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Title: Characterization of European Hazelnut (*Corylus avellana* L.) Cultivars Using SSR Markers

Abstract Approved: _____
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Twenty-one pairs of simple sequence repeat (SSR) primers were used to investigate genetic diversity in 270 accessions of European hazelnut *(Corylus avellana)* representing a wide geographic range. A capillary electrophoresis system and ABI Genescan® and Genotyper® software were used to determine the allele size generated from each PCR reaction based on an internal lane standard. A total of 211 alleles were amplified and the number of alleles detected ranged from 5 to 15 per locus with an average of 10.05. The observed heterozygosity ($H_o$) for individual loci ranged from 0.24 to 0.88, with an average of 0.67 over all loci. The presence of null alleles was detected at three loci (CAC-C010, CaT-C504 and CaT-B508) by comparing the pedigree information of some cultivars. PowerMarker software was used to generate a genetic similarity matrix based on possible pair-wise combinations of accessions using the "proportion of shared alleles". UPGMA cluster analysis was used to construct a phenogram from the genetic similarity matrix using PowerMarker and MEGA3 software. The phenogram revealed geographically tight clusters and some synonyms among European hazelnut cultivars. Of the 274 accessions in the population, 200 are unique

cultivars, 70 are suspected synonym accessions (different trees), as they are morphologically identical and 4 are duplicate DNA templates from the same tree included as checks. Of the 211 total alleles amplified, 22 were unique, as they were detected only in one cultivar. Nine of the unique alleles were amplified in cultivars that fell outside of the tightly clustered cultivar groups. A subset of 11 loci is recommended for in future hazelnut fingerprinting studies.

A total of 144 seedlings from a controlled cross of OSU 252.146 x OSU 414.062 were scored for 33 SSR markers and 29 of them were successfully integrated into the RAPD marker-based hazelnut linkage map constructed using the two-way pseudo-testcross approach, where they will serve as "anchor loci". Two loci showed aberrant segregation ratios and two loci remained unlinked. Eleven linkage groups were identified for each parent, corresponding to the haploid chromosomes number of hazelnut (2n=2x=22) and spaning a total distance of 668 cM in the susceptible parent and 813 cM in the resistant parent. The order of homologous SSR loci in the two parents was collinear in most cases. Placement of these SSR "anchor loci" on the hazelnut linkage map will make it useful in other populations.

Characterization of European Hazelnut (*Corylus avellana*) Cultivars Using SSR Markers

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Tufan Gökirmak, Author

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## CONTRIBUTION OF AUTHORS

Dr. Shawn A. Mehlenbacher was the main designer of the experiments. He assisted with data collection and writing of all parts of the thesis and provided the RAPD marker data set used in Chapter 3. Furthermore Dr. Mehlenbacher provided laboratory facilities and funding. Dr. Nahla V. Bassil helped in choosing the SSR markers used in this study and advice on laboratory procedures and data analysis.

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# CHAPTER 1

# INTRODUCTION

T. Gökirmak, S.A. Mehlenbacher, N.V. Bassil

# CHARACTERIZATION OF EUROPEAN HAZELNUT (*Corylus avellana*) CULTIVARS USING SSR MARKERS

## History and Biology of Hazelnut

The European hazelnut, *Corylus avellana* L., is grown in many regions of Europe and west Asia and has been used as a food source by humans since prehistoric times. Hazelnut became one of the species in the local flora in parts of Europe in the Boreal period, from 8000 to 5500 B.C. According to ancient Greek literature, hazelnut was brought to Greece from the shores of the Black Sea, the location of present-day Turkey (Lagerstedt, 1975). Based on a study using chloroplast DNA variation and pollen record in *Corylus*, postglacial migration of hazelnut started from southwestern France and spread to the rest of Europe except Italy and the Balkans, where another local expansion had occurred (Palmé and Vendramin, 2002)

*Corylus avellana*, the species of commerce, is native to Europe and Asia Minor and naturally grows in temperate regions of the Northern Hemisphere from the Atlantic coast of Europe as far north as Norway. The northern boundary includes the British Isles, Scandinavia and northern regions of the Russian Federation. The distribution is bounded in the east by the Ural Mountains. The southern boundary extends from Spain, Morocco and Algeria in the west through Italy, Yugoslavia, Greece, and Turkey to northwestern Iran and Transcaucasia. Although hazelnut is produced in a few regions in the southern hemisphere, it is not native to those regions (Kasapligil, 1972).

Hazelnut-producing regions have mild, moist winters and cool summers. The major production areas are located near large bodies of water at middle latitudes in the

northern hemisphere. Approximately 70% of the world's hazelnut production comes from the Black Sea region of Turkey. Italy follows Turkey with about 20 percent of world production. Spain, France, Georgia, Azerbaijan and the United States account for the remaining 10%. The moderate climate of the Pacific Northwest's coastal valleys is well-suited to hazelnut production. Approximately 99% of the U.S. hazelnut crop is produced in Oregon's Willamette Valley. The Pacific Northwest represents 3 to 5 percent of world hazelnut production (FAO Production Year Book, 2004; Hazelnut Marketing Board, 2004).

The genus name *Corylus* originated from the Greek word korys, which means a helmet or hood. The word hazelnut comes from the Anglo-Saxon word for bonnet, haesel. Hazelnut is a general name used for the nuts of all *Corylus* species. Even though hazelnut is the most commonly used name in world commerce, some growers call these nuts filberts. The name "filbert" probably originated from the German term vollbart, or full beard, descriptive of the long leafy husks of some varieties of hazelnut (Lagerstedt,1975). The genus *Corylus* is a member of the Betulaceae or Birch family and includes other important forest tree species and ornamentals (Erdogan and Mehlenbacher, 2000). The European hazelnut, *Corylus avellana*, is one of the 25 described species in the genus, all of which produce edible nuts, which are collected from the wild by humans. All *Corylus* species are diploid with 2n = 22 chromosomes (Kasapligil, 1968; Thompson et al., 1996).

The European hazelnut grows naturally as a bush or a multi-stemmed shrub. In Turkey and southern Europe, hazelnut has been grown in its natural growth habit for

centuries. In the US, however, hazelnuts are usually grown as single-trunk trees for mechanical harvesting purposes. They can reach approx. 13 m, if planted in good soil and managed with proper pruning, fertilization, and pest control practices.

Hazelnut has a unique floral biology, which was reviewed in detail by Germain (1994). It is monoecious, having separate male and female flowers on the same tree, and wind-pollinated (Thompson et al., 1996). The male flowers are borne in catkins, and female inflorescences have no perianth. They appear as a tuft of red stigmatic styles protruding from the apex of compound buds located on shoots or catkin peduncles. The hazelnut tree is self-incompatible, which enforces cross-pollination. Incompatibility is of the sporophytic type, and is under the control of a single locus (the S-locus) with multiple alleles (Mehlenbacher, 1997). If the same allele is expressed in both pollen and pistil, pollen germination is delayed and pollen tubes fail to penetrate the stigmatic surface (Hampson et al., 1993). So far, 25 unique S-alleles have been identified and the hierarchical order among these alleles determines which is expressed in the pollen and thus the direction of the crosses in breeding programs (Pomper et al., 1998; Mehlenbacher, 1997). The dichogamous nature of hazelnut is another factor that promotes cross-pollination. Male flower induction usually occurs a month before female induction. Female flower formation is affected by three main factors: the level of light received by the one-year-old shoots during the previous growing season, the vigor and the origin of these shoots. For an equivalent length, shoots receiving more light bear more inflorescences. The total number of female inflorescences per shoot increases with shoot length irrespective of tree age or variety. On the other hand, the origin of the shoot also

has a significant effect on number of flowers. On shoots originating from glomerules and bearing a nut cluster at the extremity, formation of female flowers is nearly or completely inhibited (Germain, 1994).

Like many other horticultural crops, hazelnut is clonally propagated and highly heterozygous. Controlled crosses (complementary hybridization) and the modified backcross method are used in hazelnut breeding programs (Mehlenbacher, 1995). The modified backcross method involves a series of crosses and is utilized to transfer a desirable single trait from a wild or unadapted source and combine it with other desired qualities. In the modified backcross method, a different "recurrent parent" is used in each backcross generation to avoid inbreeding. The heterozygous nature of the parents results in segregation in the $F_1$ progeny, allowing linkage map construction (Pomper et al. 1998).

## Molecular Markers

### *Applications of molecular markers*

Traditional techniques used for identification of cultivars and clones are based on morphological characters. However these techniques are not always useful due to environmental variation affecting gene expression (Marinoni et al., 2003). Molecular markers offer a direct method of identification of plant material through determination of the differences in the genetic material independent of environmental effects.

Molecular markers can be placed into two main categories: biochemical (e.g. isozymes) and DNA-based markers. Isozymes are different molecular forms of the same enzyme that are specific to a common substrate but differ in electrophoretic mobility.

They may be visualized following gel separation and show codominant inheritance. However, isozymes do not generally show a high level of polymorphism within a population. On the other hand, DNA markers are based on naturally occurring DNA polymorphism (i.e. base pair deletions, substitutions, additions or repeat patterns) throughout the genome (Gupta et al., 1999). They have different applications in plant breeding, scientific research and identification of plants in commerce. Plant breeding applications include marker-assisted selection and confirmation of parent-progeny relationships. Research applications range from taxonomic studies to population genetic analysis and linkage map construction. Molecular markers also have commercial applications such as "fingerprinting" for enforcement of legal protection, quality control in plant production (i.e. trueness to name) and processing and labeling plant-derived foods and other products.

***Types of DNA markers***

There are several different types of nucleic acid sequence-based markers. They are divided into two main categories: restriction fragment length polymorphism (RFLP) markers and PCR-based molecular markers, such as RAPDs, AFLPs, SSRs, SCARs, etc.

RFLP is one the first molecular markers and has been used in numerous DNA fingerprinting and genetic diversity analyses in species such as rice (Wang and Tanksley, 1989), sorghum (Tao et al., 1993) and wheat (Paull et al., 1998). This technique centers around Southern hybridization of restriction enzyme-digested DNA with genomic or cDNA probes. Polymorphism results from differences in the length of fragments

produced by restriction enzyme digestion. Due to probe sequence homology, such markers are perfectly suitable for phylogenetic analysis between related species, such as within the genus *Musa* (Nwakanma et al., 2003). RFLPs are co-dominant and robust markers, can be easily exchanged between labs, and have high repeatability. However, the technique has some disadvantages that include a requirement for large amounts of high-quality DNA, the use of radioisotopes, probe library construction for previously unexplored species, and difficulties in statistical evaluations and standardization.

***PCR based markers***

Polymerase chain reaction (PCR)-based markers have some advantages over biochemical and RFLP markers. They are faster than RFLPs, and only a small amount of genomic DNA (5-25 ng) is required. Additionally, exchange of primer sequence information instead of DNA is another advantage of PCR-based markers.

Random amplified polymorphic DNA (RAPD) was the first type of PCR-based marker developed. RAPDs are fragments amplified by PCR using arbitrary, usually10-12 mer primers (Williams et al., 1990). PCR products are separated by gel electrophoresis and scored for the presence or absence of an amplicon after staining with ethidium bromide (EtBr). RAPD markers are usually dominant and thus they cannot distinguish between homozygotes and heterozygotes. Polymorphism is the result of presence or absence of the priming site in the genomic DNA. RAPD is a fast, easy and inexpensive technique. However, it has some reliability problems. Results are very sensitive to reaction conditions like $MgCl_2$ concentration, primer concentration and annealing

temperature. Even different equipment in different laboratories may give different results. For these reasons, RAPDs are not as reliable as other sequence-specific markers. On the other hand, some of these problems can be overcome by cloning and sequencing the fragments, and converting these into sequence characterized amplified regions (SCAR), which are usually more robust than RAPDs (Paran and Michelmore, 1993). However, the original polymorphism may be lost with the use of longer primers. Several different approaches like PCR optimization, primer redesign and cleaved amplified polymorphic sequences (CAPS) can be used to recover the polymorphism. CAPS may reveal polymorphism lost during SCAR marker development by digesting the PCR product with a "frequent cutter" restriction enzyme that recognizes a 4 bp restriction enzyme site (Moury et al., 2000; Ohmori et al., 1996; Paran and Michelmore, 1993).

Amplified fragment length polymorphism (AFLP) is a PCR-based marker system for DNA fingerprinting developed by Vos et al. (1995). The procedure involves digestion of genomic DNA with two restriction enzymes, a frequent cutter (e.g. *MseI*) and a rare cutter (e.g. *EcoRI*). DNA templates for PCR reactions are generated by ligating oligonucleotide adapters to the restriction fragments. PCR fragments are selectively amplified in two steps using primers that are complementary to the adapter sequence and the restriction enzyme cleavage site and up to four selective nucleotides at the 3' end. Polymorphism is determined by presence or absence of the amplified fragments on high-resolution sequencing gels visualized by silver staining, fluorescent dyes or radioactivity. AFLP enables screening a much larger number of loci per PCR reaction for polymorphism than other available PCR-based techniques and has been demonstrated to

generate a large number of polymorphisms in barley (*Hordeum vulgare* L.) (Becker et al., 1995). Polymorphism produced by AFLPs is generally reproducible. Like RAPDs, AFLPs are also dominant markers. Although they are reliable and a large number of bands is generated by a single PCR, they are expensive, complicated and technologically demanding. Conversion of AFLPs to SCARs reduces their cost and advances their use in marker-assisted selection. However, as mentioned earlier in the conversion of RAPDs to SCARs, the polymorphism may be lost. This problem can be overcome by generating CAPS (Konieczny and Ausubel, 1993).

***Microsatellites***

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated 1-6 bp sequence motifs found in both eukaryotic and prokaryotic genomes. They are abundant and dispersed throughout the genome and can be found in both coding and non-coding regions. However, they are more abundant in the latter since non-coding DNA can accumulate mutations more easily than coding DNA (Ahmad et al., 2003). Furthermore, they have a high level of length polymorphism relative to other genetic markers (Zane et al., 2002). Although the exact mechanism of microsatellite evolution is not clear, it appears most likely due to the slippage of DNA polymerase during DNA replication or unequal crossing-over between homologous chromosomes during recombination (Schlötterer and Tautz, 1992). In non-coding regions of the genome the most abundant class of microsatellites contains dinucleotide repeats (Li et al., 2002). On the other hand, microsatellites found in coding regions of *Arabidopsis*, especially in the 5' Untranslated

Region (5'UTR) are mainly trinucleotide and hexanucleotide repeats, probably due to negative selection against frameshift mutations in coding regions (Morgante et al., 2002). Simple sequence length polymorphisms generated by the different number of repeat units can be easily detected as size differences obtained from DNA amplification using primers complementary to the flanking regions of the repeat motifs. According to Stallings (1992), Lagercrantz et al. (1993) and Hancock (1999), the most abundant motifs in plants are GA and AT repeats and in animals, GT repeats.

SSRs have many advantages over other marker systems and are extensively used in fingerprinting (Thomas and Scott, 1993), linkage map construction (Bowcock et al., 1994; Hazan et al., 1992), forensic DNA research, and population genetic studies (Jarne and Lagoda, 1996). Valuable characteristics of SSR markers include high polymorphism, co-dominance, sensitivity (even a small quantity of DNA can be amplified by PCR), conservation in related species, reproducibility and ease of data scoring. The exchange of primer sequences instead of probes allows other labs to work with the same loci. The only disadvantages of microsatellite markers are that they must be isolated *de novo* from most species being examined for the first time and considerable investment and technical expertise are required for the initial development (Zane et al., 2002; Jauhar, 1996).

In recent years microsatellites have been successfully used in various genetic applications such as cultivar identification and breeding record verification of apple (Cabe et al., 2005) and pistachio (Ahmad et al., 2003); phylogenetic analysis of almond (Xu et al., 2004) and grape (Fatahi et al., 2003); and evaluation of genetic diversity relationships in fruit crops such as kiwifruit (Zhen et al., 2004), apricot (Romero et al.,

2003) and peach (Aranzana et al., 2002). Microsatellites have also been extensively used for management of fruit germplasm such as olive (Khadari et al., 2003), grape (Martin et al., 2003; Dangl et al., 2001), cherry (Cantini et al., 2001) and peach (Testolin et al., 2000); in different applications such as determination of parentage of clonal crops such as cherry (Struss et al., 2003) and olive (Contento et al., 2002); identification of potential duplicates in collections of apple (Hokanson et al., 2001) and grape (Dangl et al., 2001), and detection of chimeras in grapevines (Franks et al., 2002). Furthermore, microsatellites have also been used in evolutionary studies of cacao (*Theobroma cacao* ssp. *cacao*) (Motamayor et al., 2002) and hop (*Humulus lupulus* L.) (Jakśe et al., 2001).

Isolation of SSR markers is an expensive and labor-intensive process. Different approaches have been used to isolate the DNA sequences containing the repeat motifs and their flanking regions. Conventional SSR locus isolation is based on construction of a small insert (< 1000 bp) genomic library and screening thousands of clones using oligonucleotide probes containing the repeat sequences of interest (Rassmann et al., 1991). Standard isolation methods involve fragmentation of genomic DNA with restriction enzymes or sonication. The fragmented DNA is then size-selected in order to obtain small fragments (300-700 bp). Selected fragments are ligated to plasmids and cloned in *Escherichia coli*. The constructed library is then screened by means of Southern hybridization using labeled oligonucleotide probes containing the repeat motifs. Positive clones are selected, sequenced and primers are designed from the regions flanking the repeat motifs. Finally, locus-specific polymerase chain reactions are performed and fragment sizes are determined (Paniego et al., 2002).

In plants, the frequency of positive clones containing dinucleotide repeats in the whole genome is ten times smaller than in the human genome (Powell et al., 1996). In the study of Brown et al. (1996), approximately 0.2% of the library clones of sorghum hybridized with oligonucleotide probes. A second round of hybridization was performed to eliminate false positives, reducing the time and money that would have been wasted on sequencing clones that did not contain microsatellite repeats. Sequencing revealed that 70% of the sequences were useless, because of improper flanking sequences that did not permit primer design, lack of microsatellite repeats in the clones, severely imperfect repeats or a small number of repeats. Furthermore, following oligonucleotide primer synthesis, 65% of the primers failed to produce polymorphic bands in an array of plant genotypes. This low frequency of microsatellite loci in plants generates problems for large-scale isolation of microsatellite loci. Libraries enriched for certain repeat sequences have been constructed to solve this problem.

Different enrichment methods have been developed to increase the efficiency of the microsatellite isolation. They can be categorized according to the biochemical approach used during the enrichment procedure such as enrichment by colony/plaque hybridization, enrichment by primer extension and enrichment by hybridization.

The first method, colony/plaque hybridization involves further selection by means of hybridization prior to sequencing as in the standard isolation method. This second round of hybridization decreases the cost of sequencing and increases the overall efficiency of microsatellite isolation (Scott et al., 1999).

The second method, enrichment by primer extension, was developed by Ostrander et al. (1992) and Paetkau (1999). This method involves generation of a circular single stranded DNA (ssDNA) library by phagemid or phage vectors. ssDNA clones are used as template for repeat-specific oligonucleotide primers. Circular ssDNA molecules are then converted to circular dsDNA molecules by *in vitro* primer-extension. The two methods differ from each other after this step. In Ostrander's approach, the resulting circular dsDNA molecules are transferred into a specific *E.coli* strain, which favors the replication of clones containing primer-extended products resulting in a highly microsatellite-enriched library. Colonies are then screened for the presence of microsatellite loci using labeled oligonucleotide probes. Finally, positive clones are sequenced and primers are designed from the flanking regions of the repeat motif. It has been shown that an enriched library contains 50-fold more positive clones than an unenriched library (Ostrander et al., 1992). In contrast to this first technique, Paetkau (1999) used 5' biotinylated oligonucleotides and Klenow DNA polymerase in primer extension reactions. The products of these reactions in microsatellite-containing phages are linear primer-extended DNA molecules with a biotin at the 5'-end. Microsatellite-containing clones are selected from the reaction mixtures by streptavidin-coated beads. Finally, recovered single-stranded microsatellite-containing DNA molecules are converted to double-stranded molecules by a second round of primer extension and then used in transformation for secondary library construction.

A third class of enrichment method is based on selective hybridization. This technique can be further subdivided into either streptavidin-coated magnetic bead-

mediated enrichment or nylon membrane-mediated enrichment. Streptavidin-coated beads are paramagnetic beads. They are uniformly coated with a protein called streptavidin, which has a high affinity for biotin. This high affinity provides a fast and reliable way to isolate biotin-labeled target molecules. Genomic DNA fragments containing the desired repeat sequences are selected by biotin-labeled oligonucleotides. These hybrid molecules are then captured by streptavidin-coated magnetic beads. The nylon membrane-mediated enrichment method, in contrast to other enrichment techniques, produces enrichment libraries containing a variety of different microsatellite repeats. Enrichment for a pool of different microsatellite repeats is carried out by hybridization of genomic DNA fragments containing microsatellite repeats to a nylon filter bound with different microsatellite oligonucleotides. These two techniques differ from each other only in the type of selective hybridization. Microsatellite-containing fragments are amplified and sequenced by similar protocols with minor modifications (Karagyozov et al., 1993; Armour et al., 1994; Kandpal et al., 1994; Kijas et al., 1994; Edwards et al., 1996; Fisher and Backmann, 1998; Hamilton et al., 1999).

In recent years, microsatellites have been identified from expressed sequence tag (EST) databases as an alternative to enrichment. However, this approach is limited to species for which EST databases exist. Indeed, microsatellites from EST databases have been recently developed for certain species such as rice (Cho et al., 2000), grape (Scott et al., 2000), sugarcane (Cordeiro et al., 2001), rye (Hackauf and Wehling, 2002), barley (Thiel et al., 2003) and loblolly pine (Liewlaksaneeyanawin et al., 2004). Sequences obtained from EST databases are good sources of microsatellites. Since EST-derived

microsatellites are in or near transcribed regions of the DNA, they are expected to be more conserved and more transferable than genomic sequences and to have a lower frequency of null alleles. However, Liewlaksaneeyanawin et al. (2004) reported that EST-derived microsatellites have a lower level of polymorphism than those derived from genomic libraries.

***PCR fragment detection***

There are different methods to determine the sizes of microsatellite-containing PCR products. The most commonly used ones are agarose gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), denaturing PAGE and capillary electrophoresis (CE). Accurate sizing of the fragments with agarose and PAGE gel systems is not easy because of their low resolution power, but denaturing PAGE and capillary electrophoresis can determine fragment size differences as low as a single nucleotide. PCR fragments are visualized in PAGE by silver staining or radioactive labeling. Capillary electrophoresis is the latest and the fastest detection technique. SSR fragments amplified by fluorescently labeled primers can be detected without any post-PCR treatment. Furthermore, different PCR fragments labeled with different florescent tags can be multiplexed and then the products can be run in the same lane with an internal size standard (Donini et. al., 1998).

***Data analysis and statistical terminology for fingerprinting***

Statistical analyses of molecular marker data can be performed with different software packages developed for personal computers. Their easy access, sophisticated and powerful statistical techniques, and user-friendliness make them an attractive alternative to performing calculations on spreadsheets or with self-written programs. Some of the commonly used software packages are MicroSat (Minch et al., 1997), Identity 1.0 (Wagner and Sefc, 1999) and Powermarker 3.23 (Liu and Muse, 2004). These computer programs measure the genetic diversity by calculating the number and the frequency of alleles per locus, the expected heterozygosity ($H_e$) and observed heterozygosity ($H_o$), probability of identity (PI), null allele frequency from heterozygote deficiency (r) (Brookfield, 1996; Paetkau et al., 1995; Weir, 1996), polymorphic information content (PIC) and genetic distance (D) among genotypes (Bowcock et al., 1994). Observed heterozygosity ($H_o$) is the proportion of individuals in a population that is heterozygous at a given number of loci. On the other hand, the expected heterozygosity ($H_e$) is defined as the proportion of individuals which are prospective heterozygotes based on the allele frequencies and assuming Hardy-Weinberg equilibrium. The expected heterozygosity is calculated by the formula $H_e = 1-\sum p_i^2$, where $p_i$ is the frequency of $i^{th}$ allele (Nei, 1973). The null allele frequency is calculated according to the formula of Brookfield (1996): $r = (H_e-H_o)/(1+H_e)$. Probability of identity (PI) is the probability of randomly choosing two individuals in a population that have identical genotypes (Waits et al., 2001). Polymorphic information content (PIC) is defined as the probability that one could identify which homologue of a given parent was transmitted to

a given offspring, the other parent being genotyped as well (Botstein et al., 1980). This value is often used to determine the informativeness of a genetic marker for linkage studies (Guo and Elston, 1999). The PIC value of a locus ranges from 0 (monomorphic) to 1, the latter indicating a highly informative locus. PIC is calculated by the formula $PIC = 1 - \sum_{i} pi^2 - \sum_{i=1} \sum_{j=i+1} 2pi^2 pj^2$ where $p_i$ is the frequency of $i^{th}$ allele. Genetic distance among genotypes is calculated as 1-[proportion of shared alleles] (Bowcock et al., 1994) and a corresponding phenogram can be drawn using the UPGMA (unweighted pair-group method using arithmetic averages) algorithm based on the cluster analysis of the distance data. The proportion of shared alleles method makes no assumption about the populations under study or the frequency of alleles in the population and it does not imply evolutionary divergence. Phenograms are branching phylogenetic diagrams that link entities by estimates of overall similarity. They can be created by different software programs such as TreeView (Page, 1996), PHYLIP CONSENSE (Felsenstein, 1989) and Mega3 (Kumar et al., 2004). Phenograms can be created using one of two different scales, either time or evolutionary divergence. Trees with a time scale are based on some form of physical data, such as a fossil record, that provide dating information. However, more often, the scale used in phylogenetic studies is evolutionary divergence, a measure of change among genotypes. Branches of the phenogram represent the phylogenetic pathway and genetic distance among genotypes and the length of each branch is determined by the scale in horizontal (or vertical) distance.

**Linkage Map Construction by the Double Pseudo-testcross Strategy**

Mapping and sequencing of plant genomes could provide a better understanding of gene function, gene regulation and expression. Marker-saturated high-density linkage maps have useful applications in both fundamental and applied genetic research. Molecular markers are extensively used in identification and isolation of genes of interest, and linkage analysis is one of the basic and central techniques in genetics. Linkage can define the genetic distances between polymorphic traits which may be expressed as differences in appearance of enzyme activities, restriction fragment lengths or nucleotide sequences at an allelic locus (Mohan et al., 1997). In the past few years linkage maps have been constructed for woody perennial plants like *Citrus* (Sankar and Moore, 2001), *Prunus* (Joobeur et al., 1998), cacao (Pugh et al., 2004), apple (Hemmat et al., 1994; Liebhard et al., 2003), grape (Dalbo et al., 2000, Fischer et al., 2004; Riaz et al., 2004), coniferous forest trees (Scott et al., 1999), and olive (Wu et al., 2004). Among the nut crops, linkage maps have been constructed for European hazelnut (Mehlenbacher et al., 2005), European chestnut (*Castanea sativa* L.) (Casasoli et al., 2001) and macadamia (Peace et al., 2003).

Marker-assisted selection (MAS) appears to have promise in hastening the development of disease-resistant high-quality cultivars of clonally-propagated crops such as hazelnut. Many clonally propagated tree crops have a long juvenile phase. MAS would allow selection at the seedling stage for traits not expressed until reproductive maturity. Furthermore, in the development of resistant cultivars, it would allow identification of resistant seedlings in the absence of the pathogen (Mehlenbacher, 1995). Construction of

a genetic linkage map is a useful step in identifying markers linked to genes controlling traits of interest, and genetic maps can also be used to identify loci that control quantitative traits (Paterson et al., 1991). Similar to other perennial tree crops, the juvenile period of hazelnut ranges from three to six years, with five years being the median, and genotypes exhibit sporophytic self- incompatibility (Mehlenbacher and Smith, 1992; Mehlenbacher, 1997). These problems are overcome by the 'two-way pseudo-testcross strategy'. This method involves linkage analysis of an $F_1$ population produced by crossing two highly heterozygous (diploid) parent clones. The first linkage map using this method and PCR-based markers was constructed for *Eucalyptus* (Grattapaglia and Sederoff, 1994). Genetic linkage maps can be constructed using software packages such as MapMaker (Lander et al., 1987), JoinMap (Stam, 1993) or GMendel (Holloway and Knapp, 1994). The two important issues in linkage map construction are locus order and distance. The likelihood odds (LOD) score is the test statistic used to test the hypothesis that there is no linkage against the alternative hypothesis that there is linkage. A LOD score of 3.00 is roughly equal to P=0.001. So, if LOD $\geq$ 3.00, then it is very likely that two loci are linked (Li et al., 2003). Linkage distances with the highest LOD scores are the best estimates of real linkage distances.

## Research Objectives

This research project has two main objectives. The first is to fingerprint 274 accessions of European hazelnut *(Corylus avellana)* representing a wide geographical range using 21 of the simple sequence repeat (SSR) markers recently developed by Bassil et al. (2005) and Boccacci et al. (2005) and to create a phenogram that shows the genetic relationships among them. Of the 274, 70 are suspected duplicate accessions, as they are morphologically identical, but were imported from different collections under different names and 4 are duplicate DNA samples extracted at different times from the same trees. Characterization of European hazelnut accessions by means of SSR markers and comparison of the genotype of each cultivar in the collection will provide an ample opportunity to understand the genetic diversity in hazelnut and help in utilization of available genetics resources of *Corylus avellana.* An understanding of genetic diversity in *C. avellana* will provide information useful in managing germplasm collections, choosing parents for controlled crosses and legally protecting new cultivars.

The second objective is to place 33 SSR markers on the preliminary linkage map constructed by Mehlenbacher et al. (2005) based on RAPD markers to generate a high-resolution linkage map using the 'two-way pseudo-testcross' strategy. The mapping population, a total of 144 seedlings, was generated in 1993 from a controlled cross of two heterozygous selections. The maternal parent, OSU 252.146, is susceptible to eastern filbert blight, while the paternal parent , OSU 414.062, is heterozygous resistant. Because of their ease of use, high information content and co-dominant nature, SSR markers are valuable additions to dominant markers and RFLP markers in mapping projects (Dib et

al., 1996). Placement of these SSR markers on the hazelnut linkage map would allow them to serve as "anchor loci".

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# CHAPTER 2

## CHARACTERIZATION OF EUROPEAN HAZELNUT *(Corylus avellana)* CULTIVARS USING SSR MARKERS

T. Gökirmak, S.A. Mehlenbacher, N.V. Bassil

**Abstract**

Twenty-one pairs of simple sequence repeat (SSR) primers were used to investigate genetic diversity in 270 accessions of European hazelnut *(Corylus avellana)* representing a wide geographic range. A capillary electrophoresis system and ABI Genescan® and Genotyper® software were used to determine the allele sizes generated from each PCR reaction based on an internal lane standard. A total of 211 alleles were amplified and the number of alleles detected ranged from 5 to 15 per locus with an average of 10.05. The observed heterozygosity ($H_o$) for individual loci ranged from 0.24 to 0.88, with an average of 0.67 over all loci. PowerMarker software was used to generate a genetic similarity matrix based on possible pair-wise combinations of accessions using the "proportion of shared alleles". UPGMA cluster analysis was used to construct a phenogram from the genetic similarity matrix using PowerMarker and MEGA3 software. The phenogram revealed geographically tight clusters and some synonyms among European hazelnut cultivars. Of the 270 cultivars in the population, 200 had unique fingerprints while 70 were duplicates, as suspected based on identical morphology. Of the 211 total alleles amplified, 22 were unique, as they were detected only in one cultivar. Nine of the unique alleles were amplified in cultivars that fell outside of the tightly clustered cultivar groups.

## Introduction

The European hazelnut, *Corylus avellana* L., is grown in many regions of Europe and western Asia and has been used as a food source by humans since prehistoric times. *Corylus avellana*, the species of commerce, is native to Europe and Asia Minor and it naturally grows in temperate regions of the Northern Hemisphere from the Atlantic coast of Europe as far north as Norway. The northern boundary includes the British Isles, Scandinavia and northern regions of the Russian Federation. The distribution is bounded in the east by the Ural Mountains. The southern boundary extends from Spain, Morocco and Algeria in the west through Italy, Yugoslavia, Greece, and Turkey to northwestern Iran and Transcaucasia. Although hazelnut is produced in a few regions in the southern hemisphere, it is not native to those regions (Kasapligil, 1972).

Hazelnut-producing regions have mild, moist winters and cool summers. The major production areas are located near large bodies of water at middle latitudes in the northern hemisphere. Approximately 70% of the world's hazelnut production comes from the Black Sea region of Turkey. Italy follows Turkey with about 20% of world production. The United States, Spain, France, Georgia, Azerbaijan and France account for the remaining 10%. The moderate climate of coastal valleys in the Pacific Northwest is well-suited to hazelnut production. Approximately 99% of the U.S. hazelnut crop is produced in Oregon's Willamette Valley. The Pacific Northwest represents 3 to 5 percent of world hazelnut production (FAO Production Year Book, 2004; Hazelnut Marketing Board, 2004).

Hazelnut is a general name used for the nuts of all *Corylus* species. Hazelnut is clonally propagated and a highly heterozygous tree (Mehlenbacher, 1995). It has a unique floral biology, which was reviewed in detail by Germain (1994). It is monoecious, dichogamous and wind-pollinated (Thompsonet al., 1996). The male flowers are borne in catkins, and female inflorescences have no perianth. They appear as a tuft of red stigmatic styles protruding from the apex of compound buds located on shoots or catkin peduncles. The hazelnut tree is self-incompatible, which enforces cross-pollination. Incompatibility is of the sporophytic type, and is under the control of a single locus (the S-locus) with multiple alleles (Mehlenbacher, 1997). If the same allele is expressed in both pollen and pistil, pollen germination is delayed and pollen tubes fail to penetrate the stigmatic surface (Hampson et al., 1993).

Traditional techniques used for correct identification of cultivars and clones are based on morphological characters. However, these techniques are not always useful due to environmental variations (Marinoni et al. 2003). Recently, different DNA-based molecular tools were developed that detect polymorphism and offer a reliable system to characterize plant material based on differences in the genetic material independent of environmental effects. Hence, DNA markers have been used in many different commercial and scientific applications such as marker-assisted selection, confirmation of parent-progeny relationships, taxonomic studies, population genetic analysis, linkage map construction, "fingerprinting" for enforcement of legal protection, quality control in plant production (i.e. trueness to name) and processing and labeling of plant-derived foods and other products.

Among the various DNA-based molecular markers, microsatellites have become the marker of choice because of their advantages over other marker systems. Microsatellites or simple sequence repeats (SSRs) are tandemly repeated 1-6 bp sequence motifs found in both eukaryotic and prokaryotic genomes. They are abundant and dispersed throughout the genome and can be found in both coding and non-coding regions. However, they are more abundant in the latter since non-coding DNA can accumulate mutations more easily than coding DNA (Ahmad et al., 2003). Although the exact mechanism of microsatellite evolution is not clear, it appears most likely due to the slippage of DNA polymerase during DNA replication or unequal crossing-over between homologous chromosomes during meiosis (Schlötterer and Tautz, 1992). In non-coding regions of the genome the most abundant class of microsatellites contains dinucleotide repeats (Li et al., 2002). On the other hand, microsatellites found in coding regions of *Arabidopsis*, especially in the 5' Untranslated Region (5'UTR) are mainly trinucleotide and hexanucleotide repeats, probably due to negative selection against frameshift mutations in coding regions (Morgante et al., 2002).

Microsatellite markers have been extensively used in fingerprinting (Thomas et al., 1993), linkage map construction (Bowcock et al., 1994; Hazan et al., 1992), forensic DNA research, and population genetic studies (Jarne and Lagoda, 1996). Valuable characteristics of SSR markers include high polymorphism, co-dominance, sensitivity (even a small quantity of DNA can be amplified by PCR), conservation in related species, reproducibility and ease of data scoring. The exchange of primer sequences instead of probes allows other labs to work with the same loci. The only disadvantages of

microsatellite markers are that they must be isolated *de novo* from most species being examined for the first time and considerable investment and technical expertise are required for their initial development (Zane et al., 2002; Jauhar, 1996).

In recent years microsatellites have been successfully used in various genetic applications such as cultivar identification and breeding record verification of apple (*Malus x domestica*) (Cabe et al., 2005) and pistachio (*Pistacia vera* L.) (Ahmad et al., 2003); phylogenetic analysis of almond (*Prunus dulcis* Mill.) (Xu et al., 2004) and grape (*Vitis vinifera* L.) (Fatahi et al., 2003); and evaluation of genetic diversity relationships in fruit crops such as kiwifruit cultivars (*Actinidia deliciosa* A.Chev. ) (Zhen et al., 2004), apricot (*Prunus armeniaca* L.) (Romero et al., 2003) and peach (*Prunus persica* L.) (Aranzana et al., 2002). Microsatellites have also been extensively used for management of fruit germplasm collections in olive (*Olea europaea* L.) (Khadari et al., 2003), grape (Martin et al., 2003; Dangl et al., 2001), cherry (*Prunus avium* L.) (Cantini et al., 2001) and peach (Testolin et al., 2000); in different applications such as determination of parentage of clonal crops such as cherry (Struss et al., 2003) and olive (Contento et al., 2002); identification of potential duplicates in collections of apple (Hokanson et al., 2001) and grape (Dangl et al., 2001), detection of chimeras in grapevines (Franks et al., 2002). Furthermore, microsatellites have also used in evolutionary studies of cacao (*Theobroma cacao* ssp. *cacao* L.) (Motamayor et al., 2002) and hop (*Humulus lupulus* L.) (Jaksé et al., 2001).

The objective of this study is to use 21 pairs of simple sequence repeat (SSR) primers to investigate genetic diversity in 270 accessions of European hazelnut *(Corylus*

*avellana)* representing a wide range of geographical regions, and which includes many suspected duplicates. The genotyping or fingerprinting of European hazelnut cultivars provides information important for the management of genetics resources of *Corylus avellana*.

## Materials and Methods

### *Plant material and DNA extraction*

Two sets of fresh young leaves for each of 270 accessions of European hazelnut (Table 2.1), representing a wide range of geographical regions, were collected in the spring from the collections of the United States Department of Agriculture-Agriculture Research Service-National Clonal Germplasm Repository (USDA-ARS-NCGR) and Oregon State University in Corvallis, Oregon. DNA was extracted according to Lunde et al. (2000) with minor modifications and RNA was removed by incubation with RNase A (Sigma, St. Louis, MO) at 37 °C for one hour in a shaker, followed by extraction with 25 phenol: 24 chloroform: 1 isoamyl alcohol. The DNA concentrations were determined spectrophotometrically, adjusted to 5 ng/µl and the DNA was stored at -18 °C until used for PCR. A second set of DNA samples was extracted from four trees ('Bard', 'Karidaty', 'Istarski Debeloplodna' and 'Riekchen's Zellernuss') and included as checks.

