arsin; Ozlu
FAI247	Landraces	Trabzon	Surmene; Konak
FAI248	Landraces	Trabzon	Arsin; Ozlu
FAI249	Wild	Giresun	Tirebolu; Karademir
FAI250	Wild	Giresun	Tirebolu; Seku

(Cont. on the next page)

Table 2.2. (cont.)

FAI251	Wild	?	
FAI252	Landraces	Giresun	Bulancak; Hacet
FAI253	Landraces	Giresun	Hazelnut Research Institute
FAI255	Landraces	Trabzon	Arakli; Tasonu
FAI256	Landraces	Trabzon	Yenikoy
FAI257	Landraces	?	
FAI258	Wild	Trabzon	Arakli; Ayvadere
FAI259	Landraces	?	
FAI260	Wild	Trabzon	Arakli; Tasonu
FAI262	Wild	Trabzon	Of; Dumlusu
FAI263	Wild	Trabzon	Bolumlu
FAI264	Landraces	Trabzon	Bolumlu
FAI265	Landraces	Trabzon	Hopa; Sugoren
FAI267	Landraces	Trabzon	Of; Dumlusu
FAI268	Landraces	Trabzon	Hopa; Saricayir
FAI269	Wild	Trabzon	Bolumlu
FAI270	Landraces	Rize	Findikli; Kiyicik
FAI271	Landraces	Rize	Findikli; Caglayan
FAI272	Wild	Trabzon	Hopa; Camli
FAI273	Landraces	Trabzon	Hopa; Camli
FAI274	Landraces	Trabzon	Hopa; Camli
FAI275	Landraces	Rize	Findikli; Kiyicik
FAI276	Landraces	Trabzon	Hopa; Sundura
FAI278	Landraces	Giresun	Espiye; Cibril
FAI279	Landraces	Giresun	Bulancak; Semsettin
FAI280	Landraces	Giresun	Yaglidere; Palakli
FAI283	Wild	Giresun	Espiye; Demircili
FAI284	Wild	Giresun	Tirebolu; Cegel

(Cont. on the next page)

Table 2.2. (cont.)

FAI285	Wild	Giresun	Tirebolu; Aslancik
FAI286	Landraces	Giresun	Guce
FAI287	Landraces	Giresun	Tirebolu; Isikli
FAI288	Wild	Giresun	Eynesil; Kemaliye
FAI289	Wild	Giresun	Tirebolu; Belen
FAI290	Landraces	Giresun	Tirebolu; Ortacami
FAI291	Landraces	?	
FAI292	Landraces	Giresun	Bulancak; Cindi
FAI293	Landraces	Giresun	Kesap; Bayramsah
FAI294	Landraces	Giresun	Tirebolu; Harkkoy
FAI296	Wild	Giresun	Bulancak; Kusluhan
FAI297	Wild	Giresun	Bulancak; Torcan
FAI298	Wild	Giresun	Bulancak; Inece
FAI299	Wild	Giresun	Bulancak; Kusluhan
FAI300	Landraces	Giresun	Bulancak; Eriklik
FAI301	Landraces	Giresun	Tirebolu
FAI302	Landraces	Giresun	Bulancak; Inece
FAI303	Landraces	?	
FAI304	Landraces	Giresun	Piraziz; Balcikli
FAI305	Landraces	Giresun	Bulancak; Şeyhmusa
FAI306	Landraces	?	
FAI307	Landraces	Giresun	Bulancak; Semsettin
FAI308	Wild	?	
FAI309	Wild	?	
FAI310	Wild	Giresun	Tirebolu;Avcili
FAI311	Landraces	Giresun	Tirebolu; Balcikbeleni
FAI312	Landraces	Giresun	Tirebolu; Aslancik
FAI313	Landraces	Sinop	Ayancik; Hatip

(Cont. on the next page)

Table 2.2. (cont.)

FAI314	Landraces	Giresun	Bulancak; Inece
FAI315	Wild	Giresun	Bulancak; Kucuklu
FAI316	Wild	?	
FAI317	Landraces	?	
FAI318	Landraces	?	
FAI320	Wild	Giresun	Ulper
FAI321	Landraces	Giresun	Bulancak; Seyhmusa
FAI322	Landraces	Giresun	Piraziz; Bulbullu
FAI323	Landraces	Giresun	Piraziz; Hasanseyh
FAI324	Landraces	Giresun	Piraziz; Bulbullu
FAI325	Landraces	Giresun	Piraziz; Hasanseyh
FAI327	Landraces	Giresun	Piraziz; Bulbullu
FAI328	Wild	Giresun	Bulancak; Salman
FAI329	Wild	Giresun	Bulancak;Cindi
FAI330	Wild	Giresun	Bulancak; Demircili
FAI332	Landraces	Giresun	Yazlik
FAI333	Wild	Giresun	Caykara
FAI335	Wild	Giresun	Ulper
FAI336	Landraces	Giresun	Kemaliye
FAI338	Landraces	Giresun	Yazlik
FAI339	Landraces	Giresun	Sarvan
FAI340	Landraces	Giresun	Konacik
FAI341	Landraces	Giresun	Konacik
FAI343	Wild	Giresun	Bulancak; Bozat
FAI344	Wild	Giresun	Piraziz; Balcikli
FAI345	Wild	Giresun	Kemaliye
FAI346	Wild	Giresun	Piraziz; Maden
FAI347	Landraces	Giresun	Guneykoy

(Cont. on the next page)

Table 2.2. (cont.)

FAI348	Wild	Giresun	Guneykoy
FAI349	Landraces	Giresun	Piraziz; Maden
FAI350	Landraces	Giresun	Bulancak
FAI351	Wild	Giresun	Bulancak; Seyhmusa
FAI352	Wild	Giresun	Bulancak; Ahmetli
FAI355	Landraces	Giresun	Hamidiyekoy
FAI356	Landraces	?	
FAI357	Wild	Giresun	Boztekke
FAI359	Wild	Giresun	Darikoy
FAI360	Wild	Giresun	Hamidiyekoy
FAI361	Wild	Giresun	Calis
FAI362	Landraces	Giresun	Piraziz; Şeyhli
FAI363	Landraces	Giresun	Boztekke
FAI364	Wild	Giresun	Samanlik Kirani
FAI365	Wild	Giresun	Kayadibi
FAI366	Landraces	Giresun	Darikoy
FAI369	Landraces	Giresun	Bulancak; Ucarli
FAI370	Wild	Giresun	Alinca
FAI372	Landraces	Giresun	Dogankent; Catalagac
FAI375	Landraces	Giresun	Bulancak; Hisarkaya
FAI376	Wild	Giresun	Duroglu
FAI377	Wild	Giresun	Duroglu
FAI378	Landraces	Giresun	Bulancak; Tepecik
FAI380	Wild	Giresun	Bulancak; Icilli
FAI381	Landraces	Giresun	Bulancak; Burunucu
FAI383	Wild	?	
FAI384	Wild	Giresun	Bulancak; Kizilot
FAI385	Wild	Giresun	Bulancak; Kizilot

(Cont. on the next page)

Table 2.2. (cont.)

FAI387	Wild	Giresun	Piraziz; Kilicli
FAI388	Landraces	Giresun	Bulancak; Semsettin
FAI390	Landraces	Giresun	Bulancak; Semsettin
FAI391	Landraces	Giresun	Bulancak; Semsettin
FAI392	Wild	Giresun	Bulancak; Kusluhan
FAI393	Landraces	Giresun	Bulancak; Kusluhan
FAI394	Landraces	Giresun	Canakci; Saraykoy
FAI397	Wild	Giresun	Bulancak; Semsettin
FAI398	Landraces	Giresun	Bulancak; Kusluhan
FAI399	Landraces	Giresun	Bulancak; Inece
FAI402	Landraces	Giresun	Duroglu
FAI403	Landraces	Giresun	Sarvan
FAI406	Wild	Giresun	Kesap; Yazlik
FAI408	Wild	Ordu	Kocamanbasi
FAI409	Wild	Ordu	Uzunisa
FAI410	Landraces	Ordu	Uzunisa
FAI412	Landraces	Giresun	Hazelnut Research Institute
FAI413	Landraces	Giresun	Hazelnut Research Institute
FAI414	Wild	Ordu	Terme
FAI421	Landraces	Ordu	Aybasti
FAI422	Wild	Ordu	Unye; Baskoy
FAI424	Landraces	Trabzon	Arakli; Özgen
FAI426	Landraces	Trabzon	Arakli; Yigitozu
FAI428	Landraces	Trabzon	Of; Bolumlu
FAI429	Landraces	Trabzon	Arakli; Yigitozu
FAI431	Wild	Trabzon	Arakli; Tasonu
FAI432	Landraces	Giresun	Yaglidere; Umitbuku
FAI433	Landraces	Giresun	Tirebolu; Cegel

(Cont. on the next page)

Table 2.2. (cont.)

FAI439	Landraces	Ordu	Unye; Baskoy
FAI441	Wild	Ordu	Persembe; Ortatepe
FAI442	Wild	Ordu	Unye; Baskoy
FAI443	Landraces	Giresun	Espiye; Adabuk
FAI446	Landraces	Giresun	Kesap; Guneykoy
FAI448	Landraces	Trabzon	Arakli; Yildizli
FAI451	Landraces	Giresun	Yaglidere; Omerli
FAI456	Wild	Trabzon	Carsibasi; Kavakli
FAI457	Wild	Trabzon	Carsibasi; Kucukkoy
FAI458	Landraces	Trabzon	Vakfikebir; Kucukkoy
FAI459	Wild	Trabzon	Besikduzu; Kutluca
FAI460	Wild	Trabzon	Carsibasi; Kucukkoy
FAI461	Wild	Trabzon	Besikduzu; Korkuthan
FAI465	Landraces	Giresun	Bulancak; Pazarsuyu
FAI466	Landraces	Giresun	Bulancak; Pazarsuyu
FAI468	Landraces	Giresun	Bulancak; Tepecik
FAI469	Landraces	Giresun	Bulancak; Tepecik
FAI472	Wild	Giresun	Dogankent; Sadakli
FAI473	Wild	Giresun	Tirebolu; Yaglikuyumcu
FAI474	Wild	Trabzon	Hopa; Camli
FAI475	Wild	Trabzon	Hopa; Camli
FAI476	Landraces	Giresun	Tirebolu; Ketencukur
FAI478	Landraces	Giresun	Tirebolu; Balcikbeleni
FAI479	Landraces	Giresun	Guce
FAI481	Landraces	Artvin	Hopa;Kuledibi
FAI482	Landraces	Giresun	Alinca
FAI483	Landraces	?	
FAI484	Landraces	?	

(Cont. on the next page)

Table 2.2. (cont.)

FAI485	Wild	?	
FAI486	Landraces	?	
FAI583	Landraces	Ordu	Ulubey
FAI584	Landraces	Ordu	Ulubey
FAI585	Landraces	Ordu	Fatsa/Bolaman
FAI589	Landraces	Giresun	Hazelnut Research Institute
FAI590	Landraces	Giresun	Hazelnut Research Institute
FAI591	Landraces	Giresun	Hazelnut Research Institute
FAI592	Wild	Giresun	Hazelnut Research Institute
FAI593	Wild	?	Hazelnut Research Institute
FAI594	Wild	Giresun	Hazelnut Research Institute
FAI604	Landraces	Erzurum	Hinis;Karagoz

2.1.3. World Collection

For SSR marker validation, 27 cultivars from nine countries: Italy, USA, France, UK, Croatia, Germany, Romania, Spain and Hungary (samples provided by Dr. Anita Solar, Biotechnical Faculty, Department of Agronomy, University of Ljubjana) and 19 Turkish cultivars with one wild genotype from the Hazelnut Research Institute were used (Table 2.3). The Turkish cultivar 'Tombul' obtained from the Hazelnut Research Institute (Giresun, Turkey) was used for sequencing.

Table 2.3. Hazelnut accessions used in SSR marker validation.

Name	Origin	Cultivar / Wild
101	Italy	Cultivar
119	Italy	Cultivar
Aci	Turkey	Cultivar
Allahverdi	Turkey	Cultivar
Arutela	Romania	Cultivar

(Cont. on the next page)

Table 2.3. (cont.)

Badnuss	UK	Cultivar
Bearn	France	Cultivar
Brixnut	USA	Cultivar
Cavcava	Turkey	Cultivar
Corabel	France	Cultivar
Cosford	UK	Cultivar
Cakıldak	Turkey	Cultivar
E-104	Italy	Cultivar
Ennis	USA	Cultivar
F-104	Italy	Cultivar
FAI604	Turkey	Wild
Feriale	France	Cultivar
Ferwiller	France	Cultivar
Fosa	Turkey	Cultivar
Giresun Melezi	Turkey	Cultivar
Gunslebert	Germany	Cultivar
Istrska dolgoplodna leska	Croatia	Cultivar
Istrska okrogloplodna leska	Croatia	Cultivar
Incekara	Turkey	Cultivar
Kalinkara	Turkey	Cultivar
Kan	Turkey	Cultivar
Kara	Turkey	Cultivar
Kargalak	Turkey	Cultivar
Kuş	Turkey	Cultivar
Landsberg	Germany	Cultivar
Lansing	USA	Cultivar
Lewis	USA	Cultivar
Mogul	UK	Cultivar
Negret	Spain	Cultivar
Okay28	Turkey	Cultivar
Palaz	Turkey	Cultivar

(Cont. on the next page)

Table 2.3. (cont.)

Pauetet	Spain	Cultivar
Riccadi Tlanico	Italy	Cultivar
Romoi	Hungary	Cultivar
Sivri	Turkey	Cultivar
Tombul	Turkey	Cultivar
Tonda di Giffoni	Italy	Cultivar
Uzun Musa	Turkey	Cultivar
Valcea	Romania	Cultivar
Willamette	USA	Cultivar
Yassi Badem	Turkey	Cultivar
Yuvarlak Badem	Turkey	Cultivar

2.2. Methods

2.2.1. DNA Extraction

Total genomic DNA was isolated from leaves sampled from individual trees according to Fulton et al.[99] for SSR and AFLP amplification. Total genomic DNA of Tombul was extracted using the Wizard Magnetic 96 Plant System (Promega Crop., Madison, WI, USA) and the Beckman Coulter Biomek NX Workstation for sequencing.

2.2.2. Molecular Marker Analysis

2.2.2.1. AFLP Analysis of Slovenian Germplasm

AFLP Core Reagent and AFLP Starter Primer Kits from Invitrogen (Carlsbad, CA, USA) were used according to the manufacturer's protocol [40]. Sixty-four selective EcoRI/MseI primer combinations were tested on 'Willamette' (accession S58) and the wild accession B9. Based on these results, 11 combinations (M-CAC + E-AGC, M-CAA + E-ACG, M-CAA + E-ACC, M-CAG + E-ACT, M-CTC + E-AGG, M-CTC + E-ACA, M-CAT + E-ACA, M-CAT + E-ACT, M-CTA + E-ACA, M-CTG + E-AGC and M-CTT

+ E-AGG) were chosen as the most polymorphic and subsequently applied to the 102 hazelnut accessions. After selective PCR, fragments with labeled EcoRI primer signals were detected using a Genetic Analysis System CEQ 8800 machine (Beckman-Coulter, Fullerton, CA, USA). Amplification products were diluted 1:10 in sample loading solution (SLS) with 0.5 µl size standard 600. The mixture for each accession was then run on a Beckman CEQ8800 capillary electrophoresis device using the frag2 method (capillary temperature 35 °C, denaturation 90 °C for 120 s, injection voltage 2.0 kV for 30 s, separation voltage 6.0 kV for 60 min). PCR fragments were scored binomially (presence 1, absence 0).

2.2.2.2. SSR Analysis of Slovenian Germplasm

A total of 49 SSR marker pairs was used to accession the 102 hazelnut accessions. SSR markers were selected based on their polymorphic allele content as reported by Bassil et al.[49], Boccacci et al.[48], and Gurcan et al.[53]. PCR amplification was performed with 20 ng DNA in a 20-µl reaction containing 10 pmol of each primer pair, 200 µm dNTPs, 2 µl 10× Taq polymerase buffer and 0.6 Unit Taq polymerase. The same reaction conditions were used for all primers: 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. These cycles were preceded by a denaturing step at 94 °C for 3 min and ended with an extension step at 72 °C for 5 min. The PCR amplifications were performed in a GeneAmp PCR system 9700 (Perkin Elmer Applied Biosystems). After amplification, samples were separated by capillary electrophoresis using a Fragment Analyzer™ (Applied Biosystems) with the DNF-900 dsDNA Reagent Kit (Advanced Analytical) according to the manufacturer's instructions. PCR fragments were scored binomially (presence 1, absence 0) because many of the SSR markers yielded more than two fragments and allelism could not be determined.

2.2.2.3. SSR Analysis of Turkish Germplasm

Thirty SSR markers with high levels of polymorphism as reported by Gürcan et al.[48] were used for genetic diversity determination. For all primer combinations, PCR amplification was performed with 20 ng DNA in a 20 μl reaction containing 10 pmol each primer pair, 200 μm dNTPs, 2 μl 10x Taq polymerase buffer and 0.6 Unit Taq polymerase.

A GeneAmp PCR system 9700 (Perkin Elmer Applied Biosystems) machine was used for PCR amplification. Reaction conditions were: denaturation at 94 °C for 30 sec; 30 cycles of denaturation at 94 °C, annealing at 55 °C for 30 sec and extension at 72 °C for 30 sec; and final extension at 72 °C for 5 min. PCR fragments were separated by capillary electrophoresis using a Fragment Analyzer™ (Applied Biosystems) with the DNF-900 dsDNA Reagent Kit (Advanced Analytical) according to the manufacturer's instructions. Because many of the primer pairs yielded more than two fragments and allelism could not be determined, the individual fragments were scored binomially (presence 1, absence 0).

2.2.3. Sequencing of 'Tombul' Cultivar

IIIumina Mi-Seq sequencing of 'Tombul' genomic DNA was performed at the Biotechnology Center at the University of Wisconsin-Madison, USA (https://www.biotech.wisc.edu/).

2.2.3.1. Data Pre-Processing

IIIumina Sequencing Technology depends on adapters (synthetic short DNA sequences) to sequence DNA fragments. These adapter sequences may decrease assembly quality and must be removed. Thus, adapter sequences were removed from reads using Cutadapt version 1.8.3 software using default settings [100]. At the end of this step, any reads smaller than 20 nucleotides were removed. To detect human contaminants in the dataset, cleaned reads were mapped against the human genome using Bowtie version 2.1.0 [101] and possible contaminants were removed.

2.2.3.2. Sequence Assembly

ABySS version 1.3.6 [102], a *de novo*, parallel, paired-end sequence assembler, was used to perform genomic DNA sequence assembly. To produce the best possible assembly, more than 100 runs were performed with different parameters such as changing kmer (all possible substrings of length k contained in reads) and required number of reads

to make a contig. In *de novo* genome assembly, there is not just one measurement or parameter to determine the best assembly; instead, a combination of different measurements or parameters gives an idea about the quality of the final assembly. For this purpose, N50 value (weighted median of contig length), assembly nucleotide length (closeness to estimated size of the *C. avellana* genome), and length of the largest contig were used to identify the best assembly. The settings that were finally chosen to create contigs were: (kmer=45) with default settings.

2.2.3.3. SSR Detection, Annotation and Primer Design

Contigs shorter than 1000 nucleotides were removed from the assembly. Thus, we only analyzed contigs larger than 1000 nucleotides for SSR detection using our in-house tool SiSeer (http://bioinformatics.iyte.edu.tr/index.php?n=Softwares.SiSeeR). The minimum number of repeats required to identify perfect SSRs was ten for mononucleotides, four for dinucleotides, and three for motifs comprised of three or more nucleotides. To annotate these identified SSRs, SSR sequences were extracted with their genomic context (padded with 100 nucleotides) and were converted to FASTA formatted sequences. These sequences were treated as query sequences and searched against the Uniprot non-redundant plant protein database (Taxonomy = Viridiplantae) with BLASTX version 2.2.30 [103]. The Primer 3 program (primer_core) version 2.3.6 [104] was used to design primer pairs for the SSRs with the default settings and : primer task = generic, primer optimum size = 20, primer maximum size = 24, primer minimum size = 18, primer product size = 100-300, primer minimum Tm = 50, primer maximum Tm = 60 and primer optimum Tm=55.

2.2.3.4. Sequencing of SSR Loci

To ensure that the expected SSRs were amplified by the primers, 'Tombul' DNA was used as a template and the dye-terminator sequencing method was performed to validate SSR motifs. Eight primer pairs were randomly selected and PCR fragments were purified with the DNA Clean & Concentrator–5 Kit (Zymo Research) and used as templates for sequencing using GenomeLab DTCS Quick Start Kit (Beckman Coulter). Thermal cycling conditions of the sequencing reactions were: 30 cycles of 96 °C for 20

sec, 50 °C for 20 sec, 60 °C for 4 min. The reaction mixture for each SSR amplicon was then purified using ZR DNA Sequencing Clean-up Kit (Zymo Research), DNA was resuspended in 30 µL of sample loading solution (Beckman Coulter) and run on a Beckman CEQ8800 capillary electrophoresis device using the LFR-c method (injection voltage 2.0 kV for 10–15 sec, separation temperature 60 °C, separation voltage 7.4 kV, separation time 45 min).

2.2.3.5. Marker Analysis for Validation of Genomic SSR markers in World Collection

Amplification of the hazelnut DNA with genomic SSR primers was performed with 20 ng DNA in a 20 μl reaction containing 10 pmol each primer pair, 200 μm dNTPs, 2 μl 10X Taq polymerase buffer and 0.6 Unit Taq polymerase. Thermal cycling conditions consisted of one cycle of initial denaturation for 10 min at 94 °C, followed by 30 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec, with a final extension step of 10 min at 72 °C. PCR fragments were separated by capillary electrophoresis using a Fragment Analyzer (Applied Biosystems) with the DNF-900 dsDNA Reagent Kit (Advanced Analytical) according to the manufacturer's instructions and were scored binomially (presence 1, absence 0).

2.2.3.6. Discrimination Analysis for Turkish Cultivars

The binomial data set was analyzed to discriminate Turkish cultivars from each other with a minimum number of SSR markers. The SSRs which gave two alleles after PCR amplification were chosen to ensure that the SSR was single copy in the hazelnut genome and to simplify scoring. Combinations of SSRs were tested until all standard Turkish cultivars which are grown at Hazelnut Research Institute orchard were discriminated from each other.

2.2.4. Molecular Genetic Diversity and Population Structure Analysis

2.2.4.1 Slovenian Germplasm

In the Slovenian germplasm, average gene diversity [105] was calculated for each AFLP primer combination and SSR marker with the formula: average gene diversity$_i$ =$(\sum_{i=1}^{n} 2fi(1-fi))/n$ [106], where *fi* is the frequency of band presence for the i^{th} allele and *n* is the number of alleles. Calculated in this way, the diversity value of a locus ranges from 0 (monomorphic) to 0.5 (highly informative). Cluster analysis was performed using the Dice coefficient [107] and unweighted neighbor joining algorithm in DARwin 5 software [108]. DARwin 5 was also used for principal coordinate analysis (PCoA). Population structure was determined using the computer program Structure 2.3.4 [109]. Ad hoc statistics were used to find the best reflected subpopulation number for the hazelnut genotypes [110]. For this analysis, the data were evaluated for 2 to 20 subpopulations (K= 2 to 20) with a burn-in time of 10,000 cycles. Each model was tested 10 times with 300,000 iterations per K. The probability change of each group (ΔK) was calculated using the program Structure Harvester [111]. The highest ΔK was determined to be the best fit. Clusters were determined according to a threshold of ≥ 0.70 inferred ancestry. Accessions that did not meet this threshold were considered as admixed. A second population structure computer program, InStruct [112], was used to confirm the results of Structure and to test K= 1.

2.2.4.2 Turkish Germplasm and World Collection

PowerMarker software [113] was used to calculate polymorphism information content (PIC) and observed heterozygosity (Ho) values. Polymorphic alleles were used to analyze molecular genetic diversity and determine population structure. DARwin 5 software was used to analyze the data with the Dice coefficient [107] and the unweighted neighbor joining algorithm [108]. This program was also used for principal coordinate analysis (PCoA). Structure 2.3.4 [109] software was used to determine population structure. Ad hoc statistics were used to determine the best number of subpopulations [110]. The data were evaluated for 2 to 20 subpopulations (K= 2 to 20) with 50,000 cycles. Each subpopulation model was tested 10 times with 300,000 iterations per K. The probability

change of each group (ΔK) was calculated using the program Structure Harvester [111]. The best number of subpopulations was determined from the highest ΔK. Hazelnuts were clustered using a threshold of inferred ancestry ≥ 0.70. Accessions that did not meet this threshold were considered as admixed. A second population structure program, InStruct [112], was used to confirm the results of Structure and to test K= 1.

2.2.5. Core Set Selection of Turkish Germplasm

To select core set accessions, the SSR dataset for the hazelnut accessions was analyzed with PowerCore 1.0 software which uses the M (maximization) strategy and a modified heuristic algorithm [114]. PowerCore software develops a core set by maximizing the number of alleles represented in a minimum number of individuals, thus, reducing redundancy.

2.2.6. Morphological Evaluation of Slovenian Germplasm

The hazelnut association panel was characterized over two consecutive years for 17 nut and kernel traits using 30 samples per accession. The 10 nut traits including length, width, thickness, shape index, caliber, shell thickness, weight, shape uniformity, and proportions of healthy and empty nuts. Nut length, width and thickness of randomly selected in-shell nuts from each hazelnut accession (cultivars and wild accessions) were measured using calipers in millimeters (mm). The largest value among the three dimensions (nut length, width, and thickness) was recorded as caliber. Shape index was calculated according to the following formula: width + thickness/(2×length). Shell thickness was determined on hand-cracked nuts using calipers to measure the convex side of each half of the shell. Nut weight was recorded in grams (g). Nut shape uniformity was visually determined for each hazelnut accession using a scale from 1 to 9 (1 = least uniform, 9 = most uniform). Proportions of healthy and empty nuts were calculated by cracking 30 nuts for each hazelnut accession. In addition to nut traits, the hazelnut association panel was characterized for seven kernel traits including weight, kernel percentage, shape uniformity, and proportions of kernels with brown spots, mold, deformation, and twin kernels. Kernel weight was recorded in grams (g). Kernel percentage was calculated as: (kernel weight/nut weight) × 100. Shape uniformity was

visually determined for each hazelnut accession using a scale from 1 to 9 (1 = least uniform, 9 = most uniform). Proportion of kernels with brown spots, mold, deformation, and twin kernels were recorded using 1 kg nut samples harvested for each hazelnut accession.

Means and coefficients of variation for hazelnut cultivars and wild accessions from each region of Slovenia were calculated separately for comparison. Principal component analysis (PCA) was performed with DARwin [108] and PASW software [115]. Basic statistics such as correlation analysis between traits, paired sample Student's t tests, ANOVA, and discriminant analyses were performed using PASW software. Stepwise discriminant analysis of the nut and kernel traits was done using subpopulation, dendrogram cluster, and region as grouping variables.

2.2.7. Association Mapping

The binary data generated for the SSR markers assayed on the association panel were associated to the nut and kernel trait data using the GLM and MLM models of TASSEL v2.1 (Trait Analysis by aSSociation, Evolution and Linkage software) [116]. Linkage disequilibrium (LD) values (r^2 and P values) between SSR markers were calculated using the same software. Several association mapping (AM) models were tested to identify the one with the best fit for AM of nut and kernel traits. Tested models were GLM model without correction; GLM model corrected with the Q-matrix of population structure (subgroup number = 2) [GLM (Q)], principal components (PC) [GLM (PC)] and both Q and PC [GLM (Q + PC)]; MLM model corrected with kindship matrix (K)[MLM (K)], Q-matrix [GLM (Q)], principal components (PC) [GLM (PC)], and both Q and PC [GLM (Q + PC)]. Principal components (PC) were calculated in TASSEL software. The P values of the eight models were analyzed with QVALUE [117] software using a false discovery rate (FDR) of 0.05 [118]. The model with the highest probability of significant results ($\pi 1$) was accepted as the one with the best fit and only those results are reported here. The percent value of $\pi 1$ was calculated based on the probability that a given hypothesis is null, $\pi 0$, such that $\pi 1(\%)=[100-\pi 0\ (\%)]$. Marker-trait associations with P values lower than 0.0001 [−Log (P value) = 4] were selected as significant associations.