### *Amplification and allele sizing*

Primer pairs for fourteen loci developed in Corvallis (CAC) (Bassil et al., 2005) and for seven loci in Torino (CaT) (Boccacci et al., 2005) (Table 2.2) were chosen for this study. Reverse primers were purchased from Operon Technologies (Qiagen, Valencia, CA). Forward primers fluorescently labeled with FAM and HEX were purchased from Operon Technologies (Qiagen, Valencia, CA) and with NED from Applied Biosystems (Foster City, CA) and PCR reactions were performed in a total

**Table 2.1** European Hazelnut accessions characterized using microsatellite markers.

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557207 | N04.39 | A Pellicola Bianca | Italy | 10 | 11 |
| PI 617226 | N05.11 | Acorn Hazel | Poland | 5 | 11 |
| PI 557418 | N03.67 | Alcover | Italy-Piemonte | 15 | 22 |
| — | Reps 72.02 | Alli | Estonia | 20 | ? |
| PI 617190 | W15 | Amandi | Spain | 10 | 21 |
| PI 557176 | N03.62 | Apolda | unknown | 10 | 11 |
| PI 557079 | N07.46 | Arneson's Rootstock | USA-Oregon | ? | ? |
| PI 557108 | N03.66 | Artellet | Spain | 14 | 18 |
| PI 557422 | N02.15 | Ata Baba Ganja | Azerbaijan (France) | 4 | 4 |
| PI 557195 | N05.41 | Atlas | Denmark | 1 | 10 |
| PI 557050 | N03.34 | Aurea | Germany | 6 | 9 |
| PI 557194 | N06.71 | Aveline d'Angleterre | France or England | 5 | 16 |
| PI 617174 | N06.37 | Aveline Rouge | France | 5 | 10 |
| PI 557213 | N04.37 | Avellana Speciale | Italy | 1 | 2 |
| PI 557122 | N05.25 | B-3 | Macedonia | 2 | 25 |
| PI 557125 | N06.44 | B-4 | Macedonia | 10 | 17 |
| PI 304630 | N07.17 | Badem | Turkey | 2 | 5 |
| PI 557157 | N05.67 | Bandnuss | England | 10 | 11 |
| PI 557177 | N02.60 | Barbarella | Italy | 5 | 10 |
| PI 557037 | N02.34 | Barcelona | Spain-Tarragona | 1 | 2 |
| PI 557156 | N05.71 | Barcelonner Zellernuss | England | 10 | 17 |
| PI 557241 | N02.26 | Bard | England | 5 | 11 |
| — | N04.03 | Barrettona | Italy-Lazio | 2 | 6 |
| PI 557181 | N06.61 | Barrettona (not) | Italy | 1 | 2 |
| PI 557158 | N05.70 | Barr's Zellernuss | England | 5 | 11 |
| PI 557214 | N04.48 | Bearn | France | 5 | 11 |
| PI 557239 | N02.39 | Belle di Giubilino #1 | Italy (via Spain) | 1 | 10 |
| — | N04.19 | Belle di Giubilino #2 | Italy-Campania | 1 | 10 |
| PI 557114 | N06.13 | Bergeri | Belgium | 3 | 25 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557182 | N06.62 | Bianca | Italy-Campania | 2 | ? |
| PI 557084 | 212.053 | Blumberger Zellernuss | Germany | 4 | 20 |
| PI 557131 | N01.62 | Brixley's New | England | 1 | 15 |
| PI 557031 | N04.30 | Brixnut | USA-Oregon | 1 | 14 |
| PI 557219 | N04.27 | Bulgaria XI-8 | Bulgaria | 4 | 12 |
| PI 557089 | N05.45 | Burchardt's Zellernuss | Germany | 2 | 7 |
| PI 557077 | N04.18 | Butler | USA-Oregon | 2 | 3 |
| PI 557094 | N04.21 | Buttner's Zellernuss | Germany | 11 | 27 |
| PI 617207 | N06.09 | C. avellana AL55 | Albania | 3 | ? |
| PI 637867 | N06.08 | C. avellana COR 627 | Sweden | 9 | ? |
| PI 617181 | N03.48 | C. multiflorum WIR8884 | Turkey/Russia | 3 | 11 |
| PI 296204 | N02.14 | Camponica | Italy-Campania | 1 | 2 |
| PI 557189 | N06.70 | Carello | Italy | 1 | 2 |
| PI 557033 | N03.18 | Casina | Spain-Asturias | 10 | 21 |
| PI 557235 | N03.31 | Ceret | Spain-Tarragona | 2 | 10 |
| PI 617176 | N01.47 | Cherkesskii II | Southern Russia | 4 | 24 |
| PI 617268 | LB3 | Clark | OSU : 'Tombul Ghiaghli' x 'Willamette' | 3 | 8 |
| PI 557109 | N06.02 | Closca Molla | Spain | 2 | 5 |
| PI 557178 | N03.19 | Comen | Greece | 2 | 9 |
| PI 557223 | N02.37 | Comun | Portugal | 10 | ? |
| PI 557184 | N06.60 | Comun Aleva | Spain | 9 | 23 |
| PI 557185 | N03.60 | Comune di Sicilia | Italy | 1 | 2 |
| PI 557049 | N01.15 | Contorta | England | 5 | 10 |
| PI 557424 | N06.05 | Corabel | France: Barcelona o.p. | 1 | 3 |
| PI 557039 | N06.16 | Cosford | England | 3 | 11 |
| PI 617172 | N07.43 | Cozia | Romania | 5 | 15 |
| PI 557024 | N04.20 | Creswell | USA-Oregon | 2 | 10 |
| PI 557107 | N01.21 | Culpla | Spain-Tarragona | 9 | 10 |
| PI 557306 | N03.42 | Cutleaf | England | 20 | 28 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557224 | N01.35 | Da Viega | Portugal | 10 | 21 |
| PI 617240 | N04.04 | Dal Rosso | Italy | 5 | 18 |
| PI 637876 | 780.067 | D'Algers | Netherlands | 1 | 2 |
| PI 557227 | N02.35 | Daria (104E) | Italy : 'Tonda Gentile delle Langhe' x 'Cosford' | 2 | 3 |
| PI 557040 | N05.20 | Daviana | England | 3 | 11 |
| PI 637883 | LB8.27 | Delta | OSU : 'OSU 249.159' x ('Montebello' x 'Gasaway') | 1 | 15 |
| PI 557423 | N03.17 | Des Anglais | France | 5 | 19 |
| PI 557145 | N05.66 | Dowton Long #1 COR 314 | England | 3 | 14 |
| PI 557190 | N07.66 | Dowton Long #2 COR 381 | England | 9 | 10 |
| PI 557099 | N05.29 | DuChilly | England | 10 | 14 |
| PI 557161 | N03.71 | Early Long Zellernuss | Denmark | 20 | 25 |
| PI 557045 | N02.30 | Ennis | USA-Washington | 1 | 11 |
| PI 637884 | LB11.35 | Epsilon | OSU : ('Tonda Romana' x 'Tombul Ghiaghli') x 'Zimmerman' | 1 | 4 |
| PI 557236 | N03.39 | Espinaredo | Spain | 10 | 21 |
| PI 318463 | N06.01 | Extra Ghiaghli | Greece | 4 | 12 |
| PI 557080 | N03.41 | Finland COR 18-7 | Finland | ? | ? |
| PI 557071 | N07.48 | Fitzgerald | USA-Oregon | ? | ? |
| PI 557032 | N03.30 | Fitzgerald #20 | USA-Oregon | 2 | 11 |
| PI 557205 | N04.29 | Francoli | Spain-Taragona | 17 | 22 |
| PI 617227 | N05.10 | Frango #2 | Poland | 5 | 11 |
| PI 617228 | N05.09 | Frango #4 | Poland | 15 | 25 |
| PI 617229 | N5.08 | Frango #5 | Poland | 11 | 25 |
| PI 557135 | N01.63 | Freehusker | USA-Oregon | 1 | 11 |
| PI 557097 | N07.34 | Frizzled Filbert | England | 9 | 10 |
| PI 557238 | N01.37 | Fructo Albo | France | 5 | 10 |
| PI 557208 | N04.45 | Fruttogrosso | Italy | 10 | 18 |
| PI 557047 | N01.36 | Fusco Rubra | Germany | 6 | 19 |
| PI 637882 | LB10.11 | Gamma | OSU : 'Casina' x ('Riccia di Talanico' x 'Gasaway') | 2 | 10 |
| PI 634202 | 754.001 | Ganja | Azerbaijan (via Georgia) | 4 | 4 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557162 | N04.69 | Garibaldi | England | 5 | 11 |
| PI 557165 | N06.03 | Garrofi | Spain | 1 | 6 |
| PI 557042 | N02.27 | Gasaway | USA-Washington | 3 | 26 |
| PI 557029 | N01.22 | Gem | USA-Oregon | 2 | 14 |
| PI 557115 | N05.17 | Ghirara | Italy-Sicily | 2 | 21 |
| PI 270339 | N04.71 | Gironenc Coldejou | Spain | 9 | 23 |
| PI 270339 | N03.25 | Gironenc Vermellet | Spain-Taragona | 2 | 17 |
| PI 617230 | N05.07 | Goc | Poland | 6 | 15 |
| PI 617189 | V06 | Grande | Spain | 1 | 2 |
| PI 557202 | N02.43 | Grifoll | Spain-Tarragona | 2 | 22 |
| PI 557148 | N04.60 | Gubener Barcelloner | Germany | 1 | 23 |
| — | 759.016 | Gulshishvela (not) | Georgia | 4 | 20 |
| PI 557191 | N07.70 | Gunslebert | Germany | 5 | 23 |
| PI 557085 | N06.51 | Gustav's Zellernuss | Germany | 15 | 20 |
| PI 557027 | N04.64 | Hall's Giant | Germany | 5 | 15 |
| PI 557427 | N02.55 | Henneman #3 | USA-Oregon | 6 | 10 |
| PI 557196 | N01.54 | Heynick's Zellernuss | Germany | 15 | 20 |
| — | 753.001 | Hodji (not) | Georgia | 14 | ? |
| PI 557183 | N07.63 | Iannusa Racinante | Italy-Sicily | 1 | 8 |
| PI 271105 | N01.17 | Imp. de Trebizonde #1 | Turkey | 2 | 10 |
| PI 211105 | N03.49 | Imp. de Trebizonde #2 | Turkey | 2 | 10 |
| PI 557051 | N07.69 | Imperatrice Eugenie | France | 3 | 14 |
| PI 557209 | N04.23 | Istarski Debeloplodna | Croatia | 5 | 10 |
| PI 557210 | N04.25 | Istarski Okrogloplodna | Slovenia | 10 | 18 |
| PI 557400 | N05.27 | Istrski Duguljasti | Macedonia | 10 | 17 |
| PI 557034 | N01.32 | Italian Red | Germany | 15 | 20 |
| PI 557116 | N05.31 | Jean's | Italy | 2 | 10 |
| PI 557169 | N05.64 | Jeeve's Samling | England | 12 | 20 |
| PI 557090 | N04.62 | Kadetten Zellernuss | Germany | 20 | 25 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557240 | N06.11 | Kalinkara | Turkey | 4 | 21 |
| PI 271105 | N03.37 | Karidaty | Turkey | 2 | 10 |
| PI 617231 | N05.06 | Karol | Poland | 11 | 15 |
| PI 617180 | V9a | Kerasund Dlinnyi | Russia | 8 | 10 |
| PI 557197 | N02.51 | Korthaset Zellernuss | Denmark | 10 | 14 |
| PI 557030 | N01.34 | Kruse | USA-Oregon | 1 | 2 |
| PI 617177 | N02.54 | Kudryavchik | Russia | 4 | 14 |
| — | 755.001 | Kudryavchik (not) | Russia | 4 | 14 |
| PI 557170 | N05.69 | Kunzemuller's Zellernuss | Germany | 12 | 20 |
| PI 557153 | N05.68 | Lange Landsberger | Germany | 15 | 20 |
| PI 617232 | N05.05 | Lech | Poland | 5 | 15 |
| PI 617233 | N05.04 | Lenka #3 | Poland | 3 | 5 |
| PI 617234 | N05.03 | Lenka #4 | Poland | 11 | 15 |
| PI 617210 | LB1 | Lewis | OSU : ('Barcelona' x 'Tombul Ghiaghli') x 'Willamette' | 3 | 8 |
| PI 557147 | N04.61 | Liegel's Zellernuss | Germany | 12 | 20 |
| PI 617235 | N05.02 | Little Poland | Poland | 3 | 5 |
| PI 557168 | N03.69 | Lluenta | Spain-Tarragona | 17 | 22 |
| PI 557186 | N06.69 | Locale di Piazza Armerina | Italy | 1 | 2 |
| PI 557086 | N03.52 | Louisen's Zellernuss | Germany | 10 | 25 |
| PI 557091 | N04.63 | Ludolph's Zellernuss | Germany | 5 | 20 |
| PI 557136 | N06.19 | Lyons | USA-Oregon | 2 | 14 |
| PI 557137 | N07.59 | Macrocarpa | Germany | 1 | 2 |
| PI 557174 | N06.65 | Mansa | Italy | 1 | 2 |
| PI 617236 | N05.01 | Maria | Poland | 11 | 15 |
| PI 557203 | N05.53 | Martorella | Spain-Taragona | 17 | 22 |
| — | W21 | Mincane | Turkey-Akçcakoca | 4 | 10 |
| PI 557215 | N04.31 | Minnolara | Italy | 1 | 2 |
| PI 557093 | N05.62 | Mogulnuss | England | 5 | 25 |
| PI 557225 | N03.29 | Molar | Portugal | 2 | 10 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557028 | N01.20 | Montebello | Italy-Sicily | 1 | 2 |
| PI 270338 | N02.36 | Morell | Spain-Tarragona | 1 | 2 |
| PI 339723 | N01.59 | Mortarella | Italy-Campania | 2 | 17 |
| PI 557152 | N04.67 | Multiflora | England | 3 | 11 |
| PI 557188 | N03.61 | Napoletana | Italy-Campania | 1 | 23 |
| PI 557216 | N01.14 | Napoletanedda | Italy-Campania | 2 | 14 |
| PI 270340 | N03.14 | Negret | Spain-Tarragona | 10 | 22 |
| — | 756.003 | Nemsa (not) | Georgia | 4 | 14 |
| PI 271278 | N04.26 | Neue Riesennuss | Germany | 18 | 25 |
| PI 557138 | N07.37 | Nixon | USA-Oregon | 2 | 3 |
| PI 557179 | N06.67 | Nocchiolino Sangrato | Italy-Piemonte | 7 | 17 |
| PI 557180 | N06.66 | Nocchione | Italy | 1 | 2 |
| PI 557139 | N01.65 | Noce Lungha | Italy | 10 | 17 |
| PI 557193 | N06.63 | Nociara | Italy-Sicily | 1 | 3 |
| PI 557038 | N01.18 | Nonpareil | USA-Oregon | 1 | 3 |
| PI 557259 | N04.16 | Nooksack | USA-Washington | 6 | 14 |
| PI 557172 | N03.63 | Nostrale | Italy | 1 | 2 |
| PI 557140 | N01.66 | Nottingham | England | 8 | 10 |
| PI 637875 | 780.076 | Obrovsky Novy | Slovakia | 12 | 20 |
| PI 557417 | N06.64 | Ordu | Turkey | 4 | 25 |
| PI 304632 | N03.27 | Palaz | Turkey | 2 | 4 |
| PI 557187 | N06.68 | Pallagrossa | Italy-Piemonte | 5 | 25 |
| PI 557119 | N02.17 | Pauetet | Spain-Tarragona | 18 | 22 |
| PI 557211 | N04.33 | Payrone | Italy | 10 | 18 |
| PI 557159 | N04.70 | Pearson's Prolific | England | 8 | 10 |
| PI 271110 | N06.20 | Pell. Rouge | France | 5 | 10 |
| PI 557048 | USDA Shop | Pendula | England | 3 | 9 |
| PI 557164 | N04.15 | Pere Mas | Spain | 9 | 10 |
| PI 617192 | N02.40 | Petoka (not) | England | 3 | 11 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557163 | N02.25 | Pinyolenc #1 COR 339 | Spain | 2 | ? |
| — | 212.052 | Pinyolenc #1b | Spain | 2 | ? |
| — | N07.03 | Pinyolenc #2 | Spain-Tarragona | 2 | 17 |
| — | W10b | Pinyolenc #2b | Spain-Taragona | 2 | 17 |
| PI 617178 | V14 | Pioneer | Ukraine | 2 | 4 |
| PI 557204 | N06.04 | Planeta | Spain-Tarragona | 1 | 2 |
| PI 557155 | N04.66 | Princess Royal | England | 11 | 14 |
| PI 557154 | N05.60 | Prolific Closehead | England | 5 | 11 |
| PI 557150 | N05.63 | Prolifique a Coque Serrée | France | 3 | 11 |
| PI 637873 | 780.072 | Pruhovany | Slovakia | 3 | 11 |
| PI 557105 | N06.55 | Punxenc | Spain | 1 | 10 |
| PI 637909 | GH | Purple Aveline | France | 5 | 10 |
| PI 617183 | N05.12 | Purple Fortrin | USA-Washington | 5 | 10 |
| PI 557128 | W16 | Quiros | Spain | 10 | 21 |
| PI 557217 | N04.35 | Racinante clone G | Italy | 1 | 2 |
| PI 557201 | N07.57 | Ratllada | Spain-Tarragona | 10 | 22 |
| PI 557167 | N03.70 | Ratoli | Spain-Tarragona | 2 | 10 |
| PI 557098 | N05.43 | Red Filbert | England | 11 | 14 |
| PI 557098 | N05.13 | Red Fortrin | USA- Washington | 2 | 6 |
| PI 557129 | N01.23 | Restiello | Spain-Tarragona | 10 | 22 |
| PI 557055 | N05.33 | Ribet | Spain-Tarragona | 2 | 16 |
| PI 339725 | N03.26 | Riccia di Talanico | Italy-Campania | 1 | 2 |
| PI 557199 | N04.17 | Riekchen's Zellernuss | Germany/Denmark | 5 | 25 |
| PI 637874 | 780.065 | Rimsky | Slovakia | 10 | 25 |
| PI 271280 | N07.11 | Rode Zeller | Netherlands | 6 | 11 |
| PI 557233 | N02.62 | Romai | | 10 | 18 |
| PI 617173 | N05.23 | Romavel | Romania | 2 | ? |
| PI 557171 | N05.65 | Romische Nuss | unknown | 10 | 18 |
| PI 557112 | W09 | Ros de la Selva | Spain | 2 | 9 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557113 | N07.68 | Rosetta | Spain | 2 | 6 |
| PI 557113 | N07.29 | Rosset de Valls | Spain | 14 | 18 |
| PI 557052 | N04.52 | Royal | USA-Oregon | 1 | 3 |
| PI 557390 | N01.55 | Ruby | USA-Oregon : 'Chinese Trazel G-4' x 'Fusco Rubra' | 11 | 19 |
| PI 557044 | N02.20 | Ryan | USA-Oregon | 1 | ? |
| PI 557401 | N03.65 | San Benedetto | | 4 | 12 |
| PI 557117 | N01.29 | San Giovanni | Italy-Campania | 2 | 8 |
| PI 557103 | N03.50 | Sant Jaume | Spain-Tarragona | 1 | 17 |
| — | N03.21 | Sant Joan | Spain | 2 | 25 |
| PI 55 7120 | N07.19 | Sant Pere | Spain-Tarragona | 22 | 26 |
| PI 557046 | N01.16 | Segorbe | Spain | 9 | 23 |
| | 780.059 | Shokoladny | Ukraine | 4 | 11 |
| PI 557175 | N03.68 | Siciliana | Italy | 1 | 2 |
| PI 557151 | N05.61 | Sickler's Zellernuss | Germany | 5 | 20 |
| PI 557166 | N01.25 | Simon | Spain-Tarragona | 6 | 22 |
| PI 304633 | N07.47 | Sivri Ghiaghli | Turkey-Greece | 4 | 12 |
| — | 759.025 | Sivri Ocak 5 | Turkey | 8 | 10 |
| PI 617175 | Reps 57.33 | Skorspelka | Russia | 4 | 23 |
| PI 617175 | 780.063 | Syrena | Poland | 6 | 15 |
| PI 617239 | 780.044 | Tapparona di San Colombano Cortemoli | Italy-Liguria | 2 | 24 |
| PI 557149 | N02.47 | The Shah | England | 14 | 30 |
| PI 637908 | Reps 58.32 | Tokolyi Cosford | Australia | 5 | 23 |
| PI 557200 | N05.19 | Tomasina | Spain-Tarragona | 17 | 22 |
| PI 318463 | W22 | Tombul | Turkey-Akçakoca | 4 | 10 |
| PI 304634 | N03.32 | Tombul Ghiaghli | Turkey-Greece | 4 | 8 |
| — | 759.043 | Tombul Ocak 1 | Turkey | 4 | 12 |
| PI 296206 | N07.62 | Tonda Bianca | Italy-Campania | 1 | 23 |
| PI 296207 | N01.24 | Tonda di Giffoni | Italy-Campania | 2 | 23 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557075 | N05.58 | Tonda Gentile d. Langhe COR114 | Italy-Piemonte | 2 | 7 |
| PI 557025 | N02.18 | Tonda Romana | Italy-Viterbo | 10 | 20 |
| PI 557118 | N02.21 | Tonda Rossa | Italy-Campania | 8 | 23 |
| PI 557218 | N03.45 | Tonnolella | Italy | 2 | 24 |
| PI 557110 | N07.23 | Trenet | Spain-Tarragona | 2 | 15 |
| PI 557087 | N04.65 | Truchsess' Zellernuss | Germany | 5 | 25 |
| — | 759.007 | Tskhenis Dzudzu (not) | Georgia | 4 | 20 |
| PI 557245 | N01.68 | Turk | USA-Oregon | 1 | 2 |
| PI 557100 | N06.58 | Ugbrooke | New Zealand | 5 | 9 |
| — | V13 | Unknown #3 | unknown | 4 | 8 |
| PI 617171 | N07.31 | Vilcea 22 | Romania | 2 | 10 |
| PI 617238 | N04.06 | Volski Round | Poland | 5 | 11 |
| PI 557144 | N07.39 | Wanliss Pride | Australia | 2 | 10 |
| — | N07.06 | Warsaw Red | Poland | 1 | 6 |
| PI 557160 | N04.68 | Webb's Prize Cob | England | 17 | ? |
| PI 557080 | N02.50 | White Filbert | France | 5 | 10 |
| PI 557101 | N04.43 | Whiteheart | New Zealand | 2 | 10 |
| PI 557234 | N02.71 | Willamette | OSU : 'Montebello' x 'Compton' | 1 | 3 |
| PI 557026 | N03.22 | Woodford | USA-Oregon | 1 | 3 |
| PI 637885 | LB11.40 | Zeta | OSU : 'OSU 342.019' x 'Zimmerman' | 1 | 1 |
| — | LB 8.40 | Zimmerman | USA-Oregon | 1 | 3 |
| PI 323961 | N07.60 | 26.072 | Russia-North Caucasus | 2 | 6 |
| PI 557057 | N06.18 | 54.021 | Turkey-Giresun | 4 | 5 |
| PI 557060 | N06.24 | 54.039 | Turkey-Giresun | 8 | 12 |
| PI 557061 | N06.46 | 54.041 | Turkey-Giresun | 4 | 24 |
| PI 557066 | N06.22 | 54.056 | Turkey-Giresun | 2 | 5 |
| PI 617266 | N05.40 | 408.040 | University of Minnesota | 15 | 20 |
| PI 557421 | Reps 46.02 | 495.049 | Southern Russia | 22 | 29 |

**Table 2.1** Continued

| Accession # | Location | Cultivar | Origin or Pedigree | S-Alleles | |
|---|---|---|---|---|---|
| PI 557421 | N06.53 | 495.072 | Southern Russia | 6 | ? |
| PI 637878 | N06.07 | 556.019 | Turkey-Istanbul | 8 | 17 |
| PI 617269 | Reps52.35 | 556.027 | Turkey-Istanbul | 8 | 18 |
| — | Reps50.34 | 622.051 | Turkey-Ray Clark | 4 | 25 |
| CCOR 783 | LB12.16 | 681.074 | Turkey-Akçakoca | 4 | 12 |
| PI 634203 | Reps 63.15 | 681.078 | Russia-Moscow | 1 | 14 |
| CCOR 785 | LB12.18 | 686.124 | Turkey-Giresun | 10 | 12 |
| CCOR 792 | LB12.25 | 693.073 | Turkey-Giresun | 4 | 10 |
| — | LB12.27 | 693.117 | Turkey-Yomra | 4 | 14 |
| CCOR 799 | LB12.32 | 702.041 | Turkey-Yomra | 4 | 8 |
| — | LB12.33 | 717.087 | Turkey-Akçakoca | 4 | 16 |

**Table 2.2** Description of 21 microsatellite loci: the repeat motif, sequence of the fluorescent forward primer (*FAM, NED, HEX*) and the reverse primer (*R*), the optimum annealing temperature (Tm), dilution cofactor in multiplex, allele size range, and linkage group[e]

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range (nt) | Linkage Gr[e] |
|---|---|---|---|---|---|---|
| CAC-A014a | $(CA)_{13}$ | ***FAM***-GGTTTGTTACAGAAATTCAGACG | 60°C | 1:640 | 203-251 | 5S, 5R |
| | | ***R***-GCGTGTGGTTAATGTTTTCTTT | | | | |
| CAC-A040 | $(CA)_{13}$ | ***NED***-TGCTCAAGCAAATATTGCAC | 62°C | 1:213 | 234-248 | 1S, 1R |
| | | ***R***-GTTTGGGATCCAATTAACCCTCT | | | | |
| CAC-B005 | $(GA)_{22}$ | ***FAM***-CAAACTTATGATAGGCATGCAA | 62°C | 1:320 | 277-297 | 7R |
| | | ***R***-TGTCACTTTGGAAGACAAGAGA | | | | |
| CAC-B010 | $(GA)_{16}$ | ***FAM***-AGCTTCCAAATCACACATTACC | 62°C | 1:320 | 211-227 | 3S, 3R |
| | | ***R***-GAAGAGCATCCGTATGATTCAG | | | | |
| CAC-B028 | $(AG)_{16}$ | ***NED***-ATGGACGAGGAATATTTCAGC | 55°C | 1:213 | 252-288 | 5S, 5R |
| | | ***R***-CCTGTTTCTCTTTGTTTTCGAG | | | | |
| CAC-B029b | $(GA)_{13}$ | ***NED***-CAATTTACACCTCAGGGAAGAG | 58°C | 1:160 | 114-139 | 1S, 1R |
| | | ***R***-AAGTTCACCCAAGAAATCCAC | | | | |
| CAC-B105 | $(GA)_{16}$ | ***HEX***-AAAGGAGCAAGCATGTTAGG | 62°C | 1:320 | 125-163 | 10S, 10R |
| | | ***R***-GTTTGTACGGATGATCCACTGAG | | | | |
| CAC-B111 | $(GA)_{13}$ | ***FAM***-GAAGGAGAAACAAGGGTAGTCA | 64°C | 1:320 | 170-192 | N/A |
| | | ***R***-AGAAGCGTCGTTCCATAGC | | | | |
| CAC-C010 | $(GAA)^{a}$ | ***NED***-GGAGCCACCATGAAATTATACA | 58°C | 1:320 | 272-319 | N/A |
| | | ***R***-CACTTATTGCGATTGGTTCA | | | | |

**Table 2.2** Continued

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range (nt) | Linkage Gr[e] |
|---|---|---|---|---|---|---|
| CAC-C028 | $(GAA)_{10}$ | ***NED***-CTACCCCATCGCTTGACAC | 60°C | 1:213 | 131-147 | 10R |
| | | ***R***-GGAGACTTGTTTGCCACAGA | | | | |
| CAC-C040 | $(GAA)_8(GGA)_5$ | ***FAM***-AGCCCCATTAGCCTTCTTAG | 62°C | 1:320 | 168-192 | 4R |
| | | ***R***-GTTTCCAGATCTGCCTCCATATAAT | | | | |
| CAC-C115 | $(TAA)_5(GAA)_{12}$ | ***FAM***-CATTTTCCGCAGATAATACAGG | 60°C | 1:320 | 167-225 | 4S, 4R |
| | | ***R***-GTTTCCAGATCTGCCTCCATATAAT | | | | |
| CAC-C118 | $(AAG)^b$ | ***HEX***-AGCAACAGAGGTTAGGTGTG | 60°C | 1:320 | 162-185 | 4R |
| | | ***R***-GCCCCATTAGCCTTCTTA | | | | |
| CAC-C119 | $(GA)_7(GA)_9$ | ***NED***-CTCACCTTTACCCCTTCATTTT | 62°C | 1:213 | 256-264 | 8R |
| | | ***R***-GTTTCCTCATCTTCTGAGAACCATC | | | | |
| CaT-B107 | $(CT)_{14}$ | ***NED***-GTAGGTGCACTTGATGTGCTTTAC | 58°C | 1:160 | 112-151 | 10R |
| | | ***R***-AACACCATATTGAGTCTTTCAAAGC | | | | |
| CaT-B502 | $(CT)^c$ | ***FAM***-CTCATGACTGCCCATTTCTCG | 62°C | 1:400 | 183-211 | 10S, 10R |
| | | ***R***-AGGCATGCAGGCTTCACAC | | | | |
| CaT-B504 | $(CT)_{18}$ | ***HEX***-CGCCATCTCCATTTCCCAAC | 60°C | 1:400 | 158-184 | 7R |
| | | ***R***-CGGAATGGTTTTCTGCTTCAG | | | | |
| CaT-B505 | $(CT)_{17}CC(CT)_2$ | ***NED***-AGAGAACGACTTTGTATGACAAAGA | 58°C | 1:213 | 106-139 | N/A |
| | | ***R***-TTGAACCATTAAATACATCATGTGA | | | | |

**Table 2.2** Continued

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range (nt) | Linkage Gr[e] |
|---|---|---|---|---|---|---|
| CaT-B507 | $(GA)^{d}$ | ***FAM***-CTA AGCTCACCAAGAGGAAGTTGAT | 62°C | 1:400 | 176-198 | 9S, 9R |
| | | ***R***-GCTTCTGGGTCTCCTGCTCA | | | | |
| CaT-B508 | $(GA)_{10}$ | ***HEX***-GGGTCAAGATTTGATAAAGTGGGA | 62°C | 1:213 | 142-167 | N/A |
| | | ***R***-GCACTCCACTTGTGCGTTTTC | | | | |
| CaT-C504 | $(CTT)_2T(CTT)_8$ | ***HEX***-GGTCTCCTTCGCTAACATAACCAA | 62°C | 1:400 | 152-173 | N/A |
| | | ***R***-GTTGCCCTCGAGTTGTAGTA | | | | |

[a] $(GAA)_7GGA(GAA)_2N_{21}(GAA)_2ATT(GAA)_4N_{15}(GAA)_3$
[b] $(AAG)_3(GAA)_3(AAG)_8N_6(AAG)_4$
[c] $GA)_1GC(GA)_2GC(GA)_{14}$
[d] $(CT)_{16}GCTTTTC(CT)_5$
[e] Chapter 3 (Figure 3.1)
N/A: linkage group not assigned

volume of 10 µl. The reaction mixture contained 1X Biolase $NH_4$ reaction buffer, 2 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.3 µM each of forward and reverse primers, 0.25 units of Biolase DNA polymerase (Bioline Inc., Randolph, MA), and 2.5 ng of template DNA. The PCR program consisted of 35 cycles of a 40 s denaturation step at 94 °C, a 40 s annealing step at the optimum annealing temperature (Table 2.2), and a 40 s extension step at 72 °C. Finally one 30-min extension step at 72 °C was run to maximize non-templated adenosine addition to the 5' ends. PCRs were carried out in Perkin-Elmer model 9700 thermocyclers (PE Applied Biosystems, Foster City, CA). PCR amplification and approximate fragment sizes were confirmed by agarose (3%) gel electrophoresis using 4 µl of aliquot and 5 µl of loading dye (15% Ficoll® 400, 0.03% xylene cyanol FF, 0.4% orange G, 10 mM Tris-HCL pH 7.5, and 50 mM EDTA). Gels were stained with ethidium bromide and photographed under UV-light by an imaging system (UVP, Upland, CA). Amplified PCR products were diluted forty times with nanopure water and kept as stock for multiplexing. Stock solutions were further diluted 2 to 16 times (Table 2.2) and 1 µl of a mix of three to four PCR products (Appendix A) were separated on an ABI 3100 capillary electrophoresis instrument (Applied Biosystems, Foster City, California) at the OSU Central Services Laboratory (CSL), and DNA fragments were sized using GeneScan and Genotyper software.

***Data Analysis***

The programs *IDENTITY 1.0* (Wagner and Sefc, 1999), *PowerMarker 3.2* (Liu and Muse, 2004) and *Mega3* (Kumar et al., 2004) were used to analyze the data. The number of alleles, allele frequencies, observed heterozygosity ($H_o$), expected

heterozygosity ($H_e$), probability of identity (PI) and paternity exclusion probability (PEP) (Paetkau et al., 1995; Weir, 1996) were calculated by *IDENTITY 1.0. PowerMarker 3.2* software was used to estimate the polymorphic information content (PIC). The statistical analysis was carried out twice, both with and without duplicate accessions. The statistical summary (Table 2.4) of 21 SSR loci based on 200 unique accessions is presented in this chapter. A similar table including all 274 accessions is presented in the Appendix B.

Observed heterozygosity ($H_o$) is the proportion of individuals in a population that is heterozygous at a given locus. On the other hand, the expected heterozygosity ($H_e$) was defined as the proportion of individuals that are prospective heterozygotes based on the allele frequencies and assuming Hardy-Weinberg equilibrium. The expected heterozygosity was calculated by the formula $H_e = 1-\sum p_i^2$, where $p_i$ is the frequency of the $i^{th}$ allele (Nei, 1973). The null allele frequency was calculated according to the formula of Brookfield (1996): $r = (H_e-H_o)/(1+H_e)$. Probability of identity (PI) is the probability of randomly choosing two individuals in a population that have identical genotypes (Waits et al., 2001). Polymorphic information content (PIC) is defined as the probability that one could identify which homologue of a given parent was transmitted to a given offspring, the other parent being genotyped as well (Botstein et al., 1980). This value is often used to determine the informativeness of a genetic marker for linkage studies (Guo and Elston, 1999). The PIC value of a locus ranges from 0 (monomorphic) to 1, the latter indicating a highly informative locus. PIC is calculated by the formula $PIC = 1 - \sum_i p_i^2 - \sum_{i=1} \sum_{j=i+1} 2p_i^2 p_j^2$ where $p_i$ and $p_j$ are the frequencies of the $i^{th}$ and $j^{th}$ alleles. Genetic similarities between genotypes were calculated using the "proportion of

shared alleles" estimator (Ps) of Bowcock et al. (1994) and the genetic distance ($D_{ps}$) between pairs of cultivars was calculated as (1-Ps). Cluster analysis of the distance data used the UPGMA (unweighted pair-group method using arithmetic averages) method and resulted in a phenogram that depicted the genetic relationships among the accessions (Nei, 1973). AnUPGMA tree was constructed from the genetic similarity matrix using *Mega3* (Kumar et al., 2004) and *Power Marker 3.2* software. Clusters on the UPGMA tree were further analyzed by creating a consensus tree produced from 1000 UPGMA trees from mean genetic distance ($D_{ps}$) and bootstrap values. Finally self-incompatibility alleles (Table 2.1) and phenotypic characters such as nut size, nut shape and husk length (Appendix C) were compared to confirm the results.

## Results and Discussion

### *Genetic diversity*

DNA of all genotypes was successfully amplified by all 21 SSR primer pairs and the fragment sizes were determined (Appendix D). All SSR loci were polymorphic. Of the 270 accessions, 200 showed unique fingerprinting. A total of 211 alleles were amplified and the number of alleles per locus ranged from 5 (for loci CAC-C010, CAC-C040, CAC-C119 and CAC-C118) to 15 (for loci CAC-B028, CAC-B105 and CaT-B107) with an average of 10.05 for the 21 loci. The number of alleles per locus varied according to the type of microsatellite repeat. The average number of alleles per locus for dinucleotide SSRs (11.27) was higher than for trinucleotide SSRs (7.00). The allele with the highest frequency (0.845) was 186 bp at locus CAC-C040 (Table 2.3 and Fig 2.1). The low number of alleles at some of the loci cannot be attributed to few genotypes, because a large number of genotypes were used in this study. Twenty-two of 211 alleles were unique, as they were detected only in one cultivar. Three unique alleles were found in 'Ruby' and '495.049', which are the most diverse cultivars in the population, while 'Blumberger' has two unique alleles.

The PIC values ranged from 0.26 to 0.85 with an average of 0.68. The three most polymorphic loci were CaT-B107, CaT-B507 and CAC-B028 with PIC values of 0.85, 0.82 and 0.82 respectively. The three least polymorphic loci were CAC-C040, CAC-C118 and CAC-C119 with PIC values of 0.26, 0.28 and 0.50, respectively (Table 2.3).

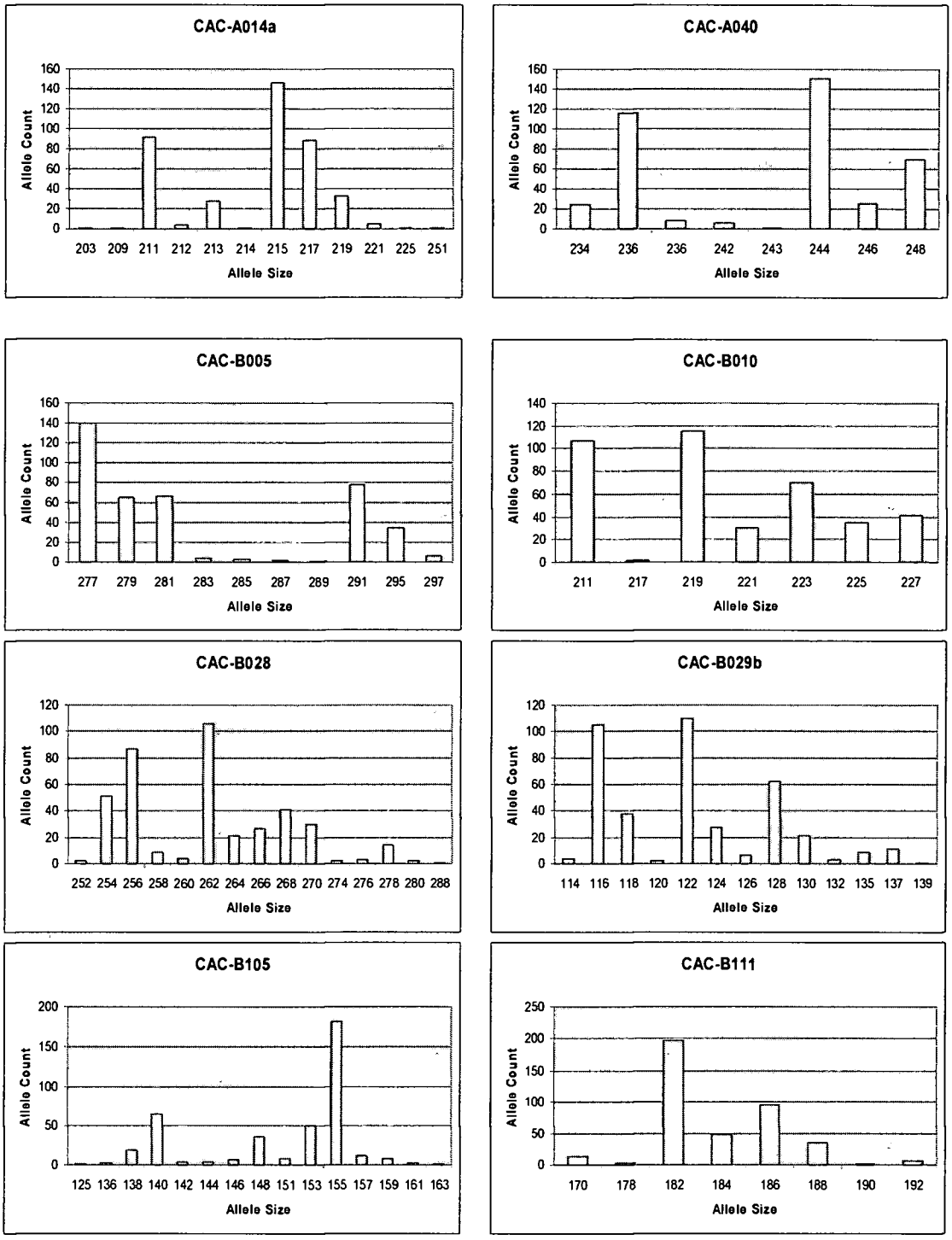


**Figure 2.1** Allele frequencies at twenty-one SSR loci in 200 unique European hazelnut cultivars

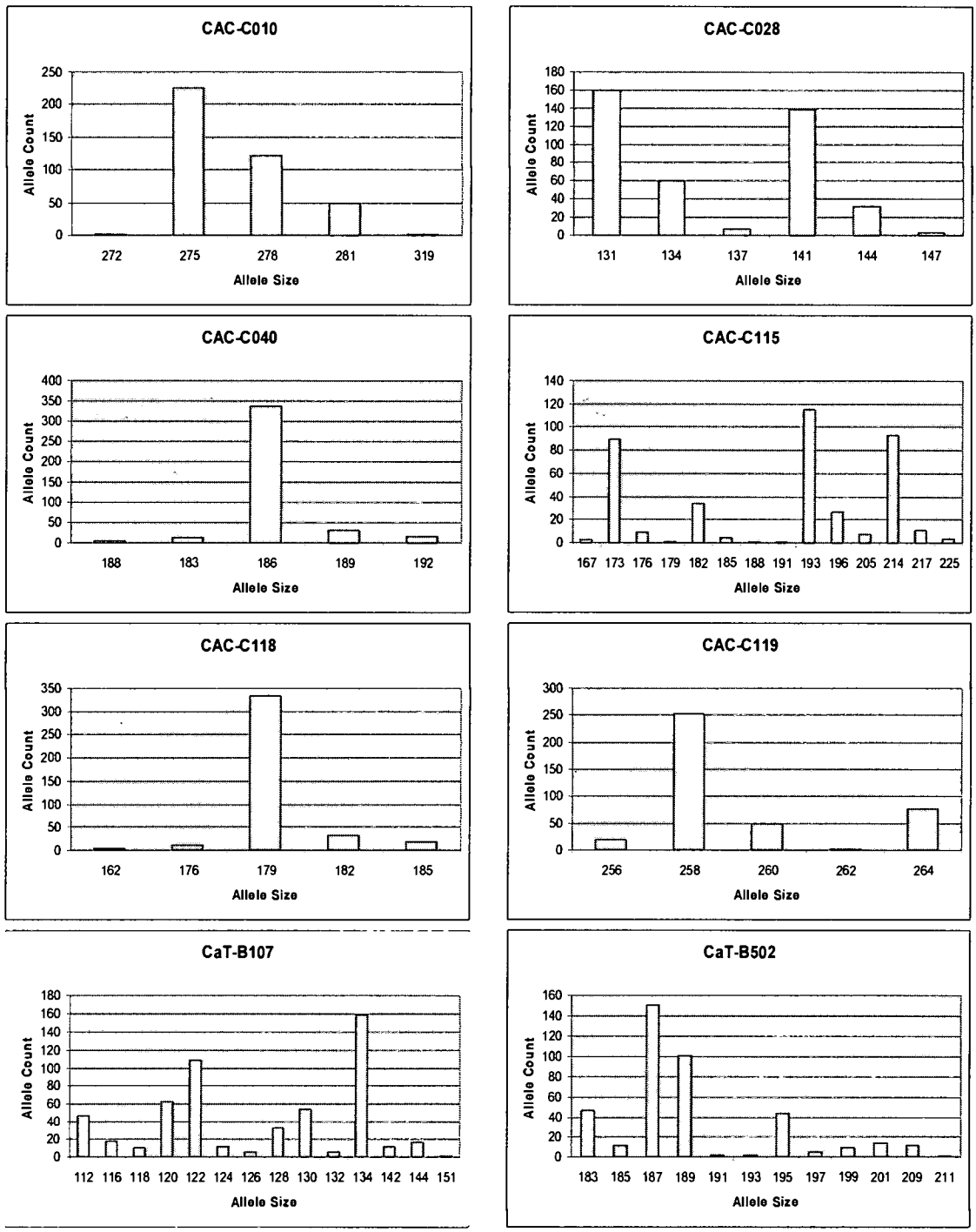


**Figure 2.1** Continued

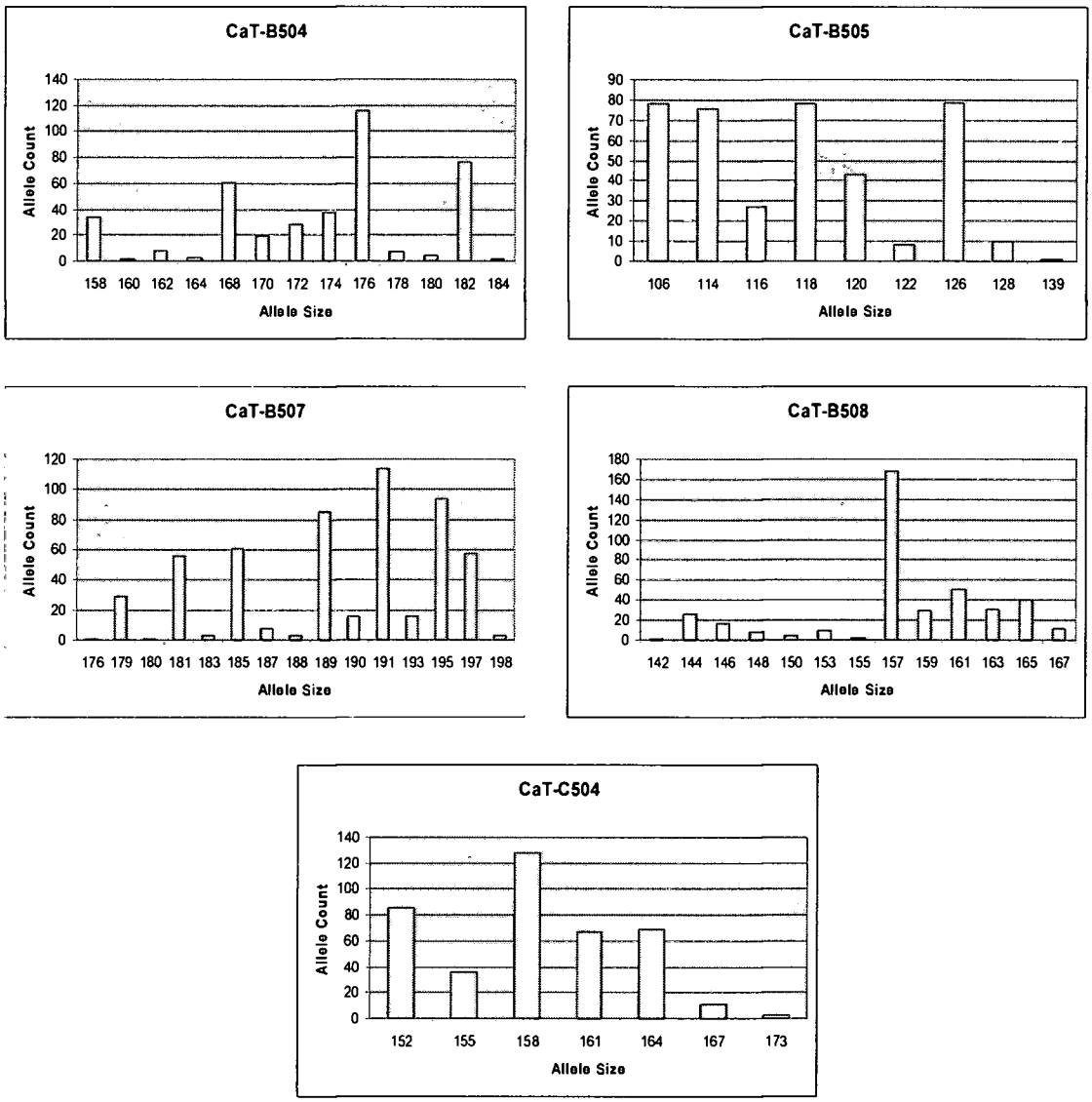


**Figure 2.1** Continued

The observed heterozygosity ($H_o$) for individual loci ranged from 0.24 to 0.88, with an average of 0.67 over all loci. Expected heterozygosity ($H_e$) ranged from 0.28 to 0.87 and averaged 0.71 (Table 2.3). The total paternity exclusion probability (PEP) was 0.9999 ranging from 0.15 to 0.73 for individual loci with a mean value of 0.52.

Although all of the 5670 PCRs carried out for genotyping 270 European hazelnut cultivars with 21 microsatellites gave PCR products, 15 of the 21 SSR markers showed a positive estimated frequency of null alleles (r). CAC-C10 had the highest positive r value of 0.214, which indicates a likely occurrence of null alleles. The probability of identity (PI) index ranged from 0.06 for CaT-B107 to 0.56 for CAC-C040 and the total probability of identity was calculated as $1.8 \times 10^{-17}$.

***Hazelnut diversity and cultivar pedigrees***

A second set of DNA samples was extracted from four trees 'Bard', 'Karidaty', 'Istarski Debeloplodna' and 'Riekchen's Zellernuss') and included as checks. In all cases these duplicates gave identical SSR allele sizes. They were not included in the statistical analyses. The phenogram constructed from an UPGMA cluster analysis of genetic distances showed that there is a large amount of diversity among European hazelnut accessions (Fig. 2.2). The phenogram revealed some tight and mixed geographical groupings. Each major group in the phenogram is condensed and discussed below separately.

**Table 2.3** Allele numbers (n), expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), polymorphic information content (PIC), probability of identity (PI), parental exclusion probability (PEP) and frequency of null alleles (r) of 21 SSR loci studied in 200 unique European hazelnut cultivars.

| SSR Locus | n | $H_e$ | $H_o$ | PIC | PI | PEP | r |
|---|---|---|---|---|---|---|---|
| **CAC-A014a** | 12 | 0.75 | 0.69 | 0.72 | 0.17 | 0.54 | 0.036 |
| **CAC-A040** | 8 | 0.74 | 0.77 | 0.69 | 0.19 | 0.51 | -0.020 |
| **CAC-B005** | 10 | 0.78 | 0.82 | 0.75 | 0.14 | 0.57 | -0.024 |
| **CAC-B010** | 7 | 0.79 | 0.80 | 0.76 | 0.13 | 0.59 | -0.002 |
| **CAC-B028** | 15 | 0.84 | 0.70 | 0.82 | 0.08 | 0.69 | 0.076 |
| **CAC-B029b** | 13 | 0.81 | 0.76 | 0.79 | 0.11 | 0.64 | 0.026 |
| **CAC-B105** | 15 | 0.74 | 0.70 | 0.71 | 0.14 | 0.55 | 0.019 |
| **CAC-B111** | 8 | 0.67 | 0.59 | 0.63 | 0.23 | 0.44 | 0.050 |
| **CAC-C010** | 5 | 0.57 | 0.24 | 0.50 | 0.40 | 0.30 | 0.214 |
| **CAC-C028** | 6 | 0.69 | 0.74 | 0.64 | 0.26 | 0.44 | -0.031 |
| **CAC-C040** | 5 | 0.28 | 0.27 | 0.26 | 0.56 | 0.15 | 0.006 |
| **CAC-C115** | 14 | 0.80 | 0.80 | 0.77 | 0.12 | 0.61 | 0.003 |
| **CAC-C118** | 5 | 0.30 | 0.28 | 0.28 | 0.53 | 0.16 | 0.010 |
| **CAC-C119** | 5 | 0.54 | 0.46 | 0.50 | 0.34 | 0.32 | 0.055 |
| **CaT-C504** | 7 | 0.78 | 0.59 | 0.75 | 0.14 | 0.58 | 0.109 |
| **CaT-B107** | 15 | 0.87 | 0.86 | 0.85 | 0.06 | 0.73 | 0.002 |
| **CaT-B508** | 13 | 0.78 | 0.69 | 0.76 | 0.10 | 0.61 | 0.049 |
| **CaT-B502** | 12 | 0.76 | 0.74 | 0.73 | 0.15 | 0.56 | 0.013 |
| **CaT-B504** | 13 | 0.83 | 0.84 | 0.81 | 0.08 | 0.67 | -0.007 |
| **CaT-B505** | 9 | 0.83 | 0.88 | 0.81 | 0.10 | 0.66 | -0.026 |
| **CaT-B507** | 14 | 0.84 | 0.80 | 0.82 | 0.07 | 0.70 | 0.025 |
| **Total** | 211 | 14.99 | 14.02 | 14.35 | 4.1 | 11.02 | |
| **Average** | 10.05 | 0.71 | 0.67 | 0.68 | | | |

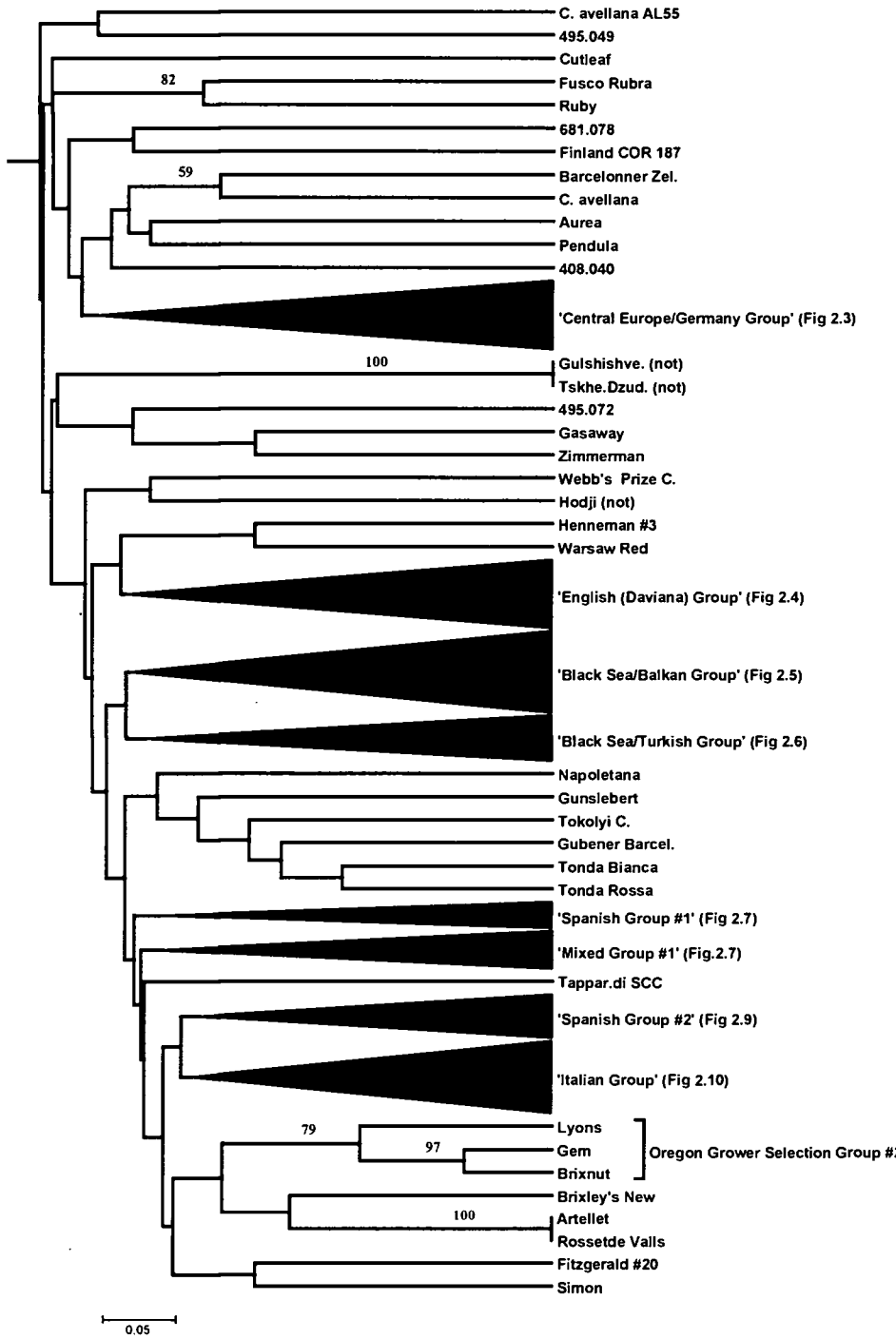


**Figure 2.2** Phenogram of 270 cultivars based on cluster analysis (UPGMA) of genetic dissimilarity

***Group 1: 'Central Europe/Germany Group'***

The first group has one Polish cluster, one English group of synonyms and one big German cluster, (Fig 2.3). The Polish cluster includes two synonyms, 'Maria' and 'Lenka #4'. They have identical incompatibility alleles $S_{11}$ and $S_{15}$ and very similar large and long nuts in intermediate length husks. They clustered with five other Polish cultivars, 'Acorn Hazelnut', 'Lenka #3', 'Frango #2', 'Volski Round' and 'Frango #5, forming a geographically distinct Polish cluster. All cultivars in the Polish cluster have medium to large nuts and adjacent cultivars share at least one incompatibility allele. SSR profiles and S-alleles of these cultivars suggested that they might be seedlings of 'Cosford' (Table 2.4).

The English group of synonyms has three cultivars: 'Bard', 'Barr's Zellernuss' and 'Bearn'. All three cultivars have identical SSR profiles and incompatibility alleles ($S_5$ and $S_{11}$). They also show similar phenotypic traits such as long nuts of small to medium size in short husks.

The German cluster includes many cultivars from Germany and nearby central European countries and has four synonym groups. The first group of synonyms consists of four cultivars: 'Obrovsky Novy' from Slovakia, 'Liegel's Zellernuss' from Germany, 'Kunzenmuller Zellernuss' from Germany and 'Jeeve's Samling' from Germany. According to Goeschke (1887), 'Jeeve's Samling' was receieved from England, but the exact origin is not known. All four cultivars have identical incompatibility alleles ($S_{12}$, $S_{20}$) and produce medium size, long and compressed nuts in short husks.

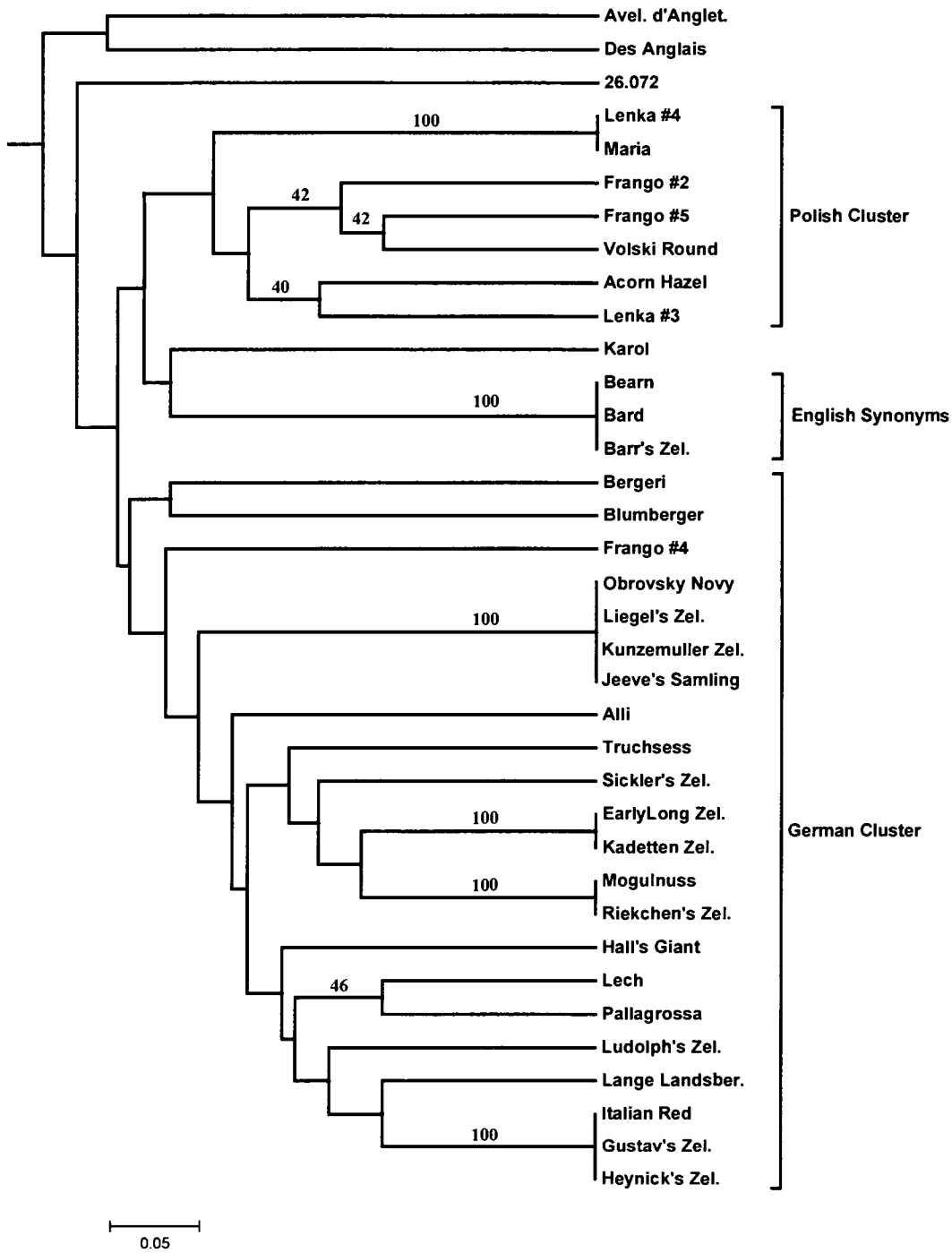


**Figure 2.3** Phenogram of 'Central Europe/Germany Group' (36 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

The second group of synonyms is a pair: 'Kadetten Zellernuss' from Germany and 'Early Long Zellernuss' from Denmark. Their incompatibility alleles are $S_{20}$ and $S_{25}$ and they have medium to large size and long nuts in short husks.

The third group of synonyms is 'Mogulnuss' from England and 'Riekchen's Zellernuss' from Landsberg-Germany. They have identical incompatibility alleles, $S_5$ and $S_{25}$. 'Riekchen's Zellernuss' has medium size long nuts in intermediate length husks and 'Mogulnuss' has large size long nuts in intermediate length husks. This slight difference may be a phenotypical measurement error or crop load might be responsible for the difference in sizes of nuts. However, 21 SSR loci detected no difference. The second and third group of synonyms clustered with two German cultivars 'Sickler's Zellernuss' from and unknown origin and 'Truchsess' from Landsberg.

The second and third groups of synonyms clustered close to each other. All four cultivars have common S-allele $S_{25}$, providing a further indication that they are genetically related.

The fourth and the last group of synonyms consists of three cultivars from Germany ['Italian Red' (Crane et al., 1937), 'Heynick's Zellernuss' and 'Gustav's Zellernuss from Landsberg-Germany]. All three have identical S-alleles ($S_{15}$ and $S_{20}$). Although there are slight differences between phenotypic characteristics of the nuts of these cultivars, they all have medium to large and long nuts in short husks. These synonyms clustered with a 'Lange Landsberger' from Landsberg-Germany and Ludolph's Zellernuss' from an unknown origin in Germany (Goeschke, 1887).

'Bergeri' from an unknown origin in Germany and 'Blumberger' from Blumberg-Germany (Goeschke, 1887) clustered together and they are the most genetically diverse cultivars in German Cluster.

***Group 2: 'English (Daviana) Group'***

The second group has three geographically tight clusters and four different synonym groups (Fig 2.4). The first and the most divergent cluster of this group is of four redleaf cultivars, 'Rote Zellernuss' from the Netherlands, 'Red Fortrin' from USA-Washington, 'Syrena' from Poland and 'Goc' from Poland. The two Polish cultivars clustered together and they have the same incompatibility alleles ($S_6$ $S_{15}$). 'Rote Zellernuss' clustered with 'Red Fortrin'. According to the SSR profiles and S-alleles, 'Red Fortrin' might have resulted from a cross of 'Barcelona' x 'Rote Zellernuss' (Table 2.4)

The first synonym group includes four cultivars. '*C. multiflorum*' from Turkey or Russia, 'Cosford' from England, 'Prolifique a Coque Serrée' from France, 'Petoka (not)' and 'Multiflora' from England. They have identical incompatibility alleles ($S_3$ and $S_{11}$) and produce similar large and long nuts in short husks. 'Little Poland' from Poland, 'Pruhovany' from Slovakia and 'Daviana' from Calcot Garden near Reading-England (Goeschke, 1887), of which the two latter are closely related to each other, clustered with 'Cosford'. Of the scored 21 SSR loci, they differ from each other only at locus CAC-C115, at which 'Daviana' carries two alleles (173/193), while 'Pruhovany' has a single allele (193/193). Additionally, the S-alleles of 'Daviana' and 'Pruhovany' are identical ($S_3$ and $S_{11}$) and both have large and long nuts in short husks. 'Little Poland' has

incompatibility alleles $S_3$ and $S_5$ and it also has large and long nuts in short husks. SSR locus profiles, S-alleles and phenotypic traits of the cultivars in this cluster suggest that they have a common origin.

Several Oregon Grower Selections clustered with 'Daviana' and 'Cosford' and placed in two adjacent clusters. These selections believed to have resulted from a cross of 'Barcelona' and 'Daviana'. 'Barcelona' has incompatibility alleles $S_1$ and $S_2$ and 'Daviana' has incompatibility alleles $S_3$ and $S_{11}$. However, SSR loci profiles suggested that some of the cultivars were selected from crosses made by 'Daviana' and some by 'Cosford' as pollenizer. 'Woodford', 'Fitzgerald, 'Nonpareil', 'Ryan'and 'Ennis' have one allele from 'Barcelona' and one allele from 'Daviana'. 'Daviana" was suggested as the pollen parent of these cultivars by the presence of null alleles at locus CaT-B508 and CAC-B028. The scored genotypes of 'Barcelona' and 'Daviana' at CaT-B508 are 157/157 and 161/161 respectively. The expected genotypes of seedlings from this cross would be 157/161. However, 157/157 was scored in these four cultivars at this locus (Table 2.4). This can be explained by the presence of a null allele in 'Daviana' at locus CaT-B508. The expected null allele frequency of locus CaT-B508 is 0.047. On the other hand, the scored genotypes of 'Barcelona' and 'Daviana' at CAC-B508 are 254/262 and 270/270 respectively. The expected genotypes of seedlings from this cross would be 254/270 and 262/270. However, 254/254 was scored in 'Ryan' and 'Ennis' at this locus (Table 2.4). This can be explained by the presence of a null allele in 'Daviana' at locus CAC-B028. The expected null allele frequency of this locus is 0.076. These two loci have a moderately high frequency of null allele. On the other hand, according to the scored

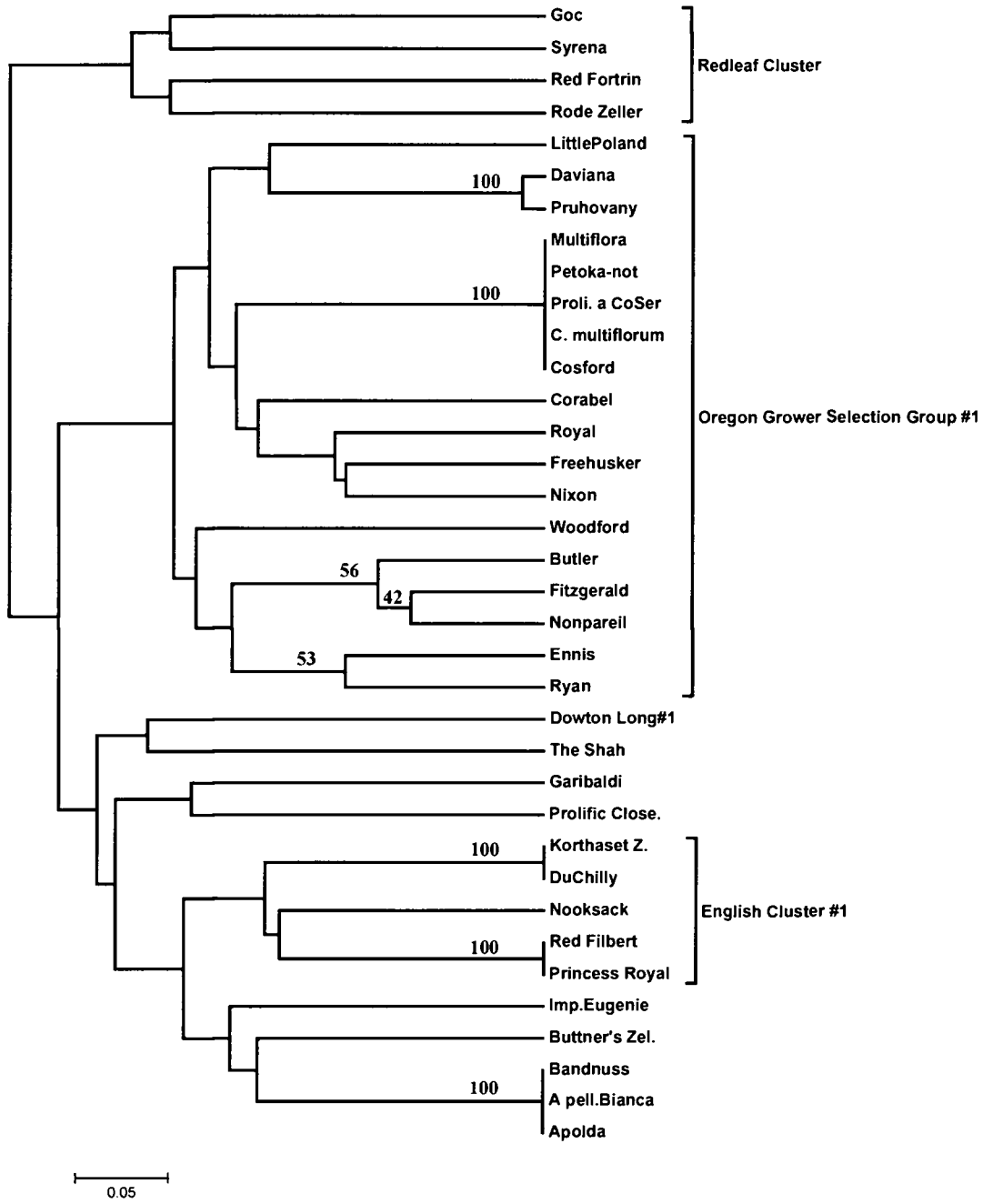


**Figure 2.4** Phenogram of 'English (Daviana) Group' (36 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

genotypes, 'Freehusker', 'Corabel', 'Nixon' and 'Royal' might have resulted from a cross of 'Barcelona' x 'Cosford'. 'Cosford' was confirmed as the pollen parent of these cultivars by the presence of unique alleles: "211" at locus CAC-A014a, "148" at CAC-B105 and "148" at CaT-B508. Furthermore, 'Cosford' also has incompatibility alleles $S_3$ and $S_{11}$. The exact pollen parent of 'Butler' could not be determined by the 21 SSR loci, as either 'Daviana' or 'Cosford' could be the pollen parent of this cultivar. However, according to the phenogram (Figure 2.3), they clustered with 'Daviana' seedlings and might be the seedlings of 'Daviana'. SSR loci profiles of 'Daviana' and 'Cosford' suggested that they might be sister seedlings (Fig.2.4).

English Cluster #1 has two groups of synonyms. The first pair of synonyms is 'Korthaset Zellernuss' from Denmark and 'Du Chilly'(syn.'Kentish Cob') from England. They have incompatibility alleles $S_{10}$ and $S_{14}$ and both produce medium size very long nuts in long tubular husks. The second synonyms of the English Cluster #2 are 'Red Filbert' from England and 'Princess Royal' from Calcot Garden near Reading-England (Goeschke, 1887), both of which have incompatibility alleles $S_{11}$ and $S_{14}$. Morphologically, these two cultivars are very similar. Both have medium size and very long nuts in short husks. The phenotypic traits and SSR-profiles suggest that these cultivars might be identical. Thisresult should be further confirmed by identifying the second S-allele of 'Red Filbert'. Two other English cultivars, 'Dowton Long #1' and 'The Shah', and 'Nooksack' from USA-Washington were placed in English Cluster #2. 'Nooksack' originated in Nooksack, Washington and was introduced in 1928 (Brooks and Olmo, 1997). It might be a chance seedling of 'DuChilly'. Indeed, the genotype of

'Nooksack' at 21 SSR loci indicates that it could be a seedling of 'Du Chilly'. Beside that, some phenotypic traits such as tree habit and large nut size are also similar to 'Du Chilly'. 'Downton Long #1' is the first of two cultivars with the same name. The second one clustered with other English cultivars in Group 2, which was mentioned earlier. According to the SSR profiles of these two trees, although they have the same name, they are genetically different. However, their clustering indicates both originated in England.

The sixth group of synonyms consists of three cultivars: 'Bandnuss' from England, 'A Pellicola Bianca' from Italy and 'Apolda' from Italy. They have identical S-alleles $S_{10}$ and $S_{11}$. They are also identical morphologically. All three have large and long nuts in short husks. Two other cultivars, 'Buttner's Zellernuss' from Germany and 'Imperatrice Eugenie' from France clustered with these synonyms.

***Group 3: 'Black Sea/Balkan Group'***

The third group on the phenogram included diverse cultivars primarily from Black Sea countries and the Balkan Peninsula (Fig 2.5). Five groups of synonyms and five tight geographical clusters were detected. The first synonym group consists of 'White Filbert' from France, 'Purple Fortrin' from USA-Washington, 'Purple Aveline' from France, 'Pellicule Rouge' from France, 'Istarski Debeloplodna' from Croatia, 'Fructo Albo' from France, 'Barbarella' from Italy and 'Aveline Rouge' from France. The identical SSR profiles of these cultivars are supported by phenotypes and S-alleles. They have small long nuts, long tubular husks and incompatibility alleles $S_5$ and $S_{10}$. 'Purple Fortrin', 'Purple Aveline', 'Pellicule Rouge' 'Barbarella' and 'Aveline Rouge', have red-purple pellicle (skin covering the kernel). This unusual pellicle color might have

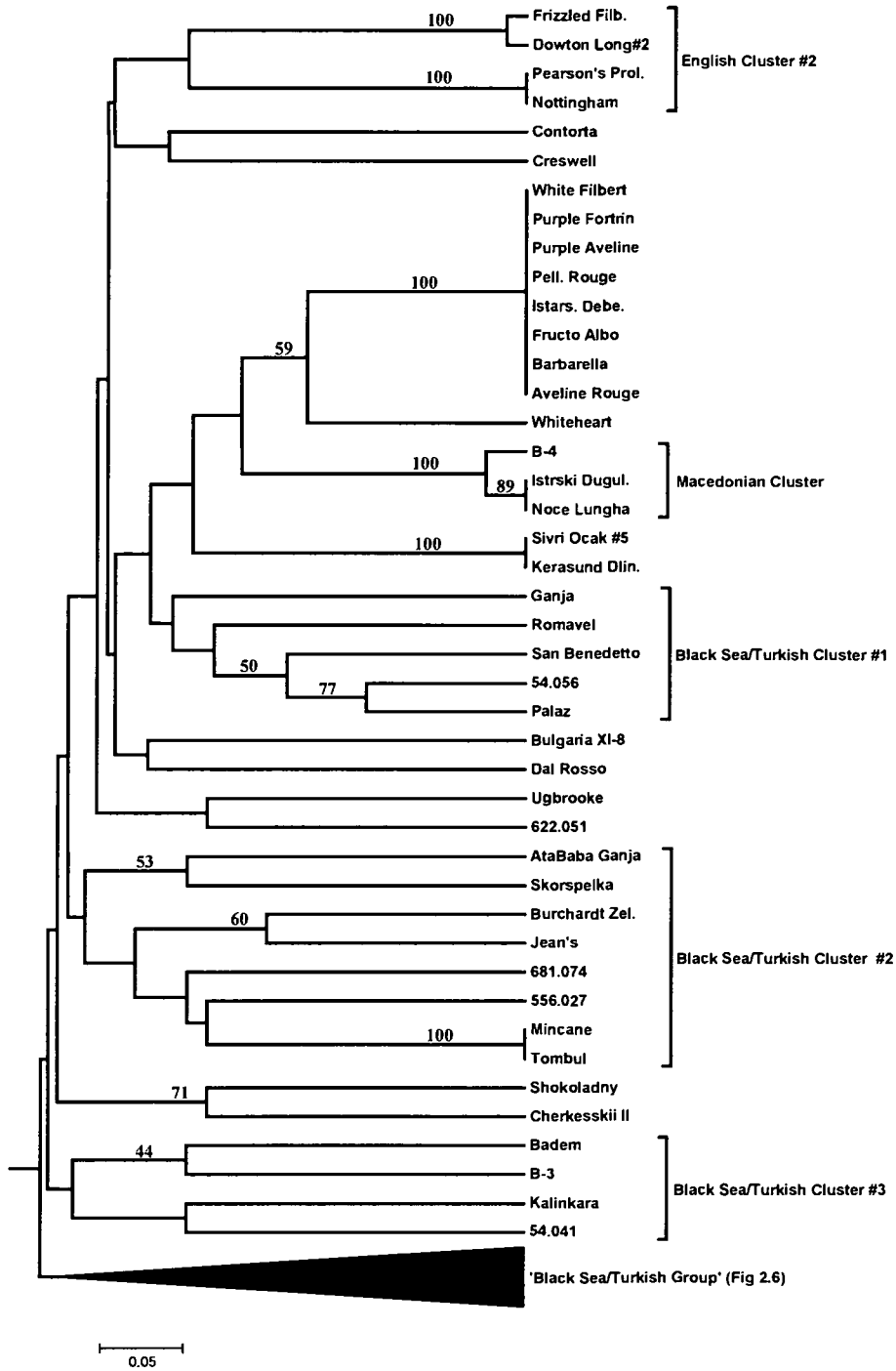


**Figure 2.5** Phenogram of 'Black Sea/Balkan Group' (44 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

arisen by mutation. However they have the same SSR alleles as 'Fructo Albo' and 'Istarski Debeloplodna' which have brown pellicles. 'Whiteheart', a cultivar from New Zealand clustered with these accessions and has incompatibility alleles $S_2$ and $S_{10}$. The SSR profiles and S-alleles suggest that 'Whiteheart' is a seedling of 'White Filbert'.

The second group of synonyms was 'Noce Lungha' from Italy and 'Istarski Duguljasti' from Croatia. They clustered with Macedonian cultivar 'B-4'. According to the SSR loci profile, 'B-4' is closely related to 'Noce Lungha' and 'Istarski Duguljasti'. It differs only at two loci. At CAC-A014a 'B-4' carries two alleles (211/215), while 'Noce Lungha' and 'Istarski Duguljasti' carry two alleles (211/217) and at the locus CaT-B507 'B-4' carries 122/134, while 'Noce Lungha' and 'Istarski Duguljasti' carry 134/134. Moreover, 'B-4' is phenotypically similar to 'Noce Lungha' and 'Istarski Duguljasti' with long large nuts and a long tubular husk. This cluster with a Croatian, a Macedonian and an Italian cultivar was geographically distinct from other cultivars in the group. Probably the synonymous cultivars 'Noce Lungha' and 'Istarski Duguljasti' had been transferred across the Adriatic Sea and different names used for the same cultivar.

The third group of synonyms was 'Sivri Ocak #5' from Turkey and 'Kerasund Dlinnyi' from the Russian Federation. Both have medium-sized long pointed nuts, very long husks and the same S-alleles ($S_8$ $S_{10}$). 'Kerasund Dlinnyi' was probably carried from Turkey to Russia. The first word "Kerasund" is similar to "Giresun" the major hazelnut producing province of Turkey, and "Dlinnyi" means "long" in Russian.

The fourth group of synonyms was two cultivars from England, 'Pearson's Prolific' and 'Nottingham', which clustered with two other English cultivars, 'Frizzled

Filbert' and 'Dowton Long #2'. Both 'Pearson's Prolific' and 'Nottingham' have incompatibility alleles $S_8$ and $S_{10}$ and are morphologically identical. Both are vigorous trees with small and very long nuts and intermediate length husks. SSR profiles show that 'Frizzled Filbert' and 'Dowton Long #2' are closely related to each other. They only differ from each other at locus CaT-B508, where 'Frizzled Filbert' has alleles 153/161, while 'Downton Long #2' has alleles 153/163. The difference in these two cultivars might be due to a mutation at this locus. Otherwise, they are phenotypically similar with small long nuts and long husks, and they have the same S-alleles ($S_9$ $S_{10}$).

In this group, three Black Sea/Turkish clusters were revealed. The first cluster consists of 'Ganja' from Azerbaijan, 'Romavel' from Romania, 'San Benedetto' from Italy-Torino, 'Palaz' from Turkey, and '54.056' from Turkey. Morphologically, they are similar, having with medium to small and slightly oblate to round nuts and long husks. 'San Bendetto' was received from Italy-Torino as a selection of *Corylus maxima.* However it seems that this cultivar did not originate in Italy. The second Black Sea/Turkish cluster includes 'Skorspelka' from the Russian Federation, 'Ata Baba Ganja' from Bordeaux, 'Jean's' of unknown origin, 'Burchardt's Zellernuss' from Germany, '681.074' from Turkey, '556.027' from Turkey, 'Tombul' from Turkey-Akçakoca, and 'Mincane' from Turkey-Akçakoca. 'Tombul' and 'Mincane' were received as scions from an orchard in Turkey-Akçakoca. The have identical SSR profiles and both have incompatibility alleles $S_4$ and $S_{10}$. Synonymy based on DNA profiles was also confirmed phenotypically.

The last Black Sea/Turkish cluster, which consists of 'Badem' from Turkey, 'B-3' from Macedonia, 'Kalinkara' from Turkey and '54.041' from Turkey, is distinct from other clusters in the group. They are phenotypically similar in that they have small to medium-sized and slightly long or long nuts.

***Group 4: 'Black Sea/Turkish Group'***

This group includes 25 cultivars that were divided into two different distinct Black Sea/Turkish clusters (Fig 2.6). In the first cluster two groups of synonyms were revealed. The first synonym group includes 'Vilcea 22' from Romania, a selected sport of 'Imperial de Trebizonde' (Turcu and Botu, 1997), 'Wanliss Pride' from Australia, two trees of the 'Imperial de Trebizonde', and 'Karidaty' from Turkey. All six cultivars have identical phenotypes (large, round nuts and intermediate husk length) and incompatibility alleles $S_2$ and $S_{10}$. In the second synonym group, 'Nemsa (not)' and 'Kudryavchik (not)' brought from Georgia and, which are not true-to-name, appeared to be genetically identical. Moreover, both trees have incompatibility alleles $S_4$ and $S_{14}$. Both trees produce large round nuts and have long husks and are otherwise phenotypically identical. Although not true-to-name, the true identity of this accession remains unknown.