CHAPTER 3

RESULTS

3.1. Molecular and Morphological Characterization of Slovenian Hazelnut Germplasm

Genetic diversity of the Slovenian germplasm's wild accessions and cultivars was determined using AFLP and SSR markers. In addition, kernel and nut traits were characterized. Together these data were used to identify genetic loci controlling the morphological traits.

3.1.1. AFLP and SSR Marker Polymorphism

A total of 532 polymorphic fragments was scored from the 11 selective AFLP primer combinations, with 27 to 69 polymorphic alleles per combination (Table 3.1). Thus, AFLP provided an average of 48.4 alleles per primer combination. Average gene diversity values, which indicate the informativeness of each combination were calculated and ranged from 0.20 (for M-CAC + E-AGC) to 0.30 (for M-CTA + E-ACA) with an average of 0.26. The 49 SSR primer pairs yielded a total of 504 polymorphic fragments in the 102 accessions with an average of 10.3 alleles per SSR marker. Number of polymorphic fragments ranged from four to 28 with SSRs B625 and B777 each yielding more than 25 fragments (Table 3.2). Average gene diversity values for the SSRs ranged from 0.20 to 0.45 with B790 and A602 as the most polymorphic markers. Average gene diversity for all 49 markers was 0.30.

Table 3.1. Average genetic diversity (GD) values for amplified fragment length polymorphism (AFLP) primer combinations used to characterize hazelnut accessions.

AFLP Selective PCR Primers	Polymorphic Fragments/ Total Fragments	Average GD ± SE
M-CAC + E-AGC	27/31	0.20 ± 0.03
M-CAA + E-ACG	51/53	0.23 ± 0.02
M-CAG + E-ACT	36/36	0.27 ± 0.02
M-CTC + E-AAG	53/56	0.28 ± 0.02
M-CAA + E-ACC	43/44	0.28 ± 0.02
M-CAT + E-ACA	54/55	0.28 ± 0.02
M-CTA + E-ACA	56/56	0.30 ± 0.01
M-CAT + E-ACT	62/62	0.28 ± 0.02
M-CTG + E-AGC	38/39	0.26 ± 0.03
M-CTC + E-ACA	69/69	0.27 ± 0.02
M-CTT + E-AGG	28/31	0.21 ± 0.03

Table 3.2. Sequences and genetic diversity (GD) values of 49 simple sequence repeat (SSR) markers for characterization of Slovenian hazelnut accessions.

Primer Name	Forward Primer	Reverse Primer	Number of polymorphic fragments	Average GD ± SE
A602	AAGAGTGGGGGTGCACTATG	GGATTCATGCCTGCGATACT	8	0.43 ± 0.02
A604	GCTCCCGAGGACTTCCAG	CCACGACATTTCCCTCTCAG	7	0.37 ± 0.04
A605	CACCCTCAAAACTGTGACGA	TGGGTCGCATTCAATAACAC	13	0.30 ± 0.04
A606	CACCTAGCTTGTTGGTGAAGC	TGACAATAATTAACCCTACACACTTTG	11	0.40 ± 0.03
A611	CACTAGCCAGCCCCTTTACA	CTGATGCCACAAACACAAGG	10	0.25 ± 0.05
A613	CACACGCCTTGTCACTCTTT	CCCCTTTCACATGTTTGCTT	11	0.35 ± 0.04
A616	CACTCATACCGCAAACTCCA	ATGGCTTTTGCTTCGTTTTG	11	0.40 ± 0.02
A622	GGAAATTAAGAGAACTGGAGATTGGATGG	GCGACCCCTACAATATGAATTGTCTAGC	5	0.32 ± 0.04
A627	AACTCTGCTGGCACTGTTACTGCCTATT	GTTCAAAGGTGTCTCAAAGCAAGCACTA	6	0.26 ± 0.06
A635	GGATCTGTGGTTGGCTTTTTGGTACTAT	TTACCCAATGGATGATGGACTAGCATT	6	0.30 ± 0.06
B602	TCAGGATGAGACACCTTTACTCT	CCACAGTGGAATAGCACATTT	7	0.28 ± 0.05
B603	TGGTGGTGATAGGGAAGGAG	TCTTTTCTTCTTCAATCAGACGA	9	0.26 ± 0.05
B604	AACAGTCAGCCCCATTTCTG	CTTCCCTAATCCCCTCAACC	10	0.32 ± 0.03
B606	TCTTGTGGTTTAGCATACTTCTCG	GAAGAAAGCAAGAAGAGAGGAGA	4	0.42 ± 0.06
B612	GCACCTCAAACTCCTTGGAC	CCCAAACACACCCTTAGTGC	9	0.35 ± 0.03
B613	CGCGTTTTGAGTCCCTTTAG	CTACCCGCCTGCGAGAAC	11	0.26 ± 0.04
B619	AGTCGGCTCCCCTTTTCTC	GCGATCTGACCTCATTTTTG	19	0.20 ± 0.02
B625	CGCAAGTCATTGCACATTTT	GTGTGCTGTGCTCCTTTGAA	28	0.22 ± 0.02
B628	AATCCCCTCTAGCCCCATTA	CACAGAATATTTGTAATTACCACCACA	13	0.33 ± 0.03
B631	TGAAGCAGACAAGCGAATAGC	TTGTGTCTCTTTGTCTTGTAAATCG	9	0.25 ± 0.05
B635	GCATCGCCAAATTATCGTCT	CTTCAACAAATCCAGGATGC	12	0.23 ± 0.04
B640	CTGCATTGATGGATTGGTTG	TTAAGAAAGGTACAAGGGCTCTC	11	0.27 ± 0.04

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Table 3.2. (cont.)

B641a	CTCCCATGAAATGATTATTCTTAG	CAAGCCATCTGTTTTGCTGA	4	0.33 ± 0.06
B641b	ATATATATAGGCTGTGTGTGTGTGTG	ACAAGCCATCTGTTTTGCTG	7	0.32 ± 0.05
B648	TGAAAGCGCCCAAAACTTAT	CTTGCGTCTTTTTGGAGAGC	15	0.29 ± 0.03
B651	TTTTCTGGAATGTCGCACAG	TCTCCTCCTTCCAACAGTGG	6	0.35 ± 0.05
B652	AGGATGCGTGGTTGTGATTT	TGGAGTAGGGTGATGAGAATGA	17	0.23 ± 0.02
B654	TCGCATGGGTAATTTTCTCAC	TCATCATTTGGGTGCTTCAA	8	0.36 ± 0.04
B655	GGGTGGCAAAATCTATGTGC	CCATTTTCTCAGATTGAATAGCAA	5	0.35 ± 0.07
B657	GAGAGTGCGTCTTCCTCTGG	AGCCTCACCTCCAACGAAC	7	0.37 ± 0.05
B660	TGTTGTAGCACAACCCTTTCA	TGCTAGCAGCAAATGGCTTA	6	0.37 ± 0.05
B709	CCAAGCACGAATGAACTCAA	GCGGGTTCTCGTTGTACACT	12	0.28 ± 0.03
B716	GAACATTGTCGTATGCGGACT	TCTGTTTGTTGCGCATGATT	13	0.31 ± 0.03
B726	GGAAATGGCAAATCCGTCTA	AACGTTTTGCCTTCCTTGTG	12	0.28 ± 0.03
B728	AGCAAGAGTTCGAGCCAGTC	TGTGGAGAAGTCCCGGATAC	18	0.23 ± 0.02
B733	CACCCTCTTCACCACCTCAT	CATCCCCTGTTGGAGTTTTC	6	0.30 ± 0.07
B735	TCCTTGCCTCCGTAGAAAAA	TCCATAGCAACCAACGTTCA	9	0.40 ± 0.03
B741	GTTCACAGGCTGTTGGGTTT	CGTGTTGCTCATGTGTTGTG	12	0.27 ± 0.03
B758	TAATTTAAGCTGCCGTGCAA	TGCAAAATTGCATTGCTCAT	12	0.28 ± 0.04
B760	AGCTAGCTCTGCATGCTGGT	TCCCTTCTTGTTTTCGGGTA	9	0.29 ± 0.05
B774	GTTTTGCGAGCTCATTGTCA	TGTGTGTGGTCTGTAGGCACT	15	0.30 ± 0.03
B776	TGTATGTACACACGGAGAGAGAGA	TGAGGGGAAGAGGTTTGATG	5	0.37 ± 0.07
B777	AGGGAAGGGTGTAGGACGTT	TCGTTTTCTCCACATCACCA	27	0.28 ± 0.02
B788	TCCCTTTCTCCGTCATCAAC	TCGTCACCGTCACCAGATAA	7	0.34 ± 0.03
B789	GCCACGTCCAGAATCAAAAT	CCTCAGGGCTGAGAAGTTGA	9	0.37 ± 0.05
B790	TGCAGGCTTATGCACATGAT	AGCCCTCACCTATAACCCTCT	8	0.45 ± 0.01

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Table 3.2. (cont.)

B791	CACCAGGACCCTGATACCAT	TCCACAATGATTTTGTGAAAAC	8	0.35 ± 0.04
CAC-B005[a]	CAAACTTATGATAGGCATGCAA	TGTCACTTTGGAAGACAAGAGA	7	0.30 ± 0.08
CAT-C504[b]	CGCCATCTCCATTTCCCAAC	CGGAATGGTTTTCTGCTTCAG	10	0.40 ± 0.03

All primers are from Gurcan et al.[52], otherwise noted: a from Bassil et al.[48] and b from Boccaci et al.[47]

3.1.2. Genetic Diversity

The AFLP and SSR data were used to construct separate distance matrices and dendrograms using the Dice coefficient and unweighted neighbor-joining algorithm. Mantel tests showed very high correlations between the dendrograms and distance matrices (r= 0.96 for both data sets). The distance matrices for the AFLP and SSR data were also tested for correlation using a Mantel test which indicated a very low correlation (r=0.33). For that reason, the two data sets were not combined.

With the AFLP data, the hazelnut accessions grouped into two main clusters: cluster A with 46 accessions and cluster B with 54 (Figure 3.1). A third cluster contained only two accessions. The minimum and maximum genetic dissimilarities between hazelnut accessions were 0.06 and 0.52, respectively, with a mean value of 0.32. All but seven of the cultivars (85%) fell in cluster A while all but five of the wild accessions (91%) fell in cluster B which also contained five cultivars. The remaining two cultivars, 'Romische Zellernuss' and 'Valcea', clustered separately (C). The cultivars in cluster A did not show any grouping based on geographical origin. In contrast, some clustering by origin was observed for the wild material. For example, a distinct subcluster of cluster B contained 18 of the 23 wild accessions from Vipava-Razdrto and Bovec (78%), 'Willamette' and a single wild accession from Maribor. In addition, seven of the eight remaining Maribor accessions were closely grouped in the AFLP dendrogram, the rest of the wild accessions in cluster B were intermixed.

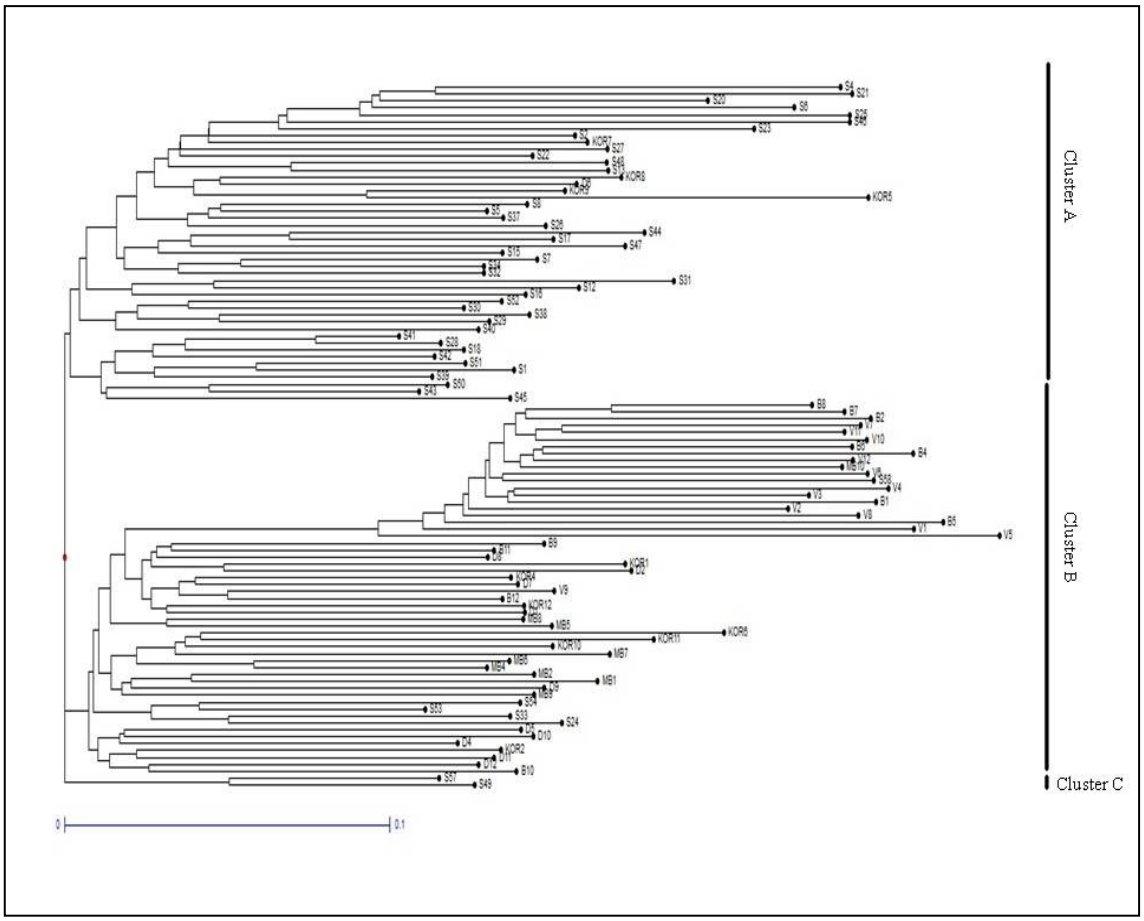

Figure 3.1 Unweighted neighbor-joining dendrogram of the 102 Slovenian National Collection genotypes based on 11 selective primer combinations of amplified fragment length polymorphism (AFLP).

The dendrogram constructed with the SSR data consisted of four clusters with 31, 46, 21, and 4 accessions in clusters A to D, respectively (Figure 3.2). The minimum genetic dissimilarity between hazelnut accessions was 0.22 and the maximum dissimilarity was 0.85 with a mean of 0.58. All but three (94%) of the cultivars ('Tonda di Giffoni', 'Pauetet' and 'Valcea') were found in cluster B which only contained one wild accession (accession 10 from Dolenjska). As with the AFLP dendrogram, the cultivars did not show any clustering by geographical origin. In addition, most of the wild accessions from Vipava-Razdrto and Bovec were intermixed and separate from the other accessions in cluster A. Similar intermixing was seen for wild accessions from Maribor, Koroska, and Dolenjska in clusters A and C.

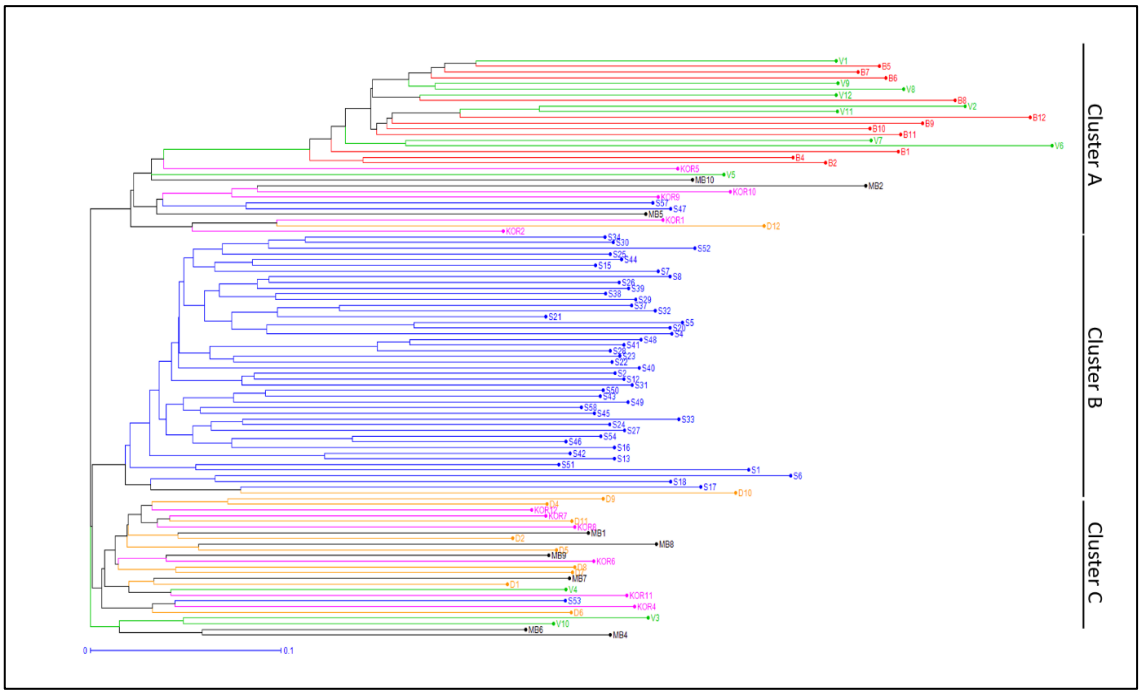

Figure 3.2. Unweighted neighbor-joining dendrogram of the 102 hazelnut accessions based on SSR data. Accessions are color coded by origin: blue = cultivar, red = Bovec, orange = Dolenjska, purple = Koroska, black = Maribor, green = Vipava-Razdrto.

Principal coordinate analysis (PCoA) of both molecular marker datasets showed clear separation of the wild accessions from the cultivars (Figure 3.3). As with the dendrogram analysis, the Vipava-Razdrto and Bovec accessions clustered together and were distinct from the other wild accessions which were intermixed in the lower half of the two-dimensional PCoA plot. Nearly all of the cultivars fell in the upper right quadrant of the PCoA plot and were more tightly clustered than the wild material. Average Dice coefficient dissimilarity values were calculated for the SSR dataset (Table 3.3) to compare the diversity present in wild vs. cultivated accessions and in accessions from different regions. As expected, the wild material was more diverse than the cultivars with mean dissimilarity values of 0.60 and 0.50, respectively. Among the different regions where wild accessions were collected, Vipava- Razdrto (0.61), Bovec (0.57), and Maribor (0.55) had the most diverse material.

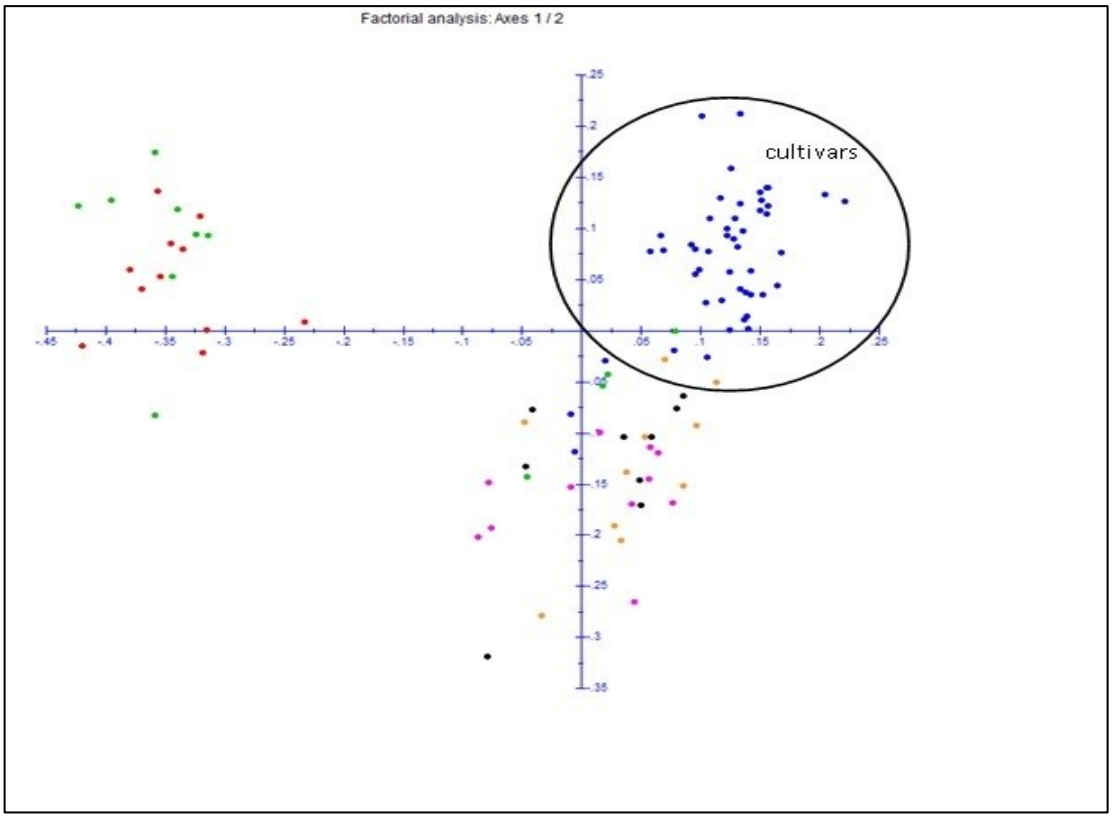

Figure 3.3. Principal coordinate analysis of hazelnut accessions based on SSR data. The first two Eigen vectors which explained 15.6 and 6.8% of the variance, respectively, are plotted. Genotypes are color coded by origin: blue = cultivar, red = Bovec, orange = Dolenjska, purple = Koroska, black = Maribor, green = Vipava-Razdrto. All but two cultivars are included in the circled region.

Table 3.3. Average Dice coefficient dissimilarity values for cultivated and wild hazelnut accessions as determined with SSR markers. Wild accessions are classified by origin, number of accessions are indicated in parenthesis after location code.

Origin	Average dissimilarity	Range
Cultivars (S, 48)	0.50	0.22-0.71
Wild material (all regions, 54)	0.60	0.36-0.83
Bovec (B, 11)	0.57	0.43-0.69
Dolenjska (D, 11)	0.50	0.37-0.64
Koroska (K, 11)	0.51	0.36-0.65
Maribor (MB, 9)	0.55	0.39-0.75
Vipava-Razdrto (V, 12)	0.61	0.38-0.78

3.1.3. Population Structure

Population structure analysis was performed with both the AFLP and SSR datasets and similar results were obtained. For that reason, only the SSR results are given here. According to the analysis, the data were best described by a K= 2 model, indicating that the material fell into two subpopulations. Based on a subpopulation identity threshold of $P \geq 0.7$, 62 individuals were assigned to subpopulation 1, 21 individuals were assigned to subpopulation 2, and 19 individuals were admixed. All but five of the hazelnut cultivars belonged to subpopulation 1 with the remaining accessions ('101', 'F-104', 'Bandnuss', 'Pauetet', and 'Valcea') showing an admixed ancestry (Figure 3.4, Table 3.4). The wild accessions were nearly equally divided between subpopulations 1 and 2 with 19 and 21 individuals in each subpopulation, respectively. The remaining 14 (26%) wild accessions were admixed. When the wild material was examined by region, all of the wild accessions from Bovec and most from Vipava-Razdrto (8 of 12 accessions) belonged to subpopulation 2 while the Dolenjska accessions (8 of 11) primarily fell into subpopulation 1 (Figure 3.4). Both Koroska and Maribor had higher incidence of admixed accessions with 36 and 56%, respectively (Figure 3.4).

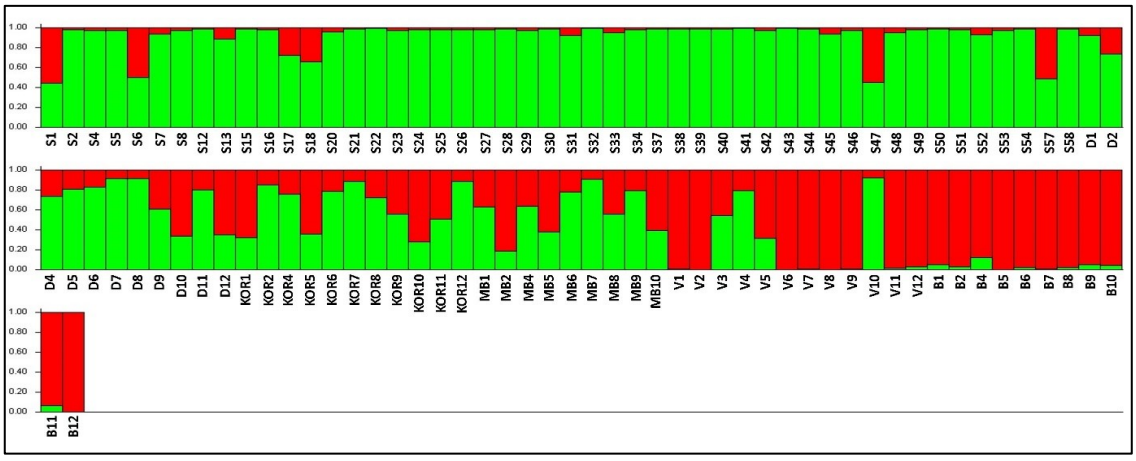

Figure 3.4. Population structure of hazelnuts according to SSR results. Each accession is represented by a vertical bar. Green sections within each vertical bar indicate membership coefficient (y-axis) of the accession to subpopulation 1 while red sections indicate membership to subpopulation 2.

Table 3.4. Hazelnut accessions and origins. Inferred subpopulation and assignment and dendrogram clustering are based on SSR results.

Name (Accession)	Type of Material	Origin	Genetic Background	Inferred Subpopulation	Dendrogram Cluster
101 (s1)	Cultivar	Italy	s54 x s13	Admixed	B
119 (s2)	Cultivar	Italy	s54 x s13	1	B
Apolda (s4)	Cultivar	Italy		1	B
Arutela (s5)	Cultivar	Romania	Merveille de Bollwiller x s54	1	B
Bandnuss (s6)	Cultivar	United Kingdom		Admixed	B
Bearn (s7)	Cultivar	France		1	B
Brixnut (s8)	Cultivar	USA		1	B
Corabel = N-473 (s12)	Cultivar	France	s21 seedling	1	B
Cosford (s13)	Cultivar	United Kingdom		1	B
Daviana (s15)	Cultivar	United Kingdom		1	B
E-104 = Daria (s16)	Cultivar	Italy	s54 x s13	1	B
Ennis (s17)	Cultivar	USA		1	B
F-104 (s18)	Cultivar	Italy	s54 x s13	Admixed	B
Feriale (s20)	Cultivar	France	s28 x Butler	1	B
Fertile de Coutard = Barcelona (s21)	Cultivar	USA		1	B
Ferwiller (s22)	Cultivar	France	Merveille de Bollwiller x Tonda G. Romana	1	B

(Cont. on the next page)

Table 3.4. (cont.)