In the second cluster, two different synonyms were detected. According to SSR loci profiles, 'Tombul Ocak 1' from Turkey and 'Extra Ghiaghli' from Greece are identical. They both have small and pointed nuts and very long husks and S-alleles 4 and 12. The second pair of synonyms in this cluster is 'Tombul Ghiaghli' from Greece and 'Unknown #3', a tree whose label was lost at planting time. According to the SSR loci profiles and S-alleles (4, 8), 'Unknown #3' and 'Tombul Ghiaghli' are synonyms.

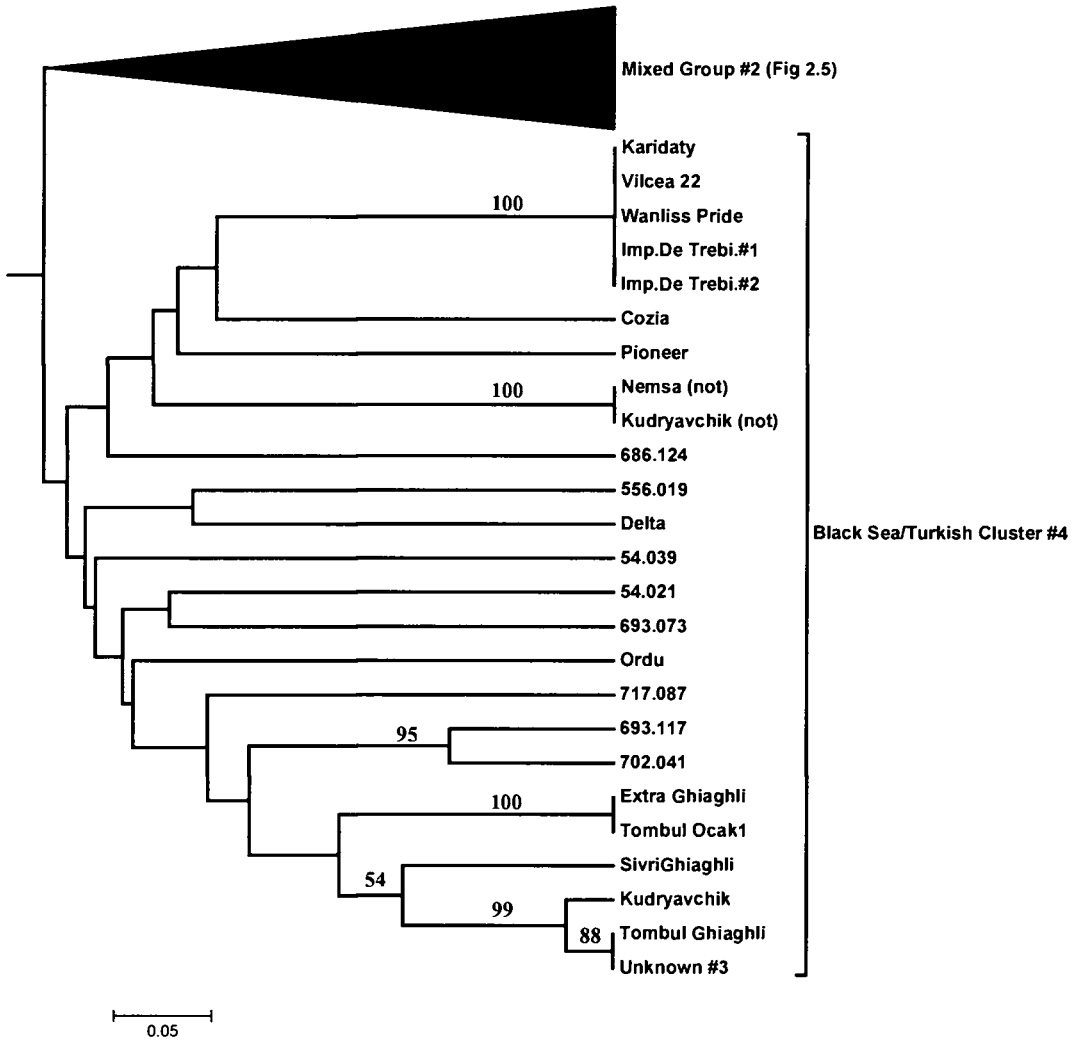


**Figure 2.6** Phenogram of 'Black Sea/Turkish Group' (25 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

'Ordu', imported as scions from Italy-Torino and selected seedlings of Turkish origin ('686.124', '556.019', '54.039', '693.073', '54.021', '717.087' '693.117', '702.041'and '693.117') clustered together. Although the two Russian cultivars, 'Kudryavchik' and 'Kudryavchik (not)' have the same name, they have different SSR

profiles and the morphology of the nuts is also different. 'Kudryavchik' has small round nuts, but 'Kudryavchik (not)' produces large round nuts. The leaf samples of 'Kudryavchik' were collected from National Clonal Germplasm Repository (NCGR) Corvallis, OR hazelnut collection and 'Kudryavchik (not)' was from the OSU hazelnut collection and propagated from scions received from the Republic of Georgia. The true 'Kudryavchik' appears to be closely related to 'Tombul Ghiaghli'. The genetic profile of 'Kudryavchik' differed from 'Tombul Ghiaghli' at locus CAC-C119, at which 'Kudryavchik" carries two alleles (258/260), while 'Tombul Ghiaghli' carries a single allele (260/260). Additionally, the S-alleles of 'Kudryavchik' are $S_4$ and $S_{14}$, while they are $S_4$ and $S_8$ for 'Tombul Ghiaghli'. So, the difference at the S-locus indicates that they are similar but different accessions.

***Group 5: Spanish Group #1***

This group consists of two and Italian cultivars, one Portuguese cultivar and 11 Spanish cultivars (Fig. 2.7). The Spanish cultivars 'Tomasina', 'Francoli', 'Martorella', 'Pauetet', 'Restiello' and 'Negret' clustered closely together. 'Restiello' and 'Negret' have identical SSR profiles and S-alleles ($S_{10}$, $S_{22}$). Both have small, slightly long nuts and short husks. 'Tomasina' and 'Francoli' have incompatibility alleles $S_{17}$ and $S_{22}$ and 'Pauetet' has incompatibility alleles $S_{18}$ and $S_{22}$. Comparison of SSR profiles of these cultivars and 'Negret' suggests that 'Tomasina' and 'Pauetet' could be seedlings of 'Negret'. However, the SSR profile of 'Francoli' is not consistent with the SSR profile of 'Negret' at four loci (Table 2.4). The second major cluster in this group includes cultivars from Italy ('Nocchiolino Sangrato' and 'Tonda Gentile delle Langhe') and Spain ('Morell',

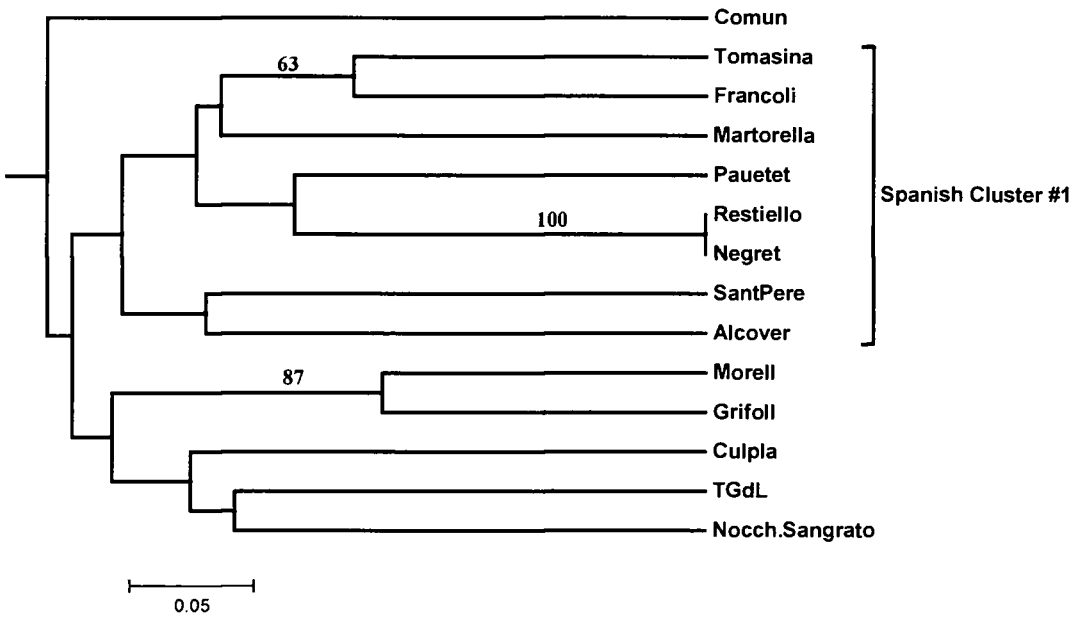


**Figure 2.7** Phenogram of 'Spanish Group #1' (14 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

'Grifoll' and 'Culpla'). These cultivars appear genetically related to each other, possibly due to exchange between Italy and Spain decades or centuries ago. The most divergent cultivars in this group is 'Comun' from Portugal, which clustered loosely with other cultivars in the group.

***Group 6: 'Mixed Group #1'***

The sixth group on the phenogram has diverse cultivars from Italy, Spain, Germany, Slovakia and four pollenizers ('Delta', 'Epsilon', 'Gamma' and 'Zeta') released by the Oregon Agricultural Experiment Station (Fig 2.8). Four groups of synonyms were detected.

The first synonym group consists of 'Fruttogrosso' from Italy, 'Istarski okrogloplodna' from Slovenia, 'Römische Nuss' from Italy, 'Payrone' from Italy and 'Romai', which might be from Italy. The identical SSR profiles of these cultivars are consistent with their phenotypes and S-alleles. They have large round nuts with short husks and incompatibility alleles $S_{10}$ and $S_{18}$. Although the name 'Römische Nuss' indicates a Roman origin, its true origin is unknown but is included in English, French, and Italian collections.

The second synonym group includes three cultivars from Spain: 'Segorbe', 'Comun Aleva' and 'Gironenc Colldejou'. These three synonyms have identical S-alleles ($S_9$ and $S_{23}$) and the same phenotype. All three cultivars have small and slightly long nuts and intermediate husk length.

The third group of synonyms is the pair 'Louisen's Zellernuss' from Germany and 'Rimsky' from Slovakia. They clustered with Italian and Spanish cultivars as mentioned earlier. They have identical S-alleles ($S_{10}$ and $S_{25}$) and produce very large round nuts with short husks. The last synonyms in this group are four Spanish cultivars ('Quiros', 'Espinaredo', 'Casina' and 'Amandi'). SSR profiles are consistent with the identical S-alleles ($S_{10}$, $S_{21}$) and phenotypes of the cultivars. All four cultivars have small, slightly long nuts and intermediate husk length. 'Gamma', a pollenizer released by the Oregon Agricultural Experiment Station, clustered together with 'Casina' and its synonyms. 'Gamma' resulted from a cross of 'Casina' x VR 6-28. VR 6-28 was selected from a cross of 'Riccia di Talanico' x 'Gasaway' (Mehlenbacher and Smith, 2004). The

genotype of 'Casina' and expected genotype of VR 6-28 ('Riccia di Talanico' x 'Gasaway') were consistent with that observed for 'Gamma' (Table 2.4).

'Epsilon', one of four pollenizers released by the Oregon Agricultural Experiment Station, clustered together with its grandparent, Italian cultivar 'Tonda Romana'. 'Epsilon' resulted from a cross of OSU 350.089 x 'Zimmerman'. OSU 350.089 was selected from the cross 'Tonda Romana' x 'Tombul Ghiaghli' (Mehlenbacher and Smith, 2004). The genotype of 'Zimmerman' and the expected genotype of OSU 350.089 based

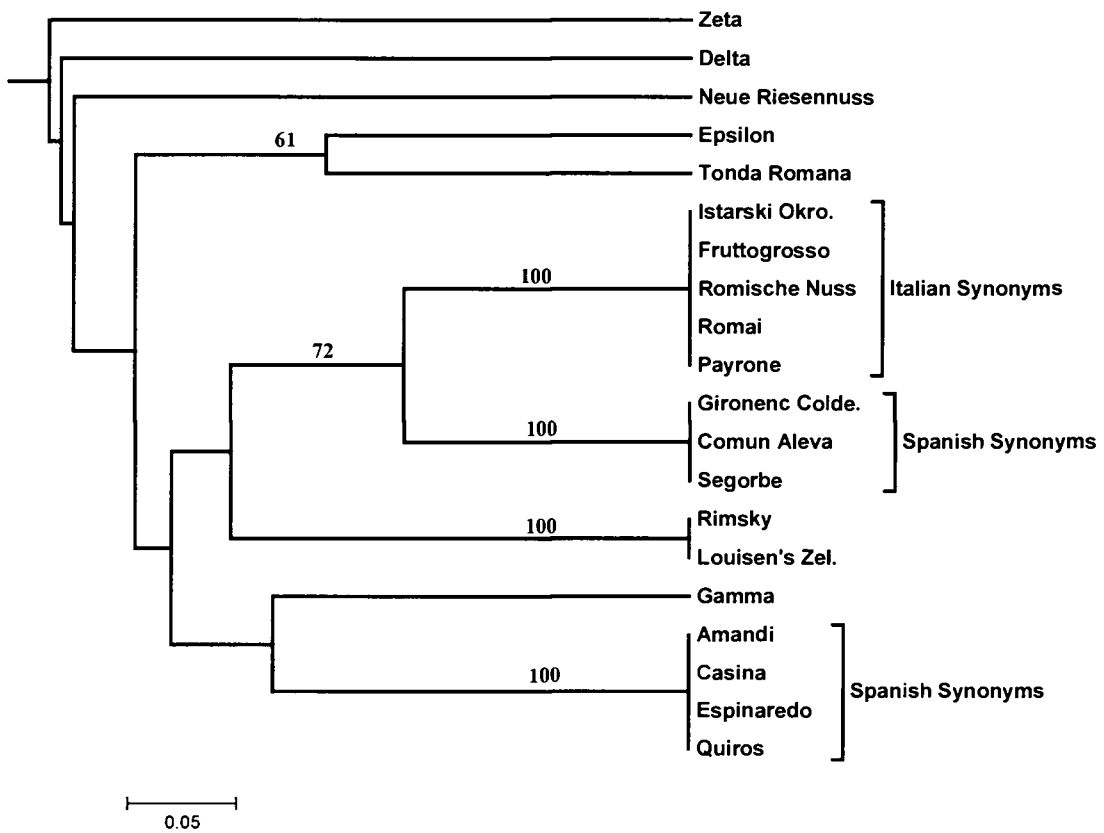


**Figure 2.8** Phenogram of 'Mixed Group #1' (21 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

on its parents 'Tonda Romana' and 'Tombul Ghiaghli' showed that the pedigree information of 'Epsilon' was correct except at locus CAC-C010 (Table 2.4). According to Mendelian segregation, the genotype of 'Epsilon' at locus CAC-C010 should be 275/278 or 278/281, but the scored genotype was 275/275. This result can be explained by the presence of null alleles in 'Tombul Ghiaghli' and/or 'Tonda Romana' and 'Zimmerman' at this locus. The expected null allele frequency of locus CAC-C010 is 0.222, a very high frequency. The other pollenizers, 'Delta' and 'Zeta' clustered outside of the group as the two most diverse accessions. 'Zeta' resulted from a cross of OSU 342.019 x 'Zimmerman'. OSU 342.019 was a selected seedling of the cross of 'Casina' x OSU 43.091 (Mehlenbacher and Smith, 2004). According to the SSR profiles, 'Zimmerman' is indeed one of parents of 'Zeta' (Table 2.4).

***Group 7: 'Spanish Group #2'***

This group includes 23 cultivars: 18 from Spain and 2 from Portugal (Figure 2.9) and three cultivars from outside the Iberian Peninsula, 'Barrettona' and 'Daria' from Italy and 'Comen' of an unknown origin and. 'Comen' was received from Italy. Manzo and Tamponi (1982) suggested that it might have originated in Greece. However, SSR profiles indicate that it has a Spanish origin, because it clustered with the three Spanish cultivars 'Sant Joan', 'Lluenta' and 'Gironenc Vermellet'.

Four groups of synonyms were identified. According to the phenogram, the Spanish cultivar 'Molar' and the Portuguese cultivar 'Ceret' are synonyms. However, the S-alleles and phenotypic traits of these two cultivars are different. 'Molar' has medium

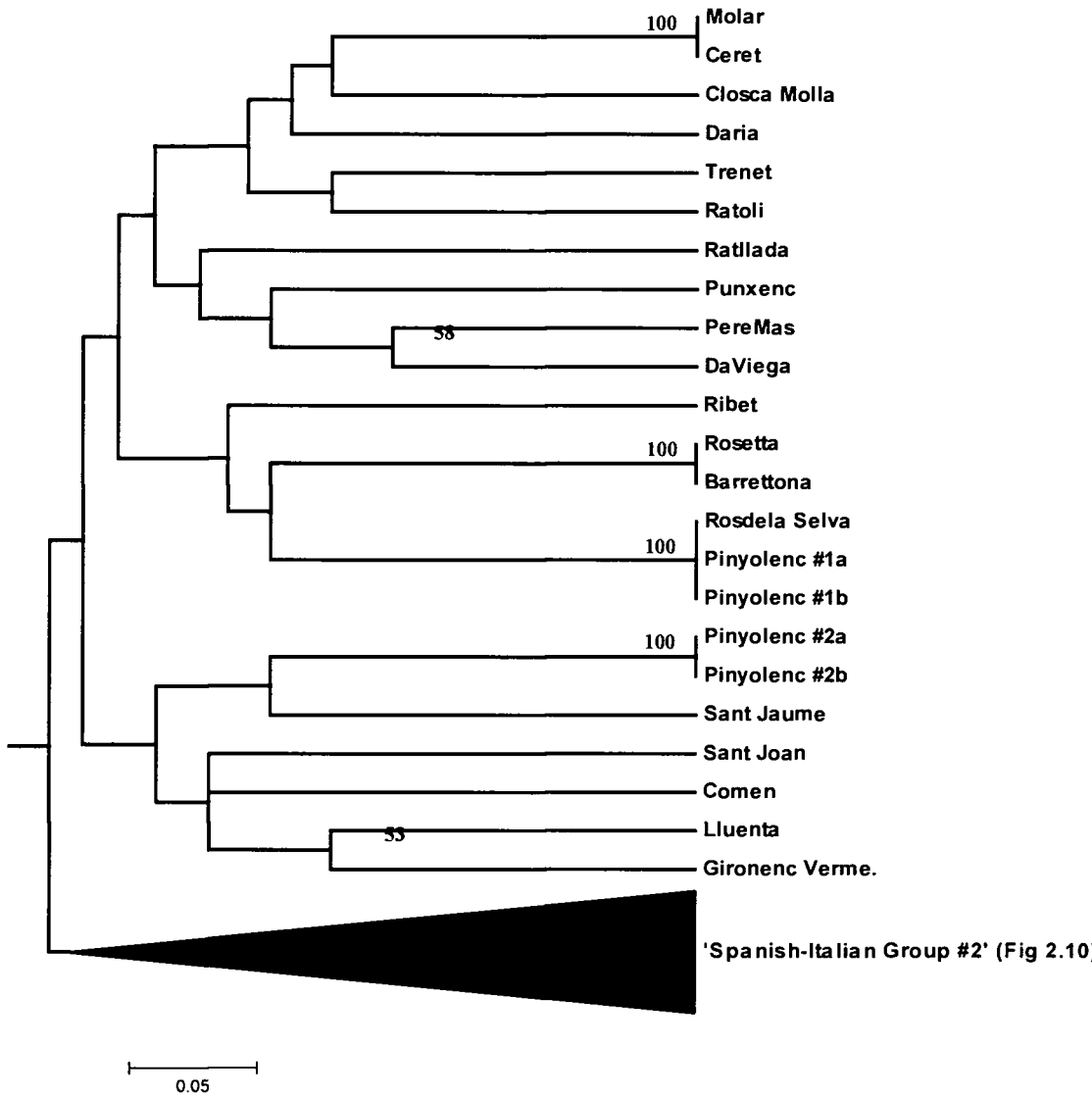


**Figure 2.9** Phenogram of 'Spanish Group #2' (23 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

size, round nuts with intermediate length husk and incompatibility alleles $S_2$ and $S_{10}$. 'Ceret' has small, round nuts with long husks and incompatibility alleles $S_1$ and $S_2$.

Italian cultivar 'Daria' resulted from a cross of 'Tonda Gentile delle Langhe' (TGdL) from Italy and 'Cosford' from England. The genotypes of 'Daria' and its parents are consistent with the pedigree information at all SSR loci except at CAC-C010. 'TGdL' and 'Cosford' carry single alleles, (275/275) and (278/278) respectively at CAC-C010. The expected genotype of 'Daria' at this locus was 275/278. However, it was scored as 278/278 (Table 2.4). This result can be explained by the presence of a null allele at CAC-C010 in 'TGdL'. Indeed, CAC-C010 has a high positive expected null allele frequency (0.214).The Spanish cultivar 'Rossetta' and the Italian cultivar 'Barrettona' have identical SSR profiles and S-alleles ($S_2$ and $S_6$). Both have large round nuts with intermediate length husks. Probably 'Barrettona' has a Spanish origin, because it clustered with other Spanish cultivars and no Italian cultivar was placed in this cluster. 'Barettona' was imported as scions from an orchard near Lago di Vico in Italy. 'Pinyolenc #1a' and 'Pinyolenc #1b' are two trees of the same genotype and have identical SSR loci profiles. Both have medium size round nuts with intermediate husk length. Their incompatibility alleles are $S_2$ and an unknown S- allele, or they may be homozygous $S_2 S_2$. 'Ros de la Selva', another cultivar from Spain, has an identical SSR profile and is morphologically indistinguishable from 'Pinyolenc #1a' and 'Pinyolenc #1b' based on nut size and shape and husk length. It has incompatibility alleles $S_2$ and $S_9$. So, this suggests that the unknown S-allele of 'Pinyolenc #1a' and 'Pinyolenc#1b' is $S_9$.

'Pinyolenc #2a' and 'Pinyolenc #2b' have identical SSR profiles, S-alleles ($S_2$ and $S_{17}$), and morphological characters. Both trees have small and slightly long nuts with intermediate length husks. Although 'Pinyolenc #1' and 'Pinyolenc #2' have the same name, they were shown to be different cultivars. They were placed in two different clusters in the 'Spanish Group #2'.

The second Portuguese cultivar, 'Da Viega' is genetically related to the Spanish cultivar 'Pere Mas' and clustered with two other Spanish cultivars, 'Ratllada' and 'Punxenc'.

***Group 8: 'Italian Group'***

This group includes cultivars mainly from Italy and a few from Spain and the OSU hazelnut breeding program (Fig. 2.10). The most divergent cultivar was 'Tonnolella' from Italy. Four groups of synonyms were revealed in this group. The first synonyms are 'Macrocarpa' from Germany, 'Kruse' from USA-Oregon and 'Turk' from USA-Oregon. All three have incompatibility alleles $S_1$ and $S_2$. Additionally, they have large round nuts with intermediate length husks. According to the SSR loci profiles, Oregon cultivars 'Turk' and 'Kruse' are related to 'Barcelona', the leading cultivar in Oregon. 'Turk' and 'Kruse' share a common allele with 'Barcelona' at 17 out 21 SSR loci. These synonyms clustered with the Oregon rootstock selection 'Arneson's Rootstock' and five Italian cultivars ('Riccia di Talanico', 'San Giovanni', 'Tonda di Giffoni' and 'Camponica').

The second group of synonyms includes 'D'Algers' from the Netherlands, 'Barcelona' from Spain and 'Grande' from Spain-Asturias. They have identical incompatibility alleles ($S_1$ and $S_2$) and large round nuts with intermediate length husks. Although according to the NCGR-Corvallis *Corylus* Catalog, 'D'Algers' was imported from the Netherlands, it might have originated in Spain. Another Spanish cultivar 'Planeta' also clustered closely with these synonyms. The third synonym group has three accessions, 'Atlas' from Denmark and two trees of 'Belle di Giubilino' from Italy. Their incompatibility alleles are $S_1$ and $S_{10}$ and they have large round nuts with short husks. 'Atlas' might have originated in Italy and been carried to the collection in Denmark.

The last group of synonyms consists of 11 cultivars from Italy-Sicily. They are 'Racinante clone G', 'Nostrale', 'Montebello', 'Minnolora', 'Barettona (not)', 'Avellana Speciale', 'Siciliana', 'Mansa', 'Locale di Piazza Armerina', 'Comune di Sicilia' and 'Carello'. They all have medium size, round-oblate nuts with short husks. Furthermore, they have identical S-alleles ($S_1$ and $S_2$). 'Nocchione' from central Italy clustered closely with the Sicilian synonyms. It has the same S-alleles, and produces morphologically similar nuts. They only differ from each other at locus CaT-C504, where 'Nocchione' has two alleles (153/164), while the Sicilian synonyms have two alleles (158/167).


**Figure 2.10** Phenogram of 'Italian Group' (38 cultivars) based on cluster analysis (UPGMA) of genetic dissimilarity

'Nocchione' might have diverged from this group by mutation at this locus. Two other Italian cultivars, 'Nociara' and 'Iannusa Racinante', were also placed in the Sicilian Cluster.

The Oregon State University releases 'Willamette' (Mehlenbacher et al., 1991), 'Clark' (Mehlenbacher et al., 2001) and 'Lewis' (Mehlenbacher et al., 2000) clustered together and they are denoted on the phenogram under the group of 'OSU Releases' (Fig. 2.10). These cultivars were released for the kernel market. 'Clark' and 'Lewis' have quantitative resistance to eastern filbert blight caused by *Anisogramma anomala* (Peck) E. Müller and are seedlings of 'Willamatte'.

According to the pedigree of 'Willamette', it resulted from a cross of 'Montebello' from Italy and 'Compton', a selection of O.C. Compton of Corvallis, Ore. (Mehlenbacher et al., 1991). 'Compton' was not included this study. However, the SSR profiles of 'Willamette' and 'Montebello' show that 'Montebello' could be one of the parents of 'Willamette' (Table 2.4).

'Clark' was selected from a progeny of 231 seedlings from a cross of 'Tombul Ghiaghli' and 'Willamette' (Mehlenbacher et al., 2001). Genotypes of 'Clark' and both parents are consistent with the pedigree information at all SSR loci except at CAC-C010. 'Tombul Ghiaghli' and 'Willamette' carry single alleles, (281/281) and (278/278) respectively at CAC-C010. The expected genotype of 'Clark' at this locus was 278/281. However, it was scored as 278/278 (Table 2.4). This result can be explained by the presence of a null allele at CAC-C010 in 'Tombul Ghiaghli'. Indeed, the CAC-C010 locus has a high positive expected null allele frequency (0.214).

'Lewis' was selected from a progeny of 428 seedlings resulting from a cross of OSU 17.028 x 'Willamette'. OSU 17.028 resulted from a cross of 'Barcelona' x 'Tombul Ghiaghli' (Mehlenbacher et al., 2000). The genotypes of 'Lewis', 'Willamette' and the expected genotype of OSU 17.028 ('Barcelona' x 'Tombul Ghiaghli') showed that the pedigree information of 'Lewis' was confirmed except at locus CaT-C504. According to Mendelian segregation, the genotype of 'Lewis' at locus CaT-C504 should be 155/167, 158/167 or 161/167. However, the scored genotype was 155/155 (Table 2.4). This can be explained by the presence of a null allele in 'Willamette' at this locus. The expected null allele frequency of locus CaT-C504 is 0.109. This locus has a moderately high frequency of null alleles and so the presence of a null allele might be expected.

***Outer Groups on UPGMA Phenogram***

The outer groups of the phenogram (Fig. 2.2) include the most genetically diverse cultivars of this study. They did not cluster in tight geographic clusters but rather sit on the outside of the major groups discussed. The first outermost cluster includes two cultivars: '*C. avellana* AL55' from Albania and '495.049' from Southern Russia.

The second outlying group includes'Cutleaf' from England and two red leaf cultivars, 'Fusco Rubra' from Germany and 'Ruby', which resulted form a cross of 'Chinese Trazel G-4' x 'Fusco Rubra' (Lagerstedt, 1990). The SSR loci data were consistent with the pedigree of 'Ruby' (Table 2.4).

The third outlying cluster includes two cultivars, '681.078' from Russia and 'COR 187' from Finland. The final outlying cluster consists of 'Aurea' from Germany,

'*C. avellana* COR 627' from Sweden, 'Barcelonner Zellernuss' from England, '408.040' received from the University of Minnesota and 'Pendula' from England.

Besides these four outlying clusters, five inner divergent clusters were revealed. The first inner cluster includes 5 cultivars: 'Zimmerman' from USA-Oregon, 'Gasaway' from USA-Washington, '495.072' from Southern Russia, 'Tskhenis Dzudzu (not)' from Georgia and 'Gulshishvela (not)' from Georgia. According to the SSR-profiles, 'Zimmerman' might be a seedling of 'Barcelona' X 'Gasaway'. This suggestion is supported by the incompatibility alleles of 'Barcelona' (S1, S2), 'Gasaway' (S3, S26) and 'Zimmerman' (S1, S3). Beside that 'Zimmerman', like 'Gasaway' has qualitative resistance to eastern filbert blight. Tskhenis Dzudzu (not)' and 'Gulshishvela (not)' have complete resistance to EFB. Although they are synonyms, their true identity remains unknown.

The second inner cluster consists of 'Hodji (not)' from Georgia and 'Webb's Prize Cob' from England. The third inner pair includes 'Warsaw Red' from Poland and 'Henneman#3' from USA-Oregon.

In the fourth inner cluster no tight geographical clustering was revealed. Cultivars from different geographical regions grouped together: 'Napoletana' from Italy, 'Gunslebert' from Germany, 'Tokolyi Cosford' from Australia, 'Gubener Barcelloner' from Germany, 'Tonda Bianca' from Italy and 'Tonda Rossa' from Italy. Although hazelnut is produced in a few regions in the southern hemisphere, it's not native to those regions. 'Tokolyi Cosford' is closely related to the German cultivar 'Gubener Barcelloner', and Italian cultivar 'Tonda Rossa', and all three cultivars have $S_{23}$ as a

common incompatibility allele. So, 'Tokolyi Cosford' might have German and/or Italian parents.

The last inner group includes Oregon Grower Selection Group # 2 ('Brixnut', 'Gem' and 'Lyons'), 'Brixley's New' and a pair of synonyms: 'Rosset de Valls' and 'Artellet' from Spain. The Oregon Grower Selection Group #2 cultivars are suggested to be selected seedlings of 'Barcelona' and 'Du Chilly', a less important cultivar occasionally used as pollenizer in Oregon. 'Barcelona' has incompatibility alleles $S_1$ and $S_2$, while 'Du Chilly' has $S_{10}$ and $S_{14}$. All cultivars in Oregon Grower Selection cluster #2 have one incompatibility allele from 'Barcelona' and one allele from'Du Chilly'. The asserted paternities of these cultivars are consistent with alleles at 21 SSR loci (Table 2.4). The synonyms 'Rosset de Valls' and 'Artellet' have incompatibility alleles $S_{14}$ and $S_{18}$ and produce phenotypically similar medium size, round and compressed nuts in short husks. Futhermore, 'Brixley's New', 'Simon' from Spain and 'Fitzgerald #20' from USA-Oregon placed in this group. 'Tapparona di San Colombano Cortemoli' from Italy clustered loosely with the last inner group, 'Spanish Group #2' and 'Italian Group' (Figure 2.2)

**Table 2.4** SSR profiles of some hazelnut accessions at 21 loci

| *Cultivars* | *CAC-A014a* | *CAC-A040* | *CAC-B005* | *CAC-B010* | *CAC-B028* | *CAC-B29b* | *CAC-B105* | *CAC-B111* | *CAC-C10* | *CAC-C28* | *CAC-C040* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cosford** | **211/217** | **236/236** | **277/281** | **211/211** | **266/270** | **116/128** | **148/153** | **182/182** | **278/278** | **131/141** | **186/186** |
| Frango #2 | 211/215 | 236/246 | 277/277 | 211/221 | 266/266 | 116/128 | 140/148 | 182/188 | 275/278 | 141/141 | 186/186 |
| Volski Round | 211/215 | 236/244 | 277/277 | 211/221 | 256/266 | 116/116 | 148/148 | 182/188 | 275/278 | 134/141 | 186/186 |
| Frango #5 | 211/215 | 236/244 | 277/281 | 211/221 | 256/266 | 116/116 | 140/148 | 182/184 | 275/278 | 141/141 | 186/186 |
| Lenka #3 | 217/217 | 236/246 | 277/281 | 211/221 | 270/270 | 128/128 | 140/153 | 182/186 | 278/278 | 141/141 | 186/186 |
| Acorn Hazel | 217/217 | 236/246 | 281/281 | 211/221 | 270/270 | 116/116 | 140/148 | 182/188 | 278/278 | 141/141 | 186/186 |
| | | | | | | | | | | | |
| **Barcelona** | **215/219** | **234/248** | **291/295** | **211/223** | **254/262** | **122/128** | **153/155** | **182/182** | **275/278** | **131/141** | **186/186** |
| **Cosford** | **211/217** | **236/236** | **277/281** | **211/211** | **266/270** | **116/128** | **148/153** | **182/182** | **278/278** | **131/141** | **186/186** |
| **Daviana** | **217/217** | **236/248** | **277/281** | **211/211** | **270/270** | **116/116** | **153/155** | **182/186** | **278/278** | **131/131** | **186/186** |
| Freehusker | 211/219 | 236/248 | 277/291 | 211/211 | 254/266 | 116/122 | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 |
| Ryan | 217/219 | 234/236 | 277/291 | 211/223 | 254/254 | 116/122 | 153/155 | 182/182 | 275/275 | 131/141 | 186/186 |
| Corabel | 211/215 | 234/236 | 277/295 | 211/223 | 262/266 | 128/128 | 148/153 | 182/182 | 275/278 | 131/141 | 186/186 |
| Nixon | 215/217 | 236/248 | 277/291 | 211/211 | 262/270 | 116/128 | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 |
| Woodford | 217/219 | 248/248 | 281/291 | 211/211 | 254/270 | 116/122 | 153/153 | 182/182 | 278/278 | 131/131 | 186/186 |
| Royal | 217/219 | 236/248 | 277/291 | 211/211 | 254/270 | 116/122 | 153/153 | 182/182 | 278/278 | 131/144 | 186/186 |
| Ennis | 217/219 | 234/248 | 277/295 | 211/223 | 254/254 | 116/128 | 153/155 | 182/182 | 278/278 | 131/141 | 186/186 |
| Fitzgerald | 217/219 | 234/236 | 277/295 | 211/223 | 254/270 | 116/128 | 155/155 | 182/186 | 278/278 | 131/131 | 186/186 |
| Nonpareil | 217/219 | 234/248 | 277/295 | 211/211 | 254/270 | 116/128 | 153/155 | 182/186 | 278/278 | 131/131 | 186/186 |
| Butler | 217/219 | 234/248 | 277/295 | 211/211 | 254/270 | 116/128 | 153/153 | 182/182 | 278/278 | 131/131 | 186/186 |
| | | | | | | | | | | | |
| **Barcelona** | **215/219** | **234/248** | **291/295** | **211/223** | **254/262** | **122/128** | **153/155** | **182/182** | **275/278** | **131/141** | **186/186** |
| **DuChilly** | **213/217** | **244/244** | **281/281** | **211/223** | **254/268** | **124/137** | **140/155** | **186/186** | **278/281** | **131/144** | **186/186** |
| Lyons | 215/217 | 234/244 | 281/291 | 223/223 | 262/268 | 122/137 | 153/155 | 182/186 | 278/281 | 131/131 | 186/186 |
| Gem | 213/215 | 234/244 | 281/291 | 223/223 | 254/262 | 128/137 | 140/153 | 182/186 | 275/278 | 131/144 | 186/186 |
| Brixnut | 213/219 | 234/244 | 281/291 | 223/223 | 254/254 | 128/137 | 140/153 | 182/186 | 275/278 | 131/144 | 186/186 |
| | | | | | | | | | | | |
| **Barcelona** | **215/219** | **234/248** | **291/295** | **211/223** | **254/262** | **122/128** | **153/155** | **182/182** | **275/278** | **131/141** | **186/186** |
| Rode Zeller | 211/217 | 236/244 | 277/281 | 211/219 | 264/266 | 116/128 | 153/155 | 182/184 | 275/278 | 131/141 | 186/189 |
| Red Fortrin | 215/217 | 234/244 | 281/295 | 211/211 | 262/264 | 128/128 | 153/155 | 182/182 | 275/275 | 141/141 | 186/189 |

**Table 2.4** Continued

| *Cultivars* | *CAC-C115* | *CAC-C118* | *CAC-C119* | *CaT-B107* | *CaT-B502* | *CaT-B504* | *CaT-B505* | *CaT-B507* | *CaT-B508* | *CaT-C504* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cosford** | **193/214** | **179/179** | **256/258** | **134/144** | **187/195** | **172/176** | **106/116** | **189/195** | **148/161** | **152/164** |
| Frango #2 | 193/214 | 179/179 | 256/264 | 130/144 | 187/187 | 172/174 | 106/118 | 189/195 | 148/165 | 152/152 |
| Volski Round | 193/214 | 179/179 | 256/264 | 130/134 | 187/201 | 172/174 | 116/118 | 189/189 | 144/161 | 152/164 |
| Frango #5 | 193/214 | 179/179 | 258/264 | 130/134 | 187/187 | 172/176 | 116/118 | 190/195 | 144/161 | 152/152 |
| Lenka #3 | 193/214 | 179/179 | 258/264 | 122/134 | 187/187 | 174/176 | 106/118 | 190/195 | 161/161 | 152/164 |
| Acorn Hazel | 214/214 | 179/179 | 256/264 | 130/134 | 187/187 | 176/176 | 116/118 | 189/189 | 144/161 | 152/164 |
| | | | | | | | | | | |
| **Barcelona** | **173/193** | **179/179** | **258/258** | **112/134** | **187/189** | **158/182** | **106/120** | **181/191** | **157/157** | **155/158** |
| **Cosford** | **193/214** | **179/179** | **256/258** | **134/144** | **187/195** | **172/176** | **106/116** | **189/195** | **148/161** | **152/164** |
| **Daviana** | **173/193** | **179/179** | **258/258** | **134/134** | **189/195** | **172/176** | **106/116** | **185/195** | **161/161** | **152/164** |
| Freehusker | 173/193 | 179/179 | 256/258 | 112/134 | 187/189 | 172/182 | 116/120 | 181/195 | 157/161 | 152/158 |
| Ryan | 173/193 | 179/179 | 258/258 | 134/134 | 187/195 | 172/182 | 116/120 | 191/195 | 157/161 | 152/158 |
| Corabel | 193/193 | 179/179 | 258/258 | 112/134 | 187/189 | 158/172 | 106/120 | 181/195 | 157/161 | 152/158 |
| Nixon | 193/193 | 179/179 | 258/258 | 134/134 | 187/189 | 172/182 | 106/116 | 181/195 | 157/161 | 152/158 |
| Woodford | 193/193 | 179/179 | 258/258 | 134/134 | 189/195 | 176/182 | 106/106 | 185/191 | 157/157 | 158/164 |
| Royal | 193/193 | 179/179 | 258/258 | 112/134 | 189/195 | 172/182 | 106/116 | 181/189 | 148/157 | 152/158 |
| Ennis | 173/193 | 179/179 | 258/258 | 134/134 | 187/195 | 158/172 | 116/120 | 185/191 | 157/157 | 152/155 |
| Fitzgerald | 173/193 | 179/179 | 258/258 | 112/134 | 189/189 | 158/172 | 106/120 | 181/195 | 157/157 | 152/155 |
| Nonpareil | 173/193 | 179/179 | 258/258 | 134/134 | 189/189 | 158/172 | 106/106 | 181/185 | 157/157 | 152/155 |
| Butler | 193/193 | 179/179 | 258/258 | 112/134 | 189/189 | 158/172 | 106/114 | 181/195 | 157/161 | 152/155 |
| | | | | | | | | | | |
| **Barcelona** | **173/193** | **179/179** | **258/258** | **112/134** | **187/189** | **158/182** | **106/120** | **181/191** | **157/157** | **155/158** |
| **DuChilly** | **173/193** | **179/179** | **258/264** | **122/130** | **189/195** | **176/176** | **106/114** | **185/189** | **157/165** | **164/164** |
| Lyons | 193/193 | 179/179 | 258/264 | 122/134 | 189/189 | 176/182 | 114/120 | 181/185 | 157/157 | 158/164 |
| Gem | 173/193 | 179/179 | 258/264 | 112/130 | 189/195 | 176/182 | 114/120 | 181/185 | 157/157 | 158/164 |
| Brixnut | 173/193 | 179/179 | 258/258 | 112/122 | 189/195 | 176/182 | 114/120 | 181/185 | 157/157 | 158/164 |
| | | | | | | | | | | |
| **Barcelona** | **173/193** | **179/179** | **258/258** | **112/134** | **187/189** | **158/182** | **106/120** | **181/191** | **157/157** | **155/158** |
| Rode Zeller | 193/214 | 179/182 | 256/264 | 130/134 | 187/195 | 168/176 | 106/126 | 189/197 | 148/157 | 158/164 |
| Red Fortrin | 173/214 | 179/182 | 258/264 | 134/134 | 187/187 | 158/176 | 106/106 | 189/191 | 157/157 | 158/164 |