Frutto Grosso (s23)	Cultivar	Italy		1	B
G1 (s24)	Cultivar	Italy	Payrone x Tonda Gentile Romana	1	B
Gem (s25)	Cultivar	USA		1	B
Gunslebert (s26)	Cultivar	Germany		1	B
Heynich's Zellernuss (s27)	Cultivar	Germany		1	B
Imperiale de Trebizonde (s28)	Cultivar	Turkey		1	B
Istrska dolgoplodna leska (s29)	Cultivar	Croatia		1	B
Istrska okrogloplodna leska (s30)	Cultivar	Croatia		1	B
Lambertskibeli (s31)	Cultivar	Germany		1	B
Landsberg (s32)	Cultivar	Germany		1	B
Lansing (s33)	Cultivar	USA		1	B
Lewis = OSU 243.002 (s34)	Cultivar	USA	(s21 x Tombul Ghiaghli) x s58	1	B
Mogul (s37)	Cultivar	United Kingdom		1	B
Morell (s38)	Cultivar	Spain		1	B
Mortarella (s39)	Cultivar	Italy		1	B
N-650 = H368-22 (s40)	Cultivar	France	Tonda Gentile Romana x s54	1	B
Negret (s41)	Cultivar	Spain		1	B

(Cont. on the next page)

Table 3.4. (cont.)

Nocchione = Montebello (s42)	Cultivar	Italy		1	B
OSU 166.034 (s43)	Cultivar	USA	Casina x Butler	1	B
OSU 167.002 (s44)	Cultivar	USA		1	B
OSU 238.125 (s45)	Cultivar	USA		1	B
OSU 244.001 (s46)	Cultivar	USA	(s21 x Tombul Ghiaghli) x s58	1	B
Pauetet (s47)	Cultivar	Spain		Admixed	A
Riccia di Talanico (s48)	Cultivar	Italy		1	B
Romische Zellernuss (s49)	Cultivar	unknown (Germany?)		1	B
Romai (s50)	Cultivar	Hungary		1	B
Segorbe (s51)	Cultivar	Spain		1	B
Sodlinger (s52)	Cultivar	unknown (Germany?)		1	B
Tonda di Giffoni (s53)	Cultivar	Italy		1	C
Tonda Gentile delle Langhe (s54)	Cultivar	Italy		1	B
Valcea (s57)	Cultivar	Romania	clonal selection of Furfulak	Admixed	A
Willamette (s58)	Cultivar	USA	s42 x Compton	1	B
d1	Wild	Dolenjska		1	C
d2	Wild	Dolenjska		1	C

(Cont. on the next page)

Table 3.4. (cont.)

d4	Wild	Dolenjska	1	C
d5	Wild	Dolenjska	1	C
d6	Wild	Dolenjska	1	C
d7	Wild	Dolenjska	1	C
d8	Wild	Dolenjska	1	C
d9	Wild	Dolenjska	Admixed	C
d10	Wild	Dolenjska	Admixed	B
d11	Wild	Dolenjska	1	C
d12	Wild	Dolenjska	Admixed	A
kor1	Wild	Koroska	Admixed	A
kor2	Wild	Koroska	1	A
kor4	Wild	Koroska	1	C
kor5	Wild	Koroska	Admixed	A
kor6	Wild	Koroska	1	C
kor7	Wild	Koroska	1	C
kor8	Wild	Koroska	1	C
kor9	Wild	Koroska	Admixed	A

(Cont. on the next page)

Table 3.4. (cont.)

kor10	Wild	Koroska	2	A
kor11	Wild	Koroska	Admixed	C
kor12	Wild	Koroska	1	C
mb1	Wild	Maribor	Admixed	C
mb2	Wild	Maribor	2	A
mb4	Wild	Maribor	Admixed	D
mb5	Wild	Maribor	Admixed	A
mb6	Wild	Maribor	1	D
mb7	Wild	Maribor	1	C
mb8	Wild	Maribor	Admixed	C
mb9	Wild	Maribor	1	C
mb10	Wild	Maribor	Admixed	A
v1	Wild	Vipava-Razdrto	2	A
v2	Wild	Vipava-Razdrto	2	A
v3	Wild	Vipava-Razdrto	Admixed	D
v4	Wild	Vipava-Razdrto	1	C
v5	Wild	Vipava-Razdrto	Admixed	A

(Cont. on the next page)

Table 3.4. (cont.)

v6	Wild	Vipava-Razdrto	2	A
v7	Wild	Vipava-Razdrto	2	A
v8	Wild	Vipava-Razdrto	2	A
v9	Wild	Vipava-Razdrto	2	A
v10	Wild	Vipava-Razdrto	1	D
v11	Wild	Vipava-Razdrto	2	A
v12	Wild	Vipava-Razdrto	2	A
b1	Wild	Bovec	2	A
b2	Wild	Bovec	2	A
b4	Wild	Bovec	2	A
b5	Wild	Bovec	2	A
b6	Wild	Bovec	2	A
b7	Wild	Bovec	2	A
b8	Wild	Bovec	2	A
b9	Wild	Bovec	2	A
b10	Wild	Bovec	2	A
b11	Wild	Bovec	2	A
b12	Wild	Bovec	2	A

3.1.4. Cultivar Origin

The cultivars were subjected to PCoA and plotted in two dimensions (Figure 3.5) to see if clustering was explained by the genetic background of the material. Seven of the cultivars were related to ‘Tonda Gentile delle Langhe’ while the ‘Cosford’ background was found in five cultivars (Table 3.4), ‘Fertile de Coutard’ and ‘Nocchione’ backgrounds were found in four cultivars each while ‘Compton’ background was found in three cultivars. ‘Tonda Gentile delle Langhe’ and ‘Cosford’-related cultivars showed no grouping in the PCoA. In contrast, ‘Fertile de Coutard’, ‘Nocchione’, and ‘Compton’-related material all showed similar clustering on the left side of the graph.

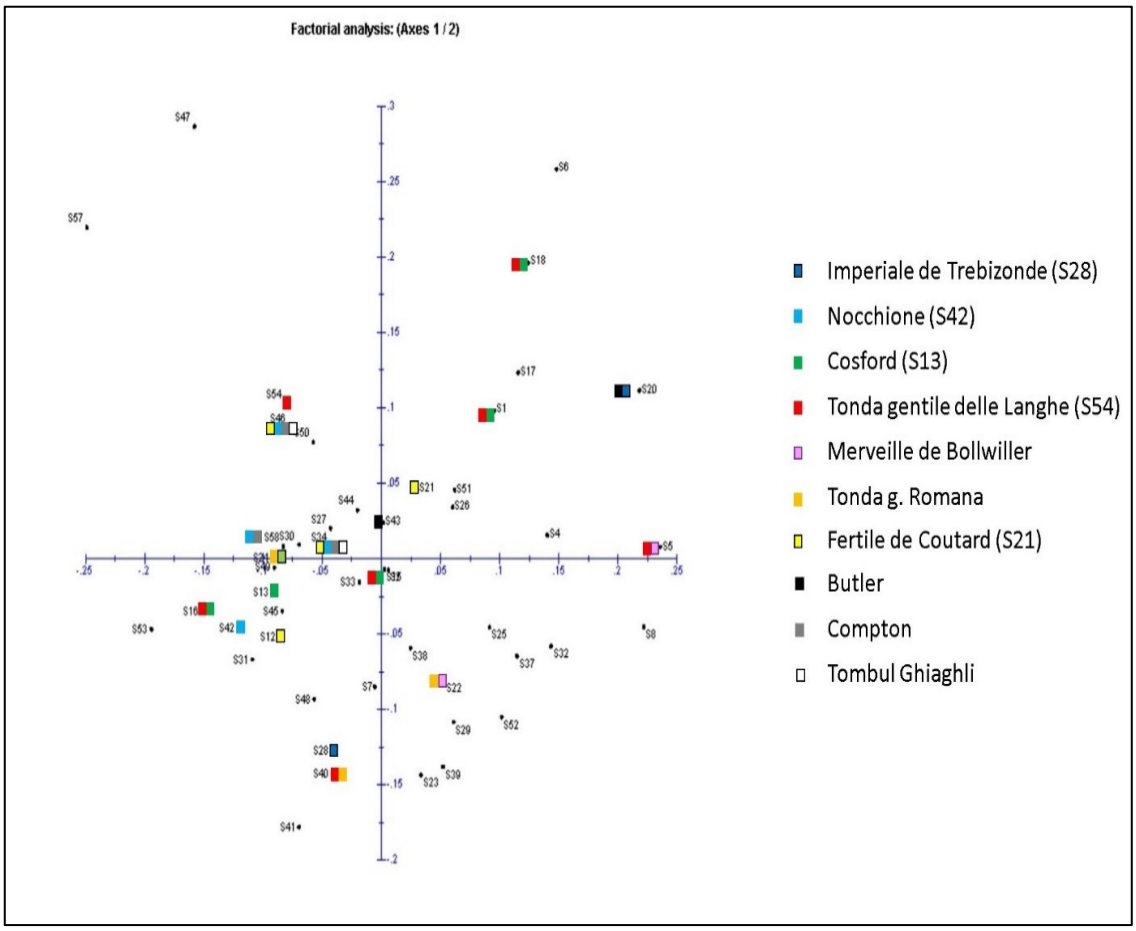

Figure 3.5. PCoA of hazelnut cultivars based on SSR results. Genetic background of cultivars is indicated by colored boxes.

3.1.5. Nut and Kernel Traits

A panel of 64 accessions was randomly selected for the association analysis including 24 cultivars and 40 wild accessions. The panel was characterized for 10 nut and seven kernel traits. Cultivars and wild accessions were analyzed separately. The wild accessions were also analyzed according to region.

3.1.5.1 Nut Traits

Mean nut length, width, and thickness were significantly higher for the cultivars (means of 21, 20, 17.5 mm, respectively) than for wild accessions (means of 17.4, 14.5, 12.3 mm, respectively) (Table 3.5). Cultivars also had slightly higher coefficient of variation (CV) than wild accessions for these traits. ANOVA and LSD (least significant difference) tests showed that there was no significant difference between the means for these three parameters for wild accessions from different regions of Slovenia ($P \leq 0.05$). Nut width and thickness had the least variation in the wild accessions from Maribor (Table 3.6).

Mean caliber of wild accessions (17.4 mm) was significantly higher than that of cultivars (15.8 mm) (Table 3.5); however, both sets of material had similar amounts of variation for this trait (CV = 10 to 12%). There were no significant differences between calibers for each region of Slovenia ($P \leq 0.05$). Wild accessions from all regions with the exception of Bovec (5%) preserved the variation of the wild accessions for caliber (Table 3.6.). Although hazelnut cultivars had slightly higher mean shape index (0.9) than wild accessions (0.8), cultivars and wild accessions had similar amounts of variation for shape index. Wild accessions from different regions had similar shape index with the least variation for this trait in Bovec and Koroska (Table 3.6).

Although mean nut shell thickness of the cultivars and wild accessions were the same (1.1 mm), wild accessions had more variation (CV = 20%) than cultivars (CV = 15%) (Table 1). There were no significant differences between wild accessions from each region for shell thickness (Table 3.6).

Hazelnut cultivars had two-fold higher mean nut weight (2.8 g) than wild accessions (1.3 g) (Table 3.5). Wild accessions had higher CV (32%) than cultivars (24%) for this trait with nuts as small as 0.6 g (Table 3.5). Hazelnuts from Maribor were the

lightest (0.9 g); however, this difference was not significant. Wild accessions from Koroska and Vipava-Razdrto had higher nut weight variation than the other regions with one accession from Koroska having nearly the same mean weight as the cultivars (2.6 vs. 2.8 g) (Table 3.6). Mean nut shape uniformity of wild accessions was higher (8) than that of the cultivars (7.5) (Table 3.5) with very little variation for this trait in both sets of material. There was no significant difference for nut shape uniformity in different regions of Slovenia (Table 3.6).

The proportion of healthy nuts for cultivars (90.8%) was much higher than for wild accessions (57.3%) with much more variation for this parameter in the wild accessions (CV = 65% vs. 7%) (Table 3.5). The Dolenjska and Vipava-Razdrto accessions had significantly healthier nuts (80 to 88%) than the Koroska accessions (12.5%). Cultivars and wild material had similarly low proportions of empty nuts. However, the wild accessions had much more variation for this trait with one accession producing 90% empty nuts. All wild accessions except those from Koroska and Maribor had no empty nuts (Table 3.6).

Table 3.5. Nut and kernel traits for hazelnut cultivars and wild accessions.

	Cultivars (S)			Wild material (all regions)		
Trait	Mean ± SE	Range	CV	Mean ± SE	Range	CV
Nut			%			%
Length, mm	21 ± 0.7a	16.5 - 29.16	15	17.4 ± 0.3b	12.9 - 21.7	11
Width, mm	20 ± 0.6a	16.98 - 27.6	14	14.5± 0.3b	12.1 - 17.5	9
Thickness, mm	17.5 ± 0.5a	14.2 - 24.37	14	12.3 ± 0.3b	10.3 - 14.9	11
Calibre, mm	15.8 ± 0.4a	12.24 - 20.25	12	17.4 ± 0.3b	13 - 21.7	10
Shape index	0.9 ± 0.1a	0.7 - 1.17	12	0.8 ± 0.1b	0.54 - 0.92	12
Shell thickness, mm	1.1 ± 0.1a	0.85 - 1.6	15	1.1 ± 0.1a	0.7 - 1.7	20
Weight, g	2.8 ± 0.1a	1.86 - 4.3	24	1.3 ± 0.1b	0.6 - 2.6	32
Shape uniformity (1-9)	7.5 ± 0.1a	6 - 8	9	8 ± 0.1b	7 - 8	2
Healthy, %	90.8 ± 1.2a	82 - 100	7	57.3 ± 5.8b	0 - 100	65
Empty, %	4.2 ± 0.8a	0 - 13.33	96	3.7± 2.4a	0 - 90	404
Kernel						
Weight, g	1.2 ± 0.1a	0.97 - 1.87	20	0.4 ± 0.1b	0.1 - 1.1	50
Kernel percentage	46.3 ± 0.1a	33.78 - 52.78	10	32.1 ± 1.8b	11.11 - 73.33	34
Shape uniformity, (1-9)	6.5 ± 0.3a	4 - 8	17	6.5 ± 0.3a	2 - 8	22
Brown spots, %	0.6 ± 0.2a	0 - 2.5	156	0	-	-
Moldy, %	0.4 ± 0a	0 - 2.5	165	0	-	-
Twins, %	2 ± 0.7a	0 - 15	179	0	-	-
Deformed, %	2.7 ± 0.5a	0 - 9	83	38.7 ± 5.4b	0 - 100	89

Table 3.6. Nut and kernel traits for wild accessions by regions.

Trait	Bovec (B)			Dolenjska (D)			Koroska (K)			Maribor (MB)			Vipava-Razdrto (V)		
	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)	Mean ± SE	Range	CV (%)
Nut															
Length, mm	16.4 ± 0.3a	14.7 - 18.1	5	18.3 ± 0.6a	15 - 21.7	11	17.6± 1.1a	12.9 -19.3	12	16.7 ± 0.1a	14.6 -20.2	13	17.5 ± 0.7a	15.9 – 20.0	9
Width, mm	14.7 ± 0.4a	13.2 - 16.8	8	14.4 ± 0.3a	12.6 - 15.8	7	14.8 ± 1.0a	12.1 -17.1	13	13.7 ± 0.4a	12.7 -14.6	5	14.6 ± 0.9a	12.3 - 17.5	15
Thickness, mm	12.7 ± 0.5a	10.9 - 14.6	11	11.8 ± 0.3a	10.7 - 13.4	9	12.7 ± 0.9a	10.4 -14.5	13	11.8 ± 0.2a	11.2 -12.4	4	12.6 ± 0.8a	10.3 - 14.9	15
Caliber, mm	16.4 ± 0.3a	14.7 - 18.1	5	18.3 ± 0.6a	15.3 - 21.7	10	17.6 ± 1.1a	13.0 -19.3	12	16.7 ± 0.1a	14.7 -20.2	13	17.5 ± 0.7a	15.9 – 20.0	9
Shape index	0.8 ± 0.1a	0.8 - 0.9	6	0.7 ± 0.1a	0.5 - 0.9	13	0.9 ± 0.1a	0.7 -0.9	9	0.8 ± 0.1a	0.6 -0.9	14	0.8 ± 0.1a	0.6 - 0.9	14
Shell thickness, mm	1.2 ± 0.1a	0.8 - 1.4	19	1.1 ± 0.1a	0.7 - 1.3	20	1.2 ± 0.2a	0.8 -1.7	20	1.0 ± 0.1a	0.7 -1.3	24	1.1 ± 0.1a	0.8 - 1.3	17
Weight, g	1.2 ± 0.1a	0.9 - 1.6	17	1.4 ± 0.1a	1.2 - 2.1	17	1.3 ± 0.4a	0.6 -2.6	49	0.9 ± 0.2a	0.6 -1.3	29	1.5 ± 0.3a	0.9 - 2.2	36
Shape uniformity	8.0 ± 0a	-	0	8.0 ± 0a	-	0	7.9 ± 0.2a	7.0 – 8.0	4	8.0 ± 0a	-	0	8.0 ± 0a	-	0
Healthy, %	55.0 ± 9.0a	0 – 90.0	52	80.7 ± 6.0b	50.0 – 100.0	23	12.5 ± 12.2c	0 -70.0	195	45.0 ± 22.2a	0 -100.0	110	88.3 ± 3.3bc	75.0 – 100.0	9
Empty, %	0a	-	-	0a	-	-	5 ± 5.3b	0 -30.0	214	22.0 ±17.5c	0 -90.0	177	0a	-	-
Kernel															
Weight, g	0.4 ± 0.1a	0.2 - 0.7	39	0.6 ± 0.1b	0.4 - 1.1	32	0.3 ± 0.1a	0.1 -0.7	63	0.3 ± 0.1a	0.2 -0.5	47	0.5 ± 0.1ab	0.2 - 0.8	48
Kernel percentage	30.2 ± 3.0a	22.2 - 46.7	25	41.4 ± 4.0b	28.6 - 73.3	28	24.2 ± 5.5a	11.1 - 43.8	45	31.3 ± 4.1a	22.2 - 45.5	29	29.6 ±1.9a	22.2 - 36.4	15
Shape uniformity	6.6 ± 0.3a	5.0 – 8.0	13	7.4 ± 0.3a	6.0 – 8.0	11	5.7 ± 0.8a	3.0 – 8.0	27	5.8 ± 1.2a	2.0 – 8.0	46	6.7 ± 0.4a	6.0 – 8.0	12
Brown spots, %	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Moldy, %	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Twins, %	0	-	-	0	-	-	-	-	-	0	-	-	0	-	-
Deformed, %	45.0 ± 9.0a	10.0 – 100.0	63	19.2 ± 5.6b	0 – 50.0	96	82.5 ±12.0c	30.0 -100.0	29	31.0 ± 18.1ab	0 - 80.0	130	11.6 ± 3.3bc	0 – 25.0	70

Means with different letters within a row are significantly different according to ANOVA (Analysis of variance) and LSD (Least Significant Difference) test ($p<0.05$)

3.1.5.2. Kernel Traits

Hazelnut cultivars had three-fold higher mean kernel weight (1.2 g) than wild accessions (0.4 g) with more variation for this trait in the wild material (Table 3.5). The wild accessions from all regions except Dolenjska (0.6 g) had similar kernel weight (0.3 to 0.4 g) with the most variation in Koroska (CV = 63%) (Table 3.6). Mean kernel percentage for cultivars (46.3%) was significantly higher than for wild accessions (32.1%) which had more variation for this trait (Table 3.5). The wild accessions from Dolenjska had significantly higher mean kernel percentage than the other regions (Table 3.6).

There was no difference between cultivars and wild accessions for kernel shape uniformity as both sets of material had moderate uniformity values of 6.5 (out of 9) (Table 3.5). Wild accessions from different regions had similar uniformity; however, those from Maribor had much higher variation (CV = 46%) than the other regions (Table 3.6).

Proportions of kernels with brown spots and mold were low for both cultivars and wild accessions (0 to 0.6%) with absolutely no variation for this trait in the wild material (Table 3.5, Table 3.6). Similarly, none of the wild accessions had twin kernels while a low percentage of the cultivars (2.0%) had this trait. Wild accessions had a 14-fold higher proportion of deformed kernels (38.7%) than cultivars (2.7%) with some wild accessions having 100% deformed kernels. Wild accessions from Dolenjska (19.2%) and Vipava-Razdrto (11.6%) had significantly fewer deformed kernels with most of the variation for this trait in Dolenjska (CV = 96%) and Maribor (CV = 130%) (Table 3.5, Table 3.6).

3.1.5.3. Trait Correlations and Principal Component Analysis

Nut length had a high positive correlation to caliber ($r^2 = 0.99$); moderate positive correlations to nut ($r^2 = 0.52$) and kernel weights ($r^2 = 0.45$); and a moderate negative correlation with nut shape index ($r^2 = -0.64$). There were high correlations between nut width and thickness ($r^2 = 0.90$) and nut weight ($r^2 = 0.72$). In addition, there were moderate positive correlations between width and nut shape index ($r^2 = 0.50$), shell thickness ($r^2 = 0.35$), and kernel weight ($r^2 = 0.56$). There were also moderate positive correlations between shell thickness and shape index ($r^2 = 0.54$) and nut weight ($r^2 = 0.60$). There was negative moderate correlation between shape index and caliber ($r^2 =$

−0.62). Positive moderate correlations were observed between caliber and nut ($r^2 = 0.53$) and kernel ($r^2 = 0.50$) weights. As expected, there was positive correlation between nut and kernel weight; however, this correlation was only moderate ($r^2 = 0.65$). Proportion of healthy nuts was correlated to four traits: kernel weight ($r^2 = 0.45$), kernel percentage ($r^2 = 0.50$), kernel shape uniformity ($r^2 = 0.70$) and proportion of empty nuts ($r^2 = -0.40$). There was negative moderate correlation between proportion of empty nuts and kernel shape uniformity ($r^2 = -0.60$). Kernel weight was highly correlated to kernel percentage ($r^2 = 0.85$).

Principal component analysis (PCA) of the nut and kernel traits was performed. The first three Eigen vectors explained 61.6% of the morphological variation. A total of 39% of the morphological variation was explained by PC1 with high positive correlations ($r^2 > 0.7$) to nut and kernel weight; nut length, width, and thickness; and kernel percentage (Table 3.7). PC2 explained 12.2% of the morphological variation with moderate positive correlations to deformed kernels, caliber, and nut length and moderate negative correlation to proportion of healthy nuts. PC3 explained 10.4% of the morphological variation with moderate positive correlations to caliber and length and high negative correlation to nut shape index (Table 3.7). The two-dimensional PCA plot of the morphological data showed that the hazelnut cultivars formed a tight cluster compared to the wild materials which were widely distributed (Figure 3.6). Tthe PCA plot did not show region-specific clustering of wild accessions based on morphological traits.

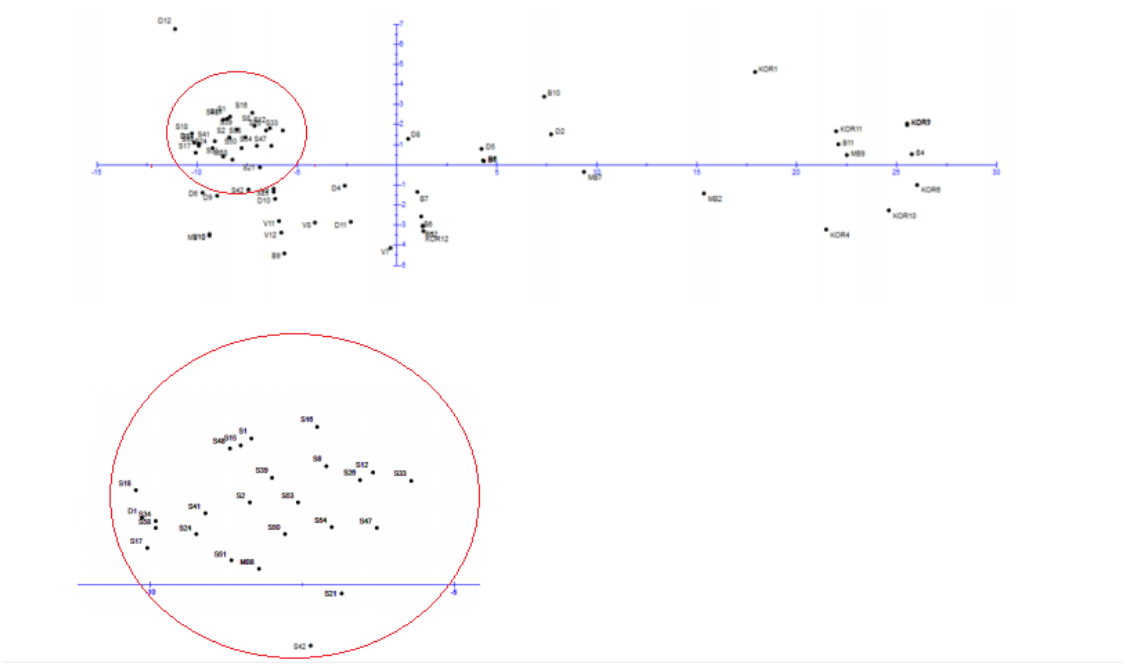

Figure 3.6. Principal component analysis (PCA) plot for nut and kernel traits. All of the hazelnut cultivars clustered together (highlighted by red circle).

Table 3.7. Principal component analysis of quantitative traits. Eigen values are given for the first three principal component (PC) axes.

Traits	PC1	PC2	PC3
Nut			
Length	0.70	0.47	0.47
Width	0.91	0.32	-0.13
Thickness	0.88	0.33	-0.15
Shape index	0.52	-0.07	-0.72
Caliber	-0.24	0.55	0.58
Shell thickness	-0.02	0.39	-0.45
Weight	0.91	0.31	0.06
Shape uniformity	-0.50	0.08	0.14
Healthy	0.62	-0.61	0.14
Empty	-0.05	0.27	-0.19
Kernel			
Weight	0.97	0.07	0.13
Kernel percentage	0.70	-0.37	0.23
Brown spots	0.51	0.01	0.10
Moldy	0.32	-0.29	0.29
Deformed	-0.65	0.53	-0.07
Twins	0.42	0.09	-0.30

Stepwise discriminant analysis was performed using sub-population (as determined from population structure) as a grouping variable and all nut and kernel traits as independents. Kernel weight and percentage made significant contributions to multivariate discrimination of the three subpopulations with moderate correlations of $r^2 = 0.71$ and $r^2 = 0.57$, respectively. Kernel weight also made significant contributions to discrimination when dendrogram cluster was used as the grouping variable. When discriminant analysis was performed using regions as the grouping variable, only the proportion of healthy nuts made a significant but moderate ($r^2 = 0.47$) contribution to discrimination of the five regions.

3.2. Association Mapping

A total of 504 SSR fragments generated from 49 SSR markers was associated with nut and kernel traits. Significant (−Log (P value) >3) linkage disequilibrium (LD) was detected for 855 (0.65%) SSR marker pairs. LD values (r^2) of these SSR marker pairs ranged from 0.17 to 1 with a mean of 0.32. Different AM models [GLM, GLM (Q), GLM (PC), GLM (Q + PC), MLM (K), MLM (Q + K), MLM (PC + K), MLM (Q + PC + K)] were compared and used to calculate the proportion of significant results (Table 3.8). The GLM models had higher proportions of significant results than the MLM models. The GML model corrected with the population structure Q-matrix had the highest proportion of significant results among the GLM models [π1 (%) = 9.9] and was used for AM of the nut and kernel traits (Table 3.8).

Table 3.8. Association models tested to determine best model for association analysis.

	π0 (%) *	π1 (%) **
GLM	92.5	7.5
GLM (Q)	90.1	9.9
GLM (PC)	92.4	7.6
GLM (Q+PC)	91.5	8.5
MLM (K)	99.5	0.5
MLM (Q+K)	97.8	2.2
MLM (PC+K)	99.0	1.0
MLM(PC+Q+K)	99.0	1.0

* Overall proportion of true null hypotheses (FDR) ** Proportion of significant results

3.2.1. Nut Traits

Nine SSR markers were associated with nut length (Table 3.9). LD values (r^2) of these SSR markers ranged from 0.22 (B791-270) to 0.46 (A622-125). A622-125 also had a significance level [−Log (P value) = 6.55]. Ten SSR markers were associated with nut thickness. The LD values (r^2) of markers associated with the trait ranged from 0.22 to 0.40. Six of these markers (A622-125, A622-130, B791-270, A604-161, B777-48 and B716-216) were also associated with length. There were no SSR markers associated with width and shape index. Ten markers were identified for nut caliber with LD values ranging from 0.23 (CAC-B005-440) to 0.41 (A613- 150). Nine of the ten caliber markers were also identified for nut length and thickness.

No SSR markers were significantly associated with shape index; however, two markers were identified for shell thickness. The LD values of these markers were 0.16 and 0.15 for B602-341 and B648 -261, respectively (Table 3.9). No markers were detected for nut weight, shape uniformity, and proportion of healthy nuts. Proportion of empty nuts was associated with the most SSRs, 22 markers. The LD values of these markers ranged from 0.22 to 0.49 with the greatest effects seen for A604-161 and A613-150.

Table 3.9. Hazelnut SSR markers associated with nut and kernel traits.

Trait	SSR locus	-Log (*P*-value)*	LD value (r^2)
Nut			
Length	A622-125	6.55	0.46
Length	B777-48	5.96	0.32
Length	A622-130	5.53	0.39
Length	A604-161	4.86	0.30
Length	A613-150	4.61	0.36
Length	B741-201	4.42	0.26
Length	B709-226	4.27	0.26
Length	B791-270	4.11	0.22
Length	B716-216	4.08	0.23
Thickness	B791-270	7.01	0.37
Thickness	B777-48	5.53	0.30
Thickness	B777-60	5.48	0.30
Thickness	A622-125	5.46	0.40
Thickness	B628-304	5.45	0.31

(Cont. on the next page)

Table 3.9. (cont.)

Thickness	A604-161	5.29	0.32
Thickness	B758-165	5.11	0.30
Thickness	A622-130	4.21	0.30
Thickness	B716-216	4.13	0.23
Thickness	CAC-B005-440	4.05	0.22
Calibre	B791-270	7.62	0.40
Calibre	A604-161	6.11	0.37
Calibre	B777-48	5.68	0.31
Calibre	B628-304	5.53	0.31
Calibre	A613-150	5.47	0.41
Calibre	B758-165	5.01	0.29
Calibre	A622-125	4.48	0.33
Calibre	B777-60	4.47	0.25
Calibre	B777-42	4.23	0.23
Calibre	CAC-B005-440	4.20	0.23
Shell thickness	B602-341	4.72	0.16
Shell thickness	B648-261	4.62	0.15
Empty	B628-304	9.10	0.48
Empty	A604-161	8.74	0.49
Empty	B791-270	8.41	0.43
Empty	A606-154	7.15	0.45
Empty	B758-165	6.80	0.39
Empty	A613-150	6.78	0.49
Empty	B640-81	6.31	0.35
Empty	B628-315	5.84	0.33
Empty	A606-146	5.60	0.37
Empty	B603-287	5.55	0.32
Empty	B777-48	5.35	0.29
Empty	B625-253	4.95	0.27
Empty	CAC-B005-440	4.87	0.26
Empty	CAT-C504-235	4.48	0.28
Empty	B791-343	4.37	0.24
Empty	CAC-B005-476	4.32	0.24
Empty	A622-172	4.26	0.31
Empty	B790-211	4.09	0.22
Empty	A606-184	4.06	0.27
Empty	A606-159	4.06	0.27
Empty	B648-55	4.05	0.23
Empty	A606-150	4.04	0.27
Kernel			
Shape Uniformity	A604-161	7.18	0.42
Shape Uniformity	B791-270	5.75	0.31

(Cont. on the next page)

Table 3.9. (cont.)

Shape Uniformity	B716-216	5.47	0.30
Shape Uniformity	B777-48	5.21	0.29
Shape Uniformity	A622-130	4.92	0.35
Shape Uniformity	A622-125	4.70	0.35
Shape Uniformity	B640-81	4.01	0.23
Moldy	B640-81	5.32	0.30
Weight	B726-281	4.76	0.30

*Negative $\log_{10}$-transformed p-values

3.2.2. Kernel Traits

Only one SSR marker (B726-281) was associated with kernel weight with an LD value of 0.30. No SSR markers were associated with kernel percentage. Seven SSR markers were associated with kernel shape uniformity with LD values ranging from 0.23 (B640-81) to 0.42 (A604-161). Association mapping was also performed for proportion of kernel with brown spots, moldy kernels, and twin kernels. Among these, a significant association was only identified for proportion of moldy kernels. This SSR marker, B640-81, had an LD value of 0.30.

3.3. Molecular Characterization of Turkish Hazelnut Germplasm

Genetic Diversity of 402 Turkish hazelnut accessions were analyzed using SSR markers and population structure of Turkish germplasm were determined. Lastly, core collection was constructed with 78 accessions from this germplasm.

3.3.1. SSR Marker Polymorphism

A total of 30 SSR marker primer pairs were used and yielded 407 fragments, 406 (99.8%) of which were polymorphic (Table 3.10). Average allele number for each SSR marker was 13.6. Marker B651 had the most polymorphic alleles (26) while B628 had the fewest (5). Observed heterozygosity for the markers varied from 0.17 to 0.42. PIC

values ranged between 0.66 and 0.99. B628 and B789 were the least polymorphic markers (PIC = 0.66 and 0.77, respectively).

Table 3.10. SSR marker sequence and polymorphism information for the Turkish hazelnut accessions.

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')	# polymorphic fragments	Ho[a]	PIC[b]
A601	TTACATGGTTCGGCAATGTG	AGATGGGAGCAGAGTGAACTG	11	0.25	0.97
A602	AAGAGTGGGGGTGCACTATG	GGATTCATGCCTGCGATACT	21	0.22	0.99
A604	GCTCCCGAGGACTTCCAG	CCACGACATTTCCCTCTCAG	15	0.37	0.99
A605	CACCCTCAAAACTGTGACGA	TGGGTCGCATTCAATAACAC	9	0.37	0.97
A606	CACCTAGCTTGTTGGTGAAGC	TGACAATAATTAACCCTACACACTTTG	9	0.24	0.95
A611	CACTAGCCAGCCCCTTTACA	CTGATGCCACAAACACAAGG	10	0.39	0.98
A613	CACACGCCTTGTCACTCTTT	CCCCTTTCACATGTTTGCTT	17	0.34	0.99
A616	CACTCATACCGCAAACTCCA	ATGGCTTTTGCTTCGTTTTG	13	0.34	0.98
A635	GGATCTGTGGTTGGCTTTTTGGTACTAT	TTACCCAATGGATGATGGACTAGCATT	12	0.33	0.98
A640	TGCCTCTGCAGTTAGTCATCAAATGTAGG	CGCCATATAATTGGGATGCTTGTTG	10	0.39	0.98
B602	AAGAGTGGGGGTGCACTATG	GGATTCATGCCTGCGATACT	14	0.30	0.97
B603	TGGTGGTGATAGGGAAGGAG	TCTTTTCTTCTTCAATCAGACGA	17	0.30	0.97
B606	TCTTGTGGTTTAGCATACTTCTCG	GAAGAAAGCAAGAAGAGAGGAGA	10	0.26	0.95
B612	GCACCTCAAACTCCTTGGAC	CCCAAACACACCCTTAGTGC	20	0.37	0.99
B613	CGCGTTTTGAGTCCCTTTAG	CTACCCGCCTGCGAGAAC	14	0.42	0.99
B625	CGCAAGTCATTGCACATTTT	GTGTGCTGTGCTCCTTTGAA	17	0.36	0.99

(Cont. on the next page)

Table 3.10. (cont.)

B628	AATCCCCTCTAGCCCCATTA	CACAGAATATTTGTAATTACCACCACA	5	0.17	0.66
B631	TGAAGCAGACAAGCGAATAGC	TTGTGTCTCTTTGTCTTGTAAATCG	13	0.27	0.95
B635	GCATCGCCAAATTATCGTCT	CTTCAACAAATCCAGGATGC	11	0.36	0.99
B640	CTGCATTGATGGATTGGTTG	TTAAGAAAGGTACAAGGGCTCTC	18	0.25	0.99
B641a	CTCCCATGAAATGATTATTCTTAG	CAAGCCATCTGTTTTGCTGA	9	0.30	0.94
B641b	ATATATATAGGCTGTGTGTGTGTGTG	ACAAGCCATCTGTTTTGCTG	18	0.31	0.99
B648	TGAAAGCGCCCAAAACTTAT	CTTGCGTCTTTTTGGAGAGC	17	0.41	0.99
B651	TTTTCTGGAATGTCGCACAG	TCTCCTCCTTCCAACAGTGG	26	0.18	0.98
B652	AGGATGCGTGGTTGTGATTT	TGGAGTAGGGTGATGAGAATGA	22	0.29	0.99
B660	TGTTGTAGCACAACCCTTTCA	TGCTAGCAGCAAATGGCTTA	8	0.39	0.96
B662	CGAAAGATGGACTTCCATGAC	CAAGTTGAGATTCTTCCTGCAA	12	0.35	0.98
B788	TCCCTTTCTCCGTCATCAAC	TCGTCACCGTCACCAGATAA	9	0.44	0.98
B789	GCCACGTCCAGAATCAAAAT	CCTCAGGGCTGAGAAGTTGA	6	0.18	0.77
CAC-B753	AAGGGTTGTTACCCATGCAC	GGTGCATTTAGTGCTTCTGG	13	0.32	0.97

All primers are from Gurcan et al.[52]. [a] Observed heterozygosity , [b] Polymorphism information content

3.3.2. Genetic Diversity

The SSR data were used to construct a distance matrix based on the Dice coefficient and to construct a dendrogram of the accessions using the unweighted neighbor joining algorithm (Figure 3.7). The Dice dissimilarity coefficient ranged from 0.10 to 0.84 with a mean of 0.49 for the pairwise comparisons between accessions. Landraces had the highest average dissimilarity coefficient (0.50) while cultivars and wild accessions had lower values (0.47). Materials from Giresun, Trabzon and Ordu (the provinces with the most trees in the collection) were also compared to cultivars. Accessions from these locations (0.48 – 0.49) were found to be only slightly more diverse than cultivars (0.47) (Table 3.11).

Table 3.11. Average Dice coefficient dissimilarity values for cultivars, landraces and wild hazelnut accessions as determined with SSR markers. Wild accessions and landraces were also combined and classified by origin for those collected in Giresun, Trabzon and Ordu (the most common locations).

Type/origin	# of accessions	Min.	Max.	Mean
Cultivar	20	0.26	0.65	0.47
Wild	143	0.12	0.77	0.47
Landrace	239	0.10	0.84	0.50
Giresun	240	0.12	0.82	0.49
Trabzon	49	0.10	0.76	0.48
Ordu	49	0.15	0.72	0.49

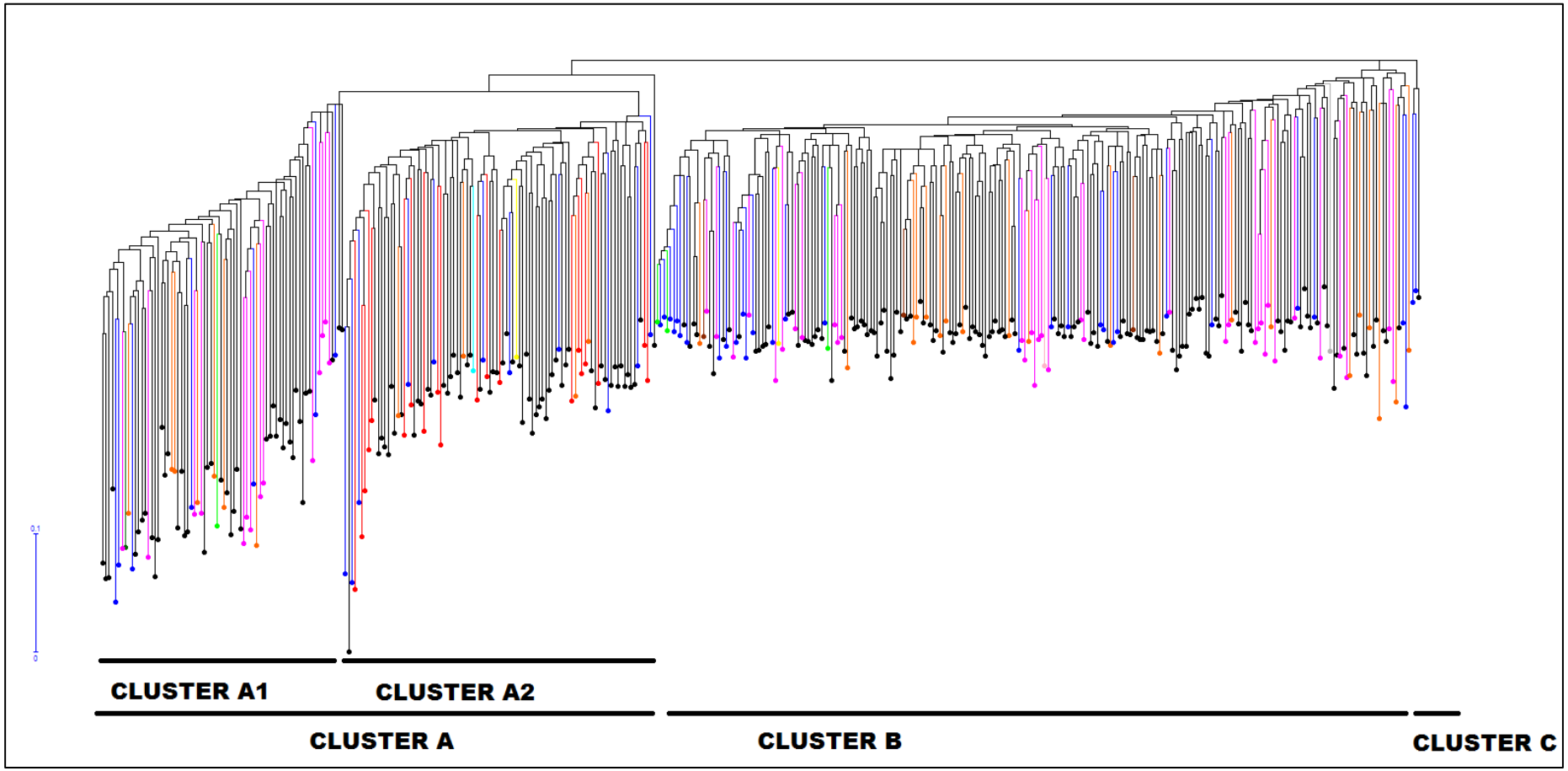

Figure 3.7. Unweighted neighbor-joining dendrogram of the 402 hazelnut accessions based on SSR data. Accessions are color coded by origin: cultivar: red, Giresun: black, Ordu: blue, Bolu: light blue, Artvin : gray, Erzurum: light pink, Kastamonu: dark green Rize: brown, Samsun: green, Sinop: yellow, Trabzon: fuchsia, Unknown: orange

A Mantel test showed a high correlation between the distance matrix and dendrogram (r=0.97). The dendrogram consisted of 3 clusters: A, B and C. Wild accessions and landraces were found in all three clusters while cultivars were limited to cluster A. Cluster A contained 169 accessions in subclusters A1 and A2 which had 74 and 95 accessions, respectively (Figures 3.8 and 3.9). Cluster A1 contained accessions from Giresun (44 accessions), Trabzon (14), Ordu (7), Samsun (1), Kastamonu (1) and unknown places (7). Cluster A2 contained all of the cultivars (20 accessions) which were distributed among the wild accessions and landraces from Giresun (58), Ordu (11), Sinop (1), Duzce (1) and unknown places (4). Cluster B was the largest with 230 accessions. It contained accessions from Giresun (137 accessions), Trabzon (35), Ordu (29), Rize (3), Samsun (3), Sinop (1), Artvin (1), Erzurum (1) and unknown places (20) (Figure 3.10). Cluster C had only three accessions: one from Giresun and two from Ordu.

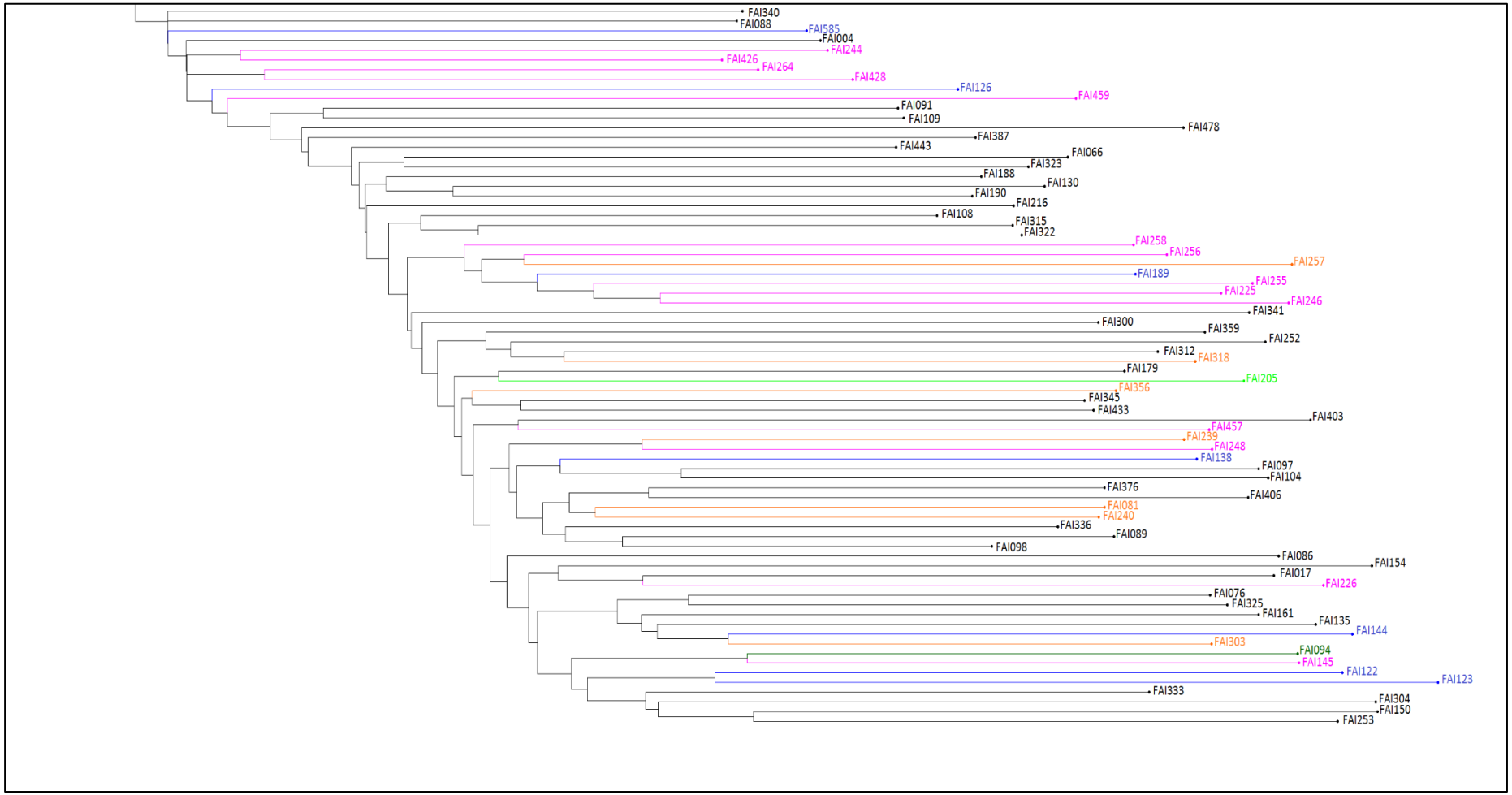

Figure 3.8. Cluster A1 of the dendrogram. Accessions are color coded by origin: Giresun: black, Ordu: blue, Kastamonu: dark green, Rize: brown, Samsun: green, Trabzon: fuchsia, Unknown: orange.

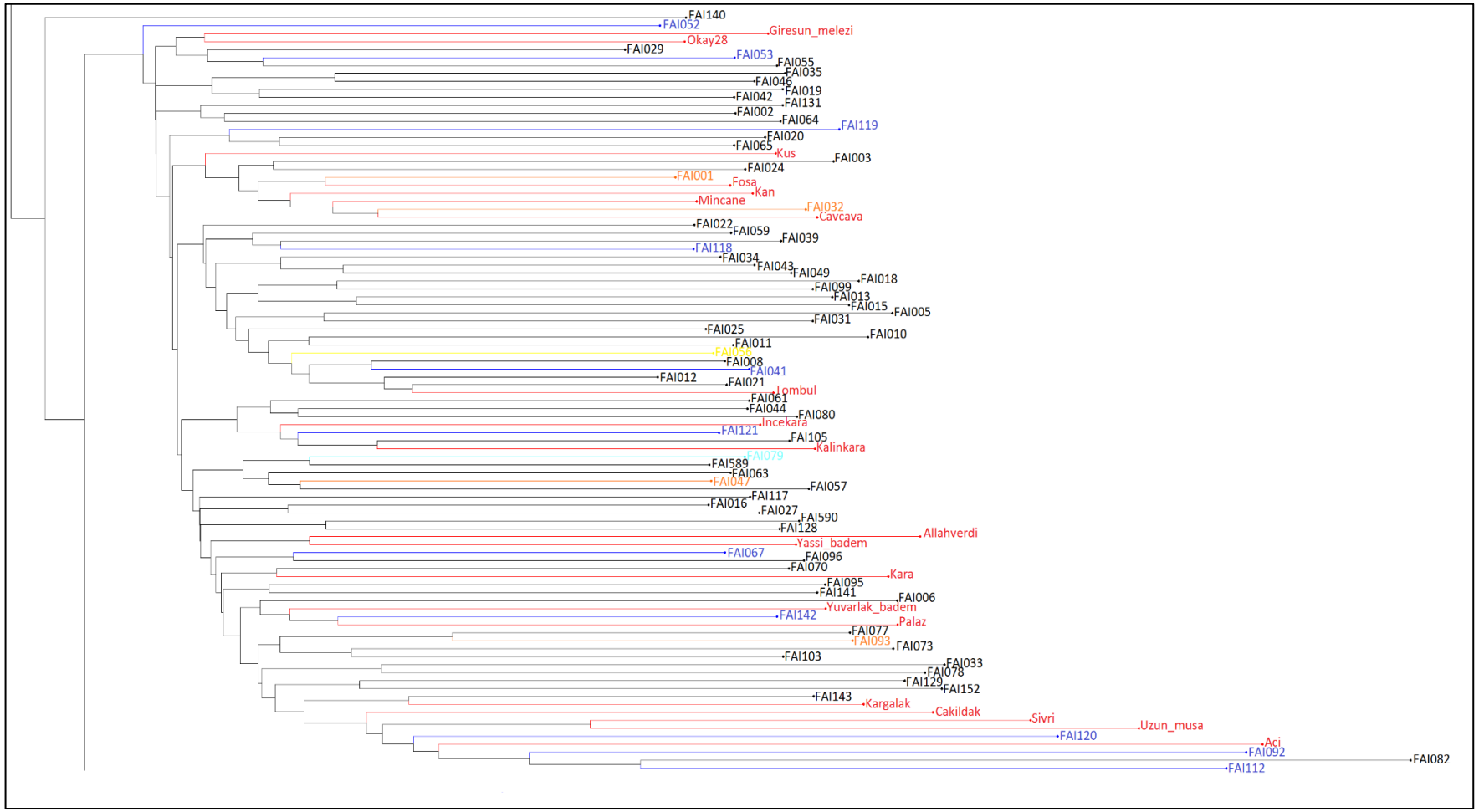

Figure 3.9. Cluster A2 of the dendrogram. Accessions are color coded by origin: cultivar: red, Giresun: black, Ordu: blue, Bolu: light blue, Sinop: yellow, Unknown: orange

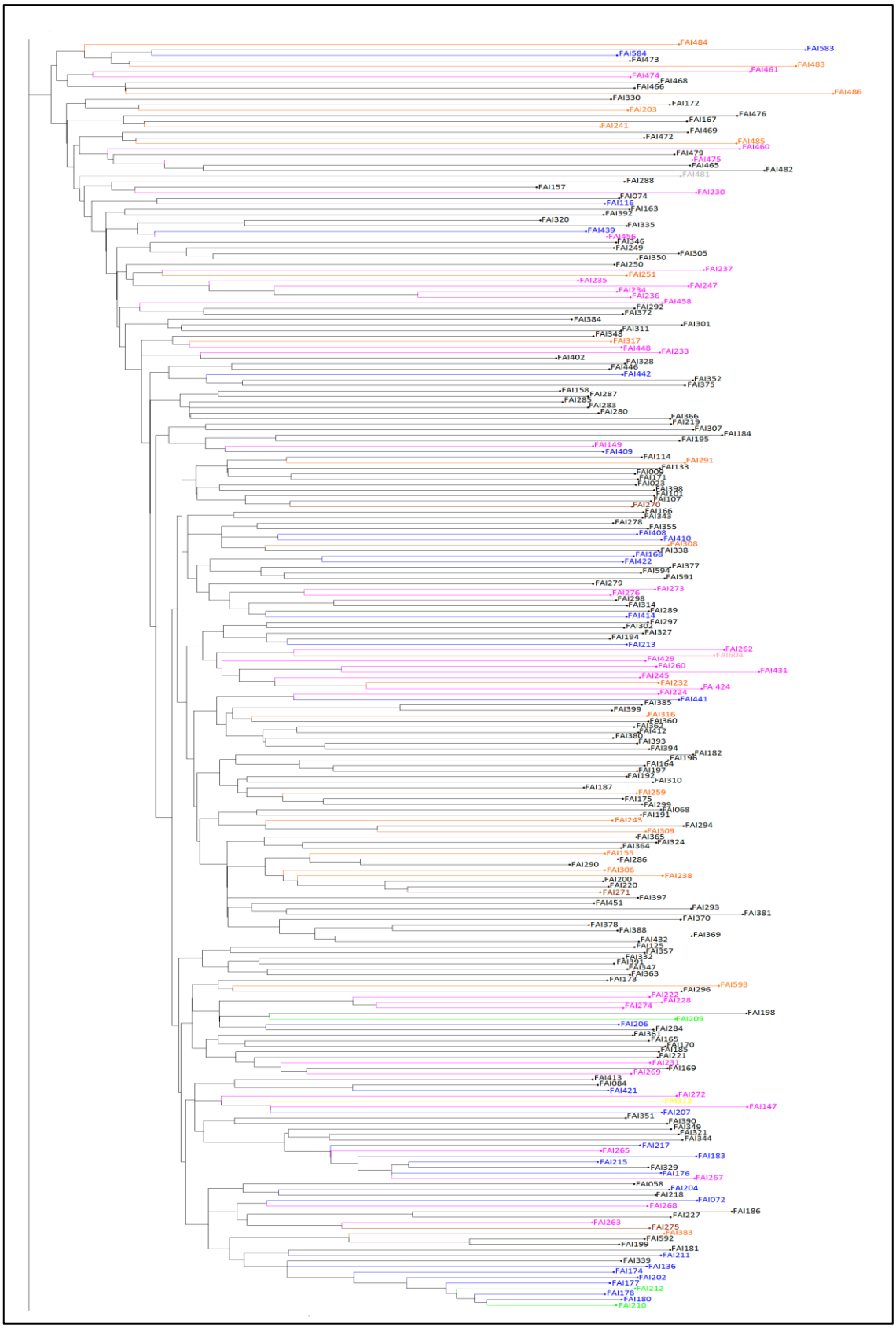

Figure 3.10. Cluster B of the dendrogram. Accessions are color coded by origin: Giresun: black, Ordu: blue, Artvin: gray, Erzurum: light pink, Rize: brown, Samsun: green, Sinop: yellow, Trabzon: fuchsia, Unknown: orange

Geographical clustering was limited; however, accessions from Trabzon were found only in clusters A1 and B while those from Giresun and Ordu were distributed throughout clusters A and B. Minor clustering of accessions from the same or neighboring locations was also observed. For example, seven accessions from Ordu and two from Samsun formed a small but distinct group in Cluster B.