**Table 2.4** Continued

| *Cultivars* | *CAC-A014a* | *CAC-A040* | *CAC-B005* | *CAC-B010* | *CAC-B028* | *CAC-B29b* | *CAC-B105* | *CAC-B111* | *CAC-C10* | *CAC-C28* | *CAC-C040* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Negret | 211/217 | 236/248 | 291/297 | 219/223 | 256/268 | 122/130 | 138/155 | 182/184 | 275/275 | 131/144 | 186/192 |
| Pauetet | 211/215 | 234/248 | 277/297 | 219/227 | 256/256 | 118/130 | 153/155 | 182/182 | 275/275 | 131/141 | 186/192 |
| Tomasina | 211/213 | 236/244 | 277/291 | 211/219 | 256/256 | 118/118 | 138/140 | 184/186 | 275/275 | 131/134 | 186/192 |
| Francoli | 211/213 | 234/248 | 277/291 | 211/223 | 256/256 | 118/122 | 140/155 | 182/184 | 275/275 | 131/134 | 186/186 |
| | | | | | | | | | | | |
| TGdL | 215/221 | 234/244 | 277/291 | 219/219 | 256/262 | 118/122 | 155/155 | 182/182 | 275/275 | 131/131 | 186/186 |
| Cosford | 211/217 | 236/236 | 277/281 | 211/211 | 266/270 | 116/128 | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 |
| Daria | 215/217 | 234/236 | 281/291 | 211/219 | 262/270 | 116/122 | 148/155 | 182/182 | 278/278 | 131/141 | 186/186 |
| | | | | | | | | | | | |
| Montebello | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 |
| Willamette | 217/219 | 236/244 | 291/295 | 219/223 | 254/270 | 122/128 | 153/155 | 182/188 | 278/278 | 131/131 | 186/186 |
| | | | | | | | | | | | |
| Tombul Ghi. | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 |
| Willamette | 217/219 | 236/244 | 291/295 | 219/223 | 254/270 | 122/128 | 153/155 | 182/188 | 278/278 | 131/131 | 186/186 |
| Clark | 215/217 | 244/244 | 279/295 | 219/225 | 262/270 | 122/128 | 153/155 | 182/184 | 278/278 | 131/141 | 186/186 |
| | | | | | | | | | | | |
| Barcelona | 215/219 | 234/248 | 291/295 | 211/223 | 254/262 | 122/128 | 153/155 | 182/182 | 275/278 | 131/141 | 186/186 |
| Tombul Ghi. | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 |
| Lewis | 215/217 | 236/244 | 279/291 | 219/223 | 262/270 | 122/122 | 155/155 | 182/182 | 278/278 | 131/134 | 186/186 |
| | | | | | | | | | | | |
| Barcelona | 215/219 | 234/248 | 291/295 | 211/223 | 254/262 | 122/128 | 153/155 | 182/182 | 275/278 | 131/141 | 186/186 |
| Gasaway | 215/215 | 236/244 | 277/297 | 211/219 | 256/278 | 116/135 | 153/157 | 192/192 | 275/278 | 144/144 | 186/189 |
| Zimmerman | 215/219 | 234/236 | 277/291 | 211/219 | 254/278 | 122/135 | 153/155 | 182/192 | 278/278 | 141/144 | 186/189 |
| | | | | | | | | | | | |
| Tonda Roma. | 211/217 | 238/248 | 277/291 | 211/219 | 262/268 | 122/122 | 155/161 | 182/182 | 275/275 | 131/141 | 183/186 |
| Tombul Ghi. | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 |
| Zimmerman | 215/219 | 234/236 | 277/291 | 211/219 | 254/278 | 122/135 | 153/155 | 182/192 | 278/278 | 141/144 | 186/189 |
| Epsilon | 215/217 | 236/248 | 277/291 | 219/225 | 262/278 | 122/122 | 153/155 | 184/192 | 275/275 | 141/144 | 183/186 |
| | | | | | | | | | | | |
| Casina | 211/217 | 244/244 | 277/281 | 219/227 | 268/278 | 118/122 | 140/155 | 182/184 | 275/275 | 131/141 | 186/192 |
| Riccia di Tala. | 215/219 | 242/244 | 277/291 | 219/223 | 254/262 | 122/122 | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 |
| Gasaway | 215/215 | 236/244 | 277/297 | 211/219 | 256/278 | 116/135 | 153/157 | 192/192 | 275/278 | 144/144 | 186/189 |
| Gamma | 215/217 | 244/244 | 277/277 | 219/223 | 262/268 | 122/135 | 140/157 | 184/192 | 275/275 | 131/144 | 186/186 |
| | | | | | | | | | | | |
| Ruby | 217/251 | 236/236 | 279/281 | 211/217 | 264/264 | 118/130 | 136/144 | 184/184 | 278/278 | 134/147 | 186/192 |
| Fusco Rubra | 215/217 | 236/246 | 277/279 | 217/219 | 264/266 | 116/118 | 136/140 | 184/186 | 272/278 | 134/134 | 186/189 |

**Table 2.4** Continued

| *Cultivars* | *CAC-C115* | *CAC-C118* | *CAC-C119* | *CaT-B107* | *CaT-B502* | *CaT-B504* | *CaT-B505* | *CaT-B507* | *CaT-B508* | *CaT-C504* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Negret** | **182/214** | **179/185** | **258/258** | **120/134** | **189/189** | **176/182** | **114/126** | **191/195** | **157/159** | **161/161** |
| Pauetet | 193/214 | 179/185 | 258/258 | 120/134 | 187/189 | 176/176 | 114/126 | 181/195 | 157/159 | 158/161 |
| Tomasina | 173/214 | 179/185 | 264/264 | 120/134 | 195/195 | 170/182 | 106/122 | 181/191 | 157/159 | 161/161 |
| Francoli | 182/214 | 179/185 | 258/264 | 120/134 | 189/195 | 170/182 | 106/114 | 191/191 | 157/159 | 161/161 |
| | | | | | | | | | | |
| **TGdL** | **173/173** | **179/179** | **258/258** | **134/151** | **185/189** | **170/182** | **114/126** | **185/191** | **146/163** | **158/161** |
| **Cosford** | **193/214** | **179/179** | **256/258** | **134/144** | **187/195** | **172/176** | **106/116** | **189/195** | **148/161** | **152/164** |
| Daria | 173/214 | 179/179 | 258/258 | 134/134 | 187/189 | 176/182 | 116/126 | 191/195 | 148/163 | 158/164 |
| | | | | | | | | | | |
| **Montebello** | **173/196** | **179/179** | **258/260** | **112/122** | **183/189** | **158/182** | **120/126** | **181/191** | **157/157** | **158/167** |
| Willamette | 193/196 | 179/179 | 258/258 | 122/144 | 189/195 | 158/182 | 120/126 | 189/191 | 157/161 | 167/167 |
| | | | | | | | | | | |
| **Tombul Ghi** | **173/182** | **179/179** | **260/260** | **116/134** | **187/187** | **168/182** | **118/126** | **189/197** | **157/163** | **158/161** |
| **Willamette** | **193/196** | **179/179** | **258/258** | **122/144** | **189/195** | **158/182** | **120/126** | **189/191** | **157/161** | **167/167** |
| Clark | 173/193 | 179/179 | 258/258 | 134/144 | 187/195 | 158/168 | 118/126 | 189/191 | 161/163 | 158/167 |
| | | | | | | | | | | |
| **Barcelona** | **173/193** | **179/179** | **258/258** | **112/134** | **187/189** | **158/182** | **106/120** | **181/191** | **157/157** | **155/158** |
| **Tombul Ghi.** | **173/182** | **179/179** | **260/260** | **116/134** | **187/187** | **168/182** | **118/126** | **189/197** | **157/163** | **158/161** |
| Lewis | 193/193 | 179/179 | 258/258 | 134/144 | 187/189 | 168/182 | 120/126 | 181/191 | 157/161 | 155/155 |
| | | | | | | | | | | |
| **Barcelona** | **173/193** | **179/179** | **258/258** | **112/134** | **187/189** | **158/182** | **106/120** | **181/191** | **157/157** | **155/158** |
| **Gasaway** | **214/217** | **179/182** | **258/264** | **122/128** | **183/195** | **174/174** | **114/120** | **179/190** | **146/165** | **161/164** |
| Zimmerman | 173/214 | 179/182 | 258/264 | 128/134 | 187/195 | 174/182 | 106/114 | 190/191 | 146/157 | 158/164 |
| | | | | | | | | | | |
| **Tonda Romana** | **173/196** | **176/179** | **258/258** | **134/142** | **187/191** | **160/182** | **118/126** | **185/189** | **157/157** | **152/158** |
| **Tombul Ghi.** | **173/182** | **179/179** | **260/260** | **116/134** | **187/187** | **168/182** | **118/126** | **189/197** | **157/163** | **158/161** |
| **Zimmerman** | **173/214** | **179/182** | **258/264** | **128/134** | **187/195** | **174/182** | **106/114** | **190/191** | **146/157** | **158/164** |
| Epsilon | 173/196 | 176/179 | 258/258 | 128/134 | 187/195 | 160/182 | 114/118 | 190/197 | 146/157 | 152/158 |
| | | | | | | | | | | |
| **Casina** | **173/196** | **179/185** | **256/258** | **130/134** | **187/187** | **176/176** | **118/126** | **189/191** | **167/161** | **158/158** |
| **Riccia di Tala.** | **182/196** | **179/179** | **258/258** | **118/134** | **187/197** | **168/182** | **118/126** | **181/185** | **157/167** | **155/158** |
| **Gasaway** | **214/217** | **179/182** | **258/264** | **122/128** | **183/195** | **174/174** | **114/120** | **179/190** | **146/165** | **161/164** |
| Gamma | 173/196 | 179/179 | 258/258 | 130/134 | 183/187 | 168/176 | 118/126 | 179/189 | 161/167 | 158/161 |
| | | | | | | | | | | |
| **Ruby** | **188/214** | **179/185** | **260/264** | **120/122** | **191/195** | **164/176** | **106/122** | **176/198** | **144/165** | **152/152** |
| Fusco Rubra | 214/214 | 179/182 | 256/264 | 120/122 | 189/195 | 164/174 | 114/122 | 189/198 | 165/165 | 152/164 |

## Conclusions

The polymorphism and somatic stability of SSR markers and UPGMA cluster analysis in combination with the proportion of shared alleles seems to be a very powerful method to analyze genetic diversity in European hazelnut and other clonally propagated crops. Unlike other distance methods, it makes no assumptions about the nature of the population under study. Especially for a clonally propagated crop like hazelnut, the 'proportion of shared alleles' distance method is appropriate (Dangl et al., 2001).

The low probability of randomly matching genotypes (PI index) in this study gives great confidence that our SSRs detected synonyms, identified mislabeled accessions and confirmed pedigree information. The phenogram revealed some tight geographically clustered groups and 70 synonyms in the population. However, SSR markers could not differentiate some synonyms that differ in pellicle color [e.g 'White Filbert' (brown pellicle), 'Purple Fortrin'(red-purple pellicle), 'Purple Aveline'(red-purple pellicle ), 'Pellicule Rouge'(red-purple pellicle), 'Istarski Debeloplodna '(brown pellicle), 'Fructo Albo'(brown pellicle), 'Barbarella' (red-purple pellicle) and 'Aveline Rouge'(red-purple pellicle)].

Some of the cultivars (e.g. 'Purple Aveline', 'White Filbert', 'Webb's Prize Cob', 'DuChilly', 'Gunslebert', 'Garibaldi', 'Ennis', 'Butler', 'Istarski Duguljasti' and 'Pellicle Rouge') are placed by various taxonomic source in a different species, *Corylus maxima.* However, these cultivars did not cluster together as a seperate group; rather they are mixed with other *C. avellana* cultivars. So, our results indicate that *C. maxima* is not a distinct taxonomic group.

SSR markers revealed a great diversity in the Turkish hazelnut cultivars. However, German and Italian cultivars are less diverse. The lack of genetic diversity was especially striking in the Sicilian group where 11 cultivars were synonyms.

This study included some low informative SSR markers with low $H_o$ and $H_e$ values and with only a few alleles. For small scale fingerprinting studies, loci with high $H_o$ and $H_e$ values and with many alleles are suggested (O'Reilly and Wright, 1995; Testolin et al., 2000).

A second set of DNA samples was extracted from four trees ('Bard', 'Karidaty', 'Istarski Debeloplodna' and 'Riekchen's Zellernuss') and included as checks. PCRs of all controversial fragment sizes such as null allele containing loci or cultivars that differ from each other only at one locus were redone at least once to make sure that the fragment size was correct. The presence of null alleles is a known problem associated with the use of SSR markers (Callen et al., 1993). Null alleles are usually not detected and individuals are scored as homozygous at that locus. This results is a loss of information. The presence of null alleles can be expected in large homogeneous populations, when the observed heterozygosity ($H_o$) is markedly less than the expected heterozygosity ($H_e$). The expected null allele frequencies at three loci (CAC-C010, CaT-C504 and CaT-B508) have high positive values and the occurrence of null alleles at these three loci was detected by comparing the pedigree information of some cultivars.

We used 21 SSR loci to characterize 270 hazelnut accessions. The map locations of 16 of these were identified (Chapter 3). We recommend that a subset of these loci be used in future fingerprinting studies, with a preference for loci with high heterozygosity

as indicated by high PIC and PEP values, low PI values, and a low frequency of null alleles. Furthermore, loci in different linkage groups are preferred as they would give better coverage of the genome. According to these criteria, we suggest CAC-B029b and CAC-A040 on chromosome 1, CAC-B010 on chromosome 3, CAC-C115 on chromosome 4, CAC-A014a and CAC-B028 on chromosome 5, CaT-B504 and CAC-C005 on chromosome 7, CAC-C119 on chromosome 8, CaT-B507 on chromosome 9 and CaT-B502 on chromosome 10. These loci generate fragments of different sizes. The use of different florescent labels would allow their use in 3 to 4 multiplexes.

Synonyms are a big problem in the management of hazelnut germplasm collections. Of the 270 accessions screened in this study, 70 (26%) turned out to be synonyms. The high percentage of synonyms might be due to mistakes during propagation and points to a need to verify that the plants in the USDA-ARS-NCGR and OSU hazelnut collections and in other collections around the world are true-to-name.. The SSR loci used in this study could be used to amplify DNA extracted from other collections, and morphological and phenological traits could be compared to those in published descriptions. We encourage the adoption of the standard set of 11 markers listed above for this purpose.

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# CHAPTER 3

# INTEGRATION OF TWENTY-NINE SSR MARKERS INTO A LINKAGE MAP OF HAZELNUT

T. Gökirmak, S.A. Mehlenbacher, N.V. Bassil

**Abstract**

Thirty-three simple sequence repeat (SSR) markers were scored in a population of European hazelnut (*Corylus avellana* L.) 144 seedlings from a controlled cross of OSU 252.146 x OSU 414.062. Twenty-nine of them were successfully integrated into the RAPD marker-based linkage map for this population. Two of these loci showed aberrant segregation ratios and were not placed on the map, and two loci remained unlinked. The linkage map, constructed using the two-way pseudo-testcross approach, identified eleven linkage groups for each parent, corresponding to the haploid chromosome number of hazelnut (2n=2x=22). The map spanned a total distance of 668 cM in the susceptible parent and 813 cM in the resistant parent. The order of homologous SSR loci in the two parents was collinear in most cases. Placement of these SSR "anchor loci" on the hazelnut linkage map will make it useful in other populations.

## Introduction

Hazelnut is a general name used for the nuts of all *Corylus* species. The genus *Corylus* is a member of the Betulaceae or Birch family and includes other important forest tree species and ornamentals (Erdogan and Mehlenbacher, 2000). The European hazelnut, *Corylus avellana* is one of the 25 described species in the genus, all of which produce edible nuts, which are collected from the wild by humans. All *Corylus* species are diploid with 2n = 22 chromosomes (Kasapligil, 1968; Thompson et al., 1996).

The European hazelnut, *Corylus avellana* L., is grown in many regions of Europe and western Asia and has been used as a food source by humans since prehistoric times. *Corylus avellana*, the species of commerce, is native to Europe and Asia Minor (Kasapligil, 1972).

Hazelnut is clonally propagated and highly heterozygous (Mehlenbacher, 1995). It has a unique floral biology, which was reviewed in detail by Germain (1994). It is monoecious, dichogamous and wind-pollinated (Thompson et al., 1996). The male flowers are borne in catkins, and female inflorescences have no perianth. They appear as a tuft of red stigmatic styles protruding from the apex of compound buds located on shoots or catkin peduncles. The hazelnut tree is self-incompatible, which enforces cross-pollination. Incompatibility is of the sporophytic type, which is under the control of a single locus (the S-locus) with multiple alleles (Mehlenbacher, 1997).

Among the various DNA-based molecular markers, random amplified polymorphic DNA (RAPD) markers are the most commonly used in hazelnut. RAPD markers linked to eastern filbert blight resistance (Davis and Mehlenbacher, 1997;

Mehlenbacher et al., 2003) and to self-incompatibility (Pomper et al., 1998) have been identified at Oregon State University. Furthermore, a linkage map based on RAPD markers was recently developed by Mehlenbacher et al. (2005 a&b). RAPDs are dominant markers. Polymorphism is the result of presence or absence of the priming site in the genomic DNA. RAPD is a fast, easy and inexpensive technique. However, it has some reliability problems. Results are very sensitive to changes in reaction conditions and even different equipment in different laboratories may give different results (Powell et al., 1996).

Microsatellites have become the marker of choice for germplasm "fingerprinting" because of their advantages over other types of markers. Microsatellites or simple sequence repeats (SSRs) are tandemly repeated 1-6 bp sequence motifs found in both eukaryotic and prokaryotic genomes. They are abundant and dispersed throughout the genome and they can be found in both coding and non-coding regions. They are present mostly in the latter due to the fact that non-coding DNA can accumulate mutations more easily than coding DNA (Ahmad et al., 2003). Although the mechanism of microsatellite evolution is not known exactly, it appears most likely due to the slippage of DNA polymerase during DNA replication or unequal crossing-over between homologous chromosomes during recombination (Schlötterer and Tautz, 1992). The majority of microsatellites are dinucleotide repeats located in non-coding regions of the genome (Li et al., 2002). On the other hand, microsatellites found in coding regions are mainly trinucleotide and hexonucleotide repeats, probably due to negative-selection against frameshift mutations in coding regions (Morgante et al., 2002).

Microsatellite markers have been extensively used in fingerprinting (Thomas and Scott, 1993), linkage map construction (Bowcock et al., 1994; Hazan et al., 1992), forensic DNA research, and population genetic studies (Jarne and Lagoda, 1996). Valuable characteristics of SSR markers include a high level of polymorphism, they are co-dominant, only a small quantity of DNA is needed for PCR, loci are usually conserved between related species and even sometimes across genera, they do not require the use of radioactivity and data are scored easily and reproducibly (Zane et al., 2002). The exchange of primer sequences instead of probes allows other labs to work with the same loci and is another advantage of SSR markers. The only disadvantages of microsatellite markers are that they must be isolated *de novo* from most species being examined for the first time and the development of SSRs requires a considerable investment and technical expertise (Zane et al., 2002; Jauhar, 1996).

***Linkage map construction by the double pseudo-testcross strategy***

Mapping and sequencing of full plant genomes would provide a more complete understanding of gene function, gene regulation and expression. Marker-saturated high-density linkage maps have useful applications in both fundamental and applied genetic research. Molecular markers are extensively used in identification and isolation of genes of interest, and linkage analysis is one of the basic and central techniques in genetics. Linkage can define the genetic distances between polymorphic loci which may be expressed as differences in appearance of enzyme activities, restriction fragment lengths or nucleotide sequences at an allelic locus (Mohan et al., 1997). In the past few years

linkage maps have been constructed for woody perennial plants including *Citrus* (Sankar and Moore, 2001), *Prunus* (Joobeur et al., 1998), cacao (*Theobroma cacao* L.) (Pugh et al., 2004), apple (*Malus x domestica*) (Hemmat et al., 1994; Liebhard et al., 2003), grape (*Vitis vinifera*) (Dalbo et al., 2000, Fischer et al., 2004; Riaz et al., 2004), coniferous forest trees (Scott et al., 1999) and olive (*Olea europae* L.) (Wu et al., 2004). Among the nut crops, linkage maps have been constructed for European hazelnut (*Corylus avellana* L.) (Mehlenbacher et al., 2005 a&b), European chestnut (*Castanea sativa* L.) (Casasoli et al., 2001) and *Macadamia* (Peace et al., 2003).

Construction of a genetic linkage map is a useful step in identifying markers linked to genes controlling traits of interest, and genetic maps can also be used to identify loci that control quantitative traits (Paterson et al., 1991). Similar to other perennial tree crops, the juvenile period of hazelnut ranges from three to six years, with five years being the median (Mehlenbacher and Smith, 1992) and genotypes exhibit sporophytic self-incompatibility (Mehlenbacher, 1997). The 'two-way pseudo-testcross strategy' involves linkage analysis of an $F_1$ population produced by crossing two highly heterozygous (diploid) parent clones. The first linkage map using this method and PCR-based markers was constructed for *Eucalyptus* (Grattapaglia and Sederoff, 1994). Genetic linkage maps can be constructed using software packages such as MapMaker (Lander et al., 1987), JoinMap 3.0 (van Ooijen and Voorrips, 2001) or GMendel (Holloway et al., 1994). The two important issues in linkage map construction are locus order and distance. The likelihood odds (LOD) score is the test statistic used to test the hypothesis that there is no linkage against the alternative hypothesis that there is linkage. A LOD score of 3.00 is

roughly equal to P=0.001. So, if LOD $\geq$ 3.00, it is very likely that two loci are linked (Li et al., 2003). Linkage distances with the highest LOD scores are the best estimates of real linkage distances.

The objective of this study was to score 33 SSR markers in a population of 144 seedlings and to place them on the RAPD marker-based linkage map constructed by Mehlenbacher et al. (2005 a&b). Because of their ease of use, high information content and co-dominant nature, SSR markers are valuable additions to dominant markers and RFLP markers in mapping projects (Dib et al., 1996). Placement of SSR markers on the hazelnut linkage map would allow them to serve as "anchor loci" that are useful in other segregating populations.

## Materials and Methods

### *Plant material and DNA extraction*

The mapping population, a total of 144 seedlings, was generated in 1993 from a controlled cross of two heterozygous clones. The maternal parent, OSU 252.146, is susceptible to eastern filbert blight, while the paternal parent, OSU 414.062, is heterozygous resistant. Two sets of fresh young leaves for each of 144 seedlings and the two parents were collected in the spring of 2004 from the collection of Oregon State University in Corvallis, Oregon. DNA was extracted according to Lunde et al. (2000) with minor modifications and RNA was removed by incubation with RNase A (Sigma, St. Louis, MO) at 37 °C for one hour in a shaker, followed by extraction with 25 phenol: 24 chloroform: 1 isoamyl alcohol. The DNA concentrations were determined spectrophotometrically, adjusted to 5 ng/μl and the DNA was stored at -18 °C until used for PCR.

### *Amplification and allele sizing*

Primer pairs for 24 loci developed in Corvallis (CAC) (Bassil et al., 2005) and for 9 loci developed in Torino (CaT) (Boccacci et al., 2005) (Table 3.1) were chosen for this study. Reverse primers were purchased from Operon Technologies (Qiagen, Valencia, CA). Forward primers fluorescently labeled with FAM and HEX were purchased from Operon Technologies and with NED from Applied Biosystems (Foster City, CA). PCR reactions were performed in a total volume of 10 μl and the reaction mixture contained 1X Biolase $NH_4$ reaction buffer, 2 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP, and

dTTP, 0.3 μM each of forward and reverse primers, 0.25 units of Biolase DNA polymerase (Bioline Inc., Randolph, MA), and 2.5 ng of template DNA. The PCR program consisted of 35 cycles of a 40 s denaturation step at 94 °C, a 40 s annealing step at the optimum annealing temperature (Table 3.1), and a 40 s extension step at 72 °C. Finally one 30-min extension step at 72 °C was run to maximize non-templated adenosine addition to the 5' ends. PCRs were run in Perkin-Elmer model 9700 thermocyclers (PE Applied Biosystems, Foster City, CA). PCR amplification and approximate fragment sizes were confirmed on 3% agarose gels using 4 μl of aliquot and 5 μl of loading dye (15% Ficoll® 400, 0.03% xylene cyanol FF, 0.4% orange G, 10mM Tris-HCL pH 7.5, and 50 mM EDTA). Gels were stained with ethidium bromide and photographed under UV-light by an imaging system (UVP, Upland, CA). Amplified PCR products were diluted forty times with nanopure water and kept as stock for multiplexing. Stock solutions were further diluted 2 to 16 (Table 3.1) times and 1 μl of mix of four to five (Appendix A) PCR products were separated on an ABI 3100 capillary electrophoresis instrument (Applied Biosystems, Foster City, California) at the OSU Central Services Laboratory (CSL), and DNA fragments were sized using GeneScan and Genotyper software.

***Integration of SSR markers into a linkage map of hazelnut***

The software package JoinMap 3.0 (van Ooijen and Voorrips, 2001) was used to construct the linkage map by integrating the SSR loci reported in this chapter with the RAPD markers previously mapped in hazelnut by Mehlenbacher et al. (2005 a&b).

**Table 3.1** Description of 33 microsatellite loci: the repeat motif, sequence of the fluorescent forward primer (*FAM, NED, HEX*) and the reverse primer (*R*), the optimum annealing temperature (Tm), dilution cofactor in multiplex, allele size range and linkage group*

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range | Linkage Gr[c] |
|---|---|---|---|---|---|---|
| CAC-A014a | $(CA)_{13}$ | ***FAM***-GGTTTGTTACAGAAATTCAGACG | 60°C | 1:640 | 203-251 | 5S, 5R |
| | | ***R***-GCGTGTGGTTAATGTTTTCTTT | | | | |
| CAC-A24b | $(GA)_{18}(AT)_7$ | ***NED***-CACAACATGCAACGTCTATGTA | 62°C | 1:120 | 118-138 | 7R |
| | | ***R***-AGGTACGTATTGACAGGCTTTT | | | | |
| CAC-A040 | $(CA)_{13}$ | ***NED***-TGCTCAAGCAAATATTGCAC | 62°C | 1:213 | 234-248 | 1S, 1R |
| | | ***R***-GTTTGGGATCCAATTAACCCTCT | | | | |
| CAC-A102 | $(AG)_{16}(AC)_{15}$ | ***HEX***-AAACTGTGACGAACGAAAACAC | 62°C | 1:80 | 269-307 | 9S, 9R |
| | | ***R***-TTGCACTTCCATAACTGTCAAA | | | | |
| CAC-B005 | $(GA)_{22}$ | ***FAM***-CAAACTTATGATAGGCATGCAA | 62°C | 1:320 | 277-297 | 7R |
| | | ***R***-TGTCACTTTGGAAGACAAGAGA | | | | |
| CAC-B010 | $(GA)_{16}$ | ***FAM***-AGCTTCCAAATCACACATTACC | 62°C | 1:320 | 211-227 | 3S, 3R |
| | | ***R***-GAAGAGCATCCGTATGATTCAG | | | | |
| CAC-B011 | $(GA)_{11}$ | ***NED***-CACTGGTGATCTCACAGGTTTA | 62°C | 1:240 | 131-157 | 2S, 2R |
| | | ***R***-GTCCTCAAAAGCTAAGCACAAG | | | | |
| CAC-B020 | $(GA)_{19}$ | ***HEX***-GGGAAAATACTCCAAATCGCT | 60°C | 1:240 | 273-289 | 7R |
| | | ***R***-TCACCGAGCCGTCATAATC | | | | |
| CAC-B028 | $(AG)_{16}$ | ***NED***-ATGGACGAGGAATATTTCAGC | 55°C | 1:213 | 252-288 | 5S, 5R |
| | | ***R***-CCTGTTTCTCTTTGTTTTCGAG | | | | |

**Table 2.2** Continued

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range | Linkage Gr[e] |
|---|---|---|---|---|---|---|
| CAC-B029b | $(GA)_{13}$ | ***NED***-CAATTTACACCTCAGGGAAGAG | 58°C | 1:160 | 114-139 | 1S, 1R |
| | | ***R***-AAGTTCACCCAAGAAATCCAC | | | | |
| CAC-B101 | $(AG)_{14}$ | ***HEX***-GCAGACCAGAGTCTGTTATTCA | 62°C | 1:480 | 135-180 | unlinked |
| | | ***R***-AGACAATTTCGTGACTGGGTAT | | | | |
| CAC-B105 | $(GA)_{16}$ | ***HEX***-AAAGGAGCAAGCATGTTAGG | 62°C | 1:320 | 125-163 | 10S, 10R |
| | | ***R***-GTTTGTACGGATGATCCACTGAG | | | | |
| CAC-B109 | $(GA)_{21}$ | ***HEX***-AATCCAAGCCTTTTCACTACC | 58°C | 1:320 | 145-155 | 9S |
| | | ***R***-ACCCATCAAGTTCACCAATC | | | | |
| CAC-B113 | $(GA)_{14}$ | ***HEX***-TTGAGGAAGTCCAGGAAAAT | 60°C | 1:320 | 167-179 | 2R |
| | | ***R***-GCCAGAGAGAGCAAGAGTTAG | | | | |
| CAC-B114 | $(GA)_{14}$ | ***HEX***-TTCCCCTCTCAAAGCCAC | 64°C | 1:480 | 138-153 | not placed |
| | | ***R***-GAAGGTTGAAGAAGAGCAACAG | | | | |
| CAC-C001a | $(CACAGAG)_3$ | ***FAM***-CCCGTAACTAACCAATCACAAT | 58°C | 1:320 | 200-220 | 9S, 9R |
| | | ***R***-TGGAGAAGAGGAGAGCTTAGTG | | | | |
| CAC-C005 | $(GAA)_8$ | ***NED***-GCTCTGAAACTATCGCTAGACG | 58°C | 1:320 | 97-127 | not placed |
| | | ***R***-GTCTGCCATTTGTGGTCTGT | | | | |
| CAC-C008 | $(AAG)_{11}$ | ***FAM***-TTTCCGCAGATAATACAGGG | 58°C | 1:320 | 200-245 | 4S, 4R |
| | | ***R***-TCCTTTGCTTTGGACCAG | | | | |

**Table 2.2** Continued

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range | Linkage Gr[c] |
|---|---|---|---|---|---|---|
| CAC-C028 | $(GAA)_{10}$ | ***NED***-CTACCCCATCGCTTGACAC | 60°C | 1:213 | 131-147 | 10R |
| | | ***R***-GGAGACTTGTTTGCCACAGA | | | | |
| CAC-C040 | $(GAA)_8(GGA)_5$ | ***FAM***-AGCCCCATTAGCCTTCTTAG | 62°C | 1:320 | 168-192 | 4R |
| | | ***R***-GTTTCCAGATCTGCCTCCATATAAT | | | | |
| CAC-C114 | $(TTC)_6$ | ***HEX***-TCTCCCTCTCCCTCTCTTCTAC | 60°C | 1:400 | 255-279 | 5S, 5R |
| | | ***R***-GAAAGGAAAAAGCACATAGCAA | | | | |
| CAC-C115 | $(TAA)_5(GAA)_{12}$ | ***FAM***-CATTTTCCGCAGATAATACAGG | 60°C | 1:320 | 167-225 | 4S, 4R |
| | | ***R***-GTTTCCAGATCTGCCTCCATATAAT | | | | |
| CAC-C118 | $(AAG)^b$ | ***HEX***-AGCAACAGAGGTTAGGTGTG | 60°C | 1:320 | 162-185 | 4R |
| | | ***R***-GCCCCATTAGCCTTCTTA | | | | |
| CAC-C119 | $(GA)_7(GA)_9$ | ***NED***-CTCACCTTTACCCCTTCATTTT | 62°C | 1:213 | 256-264 | 8R |
| | | ***R***-GTTTCCTCATCTTCTGAGAACCATC | | | | |
| CaT-A114 | $(TG)_{17}$ | ***FAM***-CGCCTTGATAGTATGTTCAAAC | 60°C | 1:320 | 165-181 | 4S, 4R |
| | | ***R***-CGGCAGAATGTAGAAGTCCCC | | | | |
| CaT-B106 | $(AG)_{17}AA(AG)_6$ | ***HEX***-CCAATCGCCAATGAATCATC | 60°C | 1:320 | 156-181 | unlinked |
| | | ***R***-CCCTTTCCAAACTGGGCAT | | | | |
| CaT-B107 | $(CT)_{14}$ | ***NED***-GTAGGTGCACTTGATGTGCTTTAC | 58°C | 1:160 | 112-151 | 10R |
| | | ***R***-AACACCATATTGAGTCTTTCAAAGC | | | | |

**Table 2.2** Continued

| SSR Locus | Motif | Primers (5' – 3') | Tm | Dilution | Allele Size Range | Linkage Gr[e] |
|---|---|---|---|---|---|---|
| CaT-B501 | $(GA)_{21}$ | ***NED***-GAAATTCAATCACACCAATAAAGCA | 64°C | 1:160 | 117-137 | 2R |
| | | ***R***-CCTCCCTTGTCCTCATCACTG | | | | |
| CaT-B502 | $(CT)^{c}$ | ***FAM***-CTCATGACTGCCCATTTCTCG | 62°C | 1:400 | 183-211 | 10S, 10R |
| | | ***R***-AGGCATGCAGGCTTCACAC | | | | |
| CaT-B504 | $(CT)_{18}$ | ***HEX***-CGCCATCTCCATTTCCCAAC | 60°C | 1:400 | 158-184 | 7R |
| | | ***R***-CGGAATGGTTTTCTGCTTCAG | | | | |
| CaT-B507 | $(GA)^{d}$ | ***FAM***-CTA AGCTCACCAAGAGGAAGTTGAT | 62°C | 1:400 | 176-198 | 9S, 9R |
| | | ***R***-GCTTCTGGGTCTCCTGCTCA | | | | |
| CaT-B509 | $(GA)_{14}$ | ***HEX***-GTCTGGCATGGTTTTGAGAAGA | 62°C | 1:320 | 109- 119 | 7R |
| | | ***R***-CTTTCCCGCCCAAACCAC | | | | |
| CaT-C502 | $(CTT)^{e}$ | ***HEX***-GCATGCAAGGTGGTCGGT | 62°C | 1:320 | 151-166 | 9R |
| | | ***R***-TTTGGCACCCAACAACTCTAGA | | | | |

[a] $(GAA)_7GGA(GAA)_2N_{21}(GAA)_2ATT(GAA)_4N_{15}(GAA)_3$
[b] $(AAG)_3(GAA)_3(AAG)_8N_6(AAG)_4$
[c] $GA)_1GC(GA)_2GC(GA)_{14}$
[d] $(CT)_{16}GCTTTTC(CT)_5$
[e] Figure 3.1

JoinMap requires a certain format for entering the data. Marker data were scored as 1 for the presence of an allele, 0 for the absence of an allele, or n for unknown, according to the parental origin of the alleles of each marker. For SSR loci, data were scored for each allele. Presence of one allele in a seedling meant absence of the other allele from that parent at that locus. Two data sets were obtained, one for the susceptible maternal parent OSU 252.146 (S) and one for the resistant paternal parent OSU 414.062 (R). Afterward, the marker data sheets were recoded as "h" for 1, "a" for 0 and "u" for n, and then saved as a tab-delimited text file and imported into JoinMap 3.0. For loci segregating with 1:2:1 ratios, the parental origin of alleles in heterozygous seedlings could not be determined. Therefore, the indicator variable for each allele was coded as "u" for unknown.

Four segregation patterns were expected for the SSR loci: 1 : 1 from the maternal parent, 1 : 1 from the paternal parent, and 1 : 2 : 1 or 1 : 1 : 1 : 1 from both parents (heterozygous in both parents). Observed segregation ratios were compared to the expected Mendelian segregation ratios using a chi-square ($\chi^2$) goodness-of-fit test. Computed $\chi^2$ values were compared to the critical values from theoretical distributions. The appropriate degrees of freedom (df) were calculated by subtracting one from the number of genotypic classes (Table 3.2). The Yates correction factor was not used.

A preliminary analysis was performed using a small group of about 250 RAPD markers for each parent to allow assignment of the SSR markers to a linkage group on the preliminary map (Mehlenbacher et al., 2005 a&b). A critical LOD value $\geq 5$ and the default recombination frequency of 0.40 were used to detect linkage and minimize the merging of groups at lower values. After linkage group assignment, individual SSR

marker data were appended to the RAPD marker data for the same linkage group. To be able to distinguish the SSR markers from RAPD markers on the map, an asterisk sign "*" was placed before the label of each SSR marker. Linkage maps were constructed independently for each group using the "LOD grouping tree" command. Following the first mapping attempt, JoinMap listed the RAPD markers and linked SSR alleles, followed by an error message, "insufficient linkage to above group", and a second group of SSR alleles. The first group includes SSR alleles linked in coupling to the RAPD markers and the second group consists of markers that are linked in repulsion. The second group was removed and JoinMap created a map for each linkage group. The Kosambi mapping function was used to convert the recombination frequencies into map distance (Kosambi, 1944). JoinMap tests each marker with a chi-square test for goodness-of-fit. The highest chi-square value allowed was arbitrarily set at 6 and markers with larger values were removed in stepwise fashion until all markers in each linkage group had a value of less than 6.

## Results and Discussion

### *Microsatellite map position*

The genotypes of each of the 144 seedlings and the two parents were successfully determined at all 33 SSR loci. Analysis of segregation ratios showed that two of thirty-three SSR markers (6%) (CAC-B114, CAC-C005) displayed a significant deviation from expected ratios ($P < 0.05$) (Table 3.2). JoinMap assigned both of these markers to linkage groups.CAC-B114 could be placed only on chromosome 11 of the susceptible parent, although both parents are heterozygous at this locus. On the other hand, CAC-C005 was mapped on chromosome 6 of both resistant and susceptible parents, but did not show perfect colinearity. Because of these aberrations, both were removed from the map. CAC-B101 and CaT-B106 had low chi-square values but could not be placed on the map and are listed as unlinked loci.