Principal coordinate analysis of the SSR dataset did not show clear separation of the wild accessions and landraces from the cultivars but all cultivars were clustered in the lower left quadrant of the two dimensional PCoA plot (Figure 3.11). The clusters in the PCoA analysis correspond to the clusters in the dendrogram analysis.

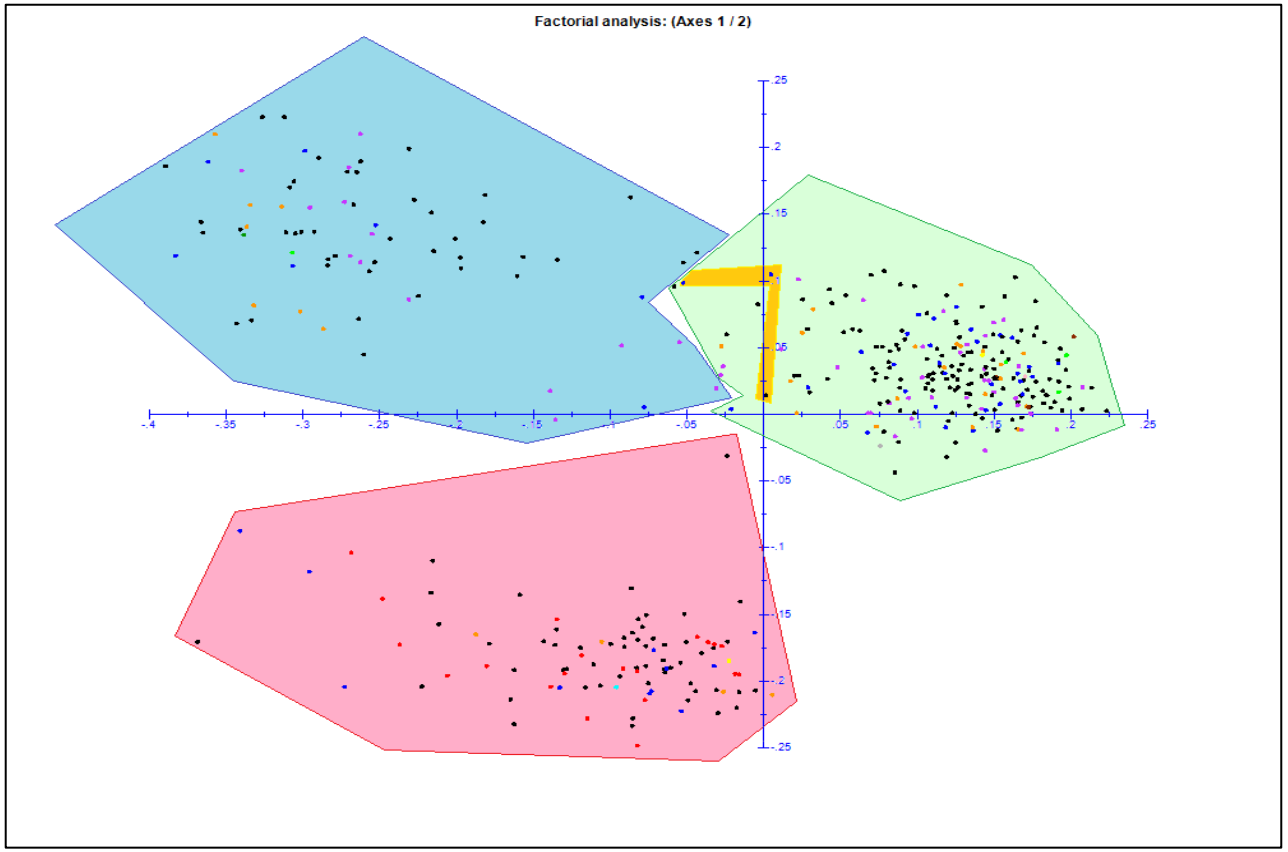

Figure 3.11. Principal coordinate analysis of hazelnut accessions according to the first two Eigen vectors which explained 12.8 and 6.0 % of the variance, respectively. Cultivars and A1 accessions were clustered in red area, A2 accessions were clustered in blue area, B accessions clustered in green area and C accessions were clustered in yellow area. Accessions are color coded by origin: cultivar: red, Giresun: black, Ordu: blue, Bolu: light blue, Artvin: gray, Erzurum: light pink, Kastamonu: dark green, Rize: brown, Samsun: green, Sinop: yellow, Trabzon: fuchsia, Unknown: orange

3.3.3. Population Structure

Population structure analysis indicated that the SSR data were best described by a model containing two subpopulations (K=2). Thus, a membership threshold of P $\geq$ 0.7 was used (Figure 3.12). In this way, 139 (35%) accessions were assigned to subpopulation 1, 185 (46%) accessions were assigned to subpopulation 2, while 78 (19%) were admixed (Figure 3.13). All but five Turkish hazelnut cultivars belonged to subpopulation 1. The exceptions were: 'Fosa,' 'Giresun Melezi,' 'Incekara,' 'Kan' and 'Okay28,' all of which had admixed ancestry (Table 3.12). Subpopulation 1 included accessions from Giresun (100), Ordu (15), Trabzon (12), Samsun (1), Kastamonu (1), Duzce (1) and unknown places (9). Similarly, subpopulation 2 had accessions from Giresun (113), Ordu (26), Trabzon (24), Rize (3), Samsun (3), Sinop (1), Erzurum (1) and unknown places (14). The 78 individuals which were admixed included accessions from Giresun (47), Trabzon (13), Ordu (8), Sinop (1), Artvin (1), and unknown places (8). When the population structure results were compared with the dendrogram and PCoA plot, cluster A corresponded to subpopulation 1 plus 30 admixed accessions and cluster B corresponded to subpopulation 2 plus 45 admixed accessions. Cluster C had only admixed accessions.

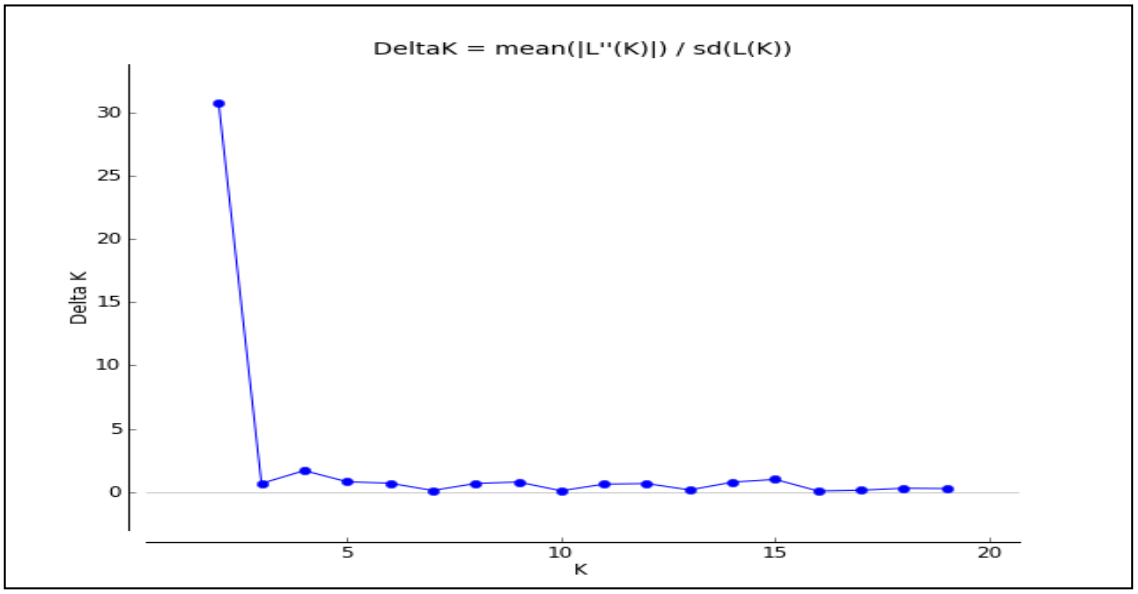

Figure 3.12. Delta K values of the Structure program outcome for each subpopulation assumption. The value of K with the highest Delta K value was chosen as the best number of subpopulations for the hazelnut accessions (K=2).

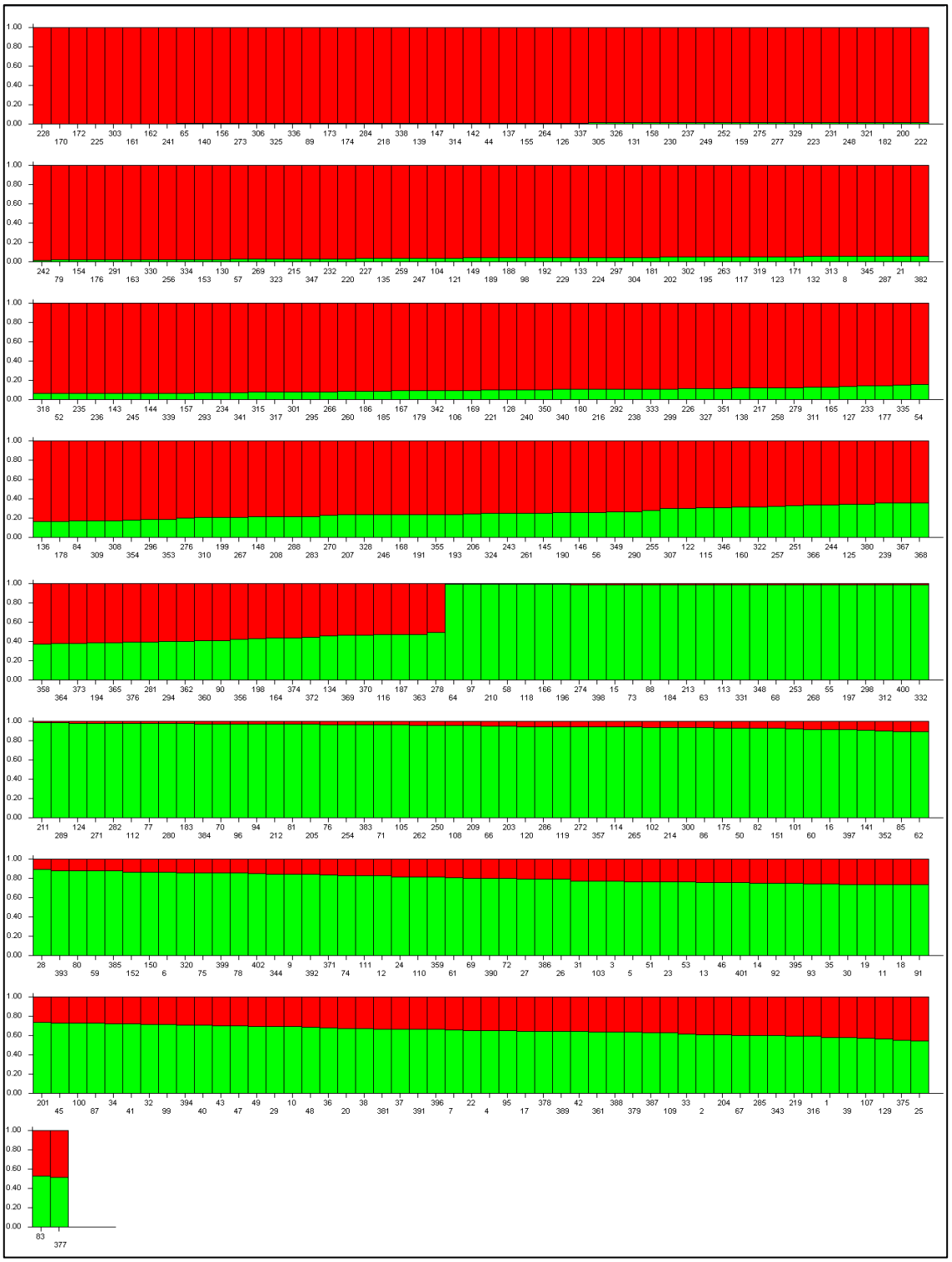

Figure 3.13. Population structure plots of the 402 hazelnut accessions. Each accession is represented by a vertical bar. Green sections within each vertical bar indicate membership coefficient (y-axis) of the accession to subpopulation 1 while red sections indicate membership to subpopulation 2. Numbers were given according to list of Table 2.

Table 3.12. Hazelnut accessions and origins. Inferred ancestry subpopulation assignment and dendrogram clustering are based on SSR results.

Accession Name	Type of Material	Province	District	Inferred Ancestry	Dendrogram Cluster
Aci	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Allahverdi	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Cakildak	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Cavcava	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Fosa	Cultivar	Giresun	Hazelnut Research Institute	Admixed	A2
Giresun Melezi	Cultivar	Giresun	Hazelnut Research Institute	Admixed	A2
Incekara	Cultivar	Giresun	Hazelnut Research Institute	Admixed	A2
Kalinkara	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Kan	Cultivar	Giresun	Hazelnut Research Institute	Admixed	A2
Kara	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Kargalak	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Kus	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Mincane	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Okay28	Cultivar	Giresun	Hazelnut Research Institute	Admixed	A2
Palaz	Cultivar	Giresun	Hazelnut Research Institute	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

Sivri	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Tombul	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Uzun Musa	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Yassibadem	Cultivar	Giresun	Hazelnut Research Institute	1	A2
Yuvarlakbadem	Cultivar	Giresun	Hazelnut Research Institute	1	A2
FAI001	Wild	?		Admixed	A2
FAI002	Wild	Giresun	Bulancak; Bostanli	Admixed	A2
FAI003	Landraces	Giresun	Bulancak;Icilli	1	A2
FAI004	Landraces	Giresun	Tekke	Admixed	A1
FAI005	Landraces	Giresun	Darikoy	1	A2
FAI006	Wild	Giresun	Dereli;Kuknarli	1	A2
FAI008	Landraces	Giresun	Konacik	Admixed	A2
FAI009	Landraces	Giresun	Gurkoy	2	B
FAI010	Landraces	Giresun	Dereli; Calca	1	A2
FAI011	Wild	Giresun	Kesap; Karabulduk	Admixed	A2
FAI012	Landraces	Giresun	Incegeris	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI013	Wild	Giresun	Dereli; Iklikci	1	A2
FAI015	Wild	Giresun	Mesudiye	1	A2
FAI016	Landraces	Giresun	Gurkoy	1	A2
FAI017	Wild	Giresun	Yagmurca	1	A1
FAI018	Landraces	Giresun	Ulper	1	A2
FAI019	Wild	Giresun	Ulper	Admixed	A2
FAI020	Landraces	Giresun	Piraziz; Gokceali	1	A2
FAI021	Landraces	Giresun	Akcali	1	A2
FAI022	Wild	Giresun	Mesudiye	Admixed	A2
FAI023	Wild	Giresun	Konacik	2	B
FAI024	Landraces	Giresun	Piraziz; Bulbullu	Admixed	A2
FAI025	Wild	Giresun	Piraziz; Kilicli	1	A2
FAI027	Landraces	Giresun	Akcali	1	A2
FAI029	Landraces	Giresun		Admixed	A2
FAI031	Wild	Giresun	Akcali	1	A2
FAI032	Landraces	?		1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI033	Wild	Giresun	Darikoy	1	A2
FAI034	Landraces	Giresun	Boztekke	Admixed	A2
FAI035	Wild	Giresun	Darikoy	1	A2
FAI039	Landraces	Giresun	Piraziz; Kilicli	1	A2
FAI041	Wild	Ordu	Eyuplu	1	A2
FAI042	Landraces	Giresun	Espiye; Orman Kirani	Admixed	A2
FAI043	Landraces	Giresun	Erikliman	1	A2
FAI044	Wild	Giresun	Alinca	1	A2
FAI046	Wild	Giresun	Darikoy	Admixed	A2
FAI047	Wild	?		Admixed	A2
FAI049	Wild	Giresun	Hisargeris	Admixed	A2
FAI052	Landraces	Ordu	Eyuplu	Admixed	A2
FAI053	Wild	Ordu	Aydinlar	1	A2
FAI055	Landraces	Giresun	Kesap; Karabulduk	1	A2
FAI056	Wild	Sinop	Ayancik; Agacli	Admixed	A2
FAI057	Wild	Giresun	Piraziz; Bulbullu	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI058	Landraces	Giresun	Piraziz; Bulbullu	2	B
FAI059	Wild	Giresun	Piraziz; Bulbullu	1	A2
FAI061	Wild	Giresun	Bulancak; Şeyhmusa	1	A2
FAI063	Wild	Giresun	Piraziz; Bulbullu	1	A2
FAI064	Wild	Giresun	Piraziz; Bulbullu	Admixed	A2
FAI065	Wild	Giresun	Piraziz; Bulbullu	Admixed	A2
FAI066	Wild	Giresun	Akkoy; Madenyani	1	A1
FAI067	Landraces	Ordu	Eyuplu	1	A2
FAI068	Landraces	Giresun	Bulancak; Şeyhmusa	2	B
FAI070	Wild	Giresun		1	A2
FAI072	Wild	Ordu	Persembe	2	B
FAI073	Landraces	Giresun	Bulancak	1	A2
FAI074	Wild	Giresun	Bulancak; Pazarsuyu	2	B
FAI076	Wild	Giresun	Ortakoy	1	A1
FAI077	Wild	Giresun	Yazlik	1	A2
FAI078	Wild	Giresun	Candir	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI079	Landraces	Bolu	Akcakoca	1	A2
FAI080	Wild	Giresun	Yazlik	1	A2
FAI081	Wild	?		1	A1
FAI082	Landraces	Giresun	Pinarcukuru	1	A2
FAI084	Wild	Giresun	Hazelnut Research Institute	2	B
FAI086	Landraces	Giresun	Bulancak	1	A1
FAI088	Landraces	Giresun	Bulancak	Admixed	A1
FAI089	Landraces	Giresun		1	A1
FAI091	Landraces	Giresun		1	A1
FAI092	Landraces	Ordu	Bayadi	1	A2
FAI093	Wild	?		1	A2
FAI094	Landraces	Kastamonu	Inebolu; Culurye	1	A1
FAI095	Wild	Giresun	Konacik	1	A2
FAI096	Landraces	Giresun	Konacik	1	A2
FAI097	Landraces	Giresun	Sarvan	1	A1
FAI098	Landraces	Giresun	Darikoy	1	A1

(Cont. on the next page)

Table 3.12. (cont.)

FAI099	Landraces	Giresun	Barca	1	A2
FAI101	Landraces	Giresun	Burhaniye	2	B
FAI103	Landraces	Giresun	Barca	1	A2
FAI104	Landraces	Giresun	Guveckoy	1	A1
FAI105	Landraces	Giresun	Hazelnut Research Institute	1	A2
FAI106	Landraces	Giresun	Sarvan	Admixed	C
FAI107	Landraces	Giresun	Barca	2	B
FAI108	Landraces	Giresun	Piraziz; Kilicli	1	A1
FAI109	Wild	Giresun	Bulancak; Bozat	1	A1
FAI112	Landraces	Ordu	Uzunisa	1	A2
FAI114	Wild	Giresun	Bulancak; Yalikoy	2	B
FAI116	Landraces	Ordu	Aydinlar	Admixed	B
FAI117	Wild	Giresun	Hazelnut Research Institute	1	A2
FAI118	Wild	Ordu	Persembe; Yumrutas	1	A2
FAI119	Landraces	Ordu	Persembe; Yumrutas	1	A2
FAI120	Landraces	Ordu	Persembe; Dogankoy	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI121	Wild	Ordu	Ulubey; Kirazli	Admixed	A2
FAI122	Landraces	Ordu	Ulubey; Findikli	1	A1
FAI123	Landraces	Ordu	Ulubey; Akpinar	1	A1
FAI125	Landraces	Giresun	Kesap; Yolagzi	2	B
FAI126	Landraces	Ordu	Ulubey; Karakoca	1	A1
FAI128	Wild	Giresun	Guneykoy	1	A2
FAI129	Landraces	Giresun	Guneykoy	1	A2
FAI130	Wild	Giresun	Guneykoy	1	A1
FAI131	Landraces	Giresun	Guneykoy	1	A2
FAI133	Wild	Giresun	Kesap; Gurpinar	2	B
FAI135	Landraces	Giresun	Kesap; Saraycik	1	A1
FAI136	Landraces	Ordu	Unye; Kalekoy	2	B
FAI137	Landraces	Ordu	Unye; Kurna Mengen	Admixed	C
FAI138	Landraces	Ordu	Caybasi; Haciali	1	A1
FAI140	Landraces	Giresun	Kesap; Saraycik	Admixed	A2
FAI141	Landraces	Giresun	Kesap; Saraycik	1	A2

(Cont. on the next page)

Table 3.12. (cont.)

FAI142	Landraces	Ordu	Unye; Kalekoy	1	A2
FAI143	Landraces	Giresun	Hazelnut Research Institute	1	A2
FAI144	Landraces	Ordu	Unye; Cinarcik	1	A1
FAI145	Landraces	Trabzon	Vakfikebir; Cumhuriyet mahallesi	1	A1
FAI147	Landraces	Trabzon	Besikduzu; Korkuthan	Admixed	B
FAI148	Landraces	Ordu	Unye	Admixed	C
FAI149	Landraces	Trabzon	Besikduzu; Turkelli	2	B
FAI150	Wild	Giresun	Kesap; Karabedir	1	A1
FAI152	Landraces	Giresun	Kesap; Guneykoy	1	A2
FAI154	Landraces	Giresun	Ergence	1	A1
FAI155	Landraces	?		2	B
FAI157	Landraces	Giresun	Bulancak; Pazarsuyu	Admixed	B
FAI158	Landraces	Giresun	Seyitkoy	2	B
FAI161	Landraces	Giresun	Yukarialinli	1	A1
FAI163	Landraces	Giresun	Kemaliye	Admixed	B
FAI164	Landraces	Giresun	Bulancak; Erdogan	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI165	Wild	Giresun	Kesap; Surmenli	2	B
FAI166	Wild	Giresun	Kesap; Surmenli	2	B
FAI167	Landraces	Giresun	Sivaci	Admixed	B
FAI168	Landraces	Ordu	Unye; Baskoy	2	B
FAI169	Landraces	Giresun	Camili	2	B
FAI170	Landraces	Giresun	Hazelnut Research Institute	2	B
FAI171	Landraces	Giresun	Kemaliye	2	B
FAI172	Landraces	Giresun	Kemaliye	Admixed	B
FAI173	Landraces	Giresun	Seyitkoy	2	B
FAI174	Landraces	Ordu	Caybasi; Egribucak	2	B
FAI175	Landraces	Giresun	Kayadibi	2	B
FAI176	Landraces	Ordu	Caybasi; Saricaerik	2	B
FAI177	Landraces	Ordu	Caybasi; Saricaerik	2	B
FAI178	Landraces	Ordu	Caybasi	2	B
FAI179	Wild	Giresun	Kesap; Karadere	1	A1
FAI180	Landraces	Ordu	Caybasi	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI181	Wild	Giresun	Kesap; Cakirli	2	B
FAI182	Landraces	Giresun	Kayadibi	2	B
FAI183	Wild	Ordu	Unye;Kalekoyu	2	B
FAI184	Landraces	Giresun	Bulancak; Ahmetli	2	B
FAI185	Landraces	Giresun	Bulancak; Kayhan	2	B
FAI186	Wild	Giresun	Bulancak; Ahmetli	2	B
FAI187	Landraces	Giresun	Bulancak;Saracli	2	B
FAI188	Landraces	Giresun	Bulancak; Kayhan	1	A1
FAI189	Landraces	Ordu	Kizilhisar	1	A1
FAI190	Wild	Giresun	Bulancak; Tepecik	1	A1
FAI191	Landraces	Giresun	Bulancak; Erdogan	2	B
FAI192	Landraces	Giresun	Bulancak; Seyhmusa	2	B
FAI194	Wild	Giresun	Bulancak; Icilli	2	B
FAI195	Landraces	Giresun	Bulancak; Hacet	2	B
FAI196	Landraces	Giresun	Bulancak; Tepecik	2	B
FAI197	Landraces	Giresun	Bulancak; Erdogan	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI198	Landraces	Giresun	Bulancak; Kuzkoy	Admixed	B
FAI199	Wild	Giresun	Bulancak; Ahmetli	2	B
FAI200	Landraces	Giresun	Bulancak; Kuzkoy	2	B
FAI202	Landraces	Ordu	Unye; Kalekoyu	2	B
FAI203	Wild	?		Admixed	B
FAI204	Landraces	Ordu	Fatsa; Oluklu	2	B
FAI205	Landraces	Samsun	Terme; Bazlamac	1	A1
FAI206	Landraces	Ordu	Fatsa; Korucuk	2	B
FAI207	Wild	Ordu	Fatsa; Evkaf	2	B
FAI209	Wild	Samsun	Terme; Bazlamac	2	B
FAI210	Landraces	Samsun	Carsamba; Kocalar	2	B
FAI211	Wild	Ordu	Fatsa; Oluklu	2	B
FAI212	Wild	Samsun	Terme; Kocamanbasi	2	B
FAI213	Landraces	Ordu	Akcatepe	2	B
FAI215	Wild	Ordu	Boztepe	2	B
FAI216	Landraces	Giresun	Bulancak; Pazarsuyu	1	A1

(Cont. on the next page)

Table 3.12. (cont.)