After adding the SSR loci to the map, two RAPD markers (487-500 on chromosome 2 of the resistant parent and 217-750 on chromosome 1 of the susceptible parent) showed high chi-square values and were removed from the maps. Of the 33 SSR primers, 29 of them (88%) were successfully integrated into a RAPD marker-based hazelnut linkage map at LOD scores of 5.0 and 7.0 (Figure 3.1). Eleven linkage groups were identified for each parent, corresponding to the haploid chromosomes number of hazelnut (2n=2x=22) and spanning a total distance of 668 cM in the susceptible parent and 813 cM in the resistant parent. 270 markers, 19 of which are SSRs, were placed on

**Table 3.2** Segregation analysis of microsatellite markers

| SSR Locus | ♀ x ♂ | Expected ratio | Progeny segregation | $\chi^2$ | df | P |
|---|---|---|---|---|---|---|
| CAC-A24b | (130/130) x (126/134) | 1 : 1 | 73 : 71 | 0.028 | 1 | 0.867 |
| CAC-B005 | (295/295) x (279/291) | 1 : 1 | 70 : 74 | 0.444 | 1 | 0.505 |
| CAC-C028 | (131/131) x (141/144) | 1 : 1 | 63 : 81 | 2.25 | 1 | 0.134 |
| CAC-B109 | (149/151) x (151/151) | 1 : 1 | 70 : 74 | 0.111 | 1 | 0.739 |
| CAC-B113 | (173/173) x (173/175) | 1 : 1 | 59 : 85 | 4.694 | 1 | 0.03 |
| CAC-B101 | (173/175) x (173/173) | 1 : 1 | 69 : 75 | 0.25 | 1 | 0.617 |
| CAC-C040 | (186/186) x (186/189) | 1 : 1 | 62 : 82 | 2.777 | 1 | 0.096 |
| CAC-C118 | (179/179) x (179/182) | 1 : 1 | 61 : 83 | 3.361 | 1 | 0.067 |
| CAC-C119 | (258/258) x (260/264) | 1 : 1 | 71 : 73 | 0.027 | 1 | 0.869 |
| CaT-C502 | (155/155) x (155/159) | 1 : 1 | 71 : 73 | 0.027 | 1 | 0.869 |
| CaT-B504 | (158/158) x (168/182) | 1 : 1 | 69 : 75 | 0.25 | 1 | 0.617 |
| CaT-B106 | (168/180) x (180/180) | 1 : 1 | 71 : 73 | 0.027 | 1 | 0.869 |
| CaT-B107 | (112/112) x (112/128) | 1 : 1 | 68 : 76 | 0.444 | 1 | 0.505 |
| CAC-A14a | (215/219) x (215/219) | 1 : 2 : 1 | 27 : 80 : 37 | 1.917 | 2 | 0.383 |
| CAC-A102 | (289/303) x (289/303) | 1 : 2 : 1 | 41 : 73 : 30 | 1.708 | 2 | 0.426 |
| CAC-B028 | (254/262) x (254/262) | 1 : 2 : 1 | 36 : 81 : 27 | 3.375 | 2 | 0.185 |
| CAC-C005 | (110/116) x (110/116) | 1 : 2 : 1 | 31 : 40 : 73 | 52.944* | 2 | <0.001 |
| CAC-A040 | (233/244) x (244/248) | 1 : 1 : 1 : 1 | 27 : 33 : 40 : 44 | 4.722 | 3 | 0.193 |
| CAC-B010 | (208/219) x (215/221) | 1 : 1 : 1 : 1 | 30 : 38 : 41 : 35 | 1.833 | 3 | 0.608 |
| CAC-B011 | (135/152) x (143/152) | 1 : 1 : 1 : 1 | 38 : 30 : 43 : 33 | 2.722 | 3 | 0.437 |

**Table 3.2** Continued

| SSR Locus | ♀ x ♂ | Expected ratio | Progeny segregation | $\chi^2$ | df | P |
|---|---|---|---|---|---|---|
| CAC-B020 | (283/285) x (277/283) | 1 : 1 : 1 : 1 | 38 : 31 : 41 : 34 | 1.611 | 3 | 0.657 |
| CAC-B029b | (116/128) x (116/122) | 1 : 1 : 1 : 1 | 35 : 38 : 37 : 34 | 0.277 | 3 | 0.964 |
| CAC-B105 | (153/155) x (155/159) | 1 : 1 : 1 : 1 | 33 : 35 : 30 : 46 | 4.055 | 3 | 0.256 |
| CAC-B114 | (144/149) x (142/144) | 1 : 1 : 1 : 1 | 55 : 9 : 69 : 11 | 77.889* | 3 | <0.001 |
| CAC-C001a | (210/212) x (210/214) | 1 : 1 : 1 : 1 | 35 : 40 : 39 : 30 | 1.722 | 3 | 0.632 |
| CAC-C008 | (206/215) x (206/239) | 1 : 1 : 1 : 1 | 32 : 45 : 29 : 38 | 4.166 | 3 | 0.244 |
| CAC-C114 | (270/273) x (264/273) | 1 : 1 : 1 : 1 | 39 : 24 : 42 : 39 | 5.5 | 3 | 0.139 |
| CAC-C115 | (182/193) x (182/214) | 1 : 1 : 1 : 1 | 33 : 46 : 29 : 36 | 4.388 | 3 | 0.223 |
| CaT-A114 | (165/171) x (169/173) | 1 : 1 : 1 : 1 | 30 : 37 : 31 : 46 | 4.5 | 3 | 0.212 |
| CaT-B501 | (115/129) x (121/129) | 1 : 1 : 1 : 1 | 37 : 31 : 40 : 36 | 1.166 | 3 | 0.761 |
| CaT-B502 | (189/195) x (183/187) | 1 : 1 : 1 : 1 | 40 : 30 : 42 : 32 | 2.888 | 3 | 0.409 |
| CaT-B507 | (181/191) x (191/197) | 1 : 1 : 1 : 1 | 38 : 31 : 34 : 41 | 1.611 | 3 | 0.657 |
| CaT-B509 | (109/111) x (107/109) | 1 : 1 : 1 : 1 | 34 : 36 : 43 : 31 | 2.166 | 3 | 0.539 |

*$P < 0.05$

eight chromosomes of the susceptible parent and 305 markers, 33 of which are SSRs, were placed on nine chromosome of the resistant parent. No SSR markers other than CAC-C-005 as mentioned above could be placed on chromosome 6, where the eastern filbert blight resistance gene from 'Gasaway' is located.

The order of homologous loci was collinear in most cases. JoinMap initially placed four SSR loci, CAC-A024b, CaT-B504, CAC-B005 and CAC-B113 on both chromosome 2 and chromosome 7 of the resistant parent. A similar problem was encountered with RAPD markers by Mehlenbacher et al. (2005 b) who reported that JoinMap created 11 linkage groups in the susceptible parent but only 10 linkage groups in the resistant parent. The RAPD markers initially placed on large linkage group 2 in the resistant parent were separated by MapMaker into linkage group 2 and linkage group 7. Indeed, CAC-B011 and CaT-B501 were placed on chromosome 2 of the susceptible parent indicating that group 2R is the correct assignment for these loci. Likewise, CaT-B509 and CAC-B020 were placed on chromosome 7 of the susceptible parent indicating that group 7R is the correct assignment for these loci. To identify the correct location of the other four loci (CAC-A024b, CAT-B504, CAC-B005 and CAC-B113), another linkage analysis was performed with MapMaker. MapMaker removed CAC-B113 from chromosome 7R and placed it on 2R. Loci CAC-B005 and CaT-B504 were positioned on both 2R and 7R. However, chi-square values for linkage on 7R were lower, which indicates a better fit, so they were placed on chromosome 7R. Locus CAC-A024b was also placed on chromosome 2R and 7R, but since it was tightly linked to loci CAC-B005 and CaT-B504 it was also placed on chromosome 7R.

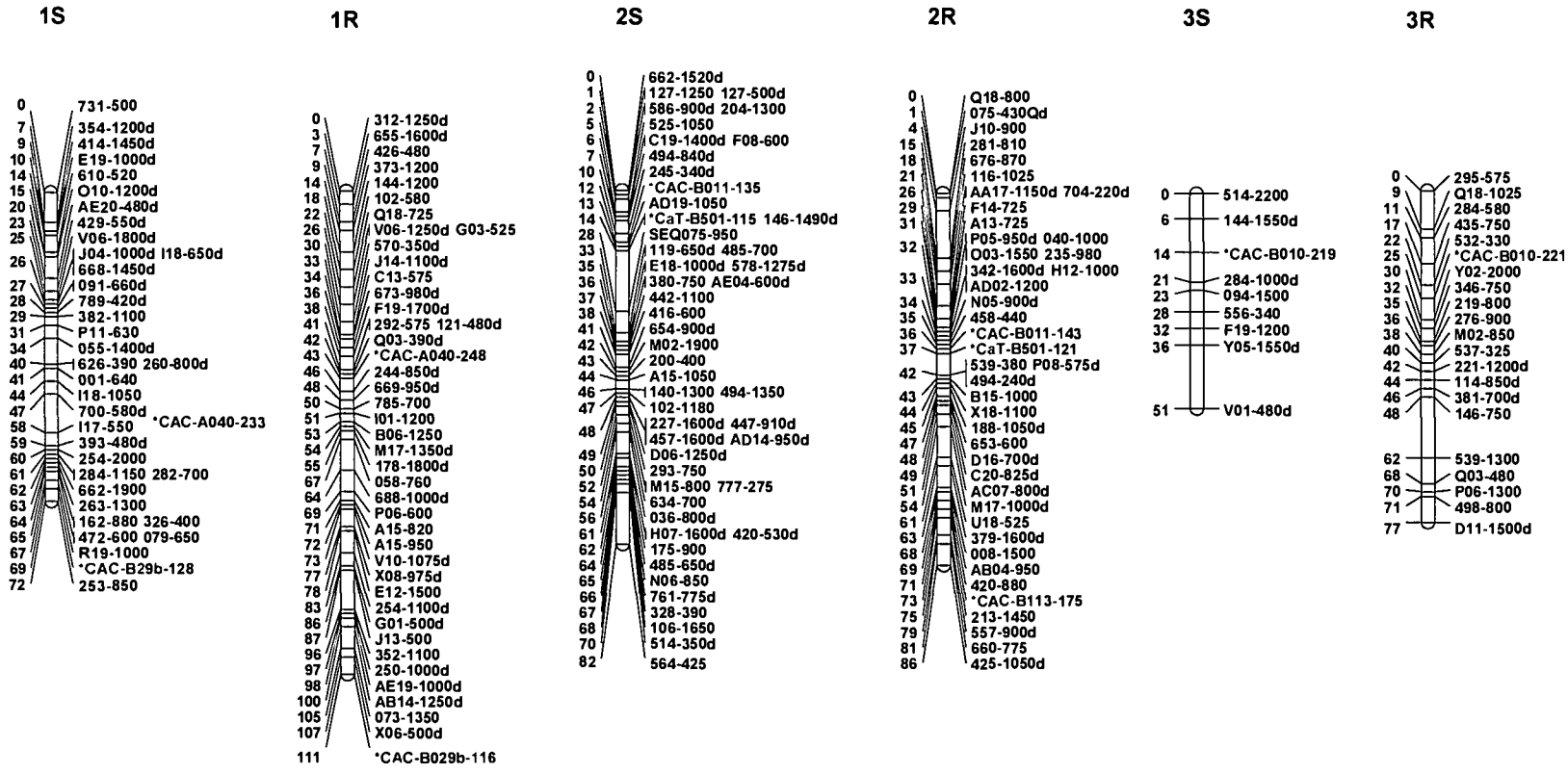


**Figure 3.1** Genetic linkage map of European hazelnut (*Corylus avellana*) based on SSR and RAPD markers. The linked allele size is shown for each SSR locus. For each linkage group marker names are shown on the right and distance between markers in cM is shown on the left. SSR markers are indicated with an asterisk (*).

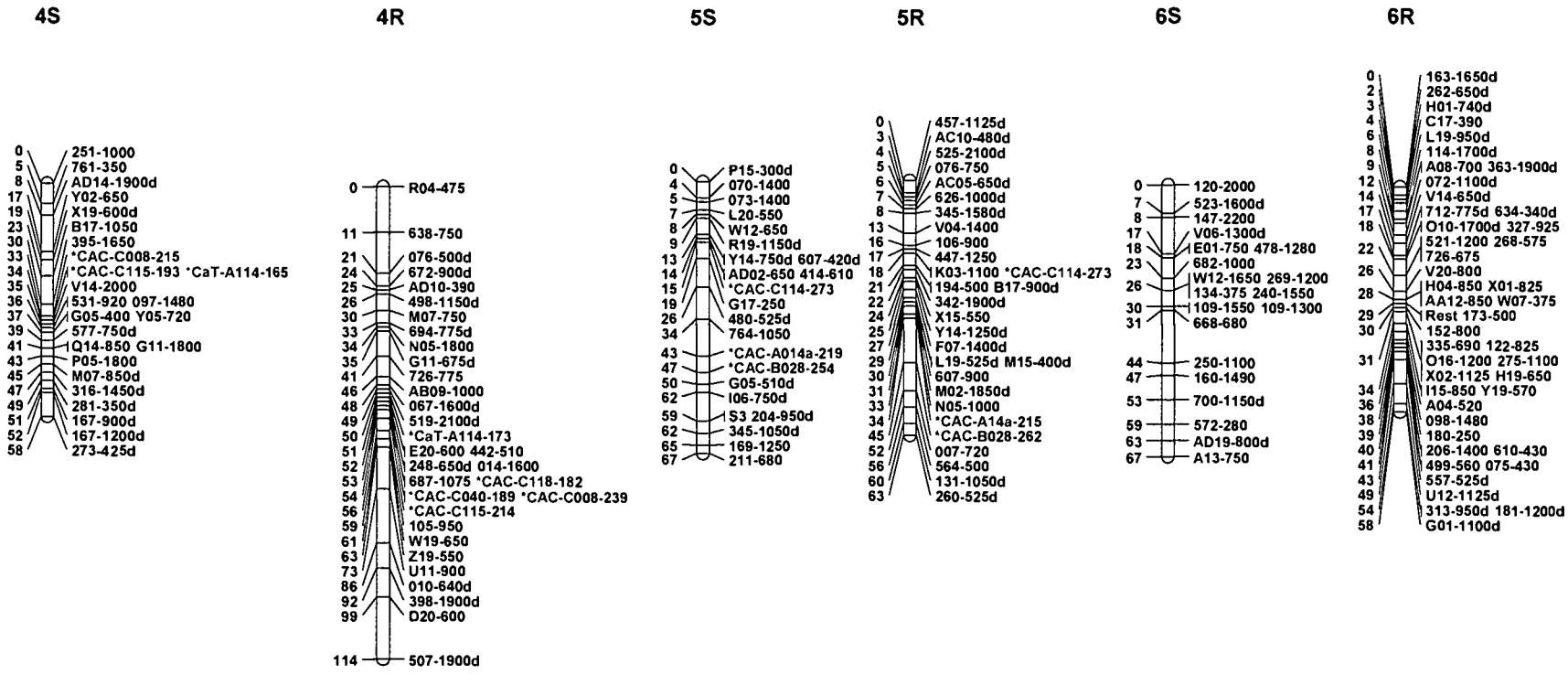


**Figure 3.1** Continued

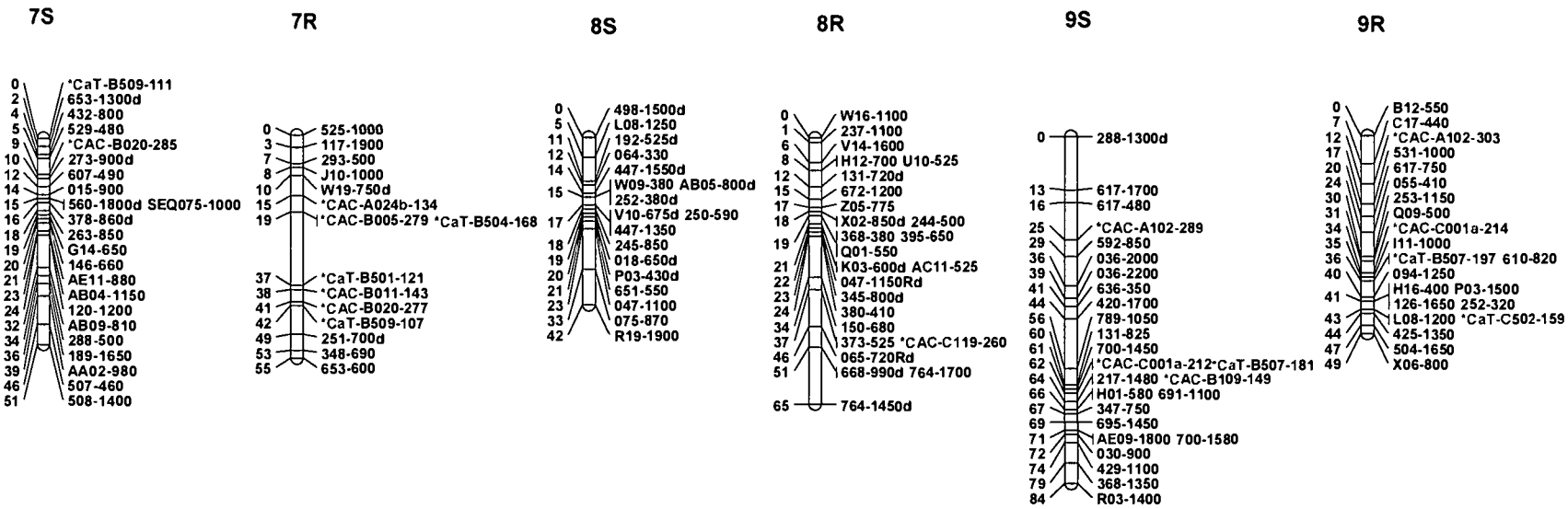


**Figure 3.1** Continued

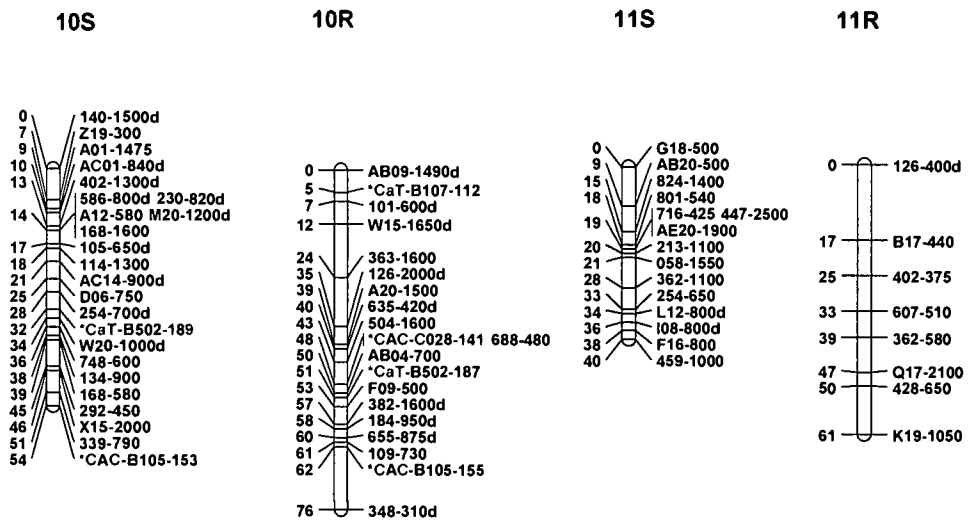


**Figure 3.1** Continued

## Conclusion

In this study we placed 29 of 33 SSR markers on the RAPD-based hazelnut linkage map. Although the percentage is high (88%), the number of loci is low and no SSR loci were placed in group 6 where the eastern filbert blight resistance gene is located. Identification of additional SSR loci and their placement on the linkage map will allow development of a more saturated microsatellite-based framework linkage map. Placement of these SSR and additional "anchor loci" on the hazelnut linkage map will make it more useful, as their locations are highly conserved and transferable to other populations. Such a map could be used in studies of genome structure, the localization of genes of interest, and allow identification of quantitative trait loci (QTLs) (Liebhard et al., 2003). SSR loci may also be useful in marker assisted selection (MAS) in breeding programs.

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# CHAPTER 4

## SUMMARY

Twenty-one pairs of simple sequence repeat (SSR) primers were used to investigate genetic diversity in 270 accessions of European hazelnut *(Corylus avellana)* representing a wide geographic range. A total of 211 alleles were amplified and the number of alleles detected ranged from 5 to 15 per locus with an average of 10.05. The observed heterozygosity ($H_o$) for individual loci ranged from 0.24 to 0.88, with an average of 0.67 over all loci. A genetic similarity matrix based on possible pair-wise combinations of accessions using the "proportion of shared alleles" was generated. UPGMA cluster analysis was used to construct a phenogram from the genetic similarity matrix using. The phenogram revealed geographically tight clusters and some synonyms among European hazelnut cultivars. Of the 274 accessions in the population, 200 are unique cultivars, 70 are suspected synonym accessions (different trees), as they are morphologically identical, but were imported from different collections under different names, and 4 are duplicate DNA templates from the same tree included as checks. 22 of 211 total alleles amplified were unique, as they were detected only in one cultivar. Nine of the unique alleles were amplified in genetically diverse cultivars, outside of the tightly clustered cultivar groups. A subset of 11 loci is recommended for use in future fingerprinting studies in hazelnut.

This study showed that the polymorphism and somatic stability of SSR markers and UPGMA cluster analysis in combination with the proportion of shared alleles seems

to be a very powerful method to analyze genetic diversity in European hazelnut. Unlike other distance methods, it makes no assumptions about the nature of the population under the study. Especially for a clonally propagated crop like hazelnut, the 'proportion of shared alleles' distance method is appropriate. The low probability of randomly matching genotypes (PI index) in this study gives great confidence that our SSRs detected synonyms, identified mislabeled accessions and confirmed pedigree information.

This study also showed that SSR markers can be successfully placed on the RAPD-based hazelnut linkage map. Total of 144 seedlings from a controlled cross OSU 252.146 x OSU 414.062 were scored for 33 SSR markers, and 29 of them (88%) were integrated into the hazelnut linkage map. Two additional loci showed aberrant segregation ratios and remained unlinked. The linkage map was constructed using the two-way pseudo-testcross approach from the mapping population. Eleven linkage groups were identified for each parent, corresponding to the haploid chromosomes number of hazelnut (2n=2x=22) and spanning a total distance of 668 cM in the susceptible parent and 813 cM in the resistant parent. The order of homologous SSR loci in the two parents was collinear in most cases.

Placement of these SSR "anchor loci" on the hazelnut linkage map will make it useful in other populations. Although most loci were assigned to a map location, only a small number of SSR loci were included in this study and no SSR was assigned to linkage group 6 where the resistance gene is located. Development of new SSR loci and their placement on the linkage map will lead to a more microsatellite-based framework linkage map. Placement of these SSR and additional "anchor loci" on the hazelnut

linkage map will make it useful in other populations, as they are highly conserved and transferable to other populations. Such a map could be used in studies of genome structure, localization of genes of interest, and identification of quantitative trait loci (QTLs). Furthermore, the map locations of SSR markers would be useful in future fingerprinting studies. SSR loci on different chromosomes rather than ones linked to each other would provide a better genomic coverage. Placement of SSR loci on the linkage map may also makes them useful in marker-assisted selection (MAS) in breeding programs.

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# APPENDICES

**Appendix A** Multiplexing of fluorescently labeled SSR primers

**Table A.1** Suggested multiplexing of florecently labeled SSR primers in Chapter 2

| Ned | Hex | Fam | Ned | Hex | Fam |
|---|---|---|---|---|---|
| **100-150** | **151-180** | **181-250** | **251-280** | **281-300** | **>300** |
| CaT-B107 (112-151) | CAC-B111 (170-192) | CAC-A014a (203-251) | | | B005 (277-297) |
| CAC-C28 (131-147) | CaT-C504 (152-173) | CaT-B502 (183-211) | | | |
| | CaT-B508 (142-167) | CaT-B507 (176-198) | CAC-C119 (256-264) | | |
| CAC-B029b (114-139) | | CAC-C040 (62,168-192) | CAC-B028 (252-288) | | |
| CaT-B505 (106-139) | CAC-B105 (125-163) | CAC-C115 (60,167-225) | CAC-A040 (234-248) | | |
| | CaT-B504 (158-184) | CAC-B010 (62, 211-227) | CAC-C010 (272-319) | | |
| | CAC-C118 (162-185) | | | | |

**Table A.2** Suggested multiplexing of fluorescently labeled SSR primers in Chapter 3

| Ned | Hex | Fam | Ned | Hex | Fam |
|---|---|---|---|---|---|
| **100-150** | **151-180** | **181-250** | **251-280** | **281-300** | **>300** |
| CAC-C005 (97-127) | CAC-B114 (138-153) | CaT-A114 (165-181) | CAC-A040 (234-248) | CAC-A102 (269-307) | |
| CaT-B107 (112-151) | CAC-B113 (167-179) | CAC-A014a (203-251) | CAC-B020 (273-289) | | B005 (277-297) |
| CAC-C028 (131-147) | CaT-B106 (156-181) | CaT-B502 (183-211) | CAC-C114 (255-279) | | |
| CAC-A024b (118-138) | CAC-B109 (145-155) | CaT-B507 (176-198) | CAC-C119 (256-264) | | |
| CAC-B029b (114-139) | CaT-B504 (158-184) | CAC-C040 (168-192) | CAC-B028 (252-288) | | |
| CaT-B501 (117-137) | CAC-C118 (162-185) | CAC-B010 (211-227) | | | |
| CAC-B011 (131-157) | CAC-B105 (125-163) | CAC-C001a (200-220) | | | |
| | CaT-B509 (109-119) | CAC-C115 (167-225) | | | |
| | CAC-B101 (135-180) | CAC-C008 (200-245) | | | |
| | CaT-C502 (151-166) | | | | |
| | | | | | |

**Appendix B** Allelic composition of 274 European hazelnut cultivars

**Table B.1** Allele numbers (n), expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), polymorphism information content (PIC), probability of identity (PI), parental exclusion probability (PEP) and frequency of null alleles (r) of 21 SSR studied in 274 European hazelnut cultivars

| SSR Locus | n | $H_e$ | $H_o$ | PIC | PI | PEP | r |
|---|---|---|---|---|---|---|---|
| **CAC-A014a** | 12 | 0.75 | 0.69 | 0.71 | 0.18 | 0.53 | 0.035 |
| **CAC-A040** | 8 | 0.72 | 0.73 | 0.68 | 0.20 | 0.49 | -0.003 |
| **CAC-B005** | 10 | 0.78 | 0.84 | 0.75 | 0.14 | 0.58 | -0.029 |
| **CAC-B010** | 7 | 0.78 | 0.78 | 0.75 | 0.14 | 0.58 | -0.002 |
| **CAC-B028** | 15 | 0.84 | 0.69 | 0.82 | 0.08 | 0.69 | 0.082 |
| **CAC-B029b** | 13 | 0.80 | 0.74 | 0.77 | 0.12 | 0.62 | 0.033 |
| **CAC-B105** | 15 | 0.70 | 0.64 | 0.68 | 0.16 | 0.51 | 0.039 |
| **CAC-B111** | 8 | 0.69 | 0.59 | 0.65 | 0.23 | 0.46 | 0.059 |
| **CAC-C010** | 5 | 0.59 | 0.23 | 0.52 | 0.39 | 0.31 | 0.222 |
| **CAC-C028** | 6 | 0.68 | 0.77 | 0.63 | 0.27 | 0.43 | -0.051 |
| **CAC-C040** | 5 | 0.27 | 0.27 | 0.26 | 0.56 | 0.15 | -0.001 |
| **CAC-C115** | 14 | 0.80 | 0.81 | 0.77 | 0.12 | 0.61 | -0.007 |
| **CAC-C118** | 5 | 0.28 | 0.29 | 0.28 | 0.54 | 0.16 | 0.002 |
| **CAC-C119** | 5 | 0.54 | 0.49 | 0.50 | 0.34 | 0.31 | 0.032 |
| **CaT-C504** | 7 | 0.78 | 0.62 | 0.75 | 0.14 | 0.58 | 0.094 |
| **CaT-B107** | 15 | 0.86 | 0.88 | 0.85 | 0.06 | 0.73 | -0.006 |
| **CaT-B508** | 13 | 0.76 | 0.68 | 0.74 | 0.11 | 0.59 | 0.045 |
| **CaT-B502** | 12 | 0.76 | 0.76 | 0.73 | 0.15 | 0.56 | 0.004 |
| **CaT-B504** | 13 | 0.82 | 0.83 | 0.80 | 0.09 | 0.65 | -0.007 |
| **CaT-B505** | 9 | 0.82 | 0.90 | 0.80 | 0.10 | 0.65 | -0.044 |
| **CaT-B507** | 14 | 0.84 | 0.82 | 0.82 | 0.08 | 0.69 | 0.012 |
| **Total** | 211 | 14.86 | 14.05 | 14.26 | | | |
| **Average** | 10.05 | 0.71 | 0.67 | 0.68 | | | |

**Appendix C** Phenotypic profiles of 110 hazelnut accessions

**Table C.1** Nut size, nut shape and husk length

| Gr. | Cultivars | Nut Size | Nut Shape | Husk Length |
|---|---|---|---|---|
| 1 | **Maria** | Large | Long | Intermediate |
| | **Lenka #4** | Large | Long | Intermediate |
| 2 | **Bard** | Small to medium | Long | Short |
| | **Barr's Zellernuss** | Small to medium | Long | Short |
| | **Bearn** | Small to medium | Long | Short |
| 3 | **Obrovsky Novy** | Medium | Long and compressed | Short |
| | **Liegel's Zellernuss** | Medium | Long and compressed | Short |
| | **Kunzenmuller Zellernuss** | Medium | Long and compressed | Short |
| | **Jeeve's Samling** | Medium | Long and compressed | Short |
| 4 | **Kadetten Zellernuss** | Medium | Large | Short |
| | **Early Long Zellernuss** | Medium | Large | Short |
| 5 | **Mogulnuss** | Large | Long | Intermediate |
| | **Riekchen's Zellernuss** | Medium to large | Long | Intermediate |
| 6 | **Italian Red** | Medium to large | Long | Short |
| | **Heynick's Zellernuss** | Medium to large | Long | Short |
| | **Gustav's Zellernuss** | Medium to large | Long | Short |
| 7 | ***C. multiflorum*** | Large | Long | Short |
| | **Cosford** | Large | Long | Short |
| | **Prolofique a Coque Serrée** | Large | Long | Short |
| | **Petoka (not)** | Large | Long | Short |
| | **Multiflora** | Large | Long | Short |
| 8 | **Little Poland** | Large | Long | Short |
| | **Pruhovany** | Large | Long | Short |
| | **Daviana** | Large | Long | Short |
| 9 | **Korthaset Zellernuss** | Medium | Very long | Long tubular |
| | **Du Chilly** | Medium | Very long | Long tubular |
| 10 | **Red Filbert** | Medium | Very long | Short |
| | **Princess Royal** | Medium | Very long | Short |
| 11 | **Bandnuss** | Large | Long | Short |
| | **A Pellicola Bianca** | Large | Long | Short |
| | **Apolda** | Large | Long | Short |
| 12 | **Pearson's Prolific** | Small | Very long | Intermediate |
| | **Nottingham** | Small | Very long | Intermediate |
| 13 | **White Filbert** | Small | Long | Long tubular |
| | **Purple Fortrin** | Small | Long | Long tubular |
| | **Purple Aveline** | Small | Long | Long tubular |
| | **Pellicle Rouge** | Small | Long | Long tubular |
| | **Istarski Debeloplodna** | Small | Long | Long tubular |
| | **Fructo Albo** | Small | Long | Long tubular |
| | **Barbarella** | Small | Long | Long tubular |
| | **Aveline Rouge** | Small | Long | Long tubular |

**Table C.1** Continued

| Gr. | Cultivars | Nut Size | Nut Shape | Husk Length |
|---|---|---|---|---|
| 14 | **Noce Lungha** | Large | Long | Long tubular |
| | **Istarski Duguljasti** | Large | Long | Long tubular |
| | **B-4** | Large | Long | Long tubular |
| 15 | **Sivri Ocak #5** | Medium | Long and pointed | Very long husk |
| | **Kerasund Dlinnyi** | Medium | Long and pointed | Very long husk |
| 16 | **Frizzled Filbert** | Small | Long | Long |
| | **Dowton Long #2** | Small | Long | Long |
| 17 | **Ganja** | Small to medium | Slightly oblate to round | Long |
| | **Romavel** | Small to medium | Slightly oblate to round | Long |
| | **San Benedetto** | Small to medium | Slightly oblate to round | Long |
| | **Palaz** | Small to medium | Slightly oblate to round | Long |
| | **54.056** | Small to medium | Slightly oblate to round | Long |
| 18 | **Karidaty** | Large | Round | Intermediate |
| | **Vilcea 22** | Large | Round | Intermediate |
| | **Wanliss Pride** | Large | Round | Intermediate |
| | **Imperial de Trabizonde #1** | Large | Round | Intermediate |
| | **Imperial de Trabizonde #2** | Large | Round | Intermediate |
| 19 | **Nemsa (not)** | Large | Round | Long |
| | **Kudryavchik (not)** | Large | Round | Long |
| | **Kudryavchik** | Small | Round | Long |
| 20 | **Tombul Ocak 1** | Small | Pointed | Very long |
| | **Extra Ghiaghli** | Small | Pointed | Very long |
| 21 | **Restiello** | Small | Slightly long | Short |
| | **Negret** | Small | Slightly long | Short |
| 22 | **Istarski Okrogloplodna** | Large | Round | Short |
| | **Fruttogrosso** | Large | Round | Short |
| | **Romische Nuss** | Large | Round | Short |
| | **Romai** | Large | Round | Short |
| | **Payrone** | Large | Round | Short |
| 23 | **Segorbe** | Small | Slightly long | Intermediate |
| | **Comun Aleva** | Small | Slightly long | Intermediate |
| | **Gironenc Colldejou** | Small | Slightly long | Intermediate |
| 24 | **Louisen's Zellernuss** | Very large | Round | Short |
| | **Rimsky** | Very large | Round | Short |
| 25 | **Quiros** | Small | Slightly long | Intermediate |
| | **Espinaredo** | Small | Slightly long | Intermediate |
| | **Casina** | Small | Slightly long | Intermediate |
| | **Amandi** | Small | Slightly long | Intermediate |
| 26 | **Molar** | Medium | Round | Intermediate |
| | **Ceret** | Small | Round | Long |
| 27 | **Rosetta** | Large | Round | Intermediate |
| | **Barrettona** | Large | Round | Intermediate |

**Table C.1** Continued

| Gr. | Cultivars | Nut Size | Nut Shape | Husk Length |
|---|---|---|---|---|
| 28 | **Pinyolenc #1a** | Medium | Round | Intermediate |
| | **Pinyolenc #1b** | Medium | Round | Intermediate |
| | **Ros de la Selva** | Medium | Round | Intermediate |
| 29 | **Pinyolenc #2a** | Small | Slightly long | Intermediate |
| | **Pinyolenc #2b** | Small | Slightly long | Intermediate |
| 30 | **Macrocarpa** | Large | Round | Intermediate |
| | **Turk** | Large | Round | Intermediate |
| | **Kruse** | Large | Round | Intermediate |
| 31 | **D'Algers** | Large | Round | Intermediate |
| | **Barcelona** | Large | Round | Intermediate |
| | **Grande** | Large | Round | Intermediate |
| 32 | **Atlas** | Large | Round | Short |
| | **Belle di Giubilino** | Large | Round | Short |
| 33 | **Racinante Clone G** | Medium | Round and oblate | Short |
| | **Nostrale** | Medium | Round and oblate | Short |
| | **Montebello** | Medium | Round and oblate | Short |
| | **Minnolora** | Medium | Round and oblate | Short |
| | **Barettona (not)** | Medium | Round and oblate | Short |
| | **Avellana Speciale** | Medium | Round and oblate | Short |
| | **Siciliana** | Medium | Round and oblate | Short |
| | **Mansa** | Medium | Round and oblate | Short |
| | **Locale di Piazza Armerina** | Medium | Round and oblate | Short |
| | **Commune di Sicilia** | Medium | Round and oblate | Short |
| | **Carello** | Medium | Round and oblate | Short |
| | **Nocchione** | Medium | Round and oblate | Short |
| 34 | **Rosset de Valls** | Medium | Round and compressed | Short |
| | **Artellet** | Medium | Round and compressed | Short |

**Appendix D** SSR profiles of 274 hazelnut accessions at 21 loci

**Table D.1** Fragment sizes of 274 hazelnut accessions determined by 21 SSR loci

| Cultivar | CAC-A14a | CAC-A40 | CAC-B005 | CAC-B010 | CAC-B028 | CAC-B29b |
|---|---|---|---|---|---|---|
| A pellicola Bianca | 211/217 | 236/244 | 277/281 | 211/211 | 266/268 | 116/124 |
| Acorn Hazel | 217/217 | 236/246 | 281/281 | 211/221 | 270/270 | 116/116 |
| Alcover | 211/217 | 236/248 | 277/291 | 219/223 | 256/256 | 122/130 |
| Alli | 215/215 | 236/244 | 277/281 | 219/227 | 256/262 | 116/132 |
| Amandi | 211/217 | 244/244 | 277/281 | 219/227 | 268/278 | 118/122 |
| Apolda | 211/217 | 236/244 | 277/281 | 211/211 | 266/268 | 116/124 |
| Arneson's Rootstock | 211/215 | 244/248 | 277/295 | 223/223 | 254/262 | 118/122 |
| Artellet | 215/215 | 234/246 | 277/291 | 211/227 | 256/256 | 118/128 |
| Ata Baba Ganja | 211/213 | 236/244 | 279/279 | 211/219 | 262/264 | 116/128 |
| Atlas | 217/219 | 244/248 | 291/295 | 211/223 | 254/268 | 118/122 |
| Aurea | 213/213 | 236/238 | 277/277 | 219/219 | 258/278 | 124/130 |
| Aveline d'Angleterre | 215/215 | 244/246 | 279/281 | 219/227 | 256/256 | 124/128 |
| Aveline Rouge | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| Avellana Speciale | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| B-3 | 213/215 | 238/248 | 277/281 | 223/225 | 262/266 | 116/124 |
| B-4 | 211/215 | 244/248 | 281/291 | 211/225 | 260/268 | 116/122 |
| Badem | 215/215 | 244/248 | 281/291 | 219/225 | 262/262 | 122/124 |
| Bandnuss | 211/217 | 236/244 | 277/281 | 211/211 | 266/268 | 116/124 |
| Barbarella | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| Barcelona | 215/219 | 234/248 | 291/295 | 211/223 | 254/262 | 122/128 |
| Barcelonner Zellernuss | 215/215 | 236/236 | 277/277 | 223/227 | 256/268 | 116/118 |
| Bard #1 | 211/215 | 236/248 | 277/277 | 211/211 | 258/266 | 124/128 |
| Bard #2 | 211/215 | 236/248 | 277/277 | 211/211 | 258/266 | 124/128 |
| Barrettona | 213/215 | 236/244 | 277/281 | 211/211 | 256/262 | 116/122 |
| Barrettona-not | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Barr's Zellernuss | 211/215 | 236/248 | 277/277 | 211/211 | 258/266 | 124/128 |
| Bearn | 211/215 | 236/248 | 277/277 | 211/211 | 258/266 | 124/128 |
| Belle di Giubilino #1 | 217/219 | 244/248 | 291/295 | 211/223 | 254/268 | 118/122 |
| Belle di Giubilino #2 | 217/219 | 244/248 | 291/295 | 211/223 | 254/268 | 118/122 |
| Bergeri | 211/217 | 236/236 | 277/281 | 211/225 | 256/268 | 116/128 |
| Bianca | 215/219 | 244/244 | 277/295 | 219/223 | 254/262 | 116/122 |
| Blumberger Zellernuss | 215/217 | 236/236 | 281/287 | 211/221 | 256/280 | 118/128 |
| Brixley's New | 215/219 | 234/246 | 277/291 | 223/227 | 254/256 | 128/137 |
| Brixnut | 213/219 | 234/244 | 281/291 | 223/223 | 254/254 | 128/137 |
| Bulgaria XI-8 | 211/215 | 244/244 | 281/297 | 211/219 | 262/262 | 116/128 |
| Burchardt's Zellernuss | 211/215 | 236/244 | 277/279 | 219/223 | 256/262 | 116/126 |
| Butler | 217/219 | 234/248 | 277/295 | 211/211 | 254/270 | 116/128 |
| Buttner's Zellernuss | 211/213 | 236/248 | 277/281 | 211/211 | 254/266 | 116/118 |
| C. multiflorum | 211/217 | 236/236 | 277/281 | 211/211 | 266/270 | 116/128 |
| C.avellana AL55 | 215/215 | 236/236 | 277/279 | 223/227 | 254/264 | 124/128 |
| C.avellana Swe. | 203/213 | 236/236 | 277/277 | 219/223 | 254/256 | 128/135 |
| Camponica | 211/215 | 236/244 | 281/295 | 219/223 | 262/278 | 118/122 |
| Carello | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Casina | 211/217 | 244/244 | 277/281 | 219/227 | 268/278 | 118/122 |
| Ceret | 215/217 | 244/248 | 281/291 | 211/219 | 262/268 | 122/122 |
| Clark | 215/217 | 244/244 | 279/295 | 219/225 | 262/270 | 122/128 |
| Closca Molla | 215/217 | 244/244 | 277/291 | 211/219 | 256/262 | 122/130 |

**Table D.1** Continued

| Cultivar | CAC-B105 | CAC-B111 | CAC-C010 | CAC-C28 | CAC-C040 | CAC-C115 |
|---|---|---|---|---|---|---|
| A pellicola Bianca | 148/155 | 182/186 | 278/281 | 131/131 | 186/186 | 193/193 |
| Acorn Hazel | 140/148 | 182/188 | 278/278 | 141/141 | 186/186 | 214/214 |
| Alcover | 138/138 | 182/182 | 275/275 | 131/131 | 186/186 | 173/182 |
| Alli | 138/146 | 182/184 | 275/275 | 131/134 | 186/186 | 214/214 |
| Amandi | 140/155 | 182/184 | 275/275 | 131/141 | 186/192 | 173/196 |
| Apolda | 148/155 | 182/186 | 278/281 | 131/131 | 186/186 | 193/193 |
| Arneson's Rootstock | 155/155 | 182/182 | 275/275 | 131/141 | 186/186 | 173/214 |
| Artellet | 140/153 | 182/182 | 275/275 | 131/141 | 186/186 | 193/193 |
| Ata Baba Ganja | 155/157 | 170/182 | 275/281 | 131/141 | 186/186 | 193/193 |
| Atlas | 155/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/196 |
| Aurea | 138/153 | 186/188 | 275/275 | 131/134 | 183/189 | 214/214 |
| Aveline d'Angleterre | 146/148 | 186/188 | 281/281 | 131/134 | 186/186 | 193/193 |
| Aveline Rouge | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| Avellana Speciale | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| B-3 | 146/155 | 182/186 | 275/275 | 131/141 | 186/189 | 173/214 |
| B-4 | 142/155 | 186/186 | 275/281 | 131/141 | 186/192 | 173/193 |
| Badem | 140/155 | 182/186 | 275/278 | 131/134 | 186/189 | 173/217 |
| Bandnuss | 148/155 | 182/186 | 278/281 | 131/131 | 186/186 | 193/193 |
| Barbarella | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| Barcelona | 153/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/193 |
| Barcelonner Zellernuss | 148/157 | 182/182 | 275/275 | 131/134 | 189/189 | 193/214 |
| Bard #1 | 148/159 | 184/186 | 275/278 | 131/134 | 186/186 | 176/193 |
| Bard #2 | 148/159 | 184/186 | 275/278 | 131/134 | 186/186 | 176/193 |
| Barrettona | 155/155 | 182/182 | 278/278 | 131/134 | 186/186 | 173/193 |
| Barrettona-not | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Barr's Zellernuss | 148/159 | 184/186 | 275/278 | 131/134 | 186/186 | 176/193 |
| Bearn | 148/159 | 184/186 | 275/278 | 131/134 | 186/186 | 176/193 |
| Belle di Giubilino #1 | 155/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/196 |
| Belle di Giubilino #2 | 155/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/196 |
| Bergeri | 138/140 | 184/186 | 275/275 | 137/141 | 186/186 | 173/214 |
| Bianca | 140/155 | 188/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Blumberger Zellernuss | 140/148 | 182/184 | 275/275 | 131/141 | 186/186 | 179/214 |
| Brixley's New | 140/153 | 182/186 | 275/275 | 131/141 | 186/186 | 173/214 |
| Brixnut | 140/153 | 182/186 | 275/278 | 131/144 | 186/186 | 173/193 |
| Bulgaria XI-8 | 155/157 | 182/186 | 275/281 | 131/144 | 186/189 | 173/182 |
| Burchardt's Zellernuss | 140/155 | 170/182 | 275/275 | 131/137 | 186/186 | 193/193 |
| Butler | 153/153 | 182/182 | 278/278 | 131/131 | 186/186 | 193/193 |
| Buttner's Zellernuss | 140/148 | 182/186 | 278/278 | 131/141 | 186/186 | 193/193 |
| C. multiflorum | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 | 193/214 |
| C.avellana AL55 | 146/159 | 182/182 | 275/275 | 131/144 | 186/186 | 193/193 |
| C.avellana Swe. | 148/148 | 182/182 | 275/275 | 131/134 | 189/189 | 214/214 |
| Camponica | 155/155 | 182/184 | 275/275 | 141/141 | 186/186 | 173/196 |
| Carello | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Casina | 140/155 | 182/184 | 275/275 | 131/141 | 186/192 | 173/196 |
| Ceret | 155/155 | 182/182 | 275/275 | 131/141 | 186/186 | 173/182 |
| Clark | 153/155 | 182/184 | 278/278 | 131/141 | 186/186 | 173/193 |
| Closca Molla | 148/155 | 182/182 | 275/275 | 131/141 | 186/186 | 182/214 |

**Table D.1** Continued

| Cultivar | CAC-C118 | CAC-C119 | CaT-B107 | CaT-B502 | CaT-B504 | CaT-B505 |
|---|---|---|---|---|---|---|
| A pellicola Bianca | 179/179 | 258/258 | 122/142 | 189/195 | 176/176 | 106/126 |
| Acorn Hazel | 179/179 | 256/264 | 130/134 | 187/187 | 176/176 | 116/118 |
| Alcover | 179/179 | 256/264 | 134/134 | 189/189 | 176/182 | 116/128 |
| Alli | 179/179 | 258/264 | 128/130 | 199/209 | 174/176 | 114/116 |
| Amandi | 179/185 | 256/258 | 130/134 | 187/187 | 176/176 | 118/126 |
| Apolda | 179/179 | 258/258 | 122/142 | 189/195 | 176/176 | 106/126 |
| Arneson's Rootstock | 179/179 | 258/258 | 112/134 | 183/189 | 158/168 | 114/118 |
| Artellet | 179/179 | 258/264 | 122/134 | 187/195 | 176/182 | 106/114 |
| Ata Baba Ganja | 179/179 | 258/264 | 122/122 | 183/189 | 168/170 | 106/120 |
| Atlas | 179/179 | 258/260 | 112/134 | 183/189 | 158/182 | 106/114 |
| Aurea | 176/182 | 260/260 | 120/120 | 187/195 | 174/176 | 118/120 |
| Aveline d'Angleterre | 179/179 | 258/264 | 130/134 | 201/201 | 170/176 | 118/126 |
| Aveline Rouge | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| Avellana Speciale | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| B-3 | 179/182 | 258/258 | 116/120 | 189/189 | 176/182 | 118/122 |
| B-4 | 179/185 | 258/258 | 134/134 | 187/189 | 176/182 | 106/118 |
| Badem | 179/182 | 258/258 | 116/122 | 187/189 | 172/176 | 118/126 |
| Bandnuss | 179/179 | 258/258 | 122/142 | 189/195 | 176/176 | 106/126 |
| Barbarella | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| Barcelona | 179/179 | 258/258 | 112/134 | 187/189 | 158/182 | 106/120 |
| Barcelonner Zellernuss | 182/182 | 256/256 | 122/134 | 187/195 | 174/176 | 116/120 |
| Bard #1 | 179/179 | 258/264 | 120/122 | 195/195 | 176/176 | 106/118 |
| Bard #2 | 179/179 | 258/264 | 120/122 | 195/195 | 176/176 | 106/118 |
| Barrettona | 179/179 | 258/258 | 122/142 | 183/189 | 176/176 | 106/122 |
| Barrettona-not | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Barr's Zellernuss | 179/179 | 258/264 | 120/122 | 195/195 | 176/176 | 106/118 |
| Bearn | 179/179 | 258/264 | 120/122 | 195/195 | 176/176 | 106/118 |
| Belle di Giubilino #1 | 179/179 | 258/260 | 112/134 | 183/189 | 158/182 | 106/114 |
| Belle di Giubilino #2 | 179/179 | 258/260 | 112/134 | 183/189 | 158/182 | 106/114 |
| Bergeri | 179/179 | 260/264 | 122/130 | 187/189 | 168/176 | 106/114 |
| Bianca | 179/179 | 258/258 | 120/122 | 183/187 | 158/176 | 114/118 |
| Blumberger Zellernuss | 179/179 | 258/260 | 128/144 | 187/195 | 176/176 | 106/139 |
| Brixley's New | 179/179 | 258/264 | 122/130 | 187/189 | 174/182 | 114/118 |
| Brixnut | 179/179 | 258/258 | 112/122 | 189/195 | 176/182 | 114/120 |
| Bulgaria XI-8 | 179/182 | 258/258 | 122/134 | 187/209 | 176/176 | 106/118 |
| Burchardt's Zellernuss | 179/179 | 258/264 | 120/130 | 183/183 | 168/178 | 126/128 |
| Butler | 179/179 | 258/258 | 112/134 | 189/189 | 158/172 | 106/~~114~~ 116 |
| Buttner's Zellernuss | 179/179 | 256/258 | 134/142 | 187/195 | 176/176 | 106/106 |
| C. multiflorum | 179/179 | 256/258 | 134/144 | 187/195 | 172/176 | 106/116 |
| C.avellana AL55 | 185/185 | 260/260 | 128/132 | 187/187 | 176/176 | 114/120 |
| C.avellana Swe. | 182/182 | 258/264 | 142/142 | 187/187 | 174/176 | 118/120 |
| Camponica | 179/179 | 258/258 | 112/134 | 183/189 | 158/176 | 118/126 |
| Carello | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Casina | 179/185 | 256/258 | 130/134 | 187/187 | 176/176 | 118/126 |
| Ceret | 179/179 | 258/258 | 122/134 | 187/189 | 176/182 | 106/126 |
| Clark | 179/179 | 258/258 | 134/144 | 187/195 | 158/168 | 118/126 |
| Closca Molla | 179/179 | 258/264 | 122/134 | 187/201 | 176/182 | 106/114 |

**Table D.1** Continued

| Cultivar | CaT-B507 | CaT-B508 | Cat-C504 |
|---|---|---|---|
| A pellicola Bianca | 185/195 | 153/165 | 158/164 |
| Acorn Hazel | 189/189 | 144/161 | 152/164 |
| Alcover | 191/191 | 157/163 | 164/164 |
| Alli | 179/195 | 161/165 | 152/152 |
| Amandi | 189/191 | 157/161 | 158/158 |
| Apolda | 185/195 | 153/165 | 158/164 |
| Arneson's Rootstock | 181/185 | 157/161 | 155/155 |
| Artellet | 181/191 | 157/165 | 152/158 |
| Ata Baba Ganja | 191/191 | 142/157 | 173/173 |
| Atlas | 181/191 | 157/157 | 158/167 |
| Aurea | 179/179 | 161/165 | 152/152 |
| Aveline d'Angleterre | 189/189 | 157/161 | 164/164 |
| Aveline Rouge | 185/197 | 157/157 | 158/164 |
| Avellana Speciale | 181/191 | 157/157 | 158/167 |
| B-3 | 185/189 | 144/163 | 158/164 |
| B-4 | 197/197 | 157/157 | 164/164 |
| Badem | 185/191 | 153/163 | 161/164 |
| Bandnuss | 185/195 | 153/165 | 158/164 |
| Barbarella | 185/197 | 157/157 | 158/164 |
| Barcelona | 181/191 | 157/157 | 155/158 |
| Barcelonner Zellernuss | 189/189 | 144/161 | 152/158 |
| Bard #1 | 195/195 | 161/161 | 152/164 |
| Bard #2 | 195/195 | 161/161 | 152/164 |
| Barrettona | 191/193 | 146/157 | 158/158 |
| Barrettona-not | 181/191 | 157/157 | 158/167 |
| Barr's Zellernuss | 195/195 | 161/161 | 152/164 |
| Bearn | 195/195 | 161/161 | 152/164 |
| Belle di Giubilino #1 | 181/191 | 157/157 | 158/167 |
| Belle di Giubilino #2 | 181/191 | 157/157 | 158/167 |
| Bergeri | 195/195 | 165/165 | 161/164 |
| Bianca | 185/191 | 161/161 | 158/167 |
| Blumberger Zellernuss | 189/195 | 159/159 | 152/152 |
| Brixley's New | 185/190 | 144/157 | 152/152 |
| Brixnut | 181/185 | 157/157 | 158/164 |
| Bulgaria XI-8 | 189/191 | 157/157 | 161/173 |
| Burchardt's Zellernuss | 187/195 | 153/159 | 158/158 |
| Butler | 181/195 | 157/161 | 152/155 |
| Buttner's Zellernuss | 195/195 | 161/165 | 158/164 |
| C. multiflorum | 189/195 | 148/161 | 152/164 |
| C.avellana AL55 | 197/197 | 157/157 | 152/152 |
| C.avellana Swe. | 195/195 | 146/161 | 161/164 |
| Camponica | 181/191 | 157/167 | 155/158 |
| Carello | 181/191 | 157/157 | 158/167 |
| Casina | 189/191 | 157/161 | 158/158 |
| Ceret | 185/191 | 157/163 | 164/164 |
| Clark | 189/191 | 161/163 | 158/167 |
| Closca Molla | 191/195 | 157/165 | 158/164 |

**Table D.1** Continued

| Cultivar | CAC-A14a | CAC-A40 | CAC-B005 | CAC-B010 | CAC-B028 | CAC-B29b |
|---|---|---|---|---|---|---|
| Comen | 215/217 | 234/248 | 277/291 | 219/223 | 262/262 | 122/128 |
| Comun | 217/217 | 236/236 | 277/291 | 219/227 | 268/268 | 116/137 |
| Comun Aleva | 211/211 | 236/248 | 277/281 | 211/219 | 266/278 | 122/135 |
| Comune di Sici. | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Contorta | 217/217 | 244/248 | 277/277 | 219/223 | 268/268 | 116/128 |
| Corabel | 211/215 | 234/236 | 277/295 | 211/223 | 262/266 | 128/128 |
| Cosford | 211/217 | 236/236 | 277/281 | 211/211 | 266/270 | 116/128 |
| Cozia | 215/215 | 244/246 | 279/291 | 219/227 | 256/256 | 116/122 |
| Creswell | 215/217 | 236/244 | 277/279 | 219/225 | 262/268 | 124/128 |
| Culpla | 213/217 | 236/244 | 277/291 | 219/227 | 256/268 | 118/122 |
| Cutleaf | 215/217 | 236/244 | 277/277 | 221/227 | 254/270 | 122/124 |
| Da Viega | 215/217 | 236/248 | 277/291 | 219/219 | 266/268 | 116/122 |
| Dal Rosso | 211/211 | 244/248 | 277/279 | 211/219 | 270/270 | 116/118 |
| D'Algers | 215/219 | 234/248 | 291/295 | 211/223 | 254/262 | 122/128 |
| Daria | 215/217 | 234/236 | 281/291 | 211/219 | 262/270 | 116/122 |
| Daviana #1 | 217/217 | 236/248 | 277/281 | 211/211 | 270/270 | 116/116 |
| Daviana #2 = Cosford | 211/217 | 236/236 | 277/281 | 211/211 | 266/270 | 116/128 |
| Delta | 215/215 | 244/244 | 281/297 | 219/221 | 256/262 | 116/122 |
| Des Anglais | 211/217 | 236/248 | 277/279 | 211/219 | 256/256 | 124/128 |
| Dowton Long #1 | 213/215 | 236/244 | 277/281 | 223/223 | 254/256 | 116/137 |
| Dowton Long #2 | 215/217 | 236/244 | 277/281 | 219/227 | 256/268 | 116/118 |
| DuChilly | 213/217 | 244/244 | 281/281 | 211/223 | 254/268 | 124/137 |
| Early Long Zel. | 215/215 | 236/246 | 277/281 | 211/227 | 256/256 | 116/128 |
| Ennis | 217/219 | 234/248 | 277/295 | 211/223 | 254/264 | 116/128 |
| Epsilon | 215/217 | 236/248 | 277/291 | 219/225 | 262/278 | 122/122 |
| Espinaredo | 211/217 | 244/244 | 277/281 | 219/227 | 268/278 | 118/122 |
| Extra Ghiaghli | 211/215 | 244/244 | 279/291 | 211/225 | 262/262 | 116/130 |
| Finland | 212/217 | 236/244 | 279/279 | 219/221 | 258/270 | 135/137 |
| Fitzgerald | 217/219 | 234/236 | 277/295 | 211/223 | 254/270 | 116/128 |
| Fitzgerald #20 | 215/215 | 234/236 | 285/295 | 211/211 | 262/262 | 116/122 |
| Francoli | 211/213 | 234/248 | 277/291 | 211/223 | 256/256 | 118/122 |
| Frango #2 | 211/215 | 236/246 | 277/277 | 211/221 | 266/266 | 116/128 |
| Frango #4 | 215/215 | 236/246 | 277/281 | 219/221 | 254/256 | 116/128 |
| Frango #5 | 211/215 | 236/244 | 277/281 | 211/221 | 256/266 | 116/116 |
| Freehusker | 211/219 | 236/248 | 277/291 | 211/211 | 254/266 | 116/122 |
| Frizzled Filbert | 215/217 | 236/244 | 277/281 | 219/227 | 256/268 | 116/118 |
| Fructo Albo | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| Fruttogrosso | 211/217 | 236/248 | 277/281 | 219/223 | 268/278 | 122/124 |
| Fusco Rubra | 215/217 | 236/246 | 277/279 | 217/219 | 264/266 | 116/118 |
| Gamma | 215/217 | 244/244 | 277/277 | 219/223 | 262/268 | 122/135 |
| Ganja | 211/215 | 236/244 | 277/279 | 211/225 | 262/276 | 122/130 |
| Garibaldi | 211/211 | 236/244 | 277/281 | 219/219 | 266/266 | 124/128 |
| Garrofi | 215/219 | 234/248 | 291/295 | 211/225 | 254/262 | 122/122 |
| Gasaway | 215/215 | 236/244 | 277/297 | 211/219 | 256/278 | 116/135 |
| Gem | 213/215 | 234/244 | 281/291 | 223/223 | 256/262 | 128/137 |
| Ghirara | 211/215 | 236/244 | 277/291 | 219/219 | 256/262 | 122/132 |
| Gironenc Coldejou | 211/211 | 236/248 | 277/281 | 211/219 | 266/278 | 122/135 |
| Gironenc Vermellet | 211/215 | 244/248 | 277/291 | 219/223 | 258/262 | 122/128 |

Petoka (not)

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**Table D.1** Continued

| Cultivar | CAC-B105 | CAC-B111 | CAC-C010 | CAC-C28 | CAC-C040 | CAC-C115 |
|---|---|---|---|---|---|---|
| Comen | 140/155 | 182/182 | 275/275 | 131/131 | 186/189 | 173/214 |
| Comun | 140/151 | 182/182 | 275/275 | 131/134 | 186/186 | 173/182 |
| Comun Aleva | 155/155 | 182/182 | 275/275 | 141/141 | 186/186 | 173/196 |
| Comune di Sici. | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Contorta | 146/155 | 186/186 | 281/281 | 131/131 | 186/186 | 185/214 |
| Corabel | 148/153 | 182/182 | 275/278 | 131/141 | 186/186 | 193/193 |
| Cosford | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 | 193/214 |
| Cozia | 140/155 | 170/188 | 275/275 | 134/134 | 186/189 | 193/217 |
| Creswell | 153/155 | 182/186 | 281/281 | 131/131 | 186/186 | 173/205 |
| Culpla | 148/155 | 182/186 | 275/278 | 131/131 | 186/186 | 182/205 |
| Cutleaf | 155/155 | 178/178 | 275/278 | 134/134 | 186/189 | 214/214 |
| Da Viega | 140/155 | 182/182 | 275/275 | 134/141 | 186/186 | 173/193 |
| Dal Rosso | 138/155 | 186/188 | 275/275 | 131/141 | 186/189 | 173/196 |
| D'Algers | 153/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/193 |
| Daria | 148/155 | 182/182 | 278/278 | 131/141 | 186/186 | 173/214 |
| Daviana #1 | 153/155 | 182/186 | 278/278 | 131/131 | 186/186 | 173/193 |
| Daviana #2 | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 | 193/214 |
| Delta | 140/157 | 182/192 | 275/275 | 141/144 | 186/186 | 196/214 |
| Des Anglais | 151/155 | 186/186 | 281/281 | 131/134 | 186/189 | 185/193 |
| Dowton Long #1 | 140/140 | 186/186 | 278/278 | 131/144 | 186/186 | 173/193 |
| Dowton Long #2 | 151/155 | 186/188 | 275/281 | 141/147 | 186/186 | 173/205 |
| DuChilly | 140/155 | 186/186 | 278/281 | 131/144 | 186/186 | 173/193 |
| Early Long Zel. | 138/140 | 184/186 | 275/275 | 131/141 | 183/186 | 214/214 |
| Ennis | 153/155 | 182/182 | 278/278 | 131/141 | 186/186 | 173/193 |
| Epsilon | 153/155 | 184/192 | 275/275 | 141/144 | 183/186 | 173/196 |
| Espinaredo | 140/155 | 182/184 | 275/275 | 131/141 | 186/192 | 173/196 |
| Extra Ghiaghli | 155/155 | 184/186 | 275/275 | 134/141 | 186/186 | 182/193 |
| Finland | 148/153 | 182/186 | 278/278 | 131/131 | 186/186 | 214/214 |
| Fitzgerald | 155/155 | 182/186 | 278/278 | 131/131 | 186/186 | 173/193 |
| Fitzgerald #20 | 153/155 | 182/186 | 275/275 | 131/131 | 186/186 | 173/193 |
| Francoli | 140/155 | 182/184 | 275/275 | 131/134 | 186/186 | 182/214 |
| Frango #2 | 140/148 | 182/188 | 275/278 | 141/141 | 186/186 | 193/214 |
| Frango #4 | 140/159 | 182/188 | 275/278 | 131/141 | 186/192 | 214/217 |
| Frango #5 | 140/148 | 182/184 | 275/278 | 141/141 | 186/186 | 193/214 |
| Freehusker | 148/153 | 182/182 | 278/278 | 131/141 | 186/186 | 173/193 |
| Frizzled Filbert | 151/155 | 186/188 | 275/281 | 141/147 | 186/186 | 173/205 |
| Fructo Albo | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| Fruttogrosso | 155/155 | 182/188 | 275/278 | 141/141 | 186/186 | 173/196 |
| Fusco Rubra | 136/140 | 184/186 | 272/278 | 134/134 | 186/189 | 214/214 |
| Gamma | 140/159 | 184/192 | 275/275 | 131/144 | 186/186 | 173/196 |
| Ganja | 155/157 | 170/186 | 281/281 | 141/141 | 186/186 | 193/193 |
| Garibaldi | 155/159 | 182/186 | 278/281 | 131/144 | 186/186 | 173/193 |
| Garrofi | 140/155 | 182/186 | 275/278 | 134/141 | 186/189 | 193/214 |
| Gasaway | 153/157 | 192/192 | 275/278 | 144/144 | 186/189 | 214/217 |
| Gem | 140/153 | 182/186 | 275/278 | 131/144 | 186/186 | 173/193 |
| Ghirara | 140/155 | 182/188 | 275/275 | 131/141 | 186/186 | 196/217 |
| Gironenc Coldejou | 155/155 | 182/182 | 275/275 | 141/141 | 186/186 | 173/196 |
| Gironenc Vermellet | 140/153 | 182/182 | 275/275 | 131/134 | 186/186 | 173/205 |

**Table D.1** Continued

| Cultivar | CAC-C118 | CAC-C119 | CaT-B107 | CaT-B502 | CaT-B504 | CaT-B505 |
|---|---|---|---|---|---|---|
| Comen | 179/185 | 258/258 | 122/134 | 189/195 | 172/182 | 120/126 |
| Comun | 179/179 | 256/258 | 122/134 | 189/195 | 174/182 | 114/122 |
| Comun Aleva | 179/179 | 258/264 | 112/134 | 183/187 | 174/176 | 106/114 |
| Comune di Sici. | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Contorta | 179/179 | 258/264 | 122/130 | 189/189 | 174/176 | 114/118 |
| Corabel | 179/179 | 258/258 | 112/134 | 187/189 | 158/172 | 106/120 |
| Cosford | 179/179 | 256/258 | 134/144 | 187/195 | 172/176 | 106/116 |
| Cozia | 179/182 | 258/264 | 130/130 | 187/201 | 168/184 | 118/126 |
| Creswell | 179/179 | 258/260 | 122/134 | 189/189 | 168/174 | 116/128 |
| Culpla | 179/179 | 258/258 | 124/134 | 183/189 | 170/182 | 114/126 |
| Cutleaf | 179/182 | 264/264 | 122/134 | 201/209 | 170/176 | 126/128 |
| Da Viega | 179/179 | 256/258 | 122/134 | 187/187 | 172/182 | 114/126 |
| Dal Rosso | 179/182 | 258/258 | 124/134 | 183/187 | 168/176 | 106/106 |
| D'Algers | 179/179 | 258/258 | 112/134 | 187/189 | 158/182 | 106/120 |
| Daria | 179/179 | 258/258 | 134/134 | 187/189 | 176/182 | 116/126 |
| Daviana #1 | 179/179 | 258/258 | 134/134 | 189/195 | 172/176 | 106/116 |
| Daviana #2 | 179/179 | 256/258 | 134/144 | 187/195 | 172/176 | 106/116 |
| Delta | 179/179 | 258/264 | 122/130 | 183/187 | 176/182 | 118/126 |
| Des Anglais | 179/182 | 258/258 | 120/134 | 189/201 | 168/174 | 106/114 |
| Dowton Long #1 | 179/179 | 260/264 | 120/122 | 195/195 | 174/176 | 114/114 |
| Dowton Long #2 | 179/179 | 258/264 | 122/130 | 183/187 | 176/176 | 118/126 |
| DuChilly | 179/179 | 258/264 | 122/130 | 189/195 | 176/176 | 106/114 |
| Early Long Zel. | 176/179 | 258/264 | 122/128 | 187/209 | 176/176 | 106/114 |
| Ennis | 179/179 | 258/258 | 134/134 | 187/195 | 158/172 | 116/120 |
| Epsilon | 176/179 | 258/258 | 128/134 | 187/195 | 160/182 | 114/118 |
| Espinaredo | 179/185 | 256/258 | 130/134 | 187/187 | 176/176 | 118/126 |
| Extra Ghiaghli | 162/179 | 260/260 | 116/116 | 187/187 | 168/182 | 106/118 |
| Finland | 179/179 | 258/264 | 126/126 | 199/199 | 168/178 | 116/118 |
| Fitzgerald | 179/179 | 258/258 | 112/134 | 189/189 | 158/172 | 106/120 |
| Fitzgerald #20 | 179/179 | 258/258 | 112/134 | 189/189 | 158/176 | 106/106 |
| Francoli | 179/185 | 258/264 | 120/134 | 189/195 | 170/182 | 106/114 |
| Frango #2 | 179/179 | 256/264 | 130/144 | 187/187 | 172/174 | 106/118 |
| Frango #4 | 179/185 | 258/258 | 120/130 | 187/187 | 162/176 | 114/118 |
| Frango #5 | 179/179 | 258/264 | 130/134 | 187/187 | 172/176 | 116/118 |
| Freehusker | 179/179 | 256/258 | 112/134 | 187/189 | 172/182 | 116/120 |
| Frizzled Filbert | 179/179 | 258/264 | 122/130 | 183/187 | 176/176 | 118/126 |
| Fructo Albo | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| Fruttogrosso | 179/179 | 258/260 | 122/134 | 183/187 | 174/176 | 114/126 |
| Fusco Rubra | 179/182 | 256/264 | 120/122 | 189/195 | 164/174 | 114/122 |
| Gamma | 179/179 | 258/258 | 130/134 | 183/187 | 168/176 | 118/126 |
| Ganja | 179/179 | 258/258 | 134/134 | 187/187 | 168/168 | 106/118 |
| Garibaldi | 179/179 | 258/258 | 134/142 | 183/187 | 176/176 | 118/126 |
| Garrofi | 179/182 | 258/258 | 112/126 | 187/195 | 158/182 | 114/120 |
| Gasaway | 179/182 | 258/264 | 122/128 | 183/195 | 174/174 | 114/120 |
| Gem | 179/179 | 258/264 | 112/130 | 189/195 | 176/182 | 114/120 |
| Ghirara | 179/179 | 258/258 | 112/112 | 183/185 | 176/182 | 118/126 |
| Gironenc Coldejou | 179/179 | 258/264 | 112/134 | 183/187 | 174/176 | 106/114 |
| Gironenc Vermellet | 179/179 | 256/258 | 112/120 | 185/189 | 176/182 | 120/126 |

**Table D.1**

| Cultivar | CaT-B507 | CaT-B508 | Cat-C504 |
|---|---|---|---|
| Comen | 191/195 | 157/165 | 158/158 |
| Comun | 191/195 | 146/157 | 155/155 |
| Comun Aleva | 179/191 | 157/165 | 155/158 |
| Comune di Sici. | 181/191 | 157/157 | 158/167 |
| Contorta | 185/189 | 146/157 | 158/161 |
| Corabel | 181/195 | 157/161 | 152/158 |
| Cosford | 189/195 | 148/161 | 152/164 |
| Cozia | 189/189 | 144/161 | 158/161 |
| Creswell | 191/197 | 146/157 | 161/161 |
| Culpla | 191/197 | 144/157 | 152/152 |
| Cutleaf | 185/195 | 159/167 | 161/161 |
| Da Viega | 191/195 | 157/165 | 158/158 |
| Dal Rosso | 197/197 | 157/157 | 161/161 |
| D'Algers | 181/191 | 157/157 | 155/158 |
| Daria | 191/195 | 148/163 | 158/164 |
| Daviana #1 | 185/195 | 161/161 | 152/164 |
| Daviana #2 | 189/195 | 148/161 | 152/164 |
| Delta | 189/189 | 144/165 | 161/161 |
| Des Anglais | 185/189 | 153/161 | 152/152 |
| Dowton Long #1 | 185/189 | 157/159 | 158/167 |
| Dowton Long #2 | 185/195 | 153/163 | 158/161 |
| DuChilly | 185/189 | 157/165 | 164/164 |
| Early Long Zel. | 193/195 | 159/165 | 152/152 |
| Ennis | 185/191 | 157/157 | 152/155 |
| Epsilon | 190/197 | 146/157 | 152/158 |
| Espinaredo | 189/191 | 157/161 | 158/158 |
| Extra Ghiaghli | 189/197 | 157/163 | 161/161 |
| Finland | 179/193 | 150/165 | 152/158 |
| Fitzgerald | 181/195 | 157/157 | 152/155 |
| Fitzgerald #20 | 181/185 | 157/157 | 155/164 |
| Francoli | 191/191 | 157/159 | 161/161 |
| Frango #2 | 189/195 | 148/165 | 152/152 |
| Frango #4 | 195/195 | 144/144 | 152/158 |
| Frango #5 | 190/195 | 144/161 | 152/152 |
| Freehusker | 181/195 | 157/161 | 152/158 |
| Frizzled Filbert | 185/195 | 153/161 | 158/161 |
| Fructo Albo | 185/197 | 157/157 | 158/164 |
| Fruttogrosso | 191/195 | 157/167 | 155/158 |
| Fusco Rubra | 189/198 | 165/165 | 152/164 |
| Gamma | 179/189 | 161/167 | 158/161 |
| Ganja | 191/198 | 144/157 | 152/158 |
| Garibaldi | 185/190 | 157/165 | 152/164 |
| Garrofi | 181/191 | 157/157 | 155/158 |
| Gasaway | 179/190 | 146/165 | 161/164 |
| Gem | 181/185 | 157/157 | 158/164 |
| Ghirara | 191/195 | 146/157 | 155/158 |
| Gironenc Coldejou | 179/191 | 157/165 | 155/158 |
| Gironenc Vermellet | 181/189 | 157/165 | 158/158 |

**Table D.1**

| Cultivar | CAC-A14a | CAC-A40 | CAC-B005 | CAC-B010 | CAC-B028 | CAC-B29b |
|---|---|---|---|---|---|---|
| Goc | 217/217 | 244/244 | 277/277 | 211/211 | 264/264 | 116/128 |
| Grande | 215/219 | 234/248 | 291/295 | 211/223 | 254/262 | 122/128 |
| Grifoll | 211/215 | 248/248 | 277/291 | 219/227 | 256/262 | 122/124 |
| Gubener Barcelloner | 211/215 | 236/242 | 277/295 | 223/225 | 256/278 | 116/118 |
| Gunslebert | 215/215 | 244/246 | 277/295 | 223/227 | 262/262 | 116/118 |
| Gustav's Zellernuss | 215/215 | 236/246 | 277/277 | 221/227 | 256/264 | 128/128 |
| Hall's Giant | 215/215 | 244/246 | 277/279 | 221/227 | 256/264 | 116/128 |
| Henneman #3 | 213/213 | 244/248 | 279/279 | 219/225 | 264/270 | 116/137 |
| Heynick's Zell. | 215/215 | 236/246 | 277/277 | 221/227 | 256/264 | 128/128 |
| Iannusa Racinante | 215/219 | 244/244 | 277/291 | 219/227 | 254/256 | 122/122 |
| Imp. de Trebizonde #1 | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| Imp. de Trebizonde #2 | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| Imperatrice Eugenie | 217/217 | 236/244 | 281/281 | 211/211 | 268/270 | 124/128 |
| Istarski Debeloplodna #1 | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| Istarski Debeloplodna #2 | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| Istarski Okrogloplodna | 211/217 | 236/248 | 277/281 | 219/223 | 268/278 | 122/124 |
| Istrski Duguljasti | 211/217 | 244/248 | 281/291 | 211/225 | 260/268 | 116/122 |
| Italian Red | 215/215 | 236/246 | 277/277 | 221/227 | 256/264 | 128/128 |
| Jean's | 215/217 | 244/248 | 277/279 | 219/223 | 262/268 | 116/116 |
| Jeeve's Samling | 215/215 | 244/246 | 279/281 | 227/227 | 256/256 | 116/116 |
| Kadetten Zellernuss | 215/215 | 236/246 | 277/281 | 211/227 | 256/256 | 116/128 |
| Kalinkara | 211/221 | 236/244 | 279/279 | 219/221 | 262/266 | 116/122 |
| Karidaty #1 | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| Karidaty #2 | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| Karol | 215/215 | 236/246 | 277/277 | 211/221 | 256/256 | 116/116 |
| Kerasund Dlin. | 217/217 | 244/244 | 279/291 | 219/225 | 262/268 | 116/122 |
| Korthaset Zellernuss | 213/217 | 244/244 | 281/281 | 211/223 | 254/268 | 124/137 |
| Kruse | 215/219 | 236/248 | 291/295 | 219/219 | 254/262 | 122/122 |
| Kudryavchik #1 | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 |
| Kudryavchik #2 | 211/215 | 244/248 | 279/279 | 211/225 | 262/262 | 114/126 |
| Kunzemuller Z. | 215/215 | 244/246 | 279/281 | 227/227 | 256/256 | 116/116 |
| Lange Landsberger | 215/215 | 236/244 | 277/279 | 221/227 | 256/256 | 128/128 |
| Lech | 215/215 | 244/246 | 277/277 | 221/227 | 256/264 | 116/116 |
| Lenka #3 | 217/217 | 236/246 | 277/281 | 211/221 | 270/270 | 128/128 |
| Lenka #4 | 211/211 | 236/246 | 277/279 | 211/227 | 256/266 | 116/116 |
| Lewis | 215/217 | 236/244 | 279/291 | 219/223 | 262/270 | 122/122 |
| Liegel's Z. | 215/215 | 244/246 | 279/281 | 227/227 | 256/256 | 116/116 |
| Liegel's Zellernuss | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Little Poland | 217/217 | 236/244 | 277/277 | 211/227 | 270/270 | 116/116 |
| Lluenta | 211/211 | 236/244 | 277/291 | 219/223 | 256/258 | 128/130 |
| Louisen's Zellernuss | 217/217 | 246/248 | 277/281 | 223/227 | 256/268 | 116/124 |
| Ludolph's Zellernuss | 215/215 | 246/246 | 279/281 | 227/227 | 256/264 | 116/116 |
| Lyons | 215/217 | 234/244 | 281/291 | 223/223 | 262/268 | 122/137 |
| Macrocarpa | 215/219 | 236/248 | 291/295 | 219/219 | 254/262 | 122/122 |
| Mansa | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Maria | 211/211 | 236/246 | 277/279 | 211/227 | 256/266 | 116/116 |
| Martorella | 211/213 | 236/248 | 277/277 | 223/227 | 256/256 | 122/130 |
| Mincane | 211/217 | 244/244 | 279/279 | 219/223 | 262/268 | 122/126 |
| Minnolara | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |

**Table D.1** Continued

| Cultivar | CAC-B105 | CAC-B111 | CAC-C010 | CAC-C28 | CAC-C040 | CAC-C115 |
|---|---|---|---|---|---|---|
| Goc | 148/155 | 182/182 | 275/275 | 141/141 | 186/186 | 173/214 |
| Grande | 153/155 | 182/182 | 275/278 | 131/141 | 186/186 | 173/193 |
| Grifoll | 155/155 | 182/184 | 275/275 | 131/144 | 186/186 | 182/185 |
| Gubener Barcelloner | 155/157 | 182/184 | 275/275 | 131/141 | 186/186 | 196/214 |
| Gunslebert | 140/155 | 184/186 | 275/275 | 141/141 | 186/186 | 196/214 |
| Gustav's Zellernuss | 138/140 | 186/188 | 275/275 | 131/141 | 186/186 | 214/214 |
| Hall's Giant | 140/148 | 186/188 | 275/275 | 134/141 | 186/186 | 193/214 |
| Henneman #3 | 148/155 | 182/186 | 278/278 | 134/141 | 186/186 | 173/214 |
| Heynick's Zell. | 138/140 | 186/188 | 275/275 | 131/141 | 186/186 | 214/214 |
| Iannusa Racinante | 155/155 | 182/182 | 278/278 | 131/141 | 183/186 | 193/196 |
| Imp. de Trebizonde #1 | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| Imp. de Trebizonde #2 | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| Imperatrice Eugenie | 148/155 | 182/186 | 278/281 | 131/141 | 186/186 | 193/193 |
| Istarski Debeloplodna #1 | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| Istarski Debeloplodna #2 | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| Istarski Okrogloplodna | 155/155 | 182/188 | 275/278 | 141/141 | 186/186 | 173/196 |
| Istrski Duguljasti | 142/155 | 186/186 | 275/281 | 131/141 | 186/192 | 173/193 |
| Italian Red | 138/140 | 186/188 | 275/275 | 131/141 | 186/186 | 214/214 |
| Jean's | 140/155 | 170/186 | 275/281 | 131/141 | 186/186 | 173/193 |
| Jeeve's Samling | 140/140 | 186/186 | 278/278 | 131/141 | 183/186 | 214/225 |
| Kadetten Zellernuss | 138/140 | 184/186 | 275/275 | 131/141 | 183/186 | 214/214 |
| Kalinkara | 146/155 | 182/182 | 275/278 | 141/144 | 186/189 | 176/182 |
| Karidaty #1 | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| Karidaty #2 | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| Karol | 148/155 | 186/186 | 275/275 | 131/134 | 186/186 | 193/193 |
| Kerasund Dlin. | 155/155 | 184/186 | 275/278 | 131/134 | 186/186 | 173/193 |
| Korthaset Zellernuss | 140/155 | 186/186 | 278/281 | 131/144 | 186/186 | 173/193 |
| Kruse | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 | 196/196 |
| Kudryavchik #1 | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 | 173/182 |
| Kudryavchik #2 | 142/155 | 170/188 | 275/275 | 134/141 | 186/189 | 167/193 |
| Kunzemuller Z. | 140/140 | 186/186 | 278/278 | 131/141 | 183/186 | 214/225 |
| Lange Landsberger | 140/140 | 186/188 | 275/275 | 131/141 | 186/186 | 214/214 |
| Lech | 140/140 | 182/188 | 275/275 | 131/144 | 186/186 | 193/214 |
| Lenka #3 | 140/153 | 182/186 | 278/278 | 141/141 | 186/186 | 193/214 |
| Lenka #4 | 140/153 | 182/188 | 278/278 | 131/144 | 186/186 | 214/214 |
| Lewis | 155/155 | 182/182 | 278/278 | 131/134 | 186/186 | 193/193 |
| Liegel's Z. | 140/140 | 186/186 | 278/278 | 131/141 | 183/186 | 214/225 |
| Liegel's Zellernuss | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Little Poland | 140/153 | 182/184 | 278/278 | 131/144 | 186/186 | 193/193 |
| Lluenta | 153/155 | 182/182 | 275/275 | 131/131 | 186/186 | 182/205 |
| Louisen's Zellernuss | 140/155 | 184/188 | 275/275 | 141/141 | 183/186 | 196/214 |
| Ludolph's Zellernuss | 138/140 | 186/186 | 275/275 | 131/134 | 186/186 | 193/214 |
| Lyons | 153/155 | 182/186 | 278/281 | 131/131 | 186/186 | 193/193 |
| Macrocarpa | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 | 196/196 |
| Mansa | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Maria | 140/153 | 182/188 | 278/278 | 131/144 | 186/186 | 214/214 |
| Martorella | 140/155 | 182/182 | 275/275 | 131/134 | 186/192 | 173/214 |
| Mincane | 144/155 | 170/186 | 275/281 | 141/144 | 186/186 | 182/193 |
| Minnolara | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |

**Table D.1** Continued

| Cultivar | CAC-C118 | CAC-C119 | CaT-B107 | CaT-B502 | CaT-B504 | CaT-B505 |
|---|---|---|---|---|---|---|
| Goc | 179/179 | 256/264 | 130/134 | 187/201 | 172/174 | 106/116 |
| Grande | 179/179 | 258/258 | 112/134 | 187/189 | 158/182 | 106/120 |
| Grifoll | 179/179 | 258/258 | 120/122 | 189/189 | 162/182 | 118/126 |
| Gubener Barcelloner | 179/179 | 258/258 | 112/130 | 187/199 | 158/176 | 118/118 |
| Gunslebert | 179/179 | 258/264 | 112/122 | 183/187 | 158/174 | 118/118 |
| Gustav's Zellernuss | 179/179 | 258/264 | 128/130 | 187/209 | 174/176 | 114/118 |
| Hall's Giant | 179/179 | 264/264 | 122/130 | 187/201 | 170/174 | 120/120 |
| Henneman #3 | 179/179 | 258/260 | 120/134 | 187/195 | 168/176 | 114/126 |
| Heynick's Zell. | 179/179 | 258/264 | 128/130 | 187/209 | 174/176 | 114/118 |
| Iannusa Racinante | 176/179 | 258/258 | 112/132 | 183/189 | 176/182 | 118/120 |
| Imp. de Trebizonde #1 | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| Imp. de Trebizonde #2 | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| Imperatrice Eugenie | 179/179 | 258/264 | 122/134 | 187/189 | 176/176 | 106/106 |
| Istarski Debeloplodna #1 | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| Istarski Debeloplodna #2 | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| Istarski Okrogloplodna | 179/179 | 258/260 | 122/134 | 183/187 | 174/176 | 114/126 |
| Istrski Duguljasti | 179/185 | 258/258 | 122/134 | 187/189 | 176/182 | 106/118 |
| Italian Red | 179/179 | 258/264 | 128/130 | 187/209 | 174/176 | 114/118 |
| Jean's | 179/179 | 258/258 | 120/122 | 183/187 | 168/178 | 126/128 |
| Jeeve's Samling | 176/179 | 258/264 | 122/128 | 187/195 | 168/176 | 114/118 |
| Kadetten Zellernuss | 176/179 | 258/264 | 122/128 | 187/209 | 176/176 | 106/114 |
| Kalinkara | 179/182 | 258/258 | 128/128 | 187/187 | 168/172 | 114/126 |
| Karidaty #1 | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| Karidaty #2 | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| Karol | 179/179 | 258/264 | 122/134 | 189/201 | 172/174 | 116/118 |
| Kerasund Dlin. | 179/179 | 258/258 | 130/130 | 187/189 | 168/182 | 118/126 |
| Korthaset Zellernuss | 179/179 | 258/264 | 122/130 | 189/195 | 176/176 | 106/114 |
| Kruse | 179/179 | 258/258 | 118/134 | 183/189 | 158/182 | 114/126 |
| Kudryavchik #1 | 179/179 | 258/260 | 116/134 | 187/187 | 168/182 | 118/126 |
| Kudryavchik #2 | 179/182 | 258/260 | 120/124 | 183/187 | 168/168 | 114/114 |
| Kunzemuller Z. | 176/179 | 258/264 | 122/128 | 187/195 | 168/176 | 114/118 |
| Lange Landsberger | 179/179 | 264/264 | 128/130 | 187/187 | 170/176 | 114/118 |
| Lech | 179/179 | 264/264 | 122/122 | 187/195 | 174/176 | 106/118 |
| Lenka #3 | 179/179 | 258/264 | 122/134 | 187/187 | 174/176 | 106/118 |
| Lenka #4 | 179/179 | 258/264 | 122/144 | 187/195 | 170/172 | 106/118 |
| Lewis | 179/179 | 258/258 | 134/144 | 187/189 | 168/182 | 120/126 |
| Liegel's Z. | 176/179 | 258/264 | 122/128 | 187/195 | 168/176 | 114/118 |
| Liegel's Zellernuss | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Little Poland | 179/179 | 258/258 | 128/134 | 187/195 | 172/174 | 106/116 |
| Lluenta | 179/179 | 258/258 | 120/134 | 189/189 | 176/182 | 120/126 |
| Louisen's Zellernuss | 176/179 | 258/260 | 122/134 | 187/187 | 174/176 | 114/114 |
| Ludolph's Zellernuss | 179/179 | 264/264 | 128/130 | 187/209 | 170/176 | 114/118 |
| Lyons | 179/179 | 258/264 | 122/134 | 189/189 | 176/182 | 114/120 |
| Macrocarpa | 179/179 | 258/258 | 118/134 | 183/189 | 158/182 | 114/126 |
| Mansa | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Maria | 179/179 | 258/264 | 122/144 | 187/195 | 170/172 | 106/118 |
| Martorella | 179/185 | 258/258 | 120/122 | 185/195 | 170/178 | 114/122 |
| Mincane | 179/179 | 258/258 | 134/134 | 187/187 | 168/168 | 126/128 |
| Minnolara | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |

**Table D.1** Continued

| Cultivar | CaT-B507 | CaT-B508 | Cat-C504 |
|---|---|---|---|
| Goc | 197/197 | 148/157 | 155/164 |
| Grande | 181/191 | 157/157 | 155/158 |
| Grifoll | 185/195 | 159/159 | 158/161 |
| Gubener Barcelloner | 179/181 | 159/159 | 155/164 |
| Gunslebert | 181/189 | 157/165 | 152/152 |
| Gustav's Zellernuss | 190/195 | 144/165 | 152/164 |
| Hall's Giant | 189/189 | 144/161 | 152/164 |
| Henneman #3 | 179/197 | 157/161 | 161/161 |
| Heynick's Zell. | 190/195 | 144/165 | 152/164 |
| Iannusa Racinante | 181/191 | 146/157 | 152/158 |
| Imp. de Trebizonde #1 | 187/191 | 157/159 | 158/161 |
| Imp. de Trebizonde #2 | 187/191 | 157/159 | 158/161 |
| Imperatrice Eugenie | 185/189 | 148/157 | 152/164 |
| Istarski Debeloplodna #1 | 185/197 | 157/157 | 158/164 |
| Istarski Debeloplodna #2 | 185/197 | 146/157 | 158/164 |
| Istarski Okrogloplodna | 191/195 | 157/167 | 155/158 |
| Istrski Duguljasti | 197/197 | 157/157 | 164/164 |
| Italian Red | 190/195 | 144/165 | 152/164 |
| Jean's | 185/195 | 153/153 | 158/158 |
| Jeeve's Samling | 195/195 | 144/159 | 152/164 |
| Kadetten Zellernuss | 193/195 | 159/165 | 152/152 |
| Kalinkara | 185/197 | 150/157 | 158/161 |
| Karidaty #1 | 187/191 | 157/159 | 158/161 |
| Karidaty #2 | 187/191 | 157/159 | 158/161 |
| Karol | 185/190 | 161/161 | 152/152 |
| Kerasund Dlin. | 179/197 | 157/157 | 161/161 |
| Korthaset Zellernuss | 185/189 | 157/165 | 164/164 |
| Kruse | 179/185 | 157/167 | 155/158 |
| Kudryavchik #1 | 189/197 | 157/163 | 161/161 |
| Kudryavchik #2 | 191/193 | 150/157 | 161/161 |
| Kunzemuller Z. | 195/195 | 144/159 | 152/164 |
| Lange Landsberger | 189/195 | 159/161 | 152/164 |
| Lech | 181/190 | 144/165 | 152/152 |
| Lenka #3 | 190/195 | 161/161 | 152/164 |
| Lenka #4 | 189/195 | 161/161 | 152/152 |
| Lewis | 181/191 | 157/161 | 155/158 |
| Liegel's Z. | 195/195 | 144/159 | 152/164 |
| Liegel's Zellernuss | 181/191 | 157/157 | 158/167 |
| Little Poland | 190/195 | 144/161 | 152/152 |
| Lluenta | 189/191 | 157/157 | 158/158 |
| Louisen's Zellernuss | 191/195 | 157/159 | 152/155 |
| Ludolph's Zellernuss | 189/195 | 144/165 | 152/164 |
| Lyons | 181/185 | 157/157 | 158/164 |
| Macrocarpa | 179/185 | 157/167 | 155/158 |
| Mansa | 181/191 | 157/157 | 158/167 |
| Maria | 189/195 | 161/161 | 152/152 |
| Martorella | 185/195 | 157/159 | 161/164 |
| Mincane | 191/197 | 150/157 | 158/158 |
| Minnolara | 181/191 | 157/157 | 158/167 |

**Table D.1** Continued

| Cultivar | CAC-A14a | CAC-A40 | CAC-B005 | CAC-B010 | CAC-B028 | CAC-B29b |
|---|---|---|---|---|---|---|
| Ratoli | 215/217 | 236/248 | 277/291 | 211/221 | 262/268 | 122/122 |
| Red Filbert | 211/213 | 236/244 | 281/281 | 211/223 | 254/266 | 124/128 |
| Red Fortrin | 215/217 | 234/244 | 281/295 | 211/211 | 262/264 | 128/128 |
| Restiello | 211/217 | 236/248 | 291/297 | 219/223 | 256/268 | 122/130 |
| Ribet | 215/215 | 246/248 | 277/281 | 211/221 | 256/262 | 122/122 |
| Riccia di Talanico | 215/219 | 242/244 | 277/291 | 219/223 | 254/262 | 122/122 |
| Riekchen's Zellernuss #2 | 215/215 | 236/246 | 277/281 | 211/227 | 256/256 | 116/128 |
| Riekchen's Zellernuss #1 | 215/215 | 236/246 | 277/281 | 211/227 | 256/256 | 116/128 |
| Rimsky | 217/217 | 246/248 | 277/281 | 223/227 | 256/268 | 116/124 |
| Rode Zeller | 211/217 | 236/244 | 277/281 | 211/219 | 264/266 | 116/128 |
| Romai | 211/217 | 236/248 | 277/281 | 219/223 | 268/278 | 122/124 |
| Romavel | 215/215 | 243/248 | 279/279 | 211/219 | 262/262 | 122/128 |
| Romische Nuss | 211/217 | 236/248 | 277/281 | 219/223 | 268/278 | 122/124 |
| Ros de la Selva | 211/215 | 236/248 | 277/291 | 211/211 | 262/262 | 122/122 |
| Rosetta | 213/215 | 236/244 | 277/281 | 211/211 | 256/262 | 116/122 |
| Rosset de Valls | 215/215 | 234/246 | 277/291 | 211/227 | 256/256 | 118/128 |
| Royal | 217/219 | 236/248 | 277/291 | 211/211 | 254/270 | 116/122 |
| Ruby | 217/251 | 236/236 | 279/281 | 211/217 | 264/264 | 118/130 |
| Ryan | 217/219 | 234/236 | 277/291 | 211/223 | 254/254 | 116/122 |
| San Benedetto | 211/215 | 238/244 | 279/279 | 211/219 | 262/262 | 116/130 |
| San Giovanni | 213/215 | 234/248 | 291/295 | 219/223 | 254/262 | 116/122 |
| Sant Jaume | 211/219 | 236/248 | 281/291 | 219/223 | 254/258 | 122/122 |
| Sant Joan | 211/215 | 236/244 | 277/291 | 219/223 | 256/262 | 122/128 |
| Sant Pere | 211/217 | 248/248 | 277/291 | 219/219 | 256/256 | 130/135 |
| Segorbe | 211/211 | 236/248 | 277/281 | 211/219 | 266/278 | 122/135 |
| Siciliana | 215/219 | 244/244 | 291/295 | 219/227 | 254/262 | 122/122 |
| Sickler's Zel. | 215/215 | 236/244 | 277/281 | 211/221 | 256/256 | 116/128 |
| Simon | 211/215 | 234/236 | 295/297 | 211/223 | 256/262 | 122/130 |
| Sivri Ghiaghli | 211/211 | 244/244 | 279/291 | 219/225 | 262/262 | 116/122 |
| Sivri Ocak 5 | 217/217 | 244/244 | 279/291 | 219/225 | 262/268 | 116/122 |
| Skorspelka | 213/217 | 236/244 | 279/279 | 211/219 | 258/262 | 116/116 |
| Syrena | 215/217 | 244/244 | 279/281 | 211/221 | 256/264 | 116/128 |
| Tapparona di SCC | 211/215 | 244/248 | 277/291 | 211/223 | 262/276 | 118/122 |
| Tonda Gentile d. Langhe | 215/221 | 234/244 | 277/291 | 219/219 | 256/262 | 118/122 |
| The Shah | 211/213 | 236/244 | 277/281 | 211/223 | 254/264 | 124/124 |
| Tokolyi Cosford | 211/211 | 242/248 | 279/295 | 219/223 | 268/268 | 116/118 |
| Tomasina | 211/213 | 236/244 | 277/291 | 211/219 | 256/256 | 118/118 |
| Tombul | 211/217 | 244/244 | 279/279 | 219/223 | 262/268 | 122/126 |
| Tombul Ghiaghli | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 |
| Tombul Ocak 1 | 211/215 | 244/244 | 279/291 | 211/225 | 262/262 | 116/130 |
| Tonda Bianca | 211/219 | 236/242 | 277/291 | 219/223 | 254/278 | 118/122 |
| Tonda di Giffoni | 211/215 | 236/248 | 291/295 | 219/223 | 262/278 | 118/122 |
| Tonda Romana | 211/217 | 238/248 | 277/291 | 211/219 | 262/268 | 122/122 |
| Tonda Rossa | 211/215 | 242/244 | 277/295 | 219/223 | 268/278 | 122/122 |
| Tonnolella | 211/215 | 236/244 | 291/295 | 219/223 | 262/266 | 118/128 |
| Trenet | 217/219 | 234/248 | 277/291 | 211/219 | 256/262 | 122/122 |
| Truchsess | 219/219 | 236/244 | 277/277 | 221/227 | 256/256 | 128/128 |
| Turk | 215/219 | 236/248 | 291/295 | 219/219 | 254/262 | 122/122 |
| Ugbrooke | 211/211 | 236/244 | 277/279 | 219/221 | 256/256 | 124/128 |

**Table D.1** Continued

| Cultivar | CAC-B105 | CAC-B111 | CAC-C010 | CAC-C28 | CAC-C040 | CAC-C115 |
|---|---|---|---|---|---|---|
| Ratoli | 140/155 | 182/182 | 275/275 | 131/144 | 186/186 | 173/182 |
| Red Filbert | 153/155 | 182/186 | 278/278 | 131/131 | 186/186 | 193/214 |
| Red Fortrin | 153/155 | 182/182 | 275/275 | 141/141 | 186/189 | 173/214 |
| Restiello | 138/155 | 182/184 | 275/275 | 131/144 | 186/192 | 182/214 |
| Ribet | 151/155 | 182/182 | 278/278 | 134/141 | 186/186 | 173/214 |
| Riccia di Talanico | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 | 182/196 |
| Riekchen's Zellernuss #2 | 138/148 | 186/188 | 275/275 | 131/134 | 183/186 | 193/214 |
| Riekchen's Zellernuss #1 | 138/148 | 186/188 | 275/275 | 131/134 | 183/186 | 193/214 |
| Rimsky | 140/155 | 184/188 | 275/275 | 141/141 | 183/186 | 196/214 |
| Rode Zeller | 153/155 | 182/184 | 275/278 | 131/141 | 186/189 | 193/214 |
| Romai | 155/155 | 182/188 | 275/278 | 141/141 | 186/186 | 173/196 |
| Romavel | 153/155 | 182/186 | 278/278 | 131/134 | 186/186 | 173/193 |
| Romische Nuss | 155/155 | 182/188 | 275/278 | 141/141 | 186/186 | 173/196 |
| Ros de la Selva | 155/159 | 182/182 | 278/278 | 134/141 | 186/186 | 173/193 |
| Rosetta | 155/155 | 182/182 | 278/278 | 131/134 | 186/186 | 173/193 |
| Rosset de Valls | 140/153 | 182/182 | 275/275 | 131/141 | 186/186 | 193/193 |
| Royal | 153/153 | 182/182 | 278/278 | 131/144 131 | 186/186 | 193/193 |
| Ruby | 136/144 | 184/184 | 278/278 | 134/147 | 186/192 | 188/214 |
| Ryan | 153/155 | 182/182 | 275/275 | 131/141 | 186/186 | 173/193 |
| San Benedetto | 155/155 | 182/184 | 281/281 | 131/141 | 186/186 | 173/196 |
| San Giovanni | 148/155 | 182/182 | 275/275 | 131/131 | 186/186 | 173/196 |
| Sant Jaume | 140/153 | 182/182 | 275/278 | 131/141 | 186/189 | 173/214 |
| Sant Joan | 148/155 | 182/182 | 275/275 | 131/141 | 186/189 | 182/214 |
| Sant Pere | 138/144 | 182/184 | 275/275 | 131/134 | 186/186 | 173/182 |
| Segorbe | 155/155 | 182/182 | 275/275 | 141/141 | 186/186 | 173/196 |
| Siciliana | 155/155 | 182/188 | 278/278 | 131/141 | 186/186 | 173/196 |
| Sickler's Zel. | 140/140 | 186/188 | 275/275 | 141/141 | 183/183 | 193/214 |
| Simon | 155/155 | 182/182 | 275/275 | 131/134 | 186/186 | 182/193 |
| Sivri Ghiaghli | 155/155 | 182/186 | 278/281 | 131/141 | 186/186 | 173/182 |
| Sivri Ocak 5 | 155/155 | 184/186 | 275/278 | 131/134 | 186/186 | 173/193 |
| Skorspelka | 144/157 | 170/186 | 275/275 | 131/141 | 186/186 | 176/182 |
| Syrena | 140/159 | 184/186 | 275/275 | 141/141 | 186/186 | 193/214 |
| Tapparona di SCC | 155/159 | 182/182 | 278/278 | 131/137 | 186/192 | 173/185 |
| Tonda Gentile d. Langhe | 155/155 | 182/182 | 275/275 | 131/131 | 186/186 | 173/173 |
| The Shah | 155/155 | 186/188 | 278/278 | 131/134 | 186/186 | 173/205 |
| Tokolyi Cosford | 155/155 | 184/186 | 275/275 | 131/141 | 186/186 | 193/214 |
| Tomasina | 138/140 | 184/186 | 275/275 | 131/134 | 186/192 | 173/214 |
| Tombul | 144/155 | 170/186 | 275/281 | 141/144 | 186/186 | 182/193 |
| Tombul Ghiaghli | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 | 173/182 |
| Tombul Ocak 1 | 155/155 | 184/186 | 275/275 | 134/141 | 186/186 | 182/193 |
| Tonda Bianca | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 | 196/214 |
| Tonda di Giffoni | 155/155 | 182/182 | 275/275 | 141/141 | 186/186 | 173/214 |
| Tonda Romana | 155/161 | 182/182 | 275/275 | 131/141 | 183/186 | 173/196 |
| Tonda Rossa | 155/155 | 184/184 | 275/275 | 141/141 | 186/186 | 196/214 |
| Tonnolella | 140/155 | 182/182 | 275/275 | 134/141 | 186/186 | 214/214 |
| Trenet | 138/140 | 182/182 | 275/278 | 131/134 | 186/186 | 173/193 |
| Truchsess | 138/148 | 186/186 | 275/275 | 131/134 | 183/186 | 214/214 |
| Turk | 155/155 | 182/184 | 275/275 | 131/141 | 186/186 | 196/196 |
| Ugbrooke | 148/155 | 178/186 | 275/275 | 131/141 | 186/186 | 193/214 |

**Table D.1** Continued

| Cultivar | CAC-C118 | CAC-C119 | CaT-B107 | CaT-B502 | CaT-B504 | CaT-B505 |
|---|---|---|---|---|---|---|
| Ratoli | 179/179 | 258/264 | 130/134 | 185/189 | 172/182 | 106/114 |
| Red Filbert | 179/179 | 258/264 | 122/144 | 189/195 | 176/176 | 106/116 |
| Red Fortrin | 179/182 | 258/264 | 134/134 | 187/187 | 158/176 | 106/106 |
| Restiello | 179/185 | 258/258 | 120/134 | 189/189 | 176/182 | 114/126 |
| Ribet | 179/179 | 258/260 | 122/134 | 185/187 | 174/176 | 106/114 |
| Riccia di Talanico | 179/179 | 258/258 | 118/134 | 187/197 | 168/182 | 118/126 |
| Riekchen's Zellernuss #2 | 176/179 | 264/264 | 128/130 | 201/209 | 174/176 | 106/118 |
| Riekchen's Zellernuss #1 | 176/179 | 264/264 | 128/130 | 201/209 | 174/176 | 106/118 |
| Rimsky | 176/179 | 258/260 | 122/134 | 187/187 | 174/176 | 114/114 |
| Rode Zeller | 179/182 | 256/264 | 130/134 | 187/195 | 168/176 | 106/126 |
| Romai | 179/179 | 258/260 | 122/134 | 183/187 | 174/176 | 114/126 |
| Romavel | 179/179 | 258/258 | 134/134 | 187/189 | 168/168 | 114/126 |
| Romische Nuss | 179/179 | 258/260 | 122/134 | 183/187 | 174/176 | 114/126 |
| Ros de la Selva | 179/179 | 258/258 | 120/134 | 189/189 | 162/182 | 106/114 |
| Rosetta | 179/179 | 258/258 | 122/142 | 183/189 | 176/176 | 106/122 |
| Rosset de Valls | 179/179 | 258/264 | 122/134 | 187/195 | 176/182 | 106/114 |
| Royal | 179/179 | 258/258 | 112/132 154 | 189/195 | 172/182 | 106/116 |
| Ruby | 179/185 | 260/264 | 120/122 | 191/195 | 164/176 | 106/122 |
| Ryan | 179/179 | 258/258 | 134/134 | 187/195 | 172/182 | 116/120 |
| San Benedetto | 179/179 | 258/260 | 134/134 | 187/189 | 168/168 | 106/126 |
| San Giovanni | 179/179 | 258/258 | 118/134 | 189/211 | 158/182 | 118/126 |
| Sant Jaume | 179/182 | 258/264 | 112/134 | 187/189 | 176/182 | 120/120 |
| Sant Joan | 179/182 | 258/260 | 122/130 | 187/189 | 176/182 | 120/126 |
| Sant Pere | 179/179 | 258/258 | 118/134 | 189/201 | 170/182 | 118/126 |
| Segorbe | 179/179 | 258/264 | 112/134 | 183/187 | 174/176 | 106/114 |
| Siciliana | 179/179 | 258/260 | 112/122 | 183/189 | 158/182 | 120/126 |
| Sickler's Zel. | 176/179 | 258/264 | 122/122 | 187/187 | 174/176 | 106/118 |
| Simon | 179/179 | 258/258 | 126/134 | 187/189 | 158/176 | 114/120 |
| Sivri Ghiaghli | 179/179 | 258/260 | 116/116 | 187/199 | 168/182 | 118/126 |
| Sivri Ocak 5 | 179/179 | 258/258 | 130/130 | 187/189 | 168/182 | 118/126 |
| Skorspelka | 179/179 | 258/264 | 120/120 | 189/197 | 168/180 | 106/106 |
| Syrena | 179/179 | 264/264 | 130/134 | 187/187 | 170/176 | 116/118 |
| Tapparona di SCC | 179/185 | 258/264 | 122/124 | 185/187 | 162/182 | 106/126 |
| Tonda Gentile d. Langhe | 179/179 | 258/258 | 134/151 | 185/189 | 170/182 | 114/126 |
| The Shah | 179/179 | 258/258 | 120/130 | 189/189 | 168/176 | 114/114 |
| Tokolyi Cosford | 179/179 | 258/258 | 118/134 | 187/197 | 158/168 | 118/126 |
| Tomasina | 179/185 | 264/264 | 120/134 | 195/195 | 170/182 | 106/122 |
| Tombul | 179/179 | 258/258 | 134/134 | 187/187 | 168/168 | 126/128 |
| Tombul Ghiaghli | 179/179 | 260/260 | 116/134 | 187/187 | 168/182 | 118/126 |
| Tombul Ocak 1 | 162/179 | 260/260 | 116/116 | 187/187 | 168/182 | 106/118 |
| Tonda Bianca | 179/179 | 258/258 | 112/118 | 183/197 | 158/168 | 114/118 |
| Tonda di Giffoni | 179/179 | 258/258 | 118/134 | 183/187 | 158/182 | 118/126 |
| Tonda Romana | 176/179 | 258/258 | 134/142 | 187/191 | 160/182 | 118/126 |
| Tonda Rossa | 179/179 | 258/258 | 112/118 | 183/183 | 158/174 | 114/114 |
| Tonnolella | 179/179 | 258/258 | 118/120 | 183/199 | 158/182 | 126/128 |
| Trenet | 179/179 | 258/264 | 130/134 | 185/189 | 176/182 | 106/126 |
| Truchsess | 176/179 | 258/264 | 128/130 | 201/209 | 174/176 | 114/118 |
| Turk | 179/179 | 258/258 | 118/134 | 183/189 | 158/182 | 114/126 |
| Ugbrooke | 179/179 | 260/260 | 122/134 | 187/199 | 168/174 | 114/126 |

**Table D.1**

| Cultivar | CaT-B507 | CaT-B508 | Cat-C504 |
|---|---|---|---|
| Ratoli | 189/191 | 157/165 | 164/164 |
| Red Filbert | 185/189 | 157/161 | 164/164 |
| Red Fortrin | 189/191 | 157/157 | 158/164 |
| Restiello | 191/195 | 157/159 | 161/161 |
| Ribet | 191/195 | 157/165 | 158/158 |
| Riccia di Talanico | 181/185 | 157/167 | 155/158 |
| Riekchen's Zellernuss #2 | 189/195 | 144/159 | 152/152 |
| Riekchen's Zellernuss #1 | 189/195 | 144/159 | 152/152 |
| Rimsky | 191/195 | 157/159 | 152/155 |
| Rode Zeller | 189/197 | 148/157 | 158/164 |
| Romai | 191/195 | 157/167 | 155/158 |
| Romavel | 191/197 | 157/157 | 158/158 |
| Romische Nuss | 191/195 | 157/167 | 155/158 |
| Ros de la Selva | 188/191 | 146/157 | 158/158 |
| Rosetta | 191/193 | 146/157 | 158/158 |
| Rosset de Valls | 181/191 | 157/165 | 152/158 |
| Royal | 181/189 | 148/157 | 152/158 |
| Ruby | 176/198 | 144/165 | 152/152 |
| Ryan | 191/195 | 157/161 | 152/158 |
| San Benedetto | 191/197 | 157/167 | 158/161 |
| San Giovanni | 181/~~190~~ | 157/167 | 155/158 |
| Sant Jaume | 181/191 | 157/165 | 158/158 |
| Sant Joan | 185/189 | 157/165 | 164/164 |
| Sant Pere | 181/191 | 157/159 | 158/158 |
| Segorbe | 179/191 | 157/165 | 155/158 |
| Siciliana | 181/191 | 157/157 | 158/167 |
| Sickler's Zel. | 189/195 | 159/161 | 152/152 |
| Simon 181/191 | ~~190/195~~ | 157/157 | 161/161 |
| Sivri Ghiaghli | 179/197 | 157/163 | 158/161 |
| Sivri Ocak 5 | 179/197 | 157/157 | 161/161 |
| Skorspelka | 191/197 | 157/161 | 158/158 |
| Syrena | 189/197 | 148/157 | 152/164 |
| Tapparona di SCC | 181/185 | 163/163 | 158/158 |
| Tonda Gentile d. Langhe | 185/191 | 146/163 | 158/161 |
| The Shah | 185/189 | 157/165 | 158/164 |
| Tokolyi Cosford | 179/197 | 157/167 | 155/158 |
| Tomasina | 181/191 | 157/159 | 161/161 |
| Tombul | 191/197 | 150/157 | 158/158 |
| Tombul Ghiaghli | 189/197 | 157/163 | 158/161 |
| Tombul Ocak 1 | 189/197 | 157/163 | 161/161 |
| Tonda Bianca | 179/181 | 167/167 | 155/155 |
| Tonda di Giffoni | 181/191 | 157/157 | 155/158 |
| Tonda Romana | 185/189 | 157/157 | 152/158 |
| Tonda Rossa | 179/181 | 146/146 | 155/155 |
| Tonnolella | 191/195 | 157/163 | 167/167 |
| Trenet | 189/191 | 157/163 | 158/164 |
| Truchsess | 189/195 | 161/165 | 152/152 |
| Turk | 179/185 | 157/167 | 155/158 |
| Ugbrooke | 189/197 | 157/159 | 158/158 |

**Table D.1** Continued

| Cultivar | CAC-A14a | CAC-A40 | CAC-B005 | CAC-B010 | CAC-B028 | CAC-B29b |
|---|---|---|---|---|---|---|
| **Unknown #3** | 211/215 | 244/244 | 279/291 | 219/225 | 262/262 | 122/122 |
| **Vilcea 22** | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| **Volski Round** | 211/215 | 236/244 | 277/277 | 211/221 | 256/266 | 116/116 |
| **Wanliss Pride** | 215/217 | 244/244 | 279/291 | 211/219 | 262/268 | 116/122 |
| **Warsaw Red** | 213/219 | 248/248 | 279/295 | 223/225 | 254/270 | 116/128 |
| **Webb's Prize Cob** | 212/214 | 236/244 | 277/283 | 211/219 | 260/276 | 116/124 |
| **White Filbert** | 217/217 | 244/248 | 279/281 | 211/219 | 268/268 | 116/124 |
| **Whiteheart** | 217/217 | 244/248 | 281/295 | 219/219 | 262/268 | 116/130 |
| **Willamette** | 217/219 | 236/244 | 291/295 | 219/223 | 254/270 | 122/128 |
| **Woodford** | 217/219 | 248/248 | 281/291 | 211/211 | 254/270 | 116/122 |
| **Zeta** | 219/219 | 236/244 | 281/291 | 211/227 | 254/254 | 118/135 |
| **Zimmerman** | 215/219 | 234/236 | 277/291 | 211/219 | 254/278 | 122/135 |
| **26.072** | 215/215 | 244/248 | 277/281 | 211/225 | 254/254 | 126/139 |
| **54.021** | 215/221 | 244/244 | 279/279 | 211/221 | 262/266 | 116/130 |
| **54.039** | 209/215 | 244/244 | 279/291 | 225/225 | 254/262 | 118/130 |
| **54.041** | 211/215 | 236/244 | 277/279 | 219/225 | 262/262 | 116/122 |
| **54.056** | 211/215 | 238/244 | 279/279 | 219/225 | 262/266 | 116/130 |
| **408.04** | 212/217 | 236/236 | 277/277 | 223/225 | 256/256 | 122/122 |
| **495.049** | 217/217 | 238/244 | 277/281 | 219/219 | 262/274 | 118/128 |
| **495.072** | 215/215 | 236/236 | 279/295 | 225/227 | 254/264 | 120/124 |
| **556.019** | 215/215 | 244/244 | 289/291 | 219/219 | 262/274 | 122/122 |
| **556.027** | 215/215 | 236/244 | 279/283 | 219/223 | 256/262 | 120/122 |
| **622.051** | 211/211 | 236/244 | 277/279 | 211/221 | 262/264 | 116/118 |
| **681.074** | 211/219 | 244/244 | 279/279 | 219/225 | 256/262 | 122/126 |
| **681.078** | 211/213 | 236/244 | 277/279 | 219/221 | 256/266 | 114/114 |
| **686.124** | 215/217 | 244/244 | 285/287 | 223/223 | 262/262 | 116/135 |
| **693.073** | 211/217 | 236/244 | 277/291 | 211/221 | 262/262 | 116/130 |
| **693.117** | 213/215 | 238/244 | 277/291 | 219/219 | 262/270 | 118/122 |
| **702.041** | 211/215 | 244/244 | 277/291 | 219/225 | 262/262 | 118/122 |
| **717.087** | 211/215 | 244/244 | 277/279 | 219/221 | 262/270 | 116/122 |

**Table D.1** Continued

| Cultivar | CAC-B105 | CAC-B111 | CAC-C010 | CAC-C28 | CAC-C040 | CAC-C115 |
|---|---|---|---|---|---|---|
| **Unknown #3** | 155/155 | 182/184 | 281/281 | 134/141 | 186/186 | 173/182 |
| **Vilcea 22** | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| **Volski Round** | 148/148 | 182/188 | 275/278 | 134/141 | 186/186 | 193/214 |
| **Wanliss Pride** | 155/155 | 170/186 | 275/275 | 134/137 | 186/189 | 193/217 |
| **Warsaw Red** | 148/153 | 182/182 | 278/278 | 131/134 | 186/186 | 173/173 |
| **Webb's Prize Cob** | 155/157 | 184/186 | 275/275 | 131/131 | 186/186 | 193/225 |
| **White Filbert** | 155/155 | 186/186 | 281/281 | 131/141 | 186/186 | 173/193 |
| **Whiteheart** | 155/155 | 182/186 | 281/281 | 131/144 | 186/186 | 193/193 |
| **Willamette** | 153/155 | 182/188 | 278/278 | 131/131 | 186/186 | 193/196 |
| **Woodford** | 153/153 | 182/182 | 278/278 | 131/131 | 186/186 | 193/193 |
| **Zeta** | 155/155 | 182/182 | 275/278 | 131/141 | 186/192 | 173/196 |
| **Zimmerman** | 153/155 | 182/192 | 278/278 | 141/144 | 186/189 | 173/214 |
| **26.072** | 140/153 | 184/188 | 275/275 | 131/141 | 186/192 | 173/214 |
| **54.021** | 155/155 | 184/186 | 275/275 | 134/141 | 168/186 | 182/193 |
| **54.039** | 155/163 | 184/190 | 278/278 | 131/141 | 168/186 | 193/193 |
| **54.041** | 155/155 | 182/182 | 281/281 | 131/144 | 186/189 | 173/176 |
| **54.056** | 138/155 | 182/186 | 275/281 | 131/144 | 186/186 | 167/193 |
| **408.04** | 140/157 | 182/182 | 275/278 | 131/134 | 183/186 | 193/214 |
| **495.049** | 136/153 | 182/182 | 275/275 | 131/144 | 186/192 | 176/191 |
| **495.072** | 153/159 | 170/186 | 275/281 | 144/144 | 186/189 | 214/217 |
| **556.019** | 155/155 | 182/184 | 275/275 | 141/141 | 186/186 | 173/214 |
| **556.027** | 140/155 | 170/184 | 278/281 | 141/141 | 186/186 | 182/217 |
| **622.051** | 125/155 | 182/186 | 275/281 | 131/141 | 186/186 | 193/214 |
| **681.074** | 155/155 | 182/184 | 278/281 | 141/144 | 186/186 | 167/182 |
| **681.078** | 148/161 | 182/182 | 275/275 | 131/131 | 186/186 | 217/225 |
| **686.124** | 155/155 | 182/190 | 275/275 | 137/141 | 168/186 | 193/193 |
| **693.073** | 155/155 | 182/186 | 275/275 | 134/141 | 168/189 | 176/193 |
| **693.117** | 155/155 | 182/182 | 281/281 | 134/141 | 186/189 | 182/217 |
| **702.041** | 155/155 | 182/186 | 278/278 | 134/141 | 186/189 | 182/217 |
| **717.087** | 153/155 | 182/184 | 281/281 | 134/141 | 186/186 | 173/182 |

**Table D.1** Continued

| Cultivar | CAC-C118 | CAC-C119 | CaT-B107 | CaT-B502 | CaT-B504 | CaT-B505 |
|---|---|---|---|---|---|---|
| **Unknown #3** | 179/179 | 260/260 | 116/134 | 187/187 | 168/182 | 118/126 |
| **Vilcea 22** | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| **Volski Round** | 179/179 | 256/264 | 130/134 | 187/201 | 172/174 | 116/118 |
| **Wanliss Pride** | 179/182 | 258/258 | 120/120 | 183/187 | 168/184 | 114/126 |
| **Warsaw Red** | 179/179 | 258/260 | 120/134 | 189/195 | 158/168 | 106/114 |
| **Webb's Prize Cob** | 179/179 | 258/258 | 120/142 | 185/189 | 178/178 | 114/120 |
| **White Filbert** | 179/179 | 258/258 | 122/134 | 187/189 | 168/176 | 106/126 |
| **Whiteheart** | 179/179 | 258/258 | 112/134 | 187/189 | 158/176 | 118/126 |
| **Willamette** | 179/179 | 258/258 | 122/144 | 189/195 | 158/182 | 120/126 |
| **Woodford** | 179/179 | 258/258 | 134/134 | 189/195 | 176/182 | 106/106 |
| **Zeta** | 179/185 | 256/256 | 128/130 | 187/189 | 176/182 | 106/118 |
| **Zimmerman** | 179/182 | 258/264 | 128/134 | 187/195 | 174/182 | 106/114 |
| **26.072** | 179/185 | 258/264 | 120/128 | 183/187 | 174/176 | 118/128 |
| **54.021** | 162/179 | 260/260 | 120/120 | 187/187 | 168/172 | 106/114 |
| **54.039** | 162/179 | 258/260 | 134/134 | 183/187 | 168/182 | 118/118 |
| **54.041** | 179/182 | 258/258 | 130/130 | 187/189 | 172/178 | 118/126 |
| **54.056** | 179/179 | 258/258 | 122/134 | 189/195 | 168/168 | 118/126 |
| **408.04** | 176/179 | 260/260 | 120/130 | 183/187 | 168/172 | 114/116 |
| **495.049** | 179/185 | 258/262 | 132/132 | 187/189 | 170/176 | 120/122 |
| **495.072** | 179/182 | 258/260 | 126/126 | 185/187 | 164/174 | 118/120 |
| **556.019** | 179/179 | 258/260 | 124/134 | 183/187 | 176/182 | 126/126 |
| **556.027** | 179/179 | 258/260 | 120/134 | 187/187 | 162/168 | 126/128 |
| **622.051** | 179/179 | 258/258 | 122/134 | 187/187 | 168/174 | 106/114 |
| **681.074** | 179/179 | 258/264 | 116/120 | 187/193 | 168/168 | 114/126 |
| **681.078** | 179/179 | 258/258 | 120/122 | 189/209 | 168/176 | 116/116 |
| **686.124** | 162/179 | 258/258 | 120/134 | 183/187 | 176/180 | 106/114 |
| **693.073** | 162/182 | 258/260 | 120/120 | 187/187 | 176/182 | 106/118 |
| **693.117** | 179/182 | 260/260 | 116/124 | 187/187 | 168/182 | 126/126 |
| **702.041** | 179/182 | 260/260 | 116/124 | 187/187 | 168/182 | 126/126 |
| **717.087** | 179/179 | 258/258 | 116/134 | 187/209 | 168/176 | 118/126 |

**Table D.1** Continued

| Cultivar | CaT-B507 | CaT-B508 | Cat-C504 |
|---|---|---|---|
| **Unknown #3** | 189/197 | 157/163 | 158/161 |
| **Vilcea 22** | 187/191 | 157/159 | 158/161 |
| **Volski Round** | 189/189 | 144/161 | 152/164 |
| **Wanliss Pride** | 187/191 | 157/159 | 158/161 |
| **Warsaw Red** | 191/197 | 157/157 | 155/158 |
| **Webb's Prize Cob** | 185/195 | 157/157 | 164/164 |
| **White Filbert** | 185/197 | 157/157 | 158/164 |
| **Whiteheart** | 185/193 | 157/157 | 158/164 |
| **Willamette** | 189/191 | 157/161 | 167/167 |
| **Woodford** | 185/191 | 157/157 | 158/164 |
| **Zeta** | 189/189 | 146/157 | 158/158 |
| **Zimmerman** | 190/191 | 146/157 | 158/164 |
| **26.072** | 181/197 | 159/159 | 152/161 |
| **54.021** | 185/195 | 157/157 | 158/161 |
| **54.039** | 179/189 | 163/163 | 161/161 |
| **54.041** | 179/197 | 157/157 | 158/164 |
| **54.056** | 191/197 | 157/157 | 158/161 |
| **408.04** | 179/179 | 150/161 | 152/158 |
| **495.049** | 179/183 | 153/157 | 152/158 |
| **495.072** | 179/191 | 157/165 | 158/164 |
| **556.019** | 189/197 | 163/163 | 158/161 |
| **556.027** | 189/197 | 157/163 | 158/158 |
| **622.051** | 185/193 | 155/157 | 158/161 |
| **681.074** | 189/195 | 163/163 | 158/158 |
| **681.078** | 183/189 | 153/165 | 152/152 |
| **686.124** | 183/191 | 157/157 | 161/164 |
| **693.073** | 189/197 | 155/163 | 161/161 |
| **693.117** | 179/197 | 159/163 | 161/161 |
| **702.041** | 179/197 | 159/163 | 161/161 |
| **717.087** | 193/197 | 144/157 | 164/164 |