FAI217	Landraces	Ordu	Boztepe	2	B
FAI218	Landraces	Giresun	Bulancak; Pazarsuyu	2	B
FAI219	Landraces	Giresun	Bulancak; Inece	2	B
FAI220	Landraces	Giresun		2	B
FAI221	Wild	Giresun	Bulancak; Eriklik	2	B
FAI222	Landraces	Trabzon	Bahcekaya	2	B
FAI224	Wild	Trabzon	Macka; Yukarikoy	2	B
FAI225	Landraces	Trabzon	Macka; Yukarikoy	1	A1
FAI226	Landraces	Trabzon	Carsibasi; Kavakli	1	A1
FAI227	Landraces	Giresun	Hazelnut Research Institute	2	B
FAI228	Landraces	Trabzon	Macka; Kaynarca	2	B
FAI230	Wild	Trabzon	Ortahisar; Caglayan	Admixed	B
FAI231	Wild	Trabzon	Kavala	2	B
FAI232	Landraces	?		2	B
FAI233	Wild	Trabzon	Cilekli	2	B
FAI234	Landraces	Trabzon	Kavala	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI235	Landraces	Trabzon	Yomra; Komurcu	2	B
FAI236	Landraces	Trabzon	Yomra; Komurcu	2	B
FAI237	Landraces	Trabzon	Ortahisar; Cukurcayir	Admixed	B
FAI238	Wild	?		2	B
FAI239	Landraces	?		1	A1
FAI240	Landraces	?		1	A1
FAI241	Landraces	?		Admixed	B
FAI243	Wild	?		2	B
FAI244	Landraces	Trabzon	Macka; Catak	1	A1
FAI245	Landraces	Trabzon	Kisarna	2	B
FAI246	Landraces	Trabzon	Arsin; Ozlu	1	A1
FAI247	Landraces	Trabzon	Surmene; Konak	Admixed	B
FAI248	Landraces	Trabzon	Arsin; Ozlu	1	A1
FAI249	Wild	Giresun	Tirebolu; Karademir	2	B
FAI250	Wild	Giresun	Tirebolu; Seku	2	B
FAI251	Wild	?		2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI252	Landraces	Giresun	Bulancak; Hacet	1	A1
FAI253	Landraces	Giresun	Hazelnut Research Institute	1	A1
FAI255	Landraces	Trabzon	Arakli; Tasonu	1	A1
FAI256	Landraces	Trabzon	Yenikoy	1	A1
FAI257	Landraces	?		1	A1
FAI258	Wild	Trabzon	Arakli; Ayvadere	1	A1
FAI259	Landraces	?		2	B
FAI260	Wild	Trabzon	Arakli; Tasonu	2	B
FAI262	Wild	Trabzon	Of; Dumlusu	2	B
FAI263	Wild	Trabzon	Bolumlu	2	B
FAI264	Landraces	Trabzon	Bolumlu	Admixed	A1
FAI265	Landraces	Trabzon	Hopa; Sugoren	2	B
FAI267	Landraces	Trabzon	Of; Dumlusu	2	B
FAI268	Landraces	Trabzon	Hopa; Saricayir	2	B
FAI269	Wild	Trabzon	Bolumlu	2	B
FAI270	Landraces	Rize	Findikli; Kiyicik	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI271	Landraces	Rize	Findikli; Caglayan	2	B
FAI272	Wild	Trabzon	Hopa; Camli	2	B
FAI273	Landraces	Trabzon	Hopa; Camli	2	B
FAI274	Landraces	Trabzon	Hopa; Camli	2	B
FAI275	Landraces	Rize	Findikli; Kiyicik	2	B
FAI276	Landraces	Trabzon	Hopa; Sundura	2	B
FAI278	Landraces	Giresun	Espiye; Cibril	2	B
FAI279	Landraces	Giresun	Bulancak; Semsettin	2	B
FAI280	Landraces	Giresun	Yaglidere; Palakli	2	B
FAI283	Wild	Giresun	Espiye; Demircili	2	B
FAI284	Wild	Giresun	Tirebolu; Cegel	2	B
FAI285	Wild	Giresun	Tirebolu; Aslancik	2	B
FAI286	Landraces	Giresun	Guce	2	B
FAI287	Landraces	Giresun	Tirebolu; Isikli	2	B
FAI288	Wild	Giresun	Eynesil; Kemaliye	Admixed	B
FAI289	Wild	Giresun	Tirebolu; Belen	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI290	Landraces	Giresun	Tirebolu; Ortacami	2	B
FAI291	Landraces	?		2	B
FAI292	Landraces	Giresun	Bulancak; Cindi	2	B
FAI293	Landraces	Giresun	Kesap; Bayramsah	Admixed	B
FAI294	Landraces	Giresun	Tirebolu; Harkkoy	2	B
FAI296	Wild	Giresun	Bulancak; Kusluhan	2	B
FAI297	Wild	Giresun	Bulancak; Torcan	2	B
FAI298	Wild	Giresun	Bulancak; Inece	2	B
FAI299	Wild	Giresun	Bulancak; Kusluhan	2	B
FAI300	Landraces	Giresun	Bulancak; Eriklik	1	A1
FAI301	Landraces	Giresun	Tirebolu	Admixed	B
FAI302	Landraces	Giresun	Bulancak; Inece	2	B
FAI303	Landraces	?		1	A1
FAI304	Landraces	Giresun	Piraziz; Balcikli	1	A1
FAI305	Landraces	Giresun	Bulancak; Şeyhmusa	2	B
FAI306	Landraces	?		2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI307	Landraces	Giresun	Bulancak; Semsettin	Admixed	B
FAI308	Wild	?		2	B
FAI309	Wild	?		2	B
FAI310	Wild	Giresun	Tirebolu;Avcili	2	B
FAI311	Landraces	Giresun	Tirebolu; Balcikbeleni	2	B
FAI312	Landraces	Giresun	Tirebolu; Aslancik	1	A1
FAI313	Landraces	Sinop	Ayancik; Hatip	2	B
FAI314	Landraces	Giresun	Bulancak; Inece	2	B
FAI315	Wild	Giresun	Bulancak; Kucuklu	1	A1
FAI316	Wild	?		2	B
FAI317	Landraces	?		2	B
FAI318	Landraces	?		1	A1
FAI320	Wild	Giresun	Ulper	2	B
FAI321	Landraces	Giresun	Bulancak; Seyhmusa	2	B
FAI322	Landraces	Giresun	Piraziz; Bulbullu	1	A1
FAI323	Landraces	Giresun	Piraziz; Hasanseyh	1	A1

(Cont. on the next page)

Table 3.12. (cont.)

FAI324	Landraces	Giresun	Piraziz; Bulbullu	2	B
FAI325	Landraces	Giresun	Piraziz; Hasanseyh	1	A1
FAI327	Landraces	Giresun	Piraziz; Bulbullu	2	B
FAI328	Wild	Giresun	Bulancak; Salman	2	B
FAI329	Wild	Giresun	Bulancak;Cindi	2	B
FAI330	Wild	Giresun	Bulancak; Demircili	Admixed	B
FAI332	Landraces	Giresun	Yazlik	2	B
FAI333	Wild	Giresun	Caykara	1	A1
FAI335	Wild	Giresun	Ulper	Admixed	B
FAI336	Landraces	Giresun	Kemaliye	1	A1
FAI338	Landraces	Giresun	Yazlik	2	B
FAI339	Landraces	Giresun	Sarvan	2	B
FAI340	Landraces	Giresun	Konacik	Admixed	A1
FAI341	Landraces	Giresun	Konacik	1	A1
FAI343	Wild	Giresun	Bulancak; Bozat	2	B
FAI344	Wild	Giresun	Piraziz; Balcikli	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI345	Wild	Giresun	Kemaliye	1	A1
FAI346	Wild	Giresun	Piraziz; Maden	2	B
FAI347	Landraces	Giresun	Guneykoy	2	B
FAI348	Wild	Giresun	Guneykoy	2	B
FAI349	Landraces	Giresun	Piraziz; Maden	2	B
FAI350	Landraces	Giresun	Bulancak	Admixed	B
FAI351	Wild	Giresun	Bulancak; Seyhmusa	2	B
FAI352	Wild	Giresun	Bulancak; Ahmetli	2	B
FAI355	Landraces	Giresun	Hamidiyekoy	2	B
FAI356	Landraces	?		1	A1
FAI357	Wild	Giresun	Boztekke	2	B
FAI359	Wild	Giresun	Darikoy	1	A1
FAI360	Wild	Giresun	Hamidiyekoy	2	B
FAI361	Wild	Giresun	Calis	2	B
FAI362	Landraces	Giresun	Piraziz; Şeyhli	2	B
FAI363	Landraces	Giresun	Boztekke	2	B

(Cont. on the next page)

Table 3.12. (cont.)

FAI364	Wild	Giresun	Samanlik Kirani	2	B
FAI365	Wild	Giresun	Kayadibi	2	B
FAI366	Landraces	Giresun	Darikoy	Admixed	B
FAI369	Landraces	Giresun	Bulancak; Ucarli	2	B
FAI370	Wild	Giresun	Alinca	2	B
FAI372	Landraces	Giresun	Dogankent; Catalagac	2	B
FAI375	Landraces	Giresun	Bulancak; Hisarkaya	2	B
FAI376	Wild	Giresun	Duroglu	1	A1
FAI377	Wild	Giresun	Duroglu	2	B
FAI378	Landraces	Giresun	Bulancak; Tepecik	2	B
FAI380	Wild	Giresun	Bulancak; Icilli	2	B
FAI381	Landraces	Giresun	Bulancak; Burunucu	Admixed	B
FAI383	Wild	?		2	B
FAI384	Wild	Giresun	Bulancak; Kizilot	2	B
FAI385	Wild	Giresun	Bulancak; Kizilot	2	B
FAI387	Wild	Giresun	Piraziz; Kilicli	1	A1

(Cont. on the next page)

Table 3.12. (cont.)

FAI388	Landraces	Giresun	Bulancak; Semsettin	2	B
FAI390	Landraces	Giresun	Bulancak; Semsettin	Admixed	B
FAI391	Landraces	Giresun	Bulancak; Semsettin	2	B
FAI392	Wild	Giresun	Bulancak; Kusluhan	2	B
FAI393	Landraces	Giresun	Bulancak; Kusluhan	2	B
FAI394	Landraces	Giresun	Canakci; Saraykoy	2	B
FAI397	Wild	Giresun	Bulancak; Semsettin	2	B
FAI398	Landraces	Giresun	Bulancak; Kusluhan	2	B
FAI399	Landraces	Giresun	Bulancak; Inece	2	B
FAI402	Landraces	Giresun	Duroglu	2	B
FAI403	Landraces	Giresun	Sarvan	1	A1
FAI406	Wild	Giresun	Kesap; Yazlik	1	A1
FAI408	Wild	Ordu	Kocamanbasi	2	B
FAI409	Wild	Ordu	Uzunisa	2	B
FAI410	Landraces	Ordu	Uzunisa	2	B
FAI412	Landraces	Giresun	Hazelnut Research Institute	2	B

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Table 3.12. (cont.)

FAI413	Landraces	Giresun	Hazelnut Research Institute	2	B
FAI414	Wild	Ordu	Terme	2	B
FAI421	Landraces	Ordu	Aybasti	2	B
FAI422	Wild	Ordu	Unye; Baskoy	2	B
FAI424	Landraces	Trabzon	Arakli; Özgen	2	B
FAI426	Landraces	Trabzon	Arakli; Yigitozu	Admixed	A1
FAI428	Landraces	Trabzon	Of; Bolumlu	1	A1
FAI429	Landraces	Trabzon	Arakli; Yigitozu	2	B
FAI431	Wild	Trabzon	Arakli; Tasonu	Admixed	B
FAI432	Landraces	Giresun	Yaglidere; Umitbuku	2	B
FAI433	Landraces	Giresun	Tirebolu; Cegel	1	A1
FAI439	Landraces	Ordu	Unye; Baskoy	2	B
FAI441	Wild	Ordu	Persembe; Ortatepe	2	B
FAI442	Wild	Ordu	Unye; Baskoy	2	B
FAI443	Landraces	Giresun	Espiye; Adabuk	1	A1
FAI446	Landraces	Giresun	Kesap; Guneykoy	2	B

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Table 3.12. (cont.)

FAI448	Landraces	Trabzon	Arakli; Yildizli	2	B
FAI451	Landraces	Giresun	Yaglidere; Omerli	2	B
FAI456	Wild	Trabzon	Carsibasi; Kavakli	Admixed	B
FAI457	Wild	Trabzon	Carsibasi; Kucukkoy	1	A1
FAI458	Landraces	Trabzon	Vakfikebir; Kucukkoy	Admixed	B
FAI459	Wild	Trabzon	Besikduzu; Kutluca	1	A1
FAI460	Wild	Trabzon	Carsibasi; Kucukkoy	Admixed	B
FAI461	Wild	Trabzon	Besikduzu; Korkuthan	Admixed	B
FAI465	Landraces	Giresun	Bulancak; Pazarsuyu	Admixed	B
FAI466	Landraces	Giresun	Bulancak; Pazarsuyu	Admixed	B
FAI468	Landraces	Giresun	Bulancak; Tepecik	Admixed	B
FAI469	Landraces	Giresun	Bulancak; Tepecik	Admixed	B
FAI472	Wild	Giresun	Dogankent; Sadakli	Admixed	B
FAI473	Wild	Giresun	Tirebolu; Yaglikuyumcu	Admixed	B
FAI474	Wild	Trabzon	Hopa; Camli	Admixed	B
FAI475	Wild	Trabzon	Hopa; Camli	Admixed	B

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Table 3.12. (cont.)

FAI476	Landraces	Giresun	Tirebolu; Ketencukur	Admixed	B
FAI478	Landraces	Giresun	Tirebolu; Balcikbeleni	1	A1
FAI479	Landraces	Giresun	Guce	Admixed	B
FAI481	Landraces	Artvin	Hopa;Kuledibi	Admixed	B
FAI482	Landraces	Giresun	Alinca	Admixed	B
FAI483	Landraces	?		Admixed	B
FAI484	Landraces	?		Admixed	B
FAI485	Wild	?		Admixed	B
FAI486	Landraces	?		Admixed	B
FAI583	Landraces	Ordu	Ulubey	Admixed	B
FAI584	Landraces	Ordu	Ulubey	Admixed	B
FAI585	Landraces	Ordu	Fatsa/Bolaman	Admixed	A1
FAI589	Landraces	Giresun	Hazelnut Research Institute	1	A2
FAI590	Landraces	Giresun	Hazelnut Research Institute	1	A2
FAI591	Landraces	Giresun	Hazelnut Research Institute	2	B
FAI592	Wild	Giresun	Hazelnut Research Institute	2	B

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Table 3.12. (cont.)

FAI593	Wild	?	Hazelnut Research Institute	2	B
FAI594	Wild	Giresun	Hazelnut Research Institute	2	B
FAI604	Landraces	Erzurum	Hinis;Karagoz	2	B

3.3.4 Core Set Selection

The SSR data were analyzed to select a core set of Turkish hazelnut accessions representing the diversity of the entire collection. Five cultivars and 29 landraces and wild hazelnuts were chosen for the core set based on their high levels of molecular genetic diversity. The remaining 15 cultivars were also added to the core set because of their economic importance and/or distinct features which merited giving them a name [66, 98]. An additional 29 hazelnuts with interesting phenotypic traits such as unusual shape, color and size of kernel and fruit were also included [64] (H.I. Balik personal communication). Thus, 78 individuals (19% of the collection) were chosen to represent the molecular genetic and morphological diversity of the entire collection (Table 3.13). Average genetic dissimilarity of the core set based on SSR markers was 0.53. The core set contained accessions from Giresun (45; 25 accessions and 20 cultivars), Trabzon (12), Ordu (9), Sinop (1), Artvin (1), Duzce (1) and unknown places (9) (Table 3.13). In terms of population structure, the core collection contained 38 accessions from subpopulation 1, 19 from subpopulation 2 and 21 admixed accessions.

Table 3.13. Accessions in the core set of Turkish hazelnuts selected based on SSR data and morphology. Subsets were selected based on molecular data (A), morphological features (B) and identity as a named cultivar (C). Subpopulation assignment for each accession is given in parenthesis.

Subset A	Subset B	Subset C
Aci (1)	FAI056 (admixed)	Allahverdi (1)
Giresun Melezi (admixed)	FAI126 (1)	Cavcava (1)
Kan (admixed)	FAI137 (admixed)	Cakıldak (1)
Kargalak (1)	FAI174 (2)	Fosa (admixed)
Mincane (1)	FAI177 (2)	Incekara (admixed)
FAI005 (1)	FAI225 (1)	Kalınkara (1)
FAI018 (1)	FAI241 (admixed)	Karafındık (1)
FAI032 (1)	FAI248 (1)	Kus (1)
FAI065 (admixed)	FAI265 (2)	Okay28 (admixed)
FAI079 (1)	FAI306 (2)	Palaz (1)
FAI081 (1)	FAI315 (1)	Sivri (1)

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Table 3.13. (cont.)

FAI093 (1)	FAI318 (1)	Tombul (1)
FAI096 (1)	FAI324 (2)	Uzunmusa (1)
FAI112 (1)	FAI333 (1)	Yassıbadem (1)
FAI144 (1)	FAI351 (2)	Yuvarlakbadem (1)
FAI145 (1)	FAI388 (2)	
FAI150 (1)	FAI406 (1)	
FAI161 (1)	FAI422 (2)	
FAI172 (admixed)	FAI457 (1)	
FAI228 (2)	FAI458 (admixed)	
FAI279 (2)	FAI459 (1)	
FAI289 (2)	FAI461 (admixed)	
FAI302 (2)	FAI469 (admixed)	
FAI314 (2)	FAI472 (admixed)	
FAI316 (2)	FAI474 (admixed)	
FAI349 (2)	FAI478 (1)	
FAI408 (2)	FAI479 (admixed)	
FAI409 (2)	FAI481 (admixed)	
FAI429 (2)	FAI485 (admixed)	
FAI451 (2)		
FAI460 (admixed)		
FAI466 (admixed)		
FAI484 (admixed)		
FAI590 (1)		

3.4. Validation of Genomic SSR Markers in World Collection

Turkish cultivar ‘Tombul’ was sequenced using next generation sequencing technology and new SSR markes were developed and applied to our hazelnut world collection for validation.

3.4.1. Sequence Assembly, Simple Sequence Repeat Identification and Primer Design

Sequencing of the hazelnut cultivar 'Tombul' produced 15,319,058 sequence reads comprising more than 4,595 Mb. Removal of adapter sequences from the raw reads resulted in 4,535 Mb sequence with an average size of 296.1 nucleotides (nt). Only contigs larger than 1000 nucleotides were further analyzed for SSR identification. As a result, 56,665 contigs were assembled which encompassed 111.85 Mb, representing 29.2% of the ~385 Mb (1C) hazelnut genome (Table 3.14).

Table 3.14. Preprocessing and assembly statistics for the *C. avellana* L. genomic sequences.

Parameter	Raw Sequence	Cleaned Sequences	Contigs
Total number of sequences	15,319,058	15,314,810	56,665
Minimum sequence length (nt)	300	20	1000
Maximum sequence length (nt)	300	300	55,633
Average sequence length (nt)	300	296.1	1973.9
Total number of bases	4,595,717,400	4,535,422,965	111,855,554

Overall, 90,142 SSRs were identified in the contigs with 1 SSR every 1240 nt in the assembly. SSR length ranged from 6 to 49 nt with an average of 15.4 nt. Among all identified SSRs, the most abundant type was mononucleotide repeats (60.9%). Dinucleotides and trinucleotides were the second and third most common type representing 26.5% and 4.4% of the SSRs, respectively (Figure 3.14). The most common motifs were A/T repeats (99.2%) for mononucleotides and AT repeats (25.4%) for dinucleotides. Among trinucleotides, the most frequent repeats were ATT/AAT repeats which accounted for 32.2% of trinucleotides. A total of 75,139 primer pairs were successfully designed for the 90,142 identified SSRs

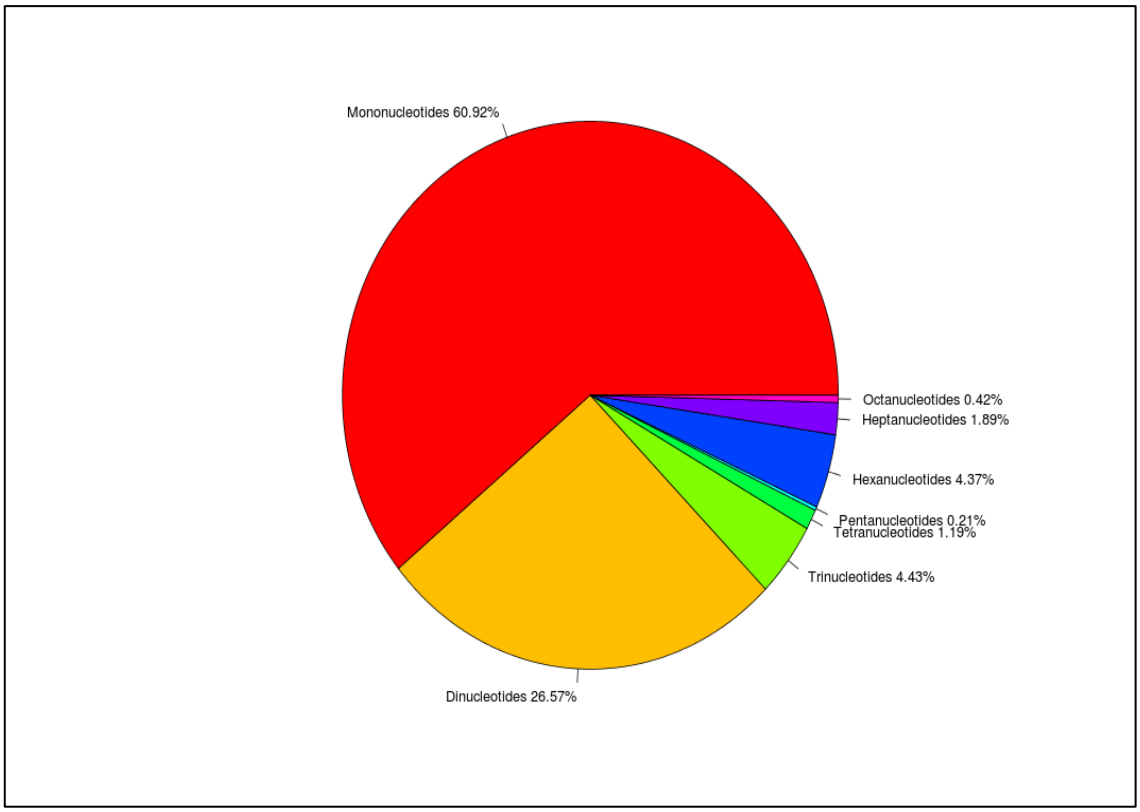

Figure 3.14. Simple sequence repeat types in *C. avellana*

3.4.2. SSR Validation

To confirm that the designed primers amplified the expected SSRs, PCR products from eight primer pairs amplified on 'Tombul' DNA were sequenced with the dye-terminator method (data not shown). All eight sequences contained the expected SSR motifs, proving that the primers amplified regions containing SSR.

Fifty of the newly developed SSR markers were validated by amplification using 47 accessions representing the hazelnuts of ten countries (Table 3.15). In all, 45 of the primers (90%) produced polymorphic bands and generated 163 alleles, 104 of which were polymorphic (64%; Table 3.16). Average allele number for each SSR marker was 3.2. The PIC value was highest for cavSSR11062 (0.97). The lowest value was 0 for two monomorphic markers: cavSSR12855, cavSSR13267.

Table 3.15. Hazelnut accessions used in study. Cluster assignments of 47 accessions according to population structure and genetic diversity analyzes

Name	Origin	Cultivar / Wild	Inferred Ancestry	Dendrogram cluster
101	Italy	Cultivar	4	B3
119	Italy	Cultivar	3	A2
Aci	Turkey	Cultivar	1	A1
Allahverdi	Turkey	Cultivar	2	B1
Arutela	Romania	Cultivar	3	B2
Badnuss	UK	Cultivar	3	B2
Bearn	France	Cultivar	3	B2
Brixnut	USA	Cultivar	admixed	B1
Cavcava	Turkey	Cultivar	2	B1
Corabel	France	Cultivar	admixed	B3
Cosford	UK	Cultivar	admixed	C
Cakıldak	Turkey	Cultivar	1	A1
E-104	Italy	Cultivar	3	C
Ennis	USA	Cultivar	admixed	C
F-104	Italy	Cultivar	3	C
FAI604	Turkey	Wild	2	B1
Feriale	France	Cultivar	3	C
Ferwiller	France	Cultivar	admixed	B3
Fosa	Turkey	Cultivar	1	A1
Giresun Melezi	Turkey	Cultivar	4	B3
Gunslebert	Germany	Cultivar	3	B2
Istrska dolgoplodna leska	Croatia	Cultivar	3	B2
Istrska okrogloplodna leska	Croatia	Cultivar	3	C
Incekara	Turkey	Cultivar	1	A1
Kalinkara	Turkey	Cultivar	1	A1
Kan	Turkey	Cultivar	2	B1
Kara	Turkey	Cultivar	1	A1
Kargalak	Turkey	Cultivar	1	A1
Kuş	Turkey	Cultivar	1	A1
Landsberg	Germany	Cultivar	3	B2
Lansing	USA	Cultivar	4	B3
Lewis	USA	Cultivar	3	C
Mogul	UK	Cultivar	3	C
Negret	Spain	Cultivar	4	A2
Okay28	Turkey	Cultivar	4	B3
Palaz	Turkey	Cultivar	1	A1
Pauetet	Spain	Cultivar	3	C
Riccadi Tlanico	Italy	Cultivar	admixed	C
Romoi	Hungary	Cultivar	3	B2
Sivri	Turkey	Cultivar	admixed	A1
Tombul	Turkey	Cultivar	1	A1
Tonda di Giffoni	Italy	Cultivar	4	B3
Uzun Musa	Turkey	Cultivar	1	A1
Valcea	Romania	Cultivar	3	C

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Table 3.15. (cont.)

Willamette	USA	Cultivar	3	C
Yassi Badem	Turkey	Cultivar	2	B1
Yuvarlak Badem	Turkey	Cultivar	admixed	B1

Table 3.16. Simple sequence repeat (SSR) markers used for the molecular genetic analysis of hazelnuts

Primer Name	Forward Sequence	Reverse Sequence	SSR Motifs	PIC	Ho	# of polymorphic fragments / total # of fragments (%)
cavSSR202	CTCAGACACGCTCTCATTTA	AGTAGTAGTGCTCCACGAAT	$(CT/GA)_{12}$	0.60	0.64	3/3 (100)
cavSSR325	GAGAGAGCTCACAGACAATT	TTCTTCTCTGGAGGGGATAG	$(AG/TC)_{16}$	0.54	0.61	2/3 (66.7)
cavSSR1361	GATATCACTCACGTCTACCG	GGTCTCTTGGTCTTGATGTT	$(AG/TC)_{14}$	0.58	0.63	1/2 (50)
cavSSR1601	TCTGGAGTTAGCTACTGTCA	ACTAGTACCTTGGAGTACCC	$(AATTT/TTAAA)_5$	0.45	0.48	1/3 (33.3)
cavSSR1632	GCCTATGTCCCTCTACAAAG	AGGAAAGTGAAGATGGTTCC	$(AG/TC)_{12}$	0.93	0.94	8/8 (100)
cavSSR1828	CGGAGTGTTTTAATGGCATC	TGGTTGGAGAACTGTACATG	$(GA/CT)_{12}$	0.08	0.08	0/5 (0)
cavSSR2135	ATGTAGCGAGCCTTGATAAG	GTTGTCAGGTAGCTTGAAGA	$(TTAA/AATT)_6$	0.41	0.46	2/2 (100)
cavSSR2527	ACCTAGTAGCTGCATTTAGC	CTACCTCCAGGAGTCAACTA	$(AAT/TTA)_8$	0.46	0.50	2/2 (100)
cavSSR2590	GGTAGGCTGTGTTTTCTGTA	CAGATAGAACGGACTGGATG	$(TC/AG)_{12}$	0.21	0.23	2/4 (50)
cavSSR2704	GCGGAGTTGGTAGTGATAAT	ATATAGGTATAAGGGGGCCC	$(TAG/ATC)_{10}$	0.72	0.75	2/2 (100)
cavSSR2975	CTGGGCATTTAGGTGTAGTT	GTAGAGAGTGGCCAAAACAT	$(CT/GA)_{12}$	0.44	0.47	4/5 (80)
cavSSR3126	CCGTGAGTTTGTAAGATTGC	AAACCTCTCACTAAGGAGGT	$(GA/CT)_{12}$	0.24	0.28	1/2 (50)
cavSSR3909	AGATGAAGCTGAAGAAAGGG	TATCGCCATCACACCATTAG	$(GGA/CCT)_8$	0.27	0.29	1/2 (50)
cavSSR4217	GACAGTTGGCATGAAAGATG	GCACTCATCAGAGAGTCAAA	$(ATT/TAA)_{10}$	0.53	0.57	2/2 (100)
cavSSR4769	CCCATGTACGTATTCTCAGG	ATACTGAACCCTTCCGTGTA	$(GCA/CGT)_8$	0.46	0.50	2/3 (66.7)
cavSSR4874	GTCTTGAGAACCTACACGTT	ACAACATCCGGATAGAAAGG	$(GA/CT)_{13}$	0.59	0.64	1/2 (50)
cavSSR4912	GTTTCCCTTTCCCTCATCAT	CAGTACTGAGGGTTGGATTG	$(GGA/CCT)_8$	0.45	0.48	2/2 (100)
cavSSR6172	TCTGCTTGGAGTGAGGTATA	TCCTTCTGAAGCTCAAGTTC	$(ATA/TAT)_8$	0.54	0.57	2/2 (100)
cavSSR6904	ATCTCCGAGAAAGTCAGAGA	AAGAGCTCTGAGGATCTGAT	$(GA/CT)_{13}$	0.54	0.61	2/5 (40)
cavSSR7457	CTTGCTTTTAGGACCTGAGT	CCTGCAATACTAGTGCTTCT	$(AG/TC)_{17}$	0.60	0.65	2/2 (100)
cavSSR7631	TTCCAGGAGCAAGAGATAGA	TTGTAGTTACAGGCAAGACC	$(CT/GA)_{14}$	0.46	0.56	1/3 (33.3)

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Table 3.16. (cont.)

cavSSR7755	TGAGTATTTGGACCTTGTGG	AAGGAGAAGCTTACACTGTG	$(CCA/GGT)_9$	0.12	0.12	0/2 (0)
cavSSR8129	GGTAATTGTTGGAGACCCAT	CTCTCTCTCCATGTGTCTTG	$(TAT/ATA)_{11}$	0.25	0.27	2/3 (66.7)
cavSSR8344	AAGTTCACGAGTCTAATCCG	GTAGTCACTGCTATGAGGTG	$(CT/GA)_{12}$	0.54	0.57	5/6 (83.3)
cavSSR8498	GCTAAATTCGCAGAGAGAGA	GCGCGCTTATATAAATAGGC	$(GA/CT)_{13}$	0.57	0.63	2/3 (66.7)
cavSSR8737	AAAGACTCAAATCTGCTCCC	GAGGTATGCCAACTGAATGA	$(AG/TC)_{13}$	0.57	0.61	2/7 (28.6)
cavSSR9999	CACTCATGGAAGGAGAAACA	TAGCAGAGGAAACAGAACAC	$(TTTC/AAAG)_6$	0.47	0.55	1/2 (50)
cavSSR10247	GGCTCGCTGTAAAGATGATA	TCCTACAAGCTGTCATGAAC	$(TC/AG)_{17}$	0.57	0.62	2/2 (100)
cavSSR10870	GGTCAATTGCATACAGTTGG	TAAAGGGTGAGGTGTAGGAA	$(GA/CT)_{16}$	0.60	0.67	2/3 (66.7)
cavSSR11062	CTCTCAGCAGGAAGAGAATC	CTGAGCTTCTTCTTAAGGCA	$(CTT/GAA)_8$	0.97	0.97	12/12 (100)
cavSSR11181	TACTACTAAGACCCCACCTG	AGTACATGTGTCAACACTCC	$(AG/TC)_{14}$	0.70	0.74	4/8 (50)
cavSSR11645	TTCTTTGGTGGATGTGAGAG	CTGAAAGAGAGCTTCCATGT	$(TC/AG)_{16}$	0.25	0.27	1/2 (50)
cavSSR12041	ATTCGGCTTGAATCTCTACC	CAATGGCTCTGGTATTCTGT	$(GAT/CTA)_8$	0.18	0.19	1/2 (50)
cavSSR12192	GGGATAACAGACCGAACTAC	GGGGGCAATTAGGTCTTTAA	$(TATG/ATAC)_7$	0.42	0.45	2/3 (66.7)
cavSSR12846	CGTCTATGGTCGTTCAATCT	GTCTCCTTTTTGTATGCACG	$(ATT/TAA)_8$	0.12	0.12	2/3 (66.7)
cavSSR12855	GGTAGTGATGATTGGGTTGT	AATAACCAGTTTCTCCGAGC	$(AG/TC)_{17}$	0.00	0.00	0/5 (0)
cavSSR12862	TAAAATGGGCCTACACTTCC	CCAGTACAGGAAGATACGAA	$(CT/GA)_{13}$	0.47	0.51	2/2 (100)
cavSSR13164	AGAAGAAAGCACTCCTCTTG	CTACCTGCTGTTCCTTTTCC	$(GA/CT)_{13}$	0.20	0.22	1/2 (50)
cavSSR13267	ATATATGCACTGTGGAGGTG	CCCTACTCACTCTATCACCA	$(AG/TC)_{15}$	0.00	0.00	0/3 (0)
cavSSR13350	TTATCCTCAATGCCTTGGAC	AACTTCTTCATCAAGACCCC	$(AG/TC)_{15}$	0.73	0.77	3/3 (100)
cavSSR13386	CCAACGAATCAAAAGACGAG	CCGCCTTCCATATAACTGAA	$(GA/CT)_{14}$	0.04	0.04	0/6 (0)
cavSSR13416	GGGCTTAGCATATGAAGTCA	AGGGTTGTACTACTAGGCAT	$(AG/TC)_{15}$	0.23	0.23	2/3 (66.7)
cavSSR13676	CATCGATGGAGAGGTTAAGG	CATACAAACCTATCCTGGGG	$(TTTC/AAAG)_6$	0.60	0.62	3/3 (100)
cavSSR13891	AAAGGTTGGGATGATGAGTC	ACTCTCCAATCGTATCCTCA	$(AAG/TTC)_{10}$	0.18	0.19	2/2 (100)
cavSSR14219	TATATGGACAGCTGACTCCA	GAGGGAGTTTGTCTGTCTTT	$(AAT/TTA)_9$	0.48	0.57	1/2 (50)
cavSSR14267	CCATCCAGGATCAAGTTGAT	TCAAAGCACCCATACTACAG	$(CATA/GTAT)_6$	0.21	0.23	2/2 (100)
cavSSR14418	GACTGCAAGAATGACAACAG	GTCCTCCTCCTTTTTCGTAG	$(TTGG/AACC)_6$	0.60	0.67	2/2 (100)
cavSSR14875	CACAAGATGATACCCATGCT	TATCAGCTCCTAAAACGACG	$(TACA/ATGT)_6$	0.53	0.61	1/2 (50)

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Table 3.16. (cont.)

cavSSR14904	GGGTTTTCGATCAGAACAAC	GTCTCGCTCTCTCTCTCTAT	$(AG/TC)_{12}$	0.25	0.26	2/2 (100)
cavSSR14937	TGAGCTCTCTGGTTTCTTTC	ACTGGATCTGCTTTTATGGG	$(CT/GA)_{12}$	0.47	0.54	2/2 (100)

The SSR data were used to construct a dendrogram using the Dice coefficient and unweighted neighbor-joining algorithm. A Mantel test showed a high correlation between the distance matrix and dendrogram (r = 0.95). The average diversity of accessions was 0.17 with the highest value (0.30) between 'Tombul' and 'FAI604' together with 'Allahverdi' and 'Fosa' and the lowest value (0.05) between Corabel (N-473) and Ferwiller. The hazelnut accessions grouped into three clusters (A, B, and C) in the dendrogram (Figure 3.15). Cluster A contained 14 accessions in two subclusters (A1 and A2). Cluster A1 contained 12 accessions and A2 contained two accessions. Genetic diversity in Cluster A ranged from 0.09 to 0.29 with an average diversity value of 0.17 (data not shown). Most of the Turkish hazelnut cultivars were found in Cluster A. Two non-Turkish accessions were found amongst the 12 Turkish accessions in Cluster A including '119' and 'Negret'. Cluster B contained 21 accessions in three subclusters (B1, B2, and B3) with genetic diversity ranging from 0.09 to 0.29 with an average of 0.18. Six Turkish accessions were found in Cluster B1 with 'Brixnut'. Cluster B2 contained seven European hazelnut cultivars from Croatia ('Istraska dolgoplodna leska'), France (Bearn), Germany ('Gunslebert', 'Landsberg'), Hungary ('Romoi'), Romania ('Arutela'), and the UK ('Badnuss'). On the other hand six European cultivars from France ('Corabel', 'Ferwiller'), Italy ('Tonda di Giffoni', '101') and the Turkish cultivars Giresun Melezi and Okay28 (Kargalak x Tombul hybrids) were found in cluster B3 with the US cultivar 'Lansing'. Cluster C had 12 accessions and genetic diversity ranged from 0.06 to 0.18 with an average diversity value of 0.11. Hazelnut cultivars from Croatia ('Istraska okrogloplodna leska'), France ('Feriale'), Italy ('Riccadi Tlanico', 'F-104', 'E-104'), Spain ('Pautet'), the UK ('Cosford', 'Mogul'), and the USA ('Lewis', 'Ennis', 'Willamette') were found in cluster C.

Population structure was also determined using the SSR data. The model with four subpopulations (K=4) was determined as the best model for population structure (Figure 3.16, Figure 3.17). Subpopulations 1 and 2 included 11 and five accessions from Turkey, respectively. A total of 17 accessions were found in subpopulation 3 and six accessions were found in subpopulation 4. The remaining eight accessions were admixed: 'Brixnut', 'Corabel', 'Cosford', 'Ennis', 'Ferwiller', 'Riccadi Tlanico', 'Sivri', 'YuvarlakBadem' (Table 3.15). Turkish accessions were distributed throughout all subpopulations (1, 2, 4 and admixed) except subpopulation 3. When the population structure results were compared with the dendrogram analysis, subcluster A1 corresponded to subpopulation 1 with the addition of one admixed accession ('Sivri'). Subcluster B1 corresponded to

subpopulation 2 with two admixed accessions (‘Yuvarlakbadem’, ‘Brixnut’). In addition, subcluster B2 and cluster C corresponded to subpopulation 3 with three admixed accessions from cluster C (‘Cosford’, ‘Ennis’, ‘Riccadi Tlanico’). Subcluster B3 corresponded to subpopulation 4 with two admixed accessions (‘Ferwiller’, ‘Corabel’). Principal coordinate analysis (PCoA) of the SSR dataset did not show a clear separation between subpopulations (Figure 3.18). In the PCoA plot, the dendrogram subclusters that were predominantly Turkish material (A1 and B1) were clearly separated from the remaining accessions.

Seven (Cav4217 Cav14875, Cav14418, Cav2704, Cav12862, Cav3909, Cav1361) of the 50 SSR markers were chosen as the minimum set of primers needed to discriminate 19 Turkish hazelnut accessions from each other (Figure 3.19). Combinations of two, three, four and five SSR markers were sufficient to discriminate the Turkish accessions. Cav4217 and Cav14875 were able to separate ‘Palaz’ and ‘Cakıldak’ cultivars from each other in two step-PCR. ‘Kan’ and ‘Giresun Melezi’, ‘Uzun Musa’ and Kargalak’ could be discriminated from each other with all primers. Cav4217, Cav14875, Cav14418 assays were common for all cultivars and determination of the heterozygosity and homozygosity of these markers was enough to discriminate several of the cultivars. However, different combinations of additional markers (Cav2704, Cav12862, Cav3909 and Cav1361) were needed for complete discrimination of the 19 Turkish cultivars.

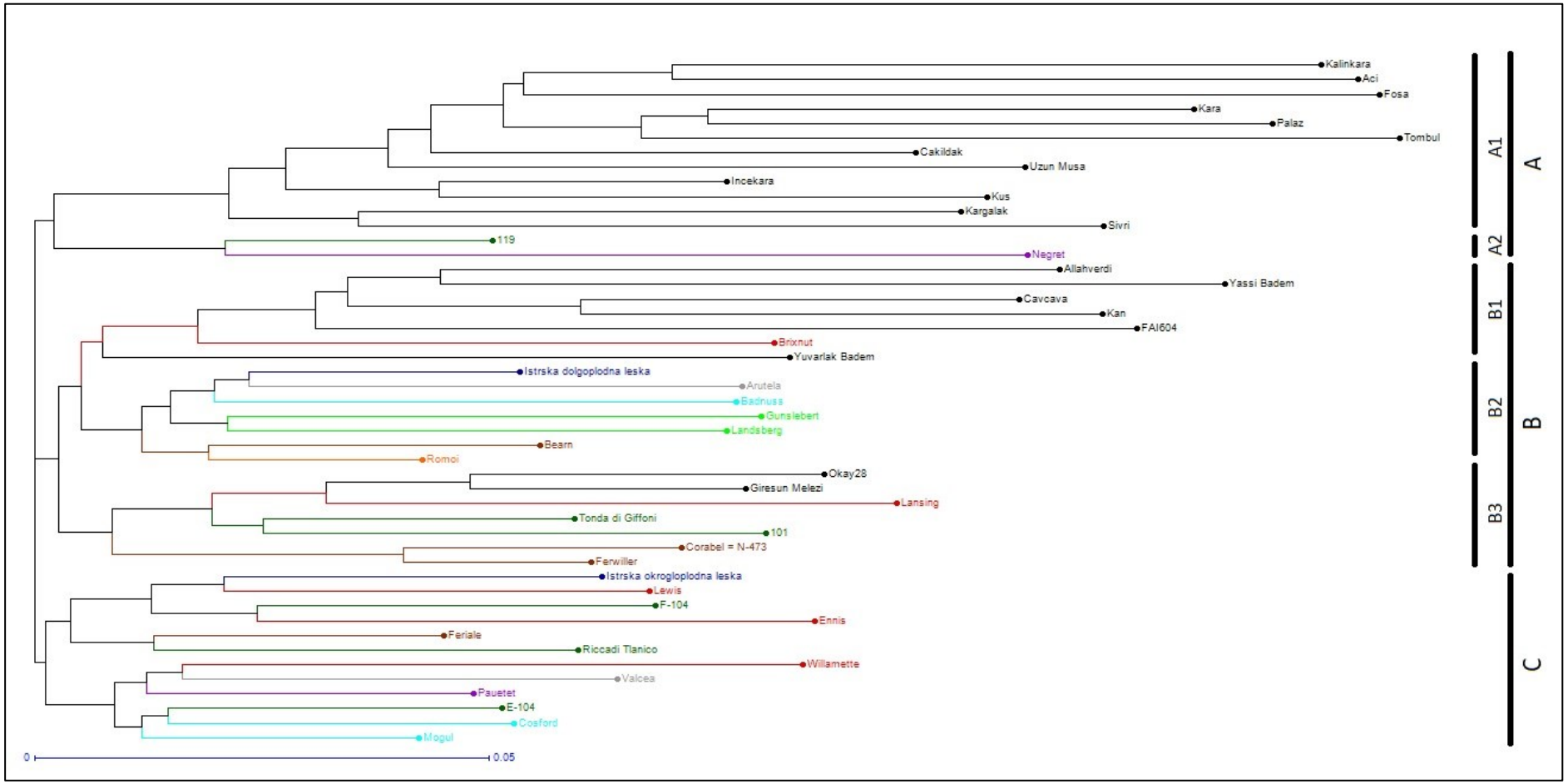

Figure 3.15. Unweighted neighbor-joining dendrogram of the 47 hazelnut accessions based on SSR data. Accessions are color coded by origin: Croatia: dark blue, France: brown, Germany: light green, Hungary: orange, Italy: dark green, Romania: gray, Spain: purple, Turkey: black, UK: light blue, USA: red.

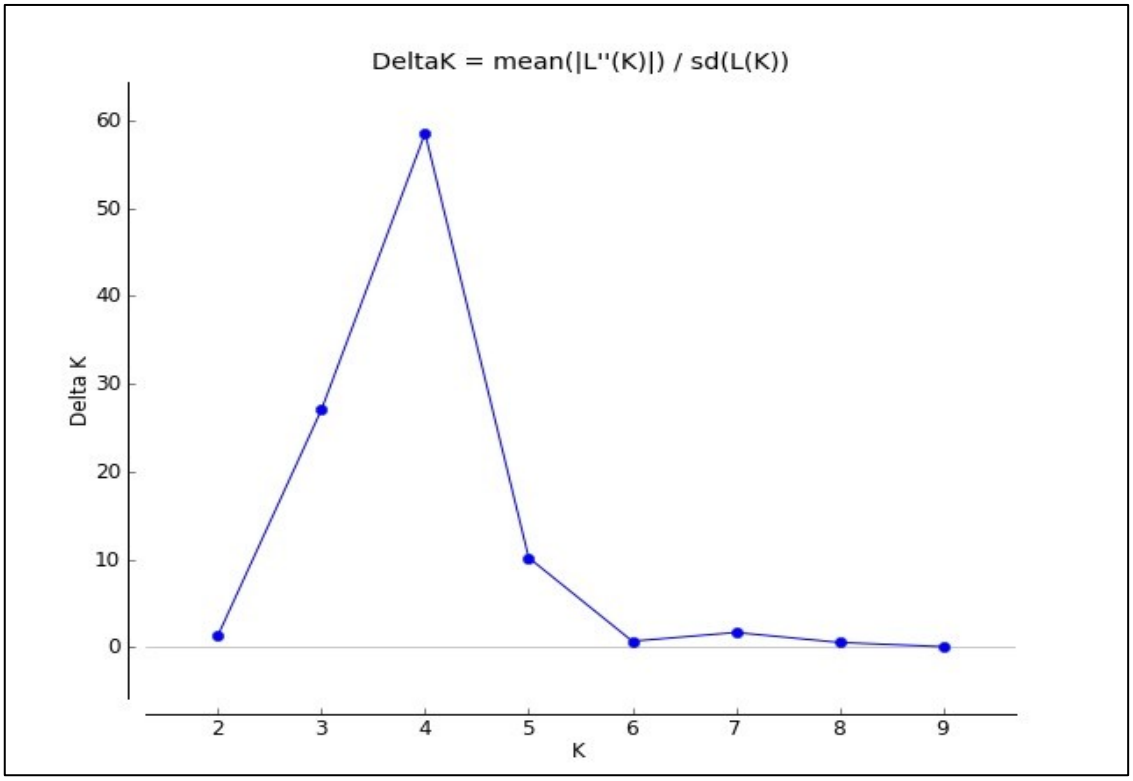

Figure 3.16. Delta K values of the Structure program outcome for each subpopulation assumption. The value of K with the highest Delta K value was chosen as the best number of subpopulations for the hazelnut accessions (K=4).

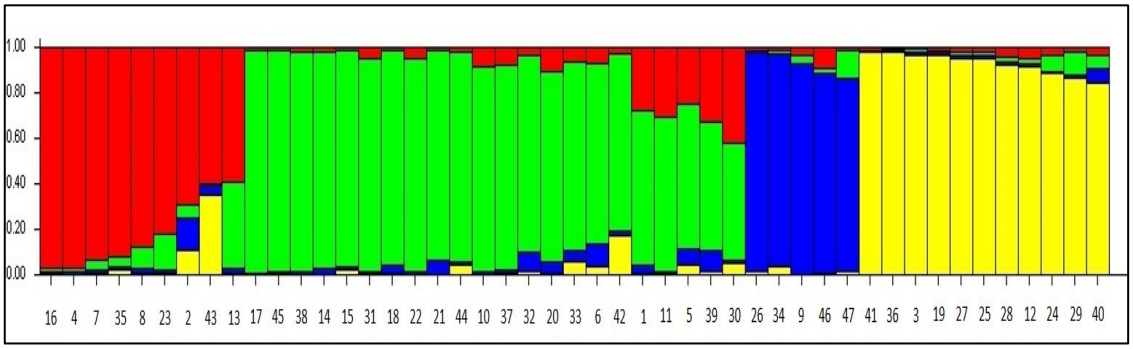

Figure 3.17. Population structure plots of the 47 hazelnut accessions. Numbers were given according to list of Table 3.15.

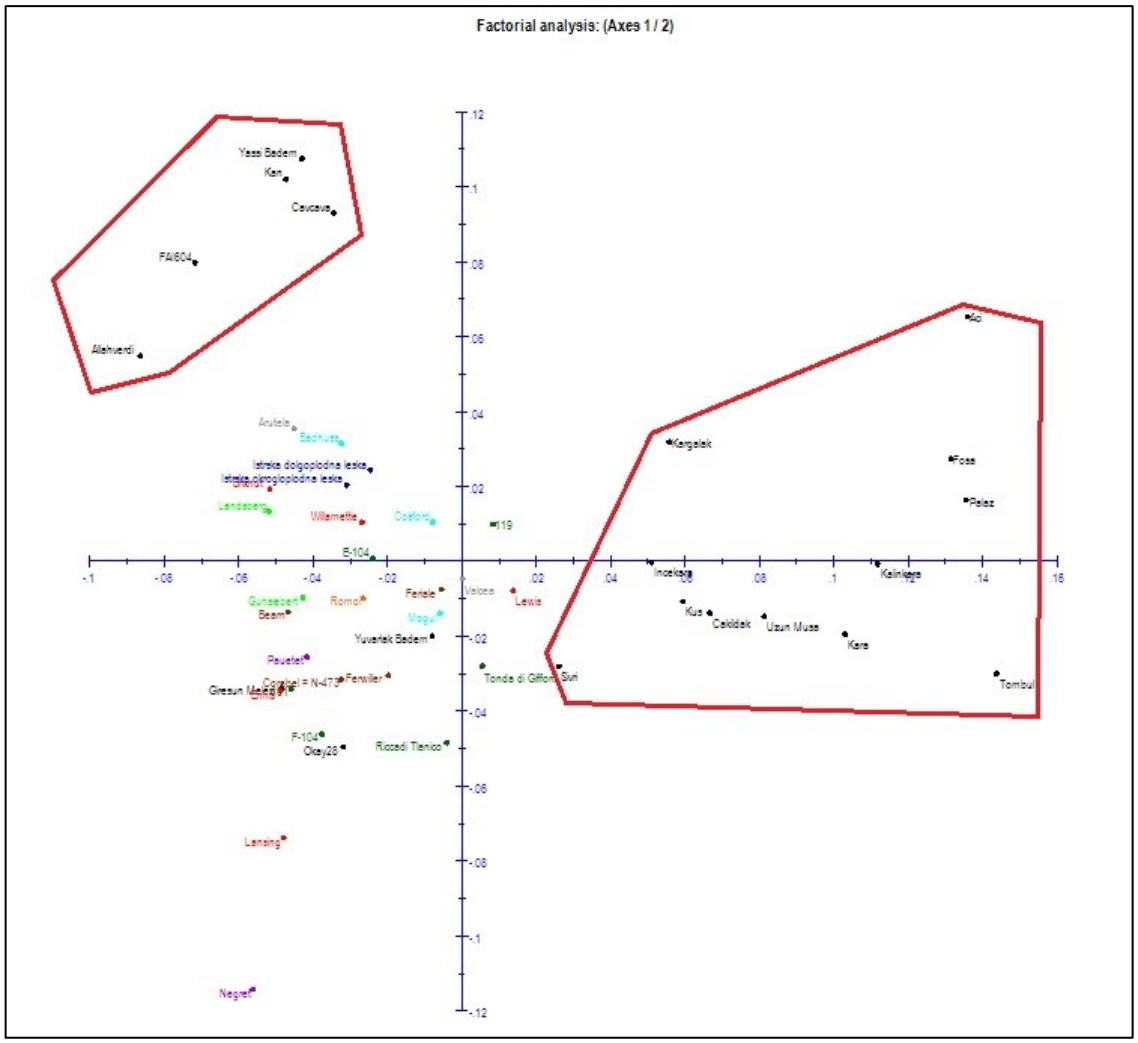

Figure 3.18. Principal coordinate analysis of hazelnut accessions according to the first two Eigen vectors which explained 17.2 and 8.7 % of the variance, respectively. Most of the Turkish accessions in Cluster A1 and B1 are grouped in red areas on PCoA plot.

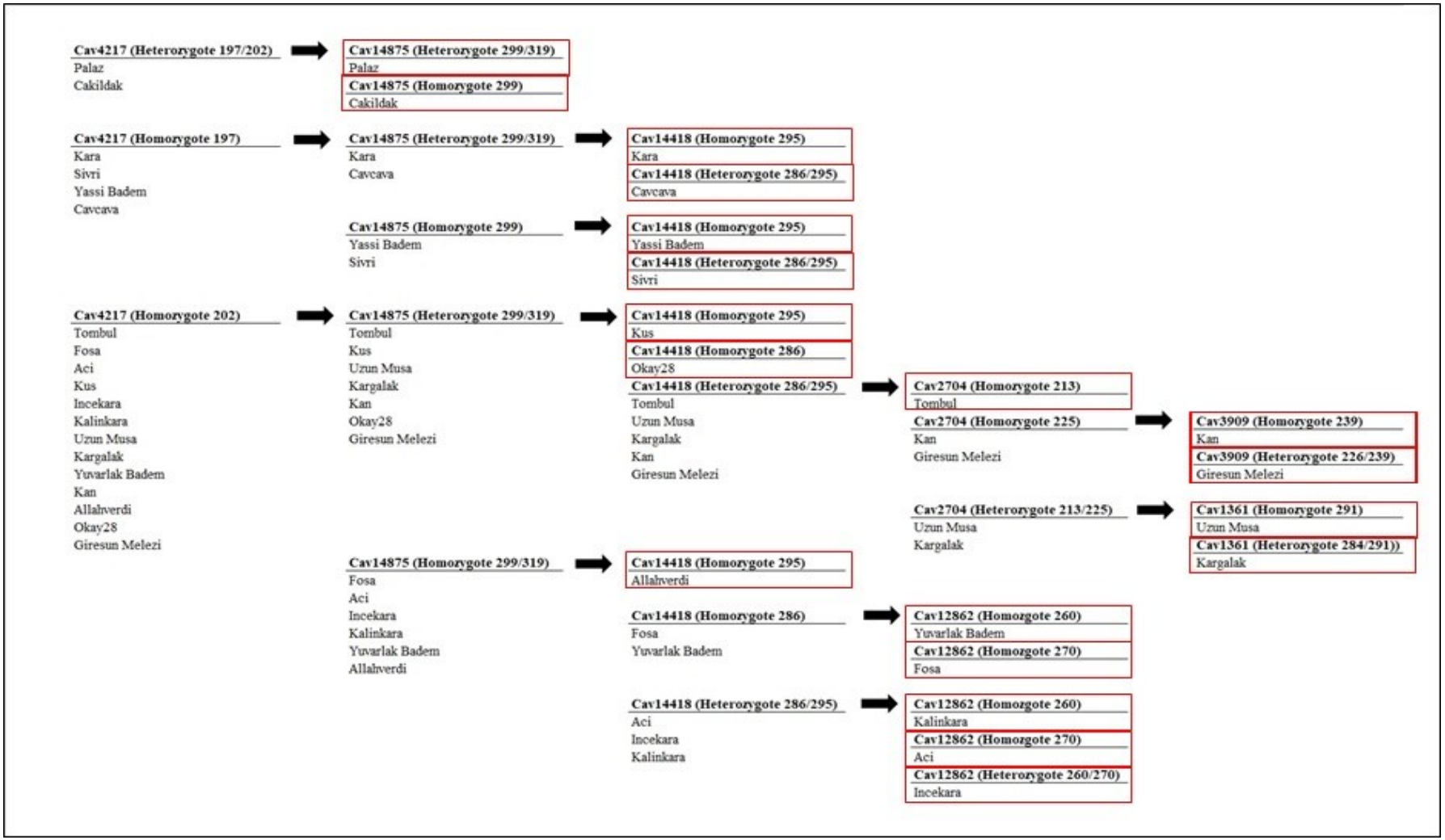

Figure 3.19. Identification key displaying the discrimination of 19 Turkish hazelnut cultivars according to seven SSR markers.

CHAPTER 4

DISCUSSION

4.1. Molecular and Morphological Characterization of Slovenian Hazelnut Germplasm

Although Slovenia is a minor hazelnut producer, it has many cultivars which were introduced in country from other countries and it also has wild accessions from regions representing Slovenia's different climatic conditions. Thus, it was important to measure the overall diversity of these materials and to see how the wild material could be distinguished from the cultivars. Moreover, morphological characterization allowed comparisons with the wild material and identification of any germplasm which could be useful in hazelnut improvement. Association of the traits with the molecular marker data also enabled association mapping of the loci controlling the traits.

4.1.1 Marker Polymorphism

Both AFLP and SSR markers had good polymorphism in the 102 hazelnut accessions. The AFLP technique gave an average of 48.4 alleles per primer combination, a value that was much higher than reported in previous work which averaged 29.9 and 24.4 polymorphic fragments, respectively. This difference may be attributed to the fact that our work examined more accessions and many more wild accessions than Kafkas et al.[37] who examined only 18 Turkish cultivars while Martins et al.[39] studied 58 accessions, only 13 of which were wild accessions. As expected, SSR markers gave fewer alleles than AFLP but proved to be more informative with an average gene diversity of 0.30 compared to 0.26 for AFLP. The 49 SSR markers had an average of 10.3 alleles per SSR which was consistent with other work with the same or similar genomic SSR markers which resulted in 3 to 10.6 alleles per marker [48-50, 52-53, 56, 58, 60, 70, 91]. The distance matrices generated from the AFLP and SSR marker data had only low correlation to each other perhaps because they sampled different parts of the hazelnut genome. While both AFLP and SSR

markers are expected to occur in coding and noncoding regions throughout the genome, the majority (96%) of SSR markers used in this study were previously selected to provide only one or two amplification products [53]. The existence of fewer alleles for these markers may indicate that they are located in coding regions under selective pressure.

4.1.2 Diversity and Population Structure of Wild and Cultivated Hazelnuts

Gene diversity values for the AFLP and SSR markers and the genetic dissimilarity matrix calculated with the Dice coefficient indicated high genetic diversity of hazelnut in agreement with many other studies [1, 30, 55-56, 58, 60, 70, 119]. Levels of diversity are expected to be high in hazelnut because it has a self-incompatible mating system which prevents self-pollination. In addition, hazelnut is a wind-pollinated species. Overall diversity of the cultivars was lower than the wild accessions with average dissimilarity coefficients of 0.50 and 0.60, respectively. Higher levels of diversity in wild accessions as compared to cultivars were also reported by Campa et al.[70] and Martins et al.[72] who studied material from northern Spain and northern Portugal, respectively. Such results are expected as hazelnut cultivars have been selected for certain similar features, are clonally propagated, and therefore, genetically fixed. In contrast, wild individuals are the result of sexual reproduction with cross-pollination which allows greater gene flow and an increased probability of recombination events leading to new alleles and greater genetic variability.

Genetic diversity and relationships among the hazelnut accessions were determined using dendrogram and PCoA analyses of the AFLP and SSR data. Based on these results, it was clear that most cultivars (91–94%, depending on dataset) grouped separately from the wild material. This was also observed in comparisons between cultivated and wild material sampled in Spain, Italy, and Portugal [39, 55, 70, 72]. Similar separation was also seen when reference cultivars were analyzed with local cultivated germplasm from northern Spain [69]. Unlike other studies [23, 55, 58, 91, 119], cultivars did not cluster according to geographic origin. This was not surprising given the relatively similar genetic origins of hazelnut breeding material which has been spread to several different countries. However, clustering by origin was observed for the wild accessions. Thus, most of the accessions from western Slovenia (Bovec and Vipava-Razdrto) formed a group which was distinct from the cluster of accessions from central and eastern Slovenia

(Dolenjska, Koroska and Maribor). Vipava-Razdrto and Bovec are located at high elevation, 533 m and 452 m above sea level, respectively. Both regions have temperate climate with dry winters, no dry season, at least 60 mm of precipitation per month and a Mediterranean influence. In such an environment, the genetically close relationship between accessions could be preserved, explaining the occurrence of all accessions from Bovec and 75% of Vipava-Razdrto accessions in the same cluster (A).

The plant material from Koroska grows in a small, isolated location in north Slovenia, characterized by a humid continental climate that is colder than the other regions examined in this work. In this area, the local forestry service protects hazelnut bushes that are at least 20 years old in order to keep the species in the region. According to foresters, hazelnut is very rarely spread in the region and of unknown origin. The intermixed clustering of some Koroska accessions with those from Bovec and Vipava-Razdrto, suggests that some Koroska hazelnuts originated from the western part of Slovenia.

The high genetic dissimilarity of the accessions from Maribor region with 33, 45, and 22% of accessions belonging to clusters A, C, and D, respectively, could be explained by the possible influence of commercial cultivars. Maribor belongs to the wider Stajerska region, one of the most important areas of commercial hazelnut growth. Therefore, wind pollination among trees could contribute to genetic variability in this region.

Population structure analysis was consistent with the dendrogram and PCoA results. For example, 95% of the subpopulation 1 accessions were located in dendrogram clusters B and C while 100% of subpopulation 2 accessions were in dendrogram cluster A. Regardless of marker type, the hazelnut accessions fell into two subpopulations which appeared to correspond to the cultivated (subpopulation 1) and wild (subpopulation 2) gene pools. Thus, with only a few cases of admixing, all but 10.4% of the cultivars fell into subpopulation 1. In contrast, the wild material fell mainly into subpopulation 2 (39%) with significant proportions belonging to subpopulation 1 (35%) and the admixed group (26%). These results may indicate that the wild accessions in subpopulation 1 originated from cultivars which escaped cultivation. Similarly, the admixed accessions may be the result of ancestral, natural crosses between wild and cultivated trees. Interestingly, the vast majority (83%) of wild accessions from the western part of Slovenia belonged to subpopulation 2. It would be useful to compare these accessions with wild material from adjacent northern Italy to see if they share the same gene pool. If so, the results may suggest the movement of wild material from Italy, a proposed origin of domestication and

diversification for hazelnut [55, 119], to parts of Slovenia. Such west to east spread of hazelnut was first proposed by Boccaci and Botta[91]. In contrast to the western accessions, most (56%) of the accessions from the northeast (Maribor) had admixed ancestry. As previously stated, Maribor is in the Stajerska region which is one of the primary areas of hazelnut cultivation in Slovenia. Thus, it is likely that the collected accessions are the result of cross-pollination between truly wild individuals and cultivars. In general, our results suggest that there is gene flow within Slovenian western and eastern hazelnut populations but very little intermixing between them. Moreover, the differentiation of wild and cultivated hazelnuts into two distinct subpopulations suggests that the wild germplasm could be a useful source of genetic diversity and new traits for hazelnut improvement [72]. Of course, it is also necessary to combine these molecular genetic results with morphological data in order to make selections that will ensure the future diversity and improvement of the crop in Slovenia and other hazelnut growing regions.

4.1.3. Morphological Evaluation

Improvement of hazelnut cultivars for nut and kernel traits is essential to increase market value. Only about 20 cultivars are grown worldwide for the confectionery industry or in-shell marketing [6]. Wild accessions could be used in hazelnut breeding to introduce new variation and improve cultivars for nut and kernel traits.

Volume of the nut and kernel is important because larger nuts are preferred for in-shell marketing, while medium and small nuts are preferred for confectionery [4]. Nut and kernel volume and uniformity is also important for correct operation of processing machines used in the food industry [81]. Cultivars had greater nut length, width, and thickness than wild accessions. This result was expected because nut volume is determined by these dimensional traits which have positive effects on increasing yield. The cultivars analyzed in the present study were, on average, longer, and wider than the 20 hybrids (from Arbor Day Farm, Nebraska City, NE) analyzed by Xu and Hanna[3]. They were also longer, wider, and thicker than four Turkish cultivars analyzed by Ozdemir and Akinci[6]. Wild Slovenian accessions had similar length and width as the aforementioned 20 hybrids but were, on average, shorter, and narrower than two Turkish cultivars (‘Palaz’ and ‘Tombul’) [3, 81]. Mean caliber of wild accessions was higher than for hazelnut cultivars because these nuts were much longer than the cultivars. The wild accessions also had

higher mean caliber (17.4) than the 16 cultivars (15.6) reported by Solar and Stampar[4]. Thus, this present study demonstrated that hazelnut wild accessions may be a good source of alleles for increased nut caliber.

Shape index is an important trait in hazelnut breeding. Globular cultivars (shape index = 1) can be more efficiently processed [81]. Cultivars and wild accessions in this study were nearly globular. Although the mean shape index of cultivars (0.9) was similar to the 16 hazelnut cultivars (0.89) studied by Solar and Stampar[4], it was lower than that for 24 Italian and foreign cultivars (1.05)[6] and four Turkish hazelnut cultivars (0.96)[81]. Some of the wild accessions had shape indices similar to the cultivars.

Thin shell is preferred for efficient processing of hazelnut. Interestingly, there was no difference between the shell thicknesses of cultivars and wild accessions. Both cultivars and wild accessions had similar mean shell thickness as 16 previously examined cultivars [4]. Two wild accessions from Dolenjska and Maribor had the thinnest shell (0.7 mm) and could be used to develop cultivars with thinner shell.

Nut weight has a direct effect on yield and cultivars are expected to have big nuts. Wild accessions did not have superior alleles for nut weight. This demonstrated that cultivars were primarily selected and adapted from wild accessions to have greater nut weight. The cultivars examined in this study had higher mean nut weight than 20 hybrids (0.6 g) [3], 24 Italian and foreign cultivars (2.4 g)[6], and four Turkish cultivars (1.8 g) [81]. The cultivars had similar mean weight as the 16 cultivars (3.0 g) analyzed by Solar and Stampar [4].

In addition to a globular shape, uniform nuts are best for processing. Thus, cultivars and wild accessions were evaluated for nut shape uniformity. Interestingly, wild accessions had more uniform nuts than the cultivars analyzed in present study and the 16 cultivars (7.5) examined by Solar and Stampar [4]. This showed that wild accessions contain good genetic potential for nut uniformity which can be introduced to cultivars.

The proportions of healthy and empty nuts are yield-related traits and are also affected by environmental conditions and can vary greatly by year. During the tested years, wild accessions did not contain high genetic potential for proportion of healthy nuts; however, some wild accessions from Vipava-Razdrto, Dolenjska, and Maribor had 100% healthy nuts and can be used in breeding programs for improvement of nut health. Both cultivars and wild accessions had very few empty nuts. Wild accessions from Bovec, Dolenjska, and Vipava-Razdrto did not contain any empty nuts and they can be used to decrease the proportion of empty nuts in cultivars.

A main objective of hazelnut breeding programs is improvement of kernel weight because of its direct effect on yield. Unfortunately, wild accessions did not have good alleles for kernel weight. The cultivars had higher mean kernel weight than the 20 hazelnut hybrids (0.6 g) studied by Xu and Hanna [3], the 24 Italian and foreign cultivars (1.1 g) examined by Cristofori et al.[6] and the four Turkish cultivars (1.0 g)[80]. These results demonstrated that the cultivars of the present study were, on average, superior to the previously studied material and/or grown under more favorable conditions.

Kernel percentage represents the proportion of nut weight that is accounted for by the kernel and is important for the food industry. Wild accessions had low genetic potential for kernel percentage. Mean kernel percentage of cultivars was higher than the 20 hazelnut hybrids (38.8) studied by Xu and Hanna[3] and slightly higher than 24 Italian and foreign cultivars (44.3%) [6] and 16 cultivars (44.6%) examined by Solar and Stampar [4].

Although kernel shape uniformity is an important trait for hazelnut processing and could be considered as a breeding goal, cultivars, and wild accessions had similar, moderate levels of uniformity. Indeed the material had similar kernel shape uniformity as the previously examined 16 hazelnut cultivars (6.2)[4]. These results indicate that new source of alleles for improved kernel shape uniformity should be introduced to breeding programs.

Hazelnut kernels with brown spots are an important problem in the processing industry because they cannot be processed because they split easily. Brown spots in kernels can decrease hazelnut production up to 30% [120]. Cultivars had a very low proportion of brown spots. More interestingly, wild hazelnut accessions did not have any kernels with brown spot. Similar results were obtained for the proportion of moldy kernels with no mold found in the wild accessions. Kernel mold is caused by fungal species, decreases quality for both the in-shell and confectionery industry markets, and is, of course, influenced by climatic conditions [121]. These results show that wild accessions can be used as allele sources to decrease the proportions of brown-spotted and moldy kernels in hazelnut. Twin kernels result from the development of two kernels in one nut. A higher proportion of twin kernels decreases kernel quality. The cultivars analyzed in the present study had extra quality according to the quality classification of the United Nations [122] because cultivars had just 2% twin kernels. According to the classification system, nuts with more than 2% twins are placed in the lower quality classes. Interestingly, wild accessions did not have any twin kernels. In contrast to the proportions of kernel with

brown spots, moldy kernels, and twin kernels, wild accessions did not have superior alleles for deformed kernels. Thus, such material is not useful for improvement of this trait.

Clustering of the hazelnut cultivars in the PCoA was expected because cultivars are more improved than wild accessions for most of the nut and kernel traits. Despite the seemingly limited genetic potential of wild accessions when examined using mean values, sizable variation was observed for all traits expect nut shape uniformity. Similarly, interesting genetic variability was seen when local cultivated germplasm from northern Spain was examined for morphological traits [69]. Thus, wild and local accessions may be useful to increase genetic diversity of hazelnut breeding material as well as improve some breeding targets.

4.1.4. Association Mapping

Breeding and improvement of hazelnut accessions requires agro-morphological, biochemical and genetic data but creation of mapping populations in trees is very difficult. Association mapping solves this problem by allowing germplasm collections to be used in QTL studies.

The proportion of SSR markers (0.65%) showing significant LD in the present study was slightly lower than the proportion of SSR markers (11 and 6.5%) with significant LD in AM panels of *Gossypium hirsutum* germplasm [123] and opium poppy [124]. This can be due to the higher genetic diversity of trees as compared to annual plants. LD identified in this study might be due to linkage of the markers but this could not be confirmed because the SSR markers used in this study are not mapped in the hazelnut genome. LD can also be due to selection and relatedness of hazelnut accessions which can lead to false positive associations between markers and traits [109, 125]. To avoid false positive associations, association mapping was corrected by the population structure Q-matrix.

In the present study, QTLs controlling nut and kernel traits were identified for the first time. A total of 49 SSR markers associated with nine of 17 traits was identified using an association mapping approach. Some of these markers could be useful for marker-assisted selection of hazelnut accessions for morphological traits. No QTLs were detected

for eight traits. This lack of QTLs for some parameters may be due to reduced genetic diversity of these traits in the AM panel.

4.2. Molecular Characterization of Turkish Hazelnut Germplasm

4.2.1 Marker Polymorphism

The 30 SSR markers provided sufficient polymorphism in the 402 hazelnut accessions with 13.6 fragments per marker. This value is slightly higher than those obtained by others using SSR markers in hazelnut which varied from 3 to 10.6 fragments per primer pair [48-50, 52-53, 56, 58, 60, 70, 119, 126-127]. In previous work, a higher annealing temperature was used for these SSR primers [53] (60 rather than 55°C). In addition, some studies used only single or low copy SSRs [50, 60, 73, 128], thereby, limiting marker polymorphism. We chose a more permissive temperature to allow amplification of additional fragments. This reduced the total number of markers needed to be analyzed, increased the efficiency and decreased the cost of the work. Such practical measures are often required for characterization of large germplasm collections. The greater number of polymorphic fragments could also be partially due to the large number and breadth of genetic material used in this study as most other studies limited themselves to cultivated material.

4.2.2. Diversity and Population Structure of Wild and Cultivated Hazelnuts

Hazelnut is a wind-pollinated species and has a self-incompatible mating system, thus genetic diversity is expected to be high in naturally-occurring plants. In Turkey, hazelnuts are clonally propagated using rooted suckers. In this way, trees which have desirable allele combinations are preserved and kept in the heterozygous condition. Average diversity of the hazelnut cultivars was similar to that of the entire collection, 0.47 and 0.49, respectively. Similarly high levels of genetic diversity were observed in cultivars and wild accessions from Spain [70], Portugal [72] and Slovenia [126]. Most of the accessions were collected in Giresun which is an area with extensive hazelnut cultivation

and production. The high level of diversity in this region may be related to its high density of trees which allowed cross-pollination of cultivars with nearby landraces and wild trees thereby resulting in 'new' material which was collected by the Hazelnut Research Institute. The importance of Giresun to the hazelnut industry in Turkey is reflected in the fact that the quality of Turkish hazelnuts is classified as Giresun (premium) or Levant (secondary). 'Tombul' is the most well-known Turkish cultivar of Giresun quality with both national and international reputations [12]. 'Tombul' and other "Giresun quality" hazelnuts were clonally propagated and distributed to other areas along the Black Sea coast.

Dendrogram and PCoA analyses of the hazelnut collection indicated that cultivars were loosely clustered but not genetically distinct from landraces and wild material. In contrast, a clear separation between wild and cultivated accessions was observed in materials from Spain, Portugal and Slovenia [39, 70, 72, 126]. The difference between these studies and ours may lie in the fact that reference cultivars were used in the other studies. Most of these references cultivars were not of local origin with the exception of some Spanish-Italian cultivars examined in the work of Campa et al.[70] which focused on wild and local materials from northern Spain. Thus, the gene pools of the cultivars and wild accessions would not be expected to overlap. In contrast, the current work examined only Turkish cultivars, all but three ('Allahverdi', 'Giresun Melezi' and 'Okay28') of which originated from selection and cultivation of formerly wild individuals. 'Giresun Melezi' and 'Okay28' are new cultivars developed from 'Kargalak' and 'Tombul' hybrids [65]. Thus, most of the materials have a common gene pool. Because hazelnut trees from Giresun were the source of most of the cultivars and other genetic resources growing in the region, clear separation of accessions by location was not observed. However there was minor clustering of hazelnut trees from geographically close regions such as those from Samsun and Ordu. In addition, accessions which were collected from same valleys tend to be in the same cluster. For example, accessions from the western part of Ordu were collected from the same valley and clustered together. In the same way, accessions from eastern valleys of Trabzon province clustered together.

Population structure analysis was consistent with the dendrogram and PCoA results. For example, 100% of the subpopulation 1 genotypes were located in dendrogram Cluster A and 100% of subpopulation 2 genotypes were in dendrogram Cluster B. Wild material and landraces fell into subpopulation 1 (33%), subpopulation 2 (49%) and the admixed group (19%). The majority of cultivars fell into subpopulation 1 (75%) with the

exception of the five admixed cultivars. Two of these five admixed cultivars were developed by hybridization, therefore, it is not surprising that they have admixed ancestry. Admixed accessions are also the result of natural hybrids due to cross-pollination.

4.2.3. Core Set Selection

A core set of hazelnut accessions was selected using the SSR data and it was found that the molecular genetic diversity of the entire collection (including all alleles) was encompassed by just 8.4% of the accessions: 29 accessions and five cultivars. Similarly, in the SAFENUT project 6.5% of 306 accessions were chosen as a core set to cover the genetic diversity in different characters [54]. Of course, molecular genetic diversity is not the sole parameter by which core sets should be selected. Morphological diversity is also an important criterion and Turkey has phenotypically diverse hazelnut resources. In addition, tree yield and quality are traits that must be preserved in a core set. For these reasons, accessions with unique phenotypes and the remaining 15 cultivars were included in the core set, thus maintaining important characters and allele combinations. The core set had representation from different geographical locations and each of the subpopulations (1, 2 and admixed). Such core sets are important in prioritizing germplasm conservation and maintenance. This is especially crucial in a long-lived tree crops like hazelnut for which wild populations are under threat from abiotic and biotic stresses and deforestation [6].

4.3. Validation of Genomic SSR Markers

Sequencing of ‘Tombul’ yielded 56,665 contigs which were assembled into 111.85 Mb, representing 29.2% of the hazelnut genome. In other work, ‘Jefferson,’ a cultivar resistant to eastern filbert blight, was sequenced and assembled to cover 345 Mb, representing 91% of the genome with 40x coverage [33]. In the same study, ‘Tombul’ was sequenced with Illumina with low coverage (10 x) because the researchers were only interested in finding the eastern filbert blight resistance gene using ‘Jefferson’ as a reference. Thus to date, our study has produced the most genome information for ‘Tombul’.

4.3.1. SSR Markers Developed by NGS

SSRs are iterations of one to six nucleotide motifs and are found in all prokaryotic and eukaryotic genomes [127]. SSR markers are very important for plant scientists because they can detect multiple alleles per locus, are highly polymorphic and can be found throughout the plant genome [129]. Thus the development of SSR markers, especially for economically important crops, is essential for more efficient plant genome analysis. Traditional SSR development techniques such as library enrichment and Sanger sequencing techniques are low-throughput, labor intensive, expensive and yield a small number of SSRs [129]. Unlike traditional methods, next generation sequencing technology is high-throughput, fast, cost-effective and produces millions of reads at once [129]. Therefore traditional technologies are already replaced with next generation technology in the area of SSR development.

A total of 90,142 non-redundant SSR markers were identified in 29.2% of the *C. avellana* genome. SSR density in these contigs was one SSR in every 1.2 kb (on average). In the sequencing study of 'Jefferson' cultivar, average SSR density was one SSR on every 1.9 kb of the contigs [33], agreeing with our results. Other results indicate one SSR in every 4.5 kb for papaver [130] every 2.9 kb for faba bean [131] and, every 4.1 kb for spinach [132]. Thus, of these different species, hazelnut has the highest density of SSRs in the examined contigs.

The most abundant SSR marker type was mononucleotides which accounted for 60.9% of the identified SSRs, followed by dinucleotides with 26.5% and trinucleotides with 4.4%. Sequencing of 'Jefferson' also indicated that mononucleotides were most common (69.3%). The results also agree with Cardle et al.[133] who also showed that the most common SSR type in many plants was mononucleotides followed by dinucleotides and trinucleotides. The most abundant mononucleotides in 'Tombul' were A/T repeats (99.2%). 'Jefferson' also had a majority of A/T mononucleotides compared to G/C ones [33]. These results agree with other studies which observed that the most common SSR repeat type in plants are A/T repeats [130-132]. According to the same studies, AT/TA repeats are the second most abundant SSR type in many plants. We also observed the same pattern in our study: 25.4% AT and 24.4% TA SSRs among dinucleotide repeats. The most common tri- and tetranucleotide motifs vary based on the plant species. In this study, the most frequent SSR type was ATT/AAT (32.2%) in trinucleotides and ATTT/AAAT

(26.5%) in tetranucleotides. AT-rich trinucleotides were also the most common (43.1%) in 'Jefferson' [33].

4.3.2. Application of Genomic SSR Markers to Population Structure and Genetic Diversity.

Fifty SSR markers were randomly chosen and tested in 47 hazelnut accessions from ten countries. In this study, the SSR markers that were used in diversity analyses were single or low copy except for markers cavSSR11062 (12 fragments), cavSSR1632 (8 fragments) and cavSSR8737 (7 fragments). The number of alleles ranged from one to 12 with an average of 3.2 alleles. This average allele number was in the range of the previous studies (from 3 to 13.6) which used SSR markers in hazelnut [48-50, 52-53, 56-58, 60, 70, 119, 126].

A dendrogram was constructed using SSR data and the accessions fell into three subclusters. All of the Turkish accessions were in Clusters A1 and B1. The genetic distinctness of the Turkish material suggests that it can be used as a source of diversity for US and European breeding programs. Accessions from Italy, France and the UK were found in both cluster C with the US cultivars and in cluster B with the other European hazelnut accessions. Trees from Germany clustered together in the dendrogram and the US cultivars were clustered with small groups of accessions from France, Italy, Turkey and the UK in (sub)clusters A1, B3 and C. This was not surprising because the US cultivars' parents are from these countries [134]. The 47 hazelnut accessions fell into four subpopulations. Turkish cultivars were found in both subpopulation 1 (11 accessions), subpopulation 2 (5 accessions) and subpopulation 3 (2 accessions) as well as in the admixed group (2 accessions).

The genetic analyses based on SSR markers revealed some interesting findings. Two Turkish cultivars ('Giresun Melezi' and 'Okay28') were developed from hybridization of 'Kargalak' and 'Tombul.' Although 'Giresun Melezi' and 'Okay28' clustered together in the dendrogram, population structure and PCoA plots, they did not group most closely with 'Kargalak' and 'Tombul'. Thus, hybridization resulted in new allelic combinations as compared to the parental lines. Such novelty may be especially pronounced in heterozygous breeding material like hazelnut. Another interesting case was the Turkish cultivar 'Yuvarlak Badem'. This cultivar was found in the same subcluster

(B1) as some of the Turkish cultivars but had admixed population structure and grouped with other countries' hazelnuts in the PCoA plot. In addition, 'Yuvarlak Badem' has distinctly different nut traits compared to other Turkish cultivars. Its nuts are round and longer than the other material and are harvested earlier [135]. Thus, this cultivar may have originated from Europe.

Hazelnut is a wind-pollinated species and has a self-incompatible mating system. As a result, high levels of genetic diversity are usually expected. However, in our study, the average diversity was low (0.17). In our previous studies, genetic diversity for Slovenian and Turkish cultivars was 0.50 [126] and 0.47 [57], and this was considered to be moderate genetic differentiation. However, these previous studies used SSRs that were selected for their high levels of polymorphism in other hazelnut accessions. In contrast, the current set of tested SSR markers was randomly selected from the thousands of primers that were designed. Low variability is not unexpected because hazelnuts have been selected for similar characters and certain allelic combinations which may reduce their diversity [57, 70, 72, 126]. Moreover, cultivars are propagated as clones, a fact that limits their diversity as compared to wild accessions.

New SSR markers were developed using next generation sequencing technology and applied to the hazelnut cultivars for validation. Because climatic conditions, altitude and soil can affect kernel, nut and agro-morphological traits which often do not become visible until hazelnut is several years old, the SSR markers were assayed on 19 Turkish cultivars to find diagnostic molecular markers. As a result, seven SSR primer were selected to discriminate Turkish cultivars so that hazelnut breeders, farmers and geneticists can identify true-type hazelnuts and use identical clones. Turkish hazelnuts can be certified using these seven SSR markers, thereby solving an important problem in hazelnut nurseries and orchards. Moreover, these markers will be useful for new cultivar development.

CHAPTER 5

CONCLUSION

European hazelnut (*Corylus avellana* L.) is an economically and nutritionally important nut crop with wild and cultivated populations found throughout Europe and in parts of Asia. In the present study hazelnut cultivars and wild accessions from Slovenia were examined for genetic diversity and morphological variation. The wild accessions were more diverse than cultivars at the molecular level with clustering of the wild material by region. Characterization of nut and kernel traits was done to assess the breeding potential of the wild germplasm. Wild accessions were shown to have breeding potential for most of the traits except nut and kernel weight and to have sizable variation for most traits. In addition, the first association mapping of hazelnut was performed with the identification of SSR markers associated with traits including the length, thickness, and caliber and nuts, as well as, kernel weight and shape uniformity. These SSR markers provide initial molecular information for marker-assisted selection in hazelnut.

In addition, this study examined molecular genetic diversity and population structure of 402 genotypes including 143 wild individuals, 239 landraces and 20 cultivars from the Turkish national hazelnut collection using SSR markers. A total of 30 SSR markers yielded 407 polymorphic fragments. Diversity analysis of the Turkish hazelnut genotypes indicated that they fell into three subpopulations according to ad hoc statistics and neighbor-joining algorithm. Although all cultivars clustered together, they overlapped with the wild accessions and landraces. Thus, the dendrogram, principal coordinate and population structure analyses suggest that they share the same gene pool. A total of 78 accessions were selected as a core set to encompass the molecular genetic and morphological diversity present in the national collection. This core set should have priority in preservation efforts and in trait characterization.

Finally, new SSR markers were developed using next generation sequencing technology and applied to the hazelnut world collection for validation. Seven SSR markers were chosen to discriminate Turkish cultivars. Hazelnut is a tree and if we do not know the name of the planted tree before it gives fruit and shows agro-morphological characteristics, these markers can be used to identify the cultivar. In addition, Turkish

hazelnuts can be certified using these seven SSR markers to solve an important problem in hazelnut nurseries and orchards. Moreover, climatic conditions, altitude and soil can affect kernel, nut and agro-morphological traits, thus, molecular markers are more reliable than morphological ones. Discrimination will also allow breeders, farmers and other people interested in hazelnuts to use identical clones.

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